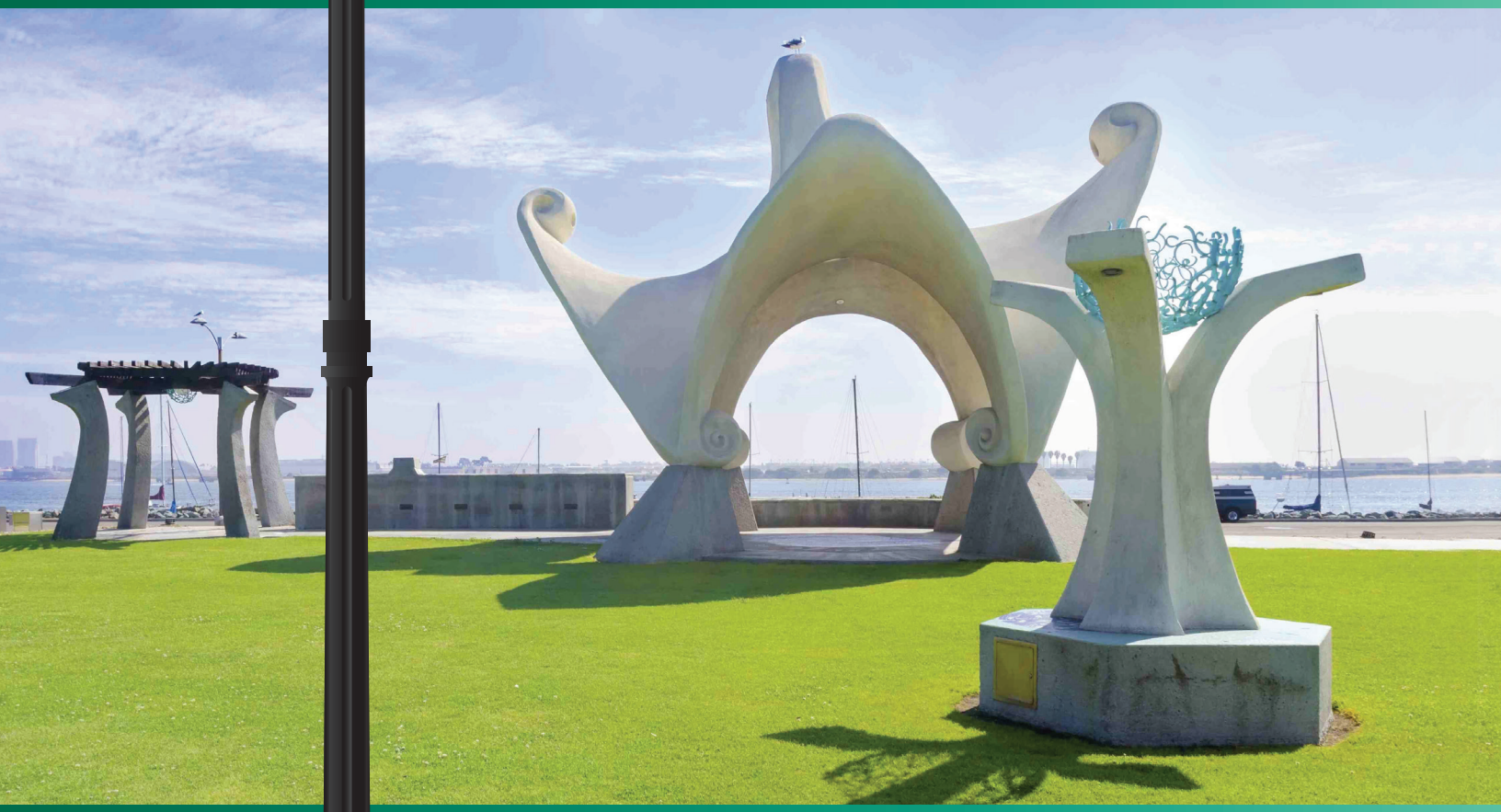
FOCiS® 2015

San Diego California

Immunology by the Bay **June 24-27, 2015**



Abstract Supplement

FOCiS® Federation of Clinical Immunology Societies www.focisnet.org

Federation of Clinical Immunology Societies
June 24 – 27, 2015
San Diego, California

FOCIS 2015
Abstract Supplement: Abstracts by Category

Table of Contents

Allergy/Asthma 2
Autoimmune Neurologic Disease 6
Autoimmune Rheumatologic Disease 15
Bone Marrow or Stem Cell Transplantation 33
Cytokines/Chemokines 36
Diabetes and Other Autoimmune Endocrine Diseases 41
General Autoimmunity 49
Genetics 58
Immune Monitoring 61
Immunity and Infection 70
Immunodefiency- Primary or Acquired 83
Immunodermatology 91
Immunology of the Eye 94
Immunoncology 95
Inflammatory Bowel Disease 103
Innate Immunity 105
Organ Transplantation 114
Reproductive Immunology 116
Therapeutics/Pharmacology 117
Transplantation 122
Other 127
Abstract Index by Subject Area 135
Abstract Index by FOCIS Pillar 137
Abstract Index by Author 138

★ = member of a FOCIS Center of Excellence
The FOCIS Centers of Excellence (FCE) provide an opportunity to build an interdisciplinary translational immunology community at your institution. Being a member of the FCE community offers an effective training environment for translational researchers and clinicians by promoting interdisciplinary innovation in research and clinical practices, and promoting new links for researchers and clinicians.

Allergy/Asthma

OR.11. Antibodies Induced by Peanut Oral Immunotherapy Suppress Heterologous IgE-mediated Degranulation when Expressed as IgG4

Johanna Steinbrecher[1], Sarita Patil[1], James Moon[1], J. Christopher Love[2] and Wayne Shreffler[1]. [1]★Massachusetts General Hospital, Boston, MA; [2]The Broad Institute of MIT and Harvard, Cambridge, MA

Rationale: In IgE-mediated peanut allergy, peanut oral immunotherapy (PNOIT) can increase clinical tolerance by incrementally increasing oral exposure. Previous research suggests tolerance may be mediated in part by induction of IgG4 antibodies that suppress allergic effector cells by blocking IgE-reactive epitopes and/or cross-linking with inhibitory IgG receptors, but there is little direct evidence. We have cloned Arah2-specific IgG4 antibodies induced during PNOIT and evaluated their suppressive activity against sensitized basophils *in vitro*.

Methods: Arah2-specific B cells from three PNOIT-treated subjects were affinity-selected from peripheral blood mononuclear cells using fluorescent Arah2. Paired heavy and light chain variable regions were cloned from single cells and used to produce 10 recombinant Arah2-specific IgG4 antibodies. Basophils from healthy donors were sensitized with serum from a peanut allergic individual and stimulated with purified Arah2 with or without prior incubation with the cocktail of IgG4 antibodies (molar ratio of Arah2:antibody = 1:34). Degranulation was quantified by flow cytometry as the percent basophils with high expression of CD63.

Results: Incubation of Arah2 with IgG4 antibodies reduced average degranulation from 20.11% to 1.76%, and 7.96% to 1.23% at Arah2 concentrations of 8.8 ng/mL and 0.88ng/mL, respectively (p=0.005, 0.003).

Conclusions: Arah2-specific antibodies induced during PNOIT and expressed as IgG4 were strongly suppressive of allergen-induced basophil activation even when sensitized with heterologous serum. Cumulated with previous knowledge of the oligoclonal B cell response to Arah2, this finding suggests that the induction of blocking antibodies to key shared epitopes may be broadly relevant to successful PNOIT outcomes.

OR.33. Critical Role of Eicosanoids in the Programming of Pulmonary NK Cells that Limit Allergic Inflammation

Zeina Jaffar, Maria Ferrini, Bryan Simons and Kevan Roberts. University of Montana, Missoula, MT

We examined the properties of NK cells in mice lacking either the eicosanoid PGI_2 receptor ($IP^{-/-}$) or cannabinoid CB2 receptor ($CB2^{-/-}$). The numbers of pulmonary $DX5^+$NK cells present in the lung tissue of either null mouse was two-fold higher than that observed in C57BL/6 wild type (WT) mice. Interestingly, an increased level of NKp46 expression by NK cells was evident in $IP^{-/-}$ mice. The increased numbers of NK cells in both null mice was associated with elevated levels of CX3CL1 (fractalkine) present in the airways and NK cells expressed its receptor CX3CR1. Remarkably, the elevated NK cell numbers were associated with a marked reduction in the development of allergic lung inflammation elicited by house dust mite allergen. This was evident from reduced number of eosinophils and $CD4^+$ T cells in the airways and depressed levels of Th2 cytokines present in bronchoalveolar lavage fluid. The attenuated allergic responses in null mice were restored to normal levels following *in vivo* depletion of $NK1.1^+$ cells. Conversely, the adoptive transfer of CD3-$NK1.1^+$ NK cells from null mice into the airways of WT mice, markedly depressed the development of allergen-induced lung inflammation. Consistent with these findings, a CB2 antagonist displayed marked anti-inflammatory properties. These data reveal a hitherto unknown role for PGI_2 and endocannabinoids in lung NK development and highlight the capacity of mucosal NK cells to prevent the development of airway inflammatory processes. The involvement of mucosal expression of CX3CL1 in influencing the properties of lung NK cells as an underlying mechanism is implicated.

T.1. Trans-generational Effect Of Di-(2-ethylhexyl) Phthalate On Allergic Lung Inflammation Under Environmentally Relevant Way and Level of Ancestral Exposures

Jau-Ling Suen[1], Tai-Ting Wu[1], Yueh-Yuan Li[1] and Shau-Ku Huang[2,3]. [1]Kaohsiung Medical University, Kaohsiung, Taiwan; [2]Johns Hopkins University, Baltimore, MD; [3]National Health Research Institutes, Miaoli Count, Taiwan

The prevalence of allergic diseases has been dramatic increasing worldwide, especially in industrialized countries, for unknown reasons. Epidemiologic studies have consistently shown that early phthalates exposure, one important environmental endocrine disruptor from diet, is associated with the increased prevalence of childhood asthma. This study tested the hypothesis that maternal exposure to di-(2-ethylhexyl) phthalate (DEHP) targets and programs offspring's dendritic cell (DC), the key cell type in immunity, leading to enhanced allergic inflammation in young offspring. We set up a transgenerational asthma model mimicking human exposure ways and observed the enhanced eosinophil infiltration and Th2-skewing cytokine pattern in bronchoalveolar lavage fluid in F1 to F3 offspring. Also, F1 to F3 offspring in DEHP group displayed altered DC homeostasis and function in spleens in response to OVA immunization, including decreased numbers of splenic conventional DCs (cDCs) and $CD8\alpha^+$ DC subset and decreased level of IL-12 secreted by cDCs. Further, the decreased frequency and increased apoptotic level of bone marrow DC precursors were also noted in young F1offspring. Our study demonstrates that trans-generational DEHP effect may result in an altered homeostasis and functions in DCs that is linked to pro-allergic function in young offspring. Our study demonstrates the causal relationship between maternal DEHP exposure and the expression of allergic lung inflammation in offspring. It provides a rational basis for the development of effective treatment and prevention strategies that will have direct and significant impact on the well-beings of our general population.

T.2. Immunological Basis for Allopurinol-induced Severe Cutaneous Adverse Reactions: HLA-B*58:01-Restricted Activation of Drug-specific T Cells and Molecular Interaction

Chia-Hsien Lin[1] and Yuan-Tsong Chen[1,2]. [1]Institute of Biomedical Science, Academia Sinica, Taipei, Taiwan; [2]★Duke University Medical Center, Durham, NC

Allopurinol is a commonly prescribed drug for symptomatic hyperuricemia and its complication, gout. The major limitation of allopurinol is that it is a frequent cause of severe cutaneous adverse reactions (SCARs). Our previous study showed that susceptibility to these life-threatening reactions induced by allopurinol is genetically determined and is strongly associated with HLA-B*58:01. The underlying mechanism remains unclear, however. To investigate the pathogenesis of allopurinol-SCAR, we aimed to study the interactions between the allopurinol or oxypurinol and HLA-B*58:01 molecules. We enrolled 14 patients with allopurinol-induced SCAR and studied the response of their cultured peripheral blood mononuclear cells (PBMCs) to allopurinol or oxypurinol, and evaluated for cell phenotype, and their interaction with HLA-B*58:01 and the drug analyzed, based on proliferation ability, cytotoxic T lymphocyte (CTL) response, surface plasmon resonance (SPR) and site-directed mutagenesis. These T cell lines (TCLs) from 14 allopurinol-SCAR patients displayed cross-reactivity between allopurinol and oxypurinol, and both $CD4^+$ and $CD8^+$ T cells were present. The expanded T cells were activated and restricted by antigen-presenting cells expressing HLA-B*58:01 in the presence of drug. The proliferation of T cells was not altered by the pre-fixation of antigen presenting cells (APC), suggesting the involvement of an antigen-processing-independent pathway. Using SPR analysis, HLA-B*58:01 was shown to exhibit specific binding affinity to allopurinol and oxypurinol. Arg97 between the C and E pocket of HLA-B*58:01 is a key residue involved in drug presentation. This study demonstrates an interaction between HLA and allopurinol or oxypurinol, and provides a molecular-level understanding of allopurinol-induced SCARs associated with HLA-B*58:01.

T.3. Endothelial Binding Pro-inflammatory T Cells (EPIC T Cells) are Elevated in Blood and Bronchoalveolar Lavage Fluid in Asthma

Pradeep K. Dagur[1], Elizabeth M. Gordon[1], Xianglan Yao[1], Rosemarie A. Cuento[1], Amisha V. Barochia[1], Angelique

Biancotto[3], Stewart J. Levine[1] and J. Philip McCoy Jr.[12]. [1]National Heart Lung and Blood Institute, Bethesda, MD; [2]National Institues of Health,Bethesda, MD

IL-17, which is secreted by both CD4 T cells (Th17) and CD8 T cells (Tc17), has been implicated in the pathogenesis of various autoimmune and inflammatory diseases in humans. CD146 is expressed on a low percentage (2 - 4%) of circulating T cells in healthy individuals, termed EPIC T cells, which secrete IL-17A, IFN-γ, and TNF-α. EPIC T cells are also found in elevated frequencies in peripheral blood of patients with various inflammatory autoimmune diseases and are significantly accumulated at the sites of inflammation (Dagur and McCoy, Autoimmun. Rev., 2015). EPIC T cells display multiple features of Th17 and Tc17 phenotypes, are capable of secreting signature cytokines without polarization, and have an enhanced ability to bind to endothelium. IL-17 has also been proposed as a possible important mediator in asthma. Here, we report that frequency of EPIC T cells is significantly elevated in blood from asthmatics and form prominent subset of T cells in bronchoalveolar lavage fluid (BALF) from both healthy individuals and asthmatics. The frequency of $CD146^+$ T cells in the circulation correlates with asthma severity. Given that healthy donors maintain a small pool of these cells in the peripheral circulation, which become elevated during active disease and correlate with disease severity, it could be speculated that these cells might act as early and persistent responders in IL-17-mediated inflammation in asthma. The finding that, on average, roughly 20% of the BALF T cells from healthy subjects and asthmatics express CD146 is unexpected and might suggest an additional role for EPIC T cells as early adaptive immune responders in the normal lung environment.

T.4. Eosinophil Activation in Response to IL-33 Stimulation is Mediated by a Soluble Factor

***Erin Stevens**[1], William Rees[2], Stephanie Matyas[3], Sean Lear[1], Chris Sheckler[1], John Ferbas[3] and Dirk Smith[1]. [1]Amgen, Seattle, WA; [2]MedImmune, Gaithersburg, MD; [3]Amgen, Thousand Oaks, CA*

IL-33 stimulation of eosinophils initiates a ST2-dependent signaling cascade that results in cell activation, enhanced survival and migration to inflammatory foci. These responses are rapid and coincident with changes in cellular morphology. Using imaging cytometry, we confirmed that whole blood stimulation with IL-33 induced a rapid and dose-dependent increase in the percentage of shape-changed eosinophils. Further, IL-33 induced expression of an activation-dependent CD11b neo-antigen on eosinophils, which is recognized by the monoclonal antibody clone CBRM1/5. To evaluate differences in donor responsiveness, whole blood from healthy volunteers was stimulated with increasing IL-33 concentrations and EC50/90 values of CBRM1/5 expression were determined. The EC50/90 values stratified individuals into EC50/90 high and low subpopulations and these differences were stable over time. Removal of soluble factors through PBS washing of whole blood cells resulted in reduction of high CBRM1/5 EC50/90 values and loss of population stratification. The concentration of serum soluble ST2 significantly correlated with the CBRM1/5 EC50. To extend this finding, whole blood from healthy volunteers was stimulated with IL-33 and eosinophil CBRM1/5 neo-epitope and basophil phospho-p38 induction levels were examined in parallel. Both assays resulted in stratification into high and low EC50/EC90 populations and EC values for both assays correlated with serum soluble ST2 levels. These results indicate that whole blood assays measuring eosinophil responses reflect IL-33 activity and suggest donor-dependent soluble ST2 levels impact eosinophil responsiveness to IL-33 stimulation. These findings may be informative in clinical trials designed to evaluate the effectiveness of IL-33 inhibitors.

T.5. Quality of Life Improvement in Allergic Rhinitis Patients Taking an Immunomodulator, a Detailed Analysis

***Toni Homberg**[1], Ivan Lara[1], Edgar Cervantes-Trujano[1], Consuelo Andaluz[1], Oscar Pineda[1], Erika Vera[1], Mayra Pérez-Tapi1[3] and María Jiménez-Martínez[2]. [1]Instituto Politecnico Nacional, Mexico City, Mexico; [2]Universidad Nacional Autónoma de México, Mexico City, Mexico*

Dialyzable leukocyte extracts (DLE) DLE are leukocyte derived peptides less than 12kDa in size, hypothesized to induce a TH1 response, useful as immunomodulators in the treatment of allergic disease. The purpose of this study

was to determine the detailed changes in quality of life of patients with allergic rhinitis, treated with standard treatment plus DLE.

Methods: We added oral DLE to standard treatment to allergic rhinitis patients who remained symptomatic. We applied the self-administered Standardized Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ(S)) before, during and after treatment with DLE. The initial DLE dose was 1 Unit (2mg/5mL/Unit) per day for 5 days, followed by 2 units per week for 5 weeks, then 1 unit per week for 5 weeks. An improved RQLQ(S) overall, group and individual symptom score of 0.5 or higher was considered as significant improvement.

Results: 30 patients (50% female) ages 14-60(33.4±11.9) added DLE to their treatment for allergic rhinitis. After 11 weeks, 26 (86.7%) patients significantly improved in the RQLQ(S) overall score, 2(6.7%) worsened and 2(6.7%) remained the same. There was additional improvement with increased treatment time in 22 patients (73.3%). Nasal (dripping, rubbing, sneezing, need for nasal tissues), sleep and emotional symptoms, as well as improved activity tolerance were improved in over 80% of patients, eye symptoms were the least affected and improved in only 63.3%.

Conclusions: DLE proved beneficial as adjuvant treatment for allergic rhinitis in this small study. Interestingly, patients showed added improvement with increased treatment time, dose reduction did not have a worsening effect.

T.6. Gastrointestinal Food Allergy Causing Spondylarthropathy-like Disease

Stephanie Finzel, Hans Baenkle and Martin Raithel. University Clinic of Erlangen, Erlangen, Germany

Gastrointestinal food allergies may cause non-erosive oligoarthritis. A 56-year old woman earlier diagnosed with seronegative spondylarthropathy presented in our Gastroenterological Outpatient Clinic in May 2014 for further investigation. Known to suffer from milk- and glutene-hypersensitivity the patient had observed a connection between intake of the respective comestibles and an exacerbation of her arthritis; after therapeutic fasting the arthritis improved.

Blood analyses gave no specific findings: C-reactive protein was low, levels of blood eosinophils, histamine, serum-ECP, tryptase and TNF-alpha were normal, as of total immunoglobuline E. There was no specific IgE detectable in peripheral blood.

An endoscopically guided segmental gut lavage including biopsies was performed. Histopathology showed no evidence of inflammatory bowel disease, but plasmacellular inflammatory infiltrates and intestinal eosinophilia in the terminal ileum in line with allergic enteritis. Food allergen-specific IgE-analysis of lavage fluid showed allergy type-I sensitisation towards egg proteine, rye flour, wheat flour, soya proteine, pork, beef, as well as nuts. The patient was thereby diagnosed with allergy type-I induced gastroenteritis with intestinal and extraintestinal manifestations.

A therapeutic diet eliminating all respective allergens lead to almost total remission of arthritis and back pain. Additionally, the patient was treated with cromoglicic acid, histamine-reducing vitamine C, and cholecalziferole. Among these measures, over 80% of the patient's complaints relieved.

Certain arthropathies such as seronegative rheumatoid arthritis or seronegative spondylarthritis should be subjected to critical examination before initializing immunosuppressive treatment. Moreover, in some patients the detection of food allergen-specific IgE in the gut might be the only way to confirm food allergy.

T.7. Role of Vasoactive Intestinal Peptide in the Induction of Regulatory T Cells in Ocular Allergic Patients

Jorge Galicia Carreon[1], Luis Alberto Salazar[2], Raúl Chávez[2], Sonia Mayra Pérez-Tapia[3], Enrique Hong[1] and María

Jiménez-Martínez[2]. [1]Cinvestav, Mexico City, Mexico; [2]National Autonomous University of Mexico, Mexico City, Mexico; [3]Instituto Politécnico Nacional, Mexico City, Mexico

Allergic conjunctivitis (AC) is one of the most common eye disorders in ophthalmology. It has been suggested that neuropeptides contribute to homeostasis in ocular surface i.e. calcitonin gene-related peptide, and substance P induce local damage; while vasoactive intestinal peptide (VIP) plays an immunomodulatory role. Although VIP contribution in ocular allergy is unclear, thus it was the aim of this study. Peripheral blood mononuclear cells (PBMC) isolated from AC-patients that were skin-positive to *Dermatophagoides pteronyssinus* (*Der p*) were stimulated with *Der p*, VIP, or *Der p*+VIP. After different times of culture (0, 24, 72 and 120 hours), PBMC were harvested and labeled with mAbs against CD4, CD25, FOXP3, and analyzed by flow cytometry. Der-p+VIP induced significant changes in the frequency of Tregs than VIP alone; it is possible that VIP pathway activation could induce an optimal microenvironment for Tregs differentiation during Ag-specific stimulation.

T.8. Transcription Factor Foxo1 Directs the Differentiation of IL-9+ T Helper Cells

***Amit Awasthi**, Sakshi Malik and Srikanth Sadhu. Translational Health Science and Technology Institute, Faridabad, Haryana, India*

Interleukin 9 (IL-9), a pleiotropic cytokine of common gamma-chain cytokine receptor family, plays a crucial role in allergic inflammation, autoimmunity and immunity to extracellular pathogens. Recent identification of anti-tumor function of IL-9 makes it an attractive target for anti-tumor therapy. Initial function of IL-9 was tested primarily in Th2-biased infection and allergic inflammation models as it thought to be a Th2 cytokine. Although Th2, Th17 cells and Foxp3+ Treg cells were shown to produce IL-9, more specialized IL-9-producing Th9 cells were identified as a distinct subset of Th cells that are proinflammatory *in vivo*. The master transcription factors that regulates IL-9 production in Th2, Th9 and Th17 cells has not been identified, although IRF-4, PU.1, Batf and IRF1 were shown to be essential for IL-9 induction in Th9 cells. Here we have identified Foxo1, a forkhead family transcription factor, predominantly required for the induction of IL-9 in Th2, Th9 and Th17 cells. We further identified AKT, an upstream kinase which regulates IL-9 induction in Th1, Th2, Th9 and Th17 cells via Foxo1. Chromatin-immunoprecipitation (Chip) analysis revealed a direct physical interaction of Foxo1 binding site in IL-9 promoter, which transactivates IL-9. Furthermore, inhibition of Foxo1 by specific siRNA, ShRNA and a Foxo1 inhibitor suppressed IL-9 production in Th2, Th9 and Th17 cells. Furthermore, in Th9 mediated asthma model, Foxo1 inhibition substantially ameliorated allergic inflammation and IL-9 induction. Our findings thus identify Foxo1 as a master transcription factor in controlling the development of Th9 and IL-9-producing Th cells.

Autoimmune Neurologic Disease

OR.13. IL-6 Signal Blockade May Suppress Plasmablast-mediated Inflammation in Multiple Sclerosis Through Skewing its Differentiating Pathways

***Masakazu Nakamura**[1], Yasuko Hirakawa[1], Manabu Araki[1] Youwei Lin[1], Wakiro Sato[1], Tomoko Okamoto[1], Miho Murata[1], Sachiko Miyake[2] and Takashi Yamamura[1]. [1]National Center of Neurology and Psychiatry, Tokyo, Japan; [2]Juntedo University Graduate School of Medicine, Tokyo, Japan*

Aberrant generation of autoreactive plasmablasts (PBs) could flare systemic lupus erythematosus through type I interferon (IFN) signature. This autoimmune texture is probably valid in the central nervous system in that neuromyelitis optica involving autoantibody-producing PBs is aggravated with exogenous IFN-beta in contrast to multiple sclerosis (MS). However, we have found that IFN-beta nonresponders in MS also expand circulating PBs which are highly responsive to IL-6 and that their expansion could be augmented through IFN-beta-induced systemic IL-6. Interestingly, it is also suggested that MS might acquire such PB-mediated pathology by upregulating the IL-6-

required differentiation of follicular helper T (T_{FH}) cells. Here, we administered anti-IL-6 receptor blocking antibody, tocilizumab, with two intractable MS patients who expand circulating PBs and analyzed peripheral blood mononuclear cells for six months. PB frequency in total B cells unexpectedly fluctuated, whereas the elevated frequency was comprised by Ki-67^{+}HLA-DR^{high} immature PBs. Concomitantly with this, secretion potential of IL-21 by memory CD4^{+} T cells was diminished, accompanying a remarkable decrease of circulating T_{FH} cells. Given that T_{FH} cells are essential for germinal center reaction through IL-21 production and second responses of memory B cells in lymphoid follicles, these results indicate that IL-6 signal blockade could suppress maturation and expansion of PBs in the follicles, which is compensated by promotion of the extra-follicular differentiation. The direct blockade in these PBs might further shorten their survival. The relative increase of immature fraction in PBs may relieve the pathology of IFN-beta nonresponsive patients with MS.

OR.15. Distinct Inflammatory Profiles of Myelin-reactive T Cells from Patients with Multiple Sclerosis

Yonghao Cao[1], Brittany A. Goods[2], Khadir Raddassi[1], Gerald T. Nepom[3], William W. Kwok[3,4], J. Christopher Love[2,5] and David A. Hafler[1,5]. [1]★Yale School of Medicine, New Haven, CT; [2]Koch Institute for Integrative Cancer Research at MIT, Cambridge, MA; [3]★Benaroya Research Institute, Seattle, WA; [4]★University of Washington, Seattle, WA; [5]The Broad Institute of MIT and Harvard, Cambridge, MA

Myelin-reactive T cells have been identified in patients with multiple sclerosis (MS) and healthy subjects, but the functional programs of self-reactive T cells that promote disease remain unknown. A total of 13,324 T cell libraries generated from blood of 23 patients and 22 healthy controls were interrogated for reactivity to myelin antigens. Libraries derived from CCR6^{+} myelin-reactive T cells from patients with MS exhibited significantly enhanced production of IFN-γ, IL-17, and GM-CSF compared to healthy controls. Single-cell clones isolated by MHC/peptide tetramers from T cell libraries also secreted more pro-inflammatory cytokines but less IL-10 relative to controls. RNA sequencing of myelin-specific T cells from patients demonstrated an unexpected transcriptome homology with encephalitogenic CD4^{+} T cells isolated from mice with experimental autoimmune encephalitis (EAE) and revealed a transcriptional profile that distinguishes MS-derived myelin-reactive T cells. The results of this unbiased approach indicate that EAE models the efferent immunologic aspects of MS.

OR.16. **Genetic Regulation of NFκB in Autoimmune Disea**se

William Housley[1] and David A. Hafler[2]. [1]★Yale University, New Haven, CT; [2]The Broad Institute of MIT and Harvard, Cambridge, MA

NF-κB is a central regulator of inflammatory responses downstream of TCRs, BCRs, TLRs, and inflammatory cytokines. Signaling through NF-κB initiates a cascade resulting in proliferation, survival, and expression of inflammatory cytokines. Multiple sclerosis is an autoimmune disorder of the central nervous system resulting from destruction of the myelin sheath around nerves. Three lines of evidence point to a central role for NF-κB in multiple sclerosis pathogenesis. First, 17% of identified genetic variants associated with MS fall within genes involved in the NF-κB signaling cascade. Secondly, we recently showed that genetic variants associated with MS fall near NF-κB binding sites. Third, we have determined that NF-κB is hyper-activated in naïve CD4 cells from relapsing remitting MS patients. As such, we hypothesized that alterations in NF-κB signaling are central to MS pathogenesis and that those changes are genetically mediated. To investigate this, we identified two variants within the Type 1 TNF-α receptor (TNFR1) and proximal to NFKB1 to determine the impact that these variants have on NF-κB signaling. Both variants increased NFκB signaling after TNF-a stimulation in naïve CD4 cells. The variant proximal to NFKB1 falls within a dense regulatory region. Consistent with changes to NFKB1 gene regulation, the MS associated risk variant results in a 20-fold increase in expression of p50 NFκB. In addition, there was a decrease in expression of the negative regulators CIAP1, TNFAIP3, and BCL3 with the risk variant, suggesting a global dysregulation of NF-κB signaling.

OR.23. A Microbiome-associated Lipid as an Immunoregulator in Multiple Sclerosis

***Emily Anstadt**, Mai Fujiwara, Frank Nichols, Reza Nemati, Xudong Yao and Robert Clark. University of Connecticut, Storrs, CT*

The role of the microbiome in multiple sclerosis (MS) remains unknown. We have identified a lipodipeptide and TLR2-agonist produced by commensal bacteria called Lipid 654 (L654). We reported that L654 is recovered from healthy human sera and that total L654 levels are significantly lower in patients with MS. The goals of the present study were to understand the functional relevance of L654 utilizing mouse models and to further delineate the relationship between serum L654 levels and disease states in MS.

L654 administration to SJL mice significantly attenuated clinical disease in an adoptive transfer model of EAE ($p < 0.0001$). Analysis of spinal cord mononuclear cells in L654-treated mice showed a significant decrease in total cell number and in the percentage of T cells producing IL-17 and IFNγ. Mechanistically, L654 administration resulted in an increase in the percentage of $CD39^+$ Tregs and IL-10-producing $CD11b^+$ cells. These results suggest that L654 can mediate an immunoregulatory and disease inhibitory effect on CNS autoimmunity.

To further characterize serum L654 levels in MS, we altered our previous approach of measuring total serum L654 to measuring the non-protein bound (free) fraction of L654. While total L654 levels are decreased in MS patients but do not differentiate among clinical subtype, levels of free L654 are significantly lower in patients with progressive disease compared to relapsing-remitting disease ($p<0.01$). Overall, our findings suggest that diminished levels of L654 may play a role in the abnormal immunoregulation seen in MS, and thus may represent a new target for therapeutic intervention.

OR.40. $FoxP3^+$ Regulatory T Cells Use Heparanase to Access IL-2 in the Extracellular Matrix

***Hedwich Kuipers**[1], Ben Falk[2], Kathy Braun[2], Mike Kinsella[2], Israel Vlodavsky[3], Gerald T. Nepom[2], Thomas Wight[2] and Paul Bollyky[2,1]. [1]★Stanford University, Stanford, CA; [2]★Benaroya Research Institute, Seattle, WA; [3]The Hebrew University of Jerusalem, Jerusalem, Israel*

$Foxp3^+$ regulatory T cells (Treg) are critically dependent on IL-2 but most IL-2 *in vivo* is rapidly sequestered within the extracellular matrix (ECM) where it is bound to heparan sulfate (HS)-containing proteoglycans. We have identified a novel role for heparanase (HPSE) and HS-bound IL-2 in the survival of Treg in peripheral tissues.

We show that HPSE is more heavily expressed on Treg than on conventional T cells and that HPSE expression is regulated by IL-2 availability. Moreover, GFP-Foxp3 Treg from $HPSE^{-/-}$ are impaired in their ability to utilize matrix-bound IL-2. Treatment of wild type Treg with the HPSE inhibitor PI88 likewise inhibits their ability to access HS-bound IL-2. Consistent with this, GFP-Foxp3.$HPSE^{-/-}$ mice have fewer Treg and Treg isolated from these animals have impaired homeostasis upon transfer *in vivo*. We therefore propose that Treg utilize HPSE to gain access to IL-2 sequestered in the ECM and that this is a fundamental mechanism for Treg survival and function in peripheral tissues.

T.9. Cbl-b-deficient Mice Demonstrate Alterations in T Cell Trafficking but Retain Sensitivity to the Multiple Sclerosis Therapeutic Agent FTY720

***Mai Fujiwara**[1], Emily Anstadt[1], Kamal Khanna[2] and Robert Clark[1]. [1]University of Connecticut School of Medicine, Farmington, CT; [2]University of Connecticut Health Center, Farmington, CT*

The variable response to treatment options in multiple sclerosis (MS) suggests the need for personalized treatment approaches based on individual clinical and genetic differences. *CBLB* gene polymorphisms have been identified in

MS, and altered T cell function and aberrant responses to interferon-β in MS patients with a *CBLB* mutation have been recently reported. Cbl-b is an E3 ubiquitin ligase that regulates T cell activation. Cbl-b-deficient (Cbl-b$^{-/-}$) mice show several T cell abnormalities such as co-stimulation-independence and resistance to Treg-mediated suppression that are also described in MS patients. This suggests that Cbl-b$^{-/-}$ mice may provide a novel approach for analyzing treatment options in patients with MS-associated *CBLB* mutations. We now report that Cbl-b$^{-/-}$ CD4^{+} T cells show significant trafficking-related abnormalities: decreased lymph node accumulation after adoptive transfer into RAG-1$^{-/-}$ mice; increased sphingosine-1-phosphate receptor 1 ($S1P_1$) expression; and decreased CD69 expression compared to wild-type cells. These data led us to ask whether *CBLB* mutations may compromise the therapeutic efficacy of FTY720, an MS-approved drug that is postulated to work by trapping T cells in lymphoid tissues. We induced EAE in Cbl-b$^{-/-}$ mice and found that despite the Cbl-b-related trafficking alterations, FTY720 significantly inhibited EAE in Cbl-b$^{-/-}$ mice.

Overall, our results: 1) document a novel role for Cbl-b in T cell trafficking; 2) indicate that FTY270 may be effective in EAE/MS through mechanisms other than T cell lymph node trapping; 3) suggest that despite potential T cell trafficking alterations, MS patients with Cbl-b abnormalities may still be excellent candidates for FTY720 treatment.

T.10. Protein Methylation Mediates Interferon β-induced Augmentation of Tumor Necrosis Factor α Secretion in Human Monocytes

Yusei Miyazaki[1], Masaaki Niino[1], Toshiyuki Fukazawa[2], Eri Takahashi[1] and Seiji Kikuchi[1]. [1]Hokkaido Medical Center, Sapporo, Japan; [2]Sapporo Neurology Clinic, Sapporo, Japan

Background: Interferon (IFN) β is widely used as a first-line treatment for multiple sclerosis (MS), but its therapeutic mechanism and the reason that some patients are unresponsive to treatment are not clearly understood. In this study, we analyzed the mechanism of cytokine regulation by IFNβ in human monocytes and CD4^{+}T cells.

Subjects and methods: Venous blood samples were obtained from 12 healthy subjects (HS), 13 untreated MS patients (UT-MS), and 9 MS patients treated with IFNβ (IFN-MS). Monocytes and CD4^{+}T cells were isolated by using magnetic beads. Monocytes were stimulated with lipopolysaccharide for 24 h and CD4^{+}T cells were stimulated with phorbol myristate acetate and ionomycin for 48 h. Interleukin (IL)-10 and tumor necrosis factor (TNF) α in the supernatant were quantified by enzyme-linked immunosorbent assay. In some experiments, cells were pre-incubated with recombinant IFNβ and methylthioadenosine as a protein methylation inhibitor before stimulation.

Results: Monocytes from IFN-MS produced less IL-10 and more TNFα compared to monocytes from UT-MS. IFNβ suppressed IL-10 and enhanced TNFα production in HS monocytes *in vitro*; however, IFNβ enhanced IL-10 and suppressed TNFα secretion in HS CD4^{+}T cells. The IFNβ-induced enhancement of TNFα production from monocytes was suppressed by addition of methylthioadenosine, but IL-10 level was not affected.

Conclusion: IFNβ differentially regulated the cytokine profile of human monocytes and CD4^{+}T cells. IFNβ-induced TNFα augmentation in monocytes was suppressed by inhibition of protein methylation. The therapeutic potential of IFNβ for MS might be enhanced by regulating protein methylation.

T.11. Effect of Natalizumab on Inflammatory and Regulatory T Cells in Multiple Sclerosis

Kimitoshi Kimura[1,2], Masakazu Nakamura[1], Wakiro Sato[1,3], Tomoko Okamoto[3], Manabu Araki[3], Youwei Lin[1,3], Miho Murata[1], Ryosuke Takahashi[2] and Takashi Yamamura[1,3]. [1]National Center of Neurology and Psychiatry, Tokyo, Japan; [2]Kyoto University Graduate School of Medicine, Kyoto, Japan; [3]National Center Hospital, Tokyo, Japan

Background: Natalizumab is known to inhibit invasion of inflammatory T cells into central nervous system by downregulation of CD49d (alpha 4 integrin) on the cell surface. CD49d is expressed not only by inflammatory but

also by regulatory T cells, which are both affected by natalizumab. In this study, the difference of the effect was investigated between inflammatory and regulatory T cells.

Methods: Peripheral blood mononuclear cells were obtained from six healthy controls and 21 multiple sclerosis patients including four treated with natalizumab. The positivity of CD49d was analyzed on Th1, Th17 and Treg cells ($CD49d^+$Th1, $CD49d^+$Th17, $CD49d^+$Treg). The positivity was compared between inflammatory and regulatory T cells.

Results: The positivity of CD49d was significantly decreased on all the three subsets in natalizumab-treated patients compared to other patients ($CD49d^+$Th1; 45% vs 74%, $CD49d^+$Th17; 40% vs 64%, $CD49d^+$Treg; 10% vs 35%, $p<0.05$). There were detected significantly higher indices of both $CD49d^+$Th1 / $CD49d^+$Treg and $CD49d^+$Th17 / $CD49d^+$Treg in natalizumab-treated patients ($p<0.05$). The indices were highest in one patient who had disease exacerbation during the natalizumab-treated period. There was no significant difference of the ratio of Th1, Th17 or Treg in total memory CD4 T cells among healthy control and two MS groups.

Discussion: The results show that natalizumab leads to larger decrease of CD49d positivity in regulatory T cells than in inflammatory T cells. This may explain the presence of poor responders to natalizumab.

T.12. Identification of Pathogenic T Helper Cells in Chronic Autoimmune Neuroinflammation

Ben Raveney, Shinji Oki, Hirohiko Hohjoh, Masakazu Nakamura, Wakiro Sato, Miho Murata and Takashi Yamamura. National Center of Neurology and Psychiatry, Tokyo, Japan

Relapsing remitting multiple sclerosis (RRMS) is associated with upregulation of NR4A2 (Nurr1) by circulating T cells. NR4A2 is also expressed by T cells infiltrating the central nervous system (CNS) following induction of experimental autoimmune encephalomyelitis (EAE). Blocking NR4A2 expression with RNAi during EAE, ameliorated clinical disease and reduced IL-17-secreting Th17 cells.

Mice with a T cell-specific NR4A2 deficiency did not develop early/acute clinical EAE symptoms, but instead developed a late-onset chronic EAE. T cells infiltrating CNS tissues during early EAE had reduced IL-17 production, but increased IFN-Y production. The absence of NR4A2-dependent T cell responses revealed a novel population of CNS-infiltrating $CD4^+$ T cells during late/chronic EAE expressing a particular molecule. Intriguingly, patients with secondary progressive MS (SPMS), but not those with RRMS, had striking increases in a similar T cell population in peripheral blood and cerebrospinal fluid. Such T helper cells in both late/chronic EAE and SPMS patients shared functional molecules and blocking the expression of this protein during EAE by RNAi knockdown or in CD4-specific conditional knockout mice significantly reduced late/chronic EAE, suggesting that these cells play a key functional role in this disease stage.

We suggest that EAE pathogenesis could be explained by two phases of immune responses: early/acute disease, which was NR4A2-dependent involving Th17 cells, and a late/chronic NR4A2-independent disease that is controlled by a previously unappreciated subset of $CD4^+$ T cells. Targeting this unusual subset of helper T cells and their function may provide a new and promising therapy for SPMS.

T.13. Avidity of the Aquaporin-4 Specific Antibody Response in Neuromyelitis Optica

Friederike Tuller[1], Kathrin Schanda[1], Thomas Berger[1], Jeffrey Bennett[2] and Markus Reindl[1]. [1]Innsbruck Medical University, Innsbruck, Austria; [2]University of Colorado, Denver, CO

Neuromyelitis optica (NMO) is an inflammatory demyelinating disease of the human central nervous system characterized by optic neuritis and longitudinally extensive transverse myelitis. The discovery of a specific autoantibody (Ab) that targets aquaporin-4 (AQP4-IgG) facilitated the distinction of NMO from multiple sclerosis. The

pathogenicity of AQP4-IgG was already proven in different experimental animal models. In the past years several immunossays for AQP-IgG have been established, but the association of serum Ab titers and their pathogenicity is still unclear. Therefore, we hypothesized that the avidity rather than the quantity (e.g. serum titers) determines the pathogenicity of AQP-IgGs. In a first step we have established a fluorescence activated cell sorting (FACS) assay using AQP4-expressing HEK cells for the fast and objective measurement of AQP4-IgG. We validated this assay using 42 AQP4-IgG seropositive patients with NMO spectrum disorders (NMOSD) and 198 control serum samples yielding an accuracy of 100%. We further validated this assay using human recombinant monoclonal AQP4-Abs (Kd-values lie in the 10-100nM range) and determined the avidity of these high-affinity AQP4-specific mAbs using increasing concentration of urea buffer solution (avidity indices 0.1-0.9, 0=low avidity, 1=high avidity). The binding ratios at a concentration of 4M urea differed and were independent from their affinity suggesting functional differences between these Abs. Currently we are analyzing the avidity of serum AQP4-IgGs in NMOSD patients in order to investigate whether they correlate with clinical and neuroradiological parameters. First results from 25 serum samples show lower avidity indices (0.3-0.7) than those seen with some of the mAbs.

T.14. DGAT1 Inhibits Retinoic Acid-dependent Regulatory T Cell Generation and Mediates Experimental Autoimmune Encephalomyelitis

Kareem Graham[1], Bonnie Werner[1], Kimberly Moyer[1], Maria Zoudilova[2] and Eugene Butcher[2]. [1]★Emory University School of Medicine, Atlanta, GA; [2]★Stanford University School of Medicine, Stanford, CA

An imbalance of regulatory versus effector $CD4^+$ T cells can contribute to autoimmune diseases including multiple sclerosis (MS), but mechanisms that govern $CD4^+$ T cell functions within the central nervous system (CNS) are poorly understood. Transcriptional profiling identified expression of *Dgat1,* a gene encoding the lipid-metabolizing enzyme diacylglycerol O-acyltransferase-1, by CNS-infiltrating memory $CD4^+$ T cells from mice with experimental autoimmune encephalomyelitis (EAE). Administration of a selective DGAT1 inhibitor attenuated EAE, and $DGAT1^-$ deficient mice developed less severe disease than wild type (WT) controls. Furthermore, $DGAT1^{-/-}$ *in vitro*-polarized encephalitogenic Th17 cells were less efficient than WT Th17 cells at transferring EAE to immunocompetent hosts. Compared to their WT counterparts, $DGAT1^{-/-}$ mice subjected to EAE induction protocols had a significantly higher proportion of $Foxp3^+$ T regulatory cells (Tregs) among T cells infiltrating the CNS and peripheral lymphoid tissues. Retinoic acid (RA) can support Treg induction, and DGAT1 functions as an acyl-CoA-dependent retinol acyltransferase, capable of sequestering RA in retinyl ester form. In *in vitro* studies, Tregs were generated at higher frequencies from $DGAT1^{-/-}$ T effector cells than WT controls; however, addition of a pharmacologic DGAT1 inhibitor during T cell activation enhanced RA-dependent Treg induction from $DGAT1^{+/+}$ T cells. Our results identify DGAT1 as an enzyme regulator of RA-dependent T cell function and a potential target for therapy of MS. These data also add to the growing appreciation of the interplay between immune and metabolic pathways.

T.15. CD4 T Cell Reactivity to Both Native and Citrullinated-MOG Epitopes Identified in HLA-DR4 Mouse Model of Demyelinating Disease

Anna Kersh and Brian Evavold. ★Emory University, Atlanta, GA

Multiple sclerosis (MS) is an autoimmune disease characterized by inflammation and demyelination in the central nervous system (CNS). HLA-DR4 is an MHC class II gene associated with increased risk for MS. Humanized HLA-DR4-transgenic mice were used to probe T cell specificity and affinity during demyelinating disease progression in response to myelin oligodendrocyte glycoprotein (MOG). Using the micropipette adhesion frequency assay to obtain sensitive, physiologically relevant measurements, only 26% of CNS-infiltrating CD4 T cells are reactive to MOG in symptomatic HLA-DR4-expressing mice. Moreover, T cells probed for specificity to a modified MOG peptide containing a citrulline residue in position 101 instead of arginine revealed T cells reactive to MOG alone, citrulline-modified MOG, or to both. Cells isolated from the cervical lymph nodes of symptomatic animals were able to proliferate to both MOG and citrullinated MOG. Of importance, the citrullinated-MOG peptide does not induce

paralytic disease on its own. We believe that these results are the first demonstration of T cell reactivity to citrullinated-MOG epitopes in the context of HLA-DR4 presentation to T cells. We suggest that T cell cross-reactivity to native and citrullinated epitopes of MOG provides a mechanism of disease progression and escape from immune tolerance. Further, reactivity to the citrullinated neoepitope has great implications for use as a potential biomarker of disease therapeutic efficacy since citrullination is the result of a deimination reaction that occurs during prolonged inflammation.

T.16. Disease Activity and Therapy Modulate Peripheral Blood Innate Lymphoid Cells (ILC) in Relapsing-Remitting Multiple Sclerosis

Florence Roan[1,2], Mariko Kita[3], Jane H. Buckner[1] and Steven Ziegler[1]. [1]★Benaroya Research Institute, Seattle, WA; [2]★University of Washington, Seattle, WA; [3]Virginia Mason Medical Center, Seattle, WA

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system characterized by inflammation, demyelination and axonal loss. The majority of patients present with a relapsing-remitting form of disease (RRMS), and experience periods of exacerbations followed by periods of remission, although many of these cases eventually evolve to secondary progressive MS (SPSS). Although the efficacy of immunomodulatory therapies in decreasing clinical relapses and disease progression provides some insight into the mechanisms of disease, the pathogenesis of MS relapse and progression remains poorly understood. Over the last several years, there has been a growing body of literature implicating innate lymphoid cells (ILC) in a variety of autoimmune diseases, including MS. Because ILC subsets parallel helper T cells in their production of effector cytokines, but do not require antigen for activation, these cells may be particularly sensitive to environmental cues that may trigger disease exacerbations. In this study, we examine peripheral blood ILC subset frequencies and activation marker expression in RRMS during active disease and remission. We also characterize changes in ILC subsets during therapy with natalizumab, a monoclonal antibody to alpha-4 integrin that blocks lymphocyte trafficking to the CNS, and demonstrate that ILC subsets in the peripheral blood are differentially affected by natalizumab treatment. These studies add to our understanding of the immune correlates of relapse, remission and therapy in MS, and may provide a foundation for the development of safer and more effective therapies.

T.17. Adenosine A_1 Receptors Contribute to Immune Regulation After Neonatal Hypoxic Ischemic Brain Injury

***Max Winerdal**, Malin Winerdal, Bertil Fredholm, Ola Winqvist. and Ulrika Ådén. ★Karolinska Institutet, Stockholm, Sweden*

Brain hypoxic ischemia outcome is greatly influenced by inflammation, and adenosine signaling is known to influence both injury development and immune cell function. Here, we focused on the importance of adenosine A_1 receptors ($A_1R^{-/-}$) after brain injury, and investigated their impact on injury size, functional outcome and key immune cell populations. Brain hypoxic ischemia was induced in ten-day-old mice. Infarctions were evaluated by MAP-2 staining and functional outcome by beam walking tests. Immune response was evaluated by flow cytometry of brain infiltrating cells. Data driven analysis of flow cytometric data was applied, principal component analysis (PCA) and expectation maximum clustering was used to predict genotype and treatment modality from the type of immune cell infiltration. Brain hypoxic ischemia in $A_1R^{-/-}$ mice resulted in significantly larger infarctions (33%) and worse results in the beam walking tests with 44% more mistakes than wild type (WT). Microglial activation after injury was enhanced in $A_1R^{-/-}$ brains. Brain infiltrating B-lymphocytes expressing IL-10 were activated in WT after injury, but unresponsive in $A_1R^{-/-}$. Moreover, $A_1R^{-/-}$ mice had significantly reduced total brain IL-10 levels compared to WT. $CD4^+$ T lymphocytes including $FoxP3^+$, i.e. T regulatory cells, were unaffected, whereas $CD8^+$ T lymphocyte responses were impaired in $A_1R^{-/-}$ mice. From the immune profile alone, we could classify $A_1R^{-/-}$ and WT genotypes and separate sham operated animals from those subjected to HI, validating the adequacy of the model in this context. In conclusion, A_1R signaling has major impact on immune cell activation and A_1R deletion results in adverse outcome after brain HI.

T.18. Gut Intraepithelial Autoimmune CD4+ T Cells Exhibit a Potential to Suppress Extraintestinal Autoimmunity in LAG-3 Dependent Manner

Atsushi Kadowaki[1,2], Sachiko Miyake[3], Ryoko Saga[1] and Takashi Yamamura[1]. [1]National Center of Neurology and Psychiatry, Tokyo, Japan; [2]Osaka University, Osaka, Japan; [3]Juntedo University, Tokyo, Japan

Increasing incidence of autoimmune diseases such as multiple sclerosis in developed countries let us question which components of the immune system are altered by environmental factors, and how the immune tolerance is subsequently disrupted or augmented. Here we demonstrate that the gut epithelium of myelin oligodendrocyte glycoprotein (MOG35-55)-specific TCR transgenic mice is inhabited by intraepithelial T lymphocytes (IEL) that are able to inhibit experimental autoimmune encephalomyelitis (EAE) upon transfer. The regulatory IEL expressed transgenic TCR brightly, had the phenotype of $CD4^+$ 'induced' IEL ($CD2^+CD5^+CD69^+CD62L^-CD44^+CD25^-$) and exhibited T_H17 cell-like cytokine profile. Unlike their $CD4^-$ counterpart or $CD2^-CD5^-$ 'natural' IEL, the $CD4^+$ 'induced' IEL had the unique ability to infiltrate the inflamed central nervous system (CNS) tissue and suppress MOG(35-55) induced EAE. The regulatory IEL constitutively expressed *Ctla4* and *Tgfb1*, whereas they markedly upregulated expression of *Lag3* after they entered the CNS. Effects of blocking antibodies revealed that immunosuppressive capability of the IEL depends on these molecules and that LAG3 has an important role for EAE suppression. We also demonstrated the suppressive capability of $CD4^+$IEL in a model of TCR transgenic arthritis. $CD4^+$IEL of wild type mice had the similar phenotype as those of the TCR-transgenic mice and $CD4^+$T cells that exhibit the similar phenotype were also found in mouse and human peripheral blood. Thus, $CD4^+$ 'induced IEL' derived from autoimmune T cell repertoire may be pivotal in the negative regulation of autoimmune diseases.

T.19. 4-methylumbelliferone, an Inhibitor of Hyaluronan Synthesis, Prevents Autoimmune Demyelination

Hedwich Kuipers, Mary Rieck, Lawrence Steinman and Paul Bollyky. ★Stanford University, Stanford, CA

The extracellular matrix polysaccharide hyaluronan (HA) is a key inflammatory mediator known to be abundant in demyelinating lesions in multiple sclerosis (MS) as well as in the mouse model of the disease, experimental autoimmune encephalomyelitis (EAE). We have identified an oral inhibitor of HA production, 4-methylumbelliferone (4-MU), that prevents development of EAE.

We show that 4-MU treatment of EAE significantly decreases the incidence, delays the onset and reduces the severity of disease. In addition, 4-MU treatment substantially reduces clinical deficits in already established EAE.

Using multi-parameter flow cytometry, we further analyzed the immunological mechanism of this therapeutic effect. We found that 4-MU treatment skews the immune response towards an anti-inflammatory profile, characterized by an increase in FoxP3 expressing regulatory T cells (Treg) and an altered T helper cell profile into the central nervous system (CNS). In addition, 4-MU treatment promotes Treg persistence *in vitro* and increases the expression of FoxP3 and the costimulatory receptor GITR in Treg *in vitro* and *in vivo*. Moreover, we observed that 4-MU treatment reduces the reactive astrogliosis response *in vivo*, characterized by a decrease of $GLAST^+$ astrocyte numbers in the spinal cord of treated EAE mice and reduced GFAP immunoreactivity in CNS tissue.

Together, our data provide the therapeutic profile and immunological mechanism of 4-MU treatment in EAE. Considering that 4-MU is already an approved therapeutic currently used in people throughout Europe and Asia, we therefore propose that 4-MU treatment has great promise for the treatment of MS.

T.20. AQP4-IgG Placental Transfer in NMO Pregnancies

Simone Mader[1], Lior Brimberg[1], Bonnin Alexandre[2], Jeffrey L. Bennett[3], Bruce T. Volpe[1], Patricio Huerta[1] and Betty

Diamond[1]. [1]★The Feinstein Institute for Medical Research, New York, NY; [2]★University of Southern California, Los Angeles, CA; [3]★University of Colorado, Denver, CO

Antibodies to astrocytic aquaporin-4 (AQP4-IgG) are a highly specific biomarker in Neuromyelitis optica spectrum disorders (NMOSD) and contribute to disease pathogenesis although it remains unresolved what regulates AQP4-IgG entry into the adult brain. Recently, it was suggested that AQP4-IgG results in an increased frequency of miscarriages in seropositive patients, raising the possibility that AQP4-IgG affects the placenta and perhaps the developing fetus. Therefore we wanted to understand whether AQP4-IgG acts on the placenta or directly on the embryo (brain, spinal cord or other organs). We retro-orbitally injected different concentrations (60 to 200 ug) of human monoclonal AQP4-IgG, monoclonal AQP4-IgG which cannot activate the complement system (comp def AQP4-IgG) or isotype control IgG into pregnant mice at embryonic day E14.5. Our results show that AQP4 is expressed at a significantly higher level in the fetal brain compared to the placenta throughout all gestational stages. Maternal AQP4-IgG results in death of the offspring at high concentrations, while the complement deficient antibody as well as an isotype control human monoclonal antibody did not lead to fetal death. At lower AQP4-IgG concentration, pregnant mice have living offspring. Our behavioral analysis of those offspring indicates that mice born to pregnant dams injected with low concentration of AQP4 IgG exhibit an impairment in flexible learning. This animal model will help us to understand the risk of pregnancies for AQP4-IgG seropositive NMOSD patients.

T.21. Dysregulated B Cells in Multiple Sclerosis and Their Impact on T Cell Function

Sara Ireland, Alyssa Guzman, Nancy Monson and Ding Chen. ★UT Southwestern Medical Center, Dallas, TX;

The role of B cells in relapsing-remitting multiple sclerosis (MS) is not well understood, but increasing evidence implicates B cells in supporting disease though production of pro-inflammatory cytokines and serving as antigen presenting cells. Moreover, several studies have identified B cells as a critical target for disease modifying therapies. We previously demonstrated that B cells from treatment naïve MS patients secrete higher levels of IL-6; however, the identity and role of human IL-6 producing B cells is unknown. We hypothesized that B cells from MS patients express a unique set of genes, including elevated IL-6, which contributes to pro-inflammatory activity and support of T cell responses. To address this hypothesis, we used RNA-seq and flow cytometry to interrogate B cell phenotype and gene expression. Additionally, we blocked IL-6 receptor signaling in cultures of autologous B and T cells in the presence of neuroantigens. We found that the naïve B cell pool is enriched for IL-6$^+$ cells that express intermediate levels of both CD24 and CD1d. By RNA-seq, we identified perturbations in the major activation signaling pathways in B cells from MS patients including CD40, B cell receptor, and toll-like receptors that could contribute to dysregulated B cell responses. Finally, we found that B cells from MS patients support neuroantigen specific TH1 and TH17 responses, but this does not uniquely require IL-6. The data presented here provide a platform for a more nuanced dissection of the role for B cells in MS and insight in guiding future therapeutic targets.

T.22. Insulin-like Growth Factors are Essential for the Differentiation of Th17s in EAE

Daniel DiToro, Casey T. Weaver and Stuart Frank. ★University of Alabama at Birmingham, Birmingham, AL

Insulin-like growth factors (Igfs) are highly conserved proteins similar to pro-insulin in both sequence and structure. They function primarily by binding the Igf1 receptor (Igf1R) and exert pleiotropic effects on many tissues throughout life. These factors were shown to be essential for the normal development and proliferation of B and T lymphocytes, and to positively influence their proliferation and survival following activation. But early attempts to identify potential roles in T cell differentiation and function lead to conflicting results, and the field was largely abandoned. More recent advances in our understanding of T cell biology, including the discovery of Th17 and Treg C4s, permit a more sophisticated approach. Our data indicate that Th17 and Treg CD4s express Igf1R and insulin-like growth factor binding protein 4 (Igfbp4), a critical regulator of Igf activity, at much higher levels than other CD4 T cells. Exogenous Igfs promote Th17 differentiation while suppressing Treg differentiation *in-vitro* at least in part through induction of the

Akt/mTOR pathway. Deletion of Igf1R on T cells leads to a defect in Stat3 phosphorylation and IL17a production and enhanced Treg differentiation *in-vitro*. Moreover, mice lacking Igf1R on T cells are substantially protected from Experimental Autoimmune Encephalomyelitis, an effect can be recapitulated through the in-vivo administration of Igf1R blocking antibodies.

T.23. Oxidized Mitochondrial DNA is a Powerful Activator of Human pDCs and Induces a Lupus-Specific CD4 T Cell Phenotype

Simone Caielli, *Romain Banchereau, and M. Virginia Pascual. Baylor Institute for Immunology Research, Dallas TX*

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by breakdown of tolerance to nuclear antigens, immune complex (IC) deposition in tissues and multiorgan involvement. The skin, blood vessels, kidneys, central nervous system, and joints become targets of inflammation at onset or during the course of the disease.

Genomic approaches have shown that >95% of children with SLE display two peculiar blood signatures, namely type I IFN- and neutrophil-related. Indeed, over-expression of neutrophil-related genes is more pronounced in patients with severe disease. A connection between these two signatures seems to exist, as stimulation of SLE neutrophils with RNA-containing IC induces the release of oxidized mitochondrial DNA (Ox mtDNA) that triggers a massive IFNa production by pDCs.

We now describe that Ox mtDNA activates the early endosome-TLR9 pathway in pDCs and rearranges their pattern of chemokine receptor expression. In particular, they up-regulate CCR7 and down-regulate CXCR4 and CXCR3, therefore acquiring the capacity to migrate towards the T cell area of peripheral lymphoid organs.

Furthermore, Ox mtDNA-activated pDCs skew naïve CD4 T cell differentiation toward a phenotype that closely resembles the one described for circulating CD4 T cells in SLE patients, including high IL-10 production, low IL-2 transcription and hyperproliferation upon restimulation. Importantly, those CD4 T cells acquire a potent B cell helper phenotype.

Our findings demonstrate that the unique phenotype of blood SLE CD4 T cells might not be due to intrinsic defects, but could result from their encounter with Ox mtDNA-activated APCs.

Autoimmune Rheumatologic Disease

OR.04. Baseline Serum Interferon Beta/Alpha Ratio Predicts Response to Tumor Necrosis Factor Alpha Inhibition in Rheumatoid Arthritis

Theresa Wampler Muskardin*[1], Priyanka Vashisht[2], Jessica Dorschner[1], Mark Jensen[1], Beverly Chrabot[3], Marlena Kern[4], S. Louis Bridges Jr.[5], Peter K. Gregersen[4] and Timothy Niewold[1]. [1]★Mayo Clinic, Rochester, MN; [2]University of Nebraska Medical Center, Omaha, NE; [3]★University of Chicago,* Chicago, IL; [4]★The Feinstein Institute for Medical Research, Manhasset, NY; [5]★University of Alabama at Birmingham, Birmingham, AL*

Objective: Studies suggest that circulating type I interferon (IFN) levels may predict response to biological agents in rheumatoid arthritis (RA). Prediction of response prior to initiating therapy would represent a major advancement.

Methods: We studied sera from a test set of 32 RA patients from the ABCoN Consortium and a validation set of 92 RA patients from the TETRAD registry. The test set included those with a good response or no response at 14 weeks

by EULAR criteria. The validation set included subjects with good, moderate, or no response at 12 weeks. Total serum type I IFN activity, IFN-α and IFN-β activity were measured using a functional reporter cell assay.

Results: In the test set, an increased ratio of IFN-β to IFN-α (IFN β/α ratio) in pre-treatment serum associated with lack of response to TNF-α inhibition (p=0.013). A receiver-operator curve supported a ratio of 1.3 as the optimal cut-off. In the validation set, subjects with an IFN β/α ratio >1.3 were significantly more likely to have non-response (p=0.020). IFN β/α ratio >1.3 was a strong discriminator of non-response vs. either moderate or good response (OR=3.32, 95% CI 1.290-8.524). Anti-CCP antibody titer and mechanism of action of TNF-α inhibitor did not influence this relationship. In meta-analysis, pre-treatment IFN β/α ratio >1.3 associated with non-response (p=0.004).

Conclusion: Increased pre-treatment serum IFN β/α ratio strongly associated with non-response to TNF-α inhibition. This study supports the potential utility of serum type I IFN in predicting outcome of TNF-α inhibition in RA.

OR.08. ROCK2 Signaling is Required for the Development and Function of T Follicular Helper Cells in SLE

Jonathan Weiss[1], Wei Chen[1], Melanie Nyuydzefe[1], Ryan Flynn[2], James Tonra[1], Suzana Marusic[3], Bruce Blazar[2], Samuel Waksal[1] and Alexandra Zanin-Zhorov[1]. [1]Kadmon Research Institute, New York, NY; [2]University of Minnesota, Minneapolis, MN; [3]Hooke Labs, Lawrence, MA

PD1$^+$CXCR5$^+$ T follicular helper (Tfh) cells are essential for generating high-affinity antibodies and B cell memory. But aberrant Tfh activity can drive autoimmune disease such as systemic lupus erythematosus (SLE) which is characterized by high levels of auto-antibodies and increased frequencies of IL-21$^+$ T cells. Rho-associated kinase (ROCK) 2 was recently shown to be critical in regulating IL-21 secretion in both mice and humans. Here, we show that the PD1$^+$CXCR5$^+$ subset is a major source of IL-21 in human CD4$^+$ T cells stimulated *in vitro*. By using KD025, a Kadmon orally available, selective ROCK2 inhibitor, we attenuated the development of human PD1$^+$CXCR5$^+$ T cells by over 60% and the perpetuation of existing Tfh cells in a dose dependent manner. Targeting of ROCK2 concurrently regulates STAT3/STAT5 phosphorylation and transcription activity followed by a reduction of Bcl6 and up-regulation of Blimp1 protein levels in human T cells. Oral administration of KD025 reduced the percent of both Tfh and plasma B cells in spleens by more than 2 fold as well as germinal center size in the Mrl/lpr murine model of SLE. KD025 treated animals had a substantial improvement in both histological and clinical scores complimented by a reduction in pSTAT3 and BCL6 expression in addition to an increase in STAT5 phosphorylation. This data suggests that ROCK2 controls the function of Tfh cells and underscores the therapeutic potential of targeted ROCK2 inhibition in the clinic.

OR.12. Sequencing Anti-citrullinated Protein Antibody Repertoires in Rheumatoid Arthritis

Sarah Kongpachith, Lisa Blum, Chia-Hsin Ju, Daniel Lu, Sisi Zhang, Lauren Lahey, Monica Chin, Jeremy Sokolove and William Robinson. ★Stanford University, Palo Alto, CA

A hallmark of rheumatoid arthritis (RA) is the production of autoantibodies, including anti-citrullinated protein antibodies (ACPAs). However, the specific targets of these autoantibodies and their role in RA pathogenesis remain ill-defined. Furthermore, little is understood about how the ACPA response arises and evolves over time. We generated fluorescent citrullinated-peptide tetramers to identify and sort individual ACPA-producing B cells in RA patient blood. Then by utilizing an antibody repertoire capture methodology developed in our lab, we were able to sort and sequence the cognate heavy- and light-chain pairs of the antibodies expressed by the cells. Repertoire analysis reveals evidence of high mutation rates and convergent evolution amidst ACPA sequences. In addition, recombinant antibodies derived from the repertoires target a variety of citrullinated proteins, including a-enolase, fibrinogen, and histones. Our data provide insight into the evolution of the ACPA repertoire and suggest that these autoantibodies are produced as part of the ongoing activated B cell response in RA.

★ = member of a FOCIS Center of Excellence

OR.20. Mitochondrial ROS Generates NETs Enriched in Oxidized Interferogenic Mitochondrial DNA

Christian Lood[1], Luz Blanco[2], Monica Purmalek[2], Suk De Ravin[3], Carolyne Smith[2], Carmelo Carmona-Rivera[2], Harry Malech[3], Jeffrey Ledbetter[4], Keith Elkon[1,4] and Mariana Kaplan[2]. [1]University of Washington, Seattle, WA; [2]Systemic Autoimmunity Branch, National Institutes of Health, Bethesda, MD; [3]Laboratory of Host Defenses, National Institutes of Health, Bethesda, MD; [4]★University of Washington, Seattle, WA

DNA released through formation of neutrophil extracellular traps (NETs) may promote inflammatory responses in systemic lupus erythematosus (SLE) and other autoimmune diseases. However, the involvement of mitochondria in the generation of ROS necessary for NETosis as well as their inflammatory potential is not clear. Following RNP immune complex (IC) activation, mitochondria generated ROS, translocated to the cell surface and released 8-OHdG-oxidized mitochondrial DNA (mitDNA) that supported the induction of type I interferons ($p<0.001$). Lupus low-density granulocytes (LDGs), a distinct proinflammatory subset, displayed enhanced synthesis of mitochondrial ROS. Inhibition of mitochondrial ROS production following RNP IC-mediated NETosis or spontaneous NETosis in LDGs decreased release of mitDNA ($p<0.001$), as well as its oxidation ($p<0.001$), thus limiting the inflammatory potential of the released material. Finally, patients with chronic granulomatous disease (CGD), a disorder caused by impaired NADPH oxidase activity and associated with a risk for autoimmunity, had circulating LDGs that spontaneously produced NETs requiring mitochondrial ROS synthesis. Therefore, certain immune stimuli induce NETosis, at least in part, by mitochondrial ROS production resulting in the release of oxidized inflammatory mitDNA. These findings suggest a novel and important role of mitochondria in immune-mediated NETosis and may enable new therapeutic targets for patients with SLE and CGD.

OR.21. Integrated, Multi-cohort Analysis of Systemic Lupus Erythematosus Identifies Clinically Relevant, Interferon-Independent Disease Signature

Winston Haynes, Paul Utz, Erika Bongen and Purvesh Khatri. ★Stanford University, Stanford, CA,

Systemic Lupus Erythematosus (SLE) is a severe autoimmune disease characterized by inflammation, swelling, and damage to many organs, including the skin and kidneys. Despite decades of research and millions of dollars of R&D, therapeutic options for SLE treatment remain inadequate. Studies which have attempted to characterize gene expression in SLE are based on single experiments and tend to focus on interferon-inducible genes. Here we show that data-driven analysis of SLE identifies a highly persistent gene expression signature. By leveraging the corpus of SLE gene expression data already deposited in the public realm, we identify a highly robust SLE signature which distinguishes SLE patients from healthy controls across diverse experiments, tissues, cell types, and organisms. Our SLE signature significantly correlates with the SLE disease activity index (SLEDAI). Further, we show that an important component of our signature is independent of interferona. Integrating our signature with other data sources, we identify biologically meaningful pathway dysregulation in SLE and propose 31 drugs for the treatment of SLE, validating six with perfect accuracy. Our research methodology presents a general framework for utilizing integrated, multi-cohort analyses of immunological diseases to drive more targeted and effective therapeutic development.

OR.22. $CD8^+PD1^+$ T Cells Enriched at the Site of Chronic Autoimmune Inflammation Show Exhaustion Signature

Alessandra Petrelli, Michal Mokry, Berent J. Prakken and Femke van Wijk. ★UMC Utrecht, Utrecht, Netherlands

Introduction: The site of chronic autoimmune inflammation (i.e., the synovial fluid, SF) of patients affected by autoimmune arthritis is enriched in $CD8^+PD1^+$ T cells, which have been described to have poor effector function (namely exhausted) in chronic infectious and tumor environments. We hypothesized that $CD8^+PD1^+$ T cells from the

SF of Juvenile Idiopathic Arthritis (JIA) patients are exhausted, thus we aimed to investigate the molecular signature of this cell population.

Methods: Phenotypic characterization of $CD8^+PD1^+$ and $CD8^+PD1^-$ cells from the peripheral blood (PB) of healthy controls (HC) and SF of JIA patients was performed by flow cytometry. Whole-transcriptome sequencing was used to identify specific gene expression signatures.

Results: $CD8^+PD1^+$ T cells from both PB and SF were endowed with a memory phenotype. SF-derived $CD8^+PD1^+$ T cell population showed later stage of differentiation as wells as increased expression of negative co-stimulatory markers (i.e. TIM-3, LAG-3). RNA-sequencing data indicated a clear segregation of the PD1+ and $PD1^-$ cell subsets in the SF, which was less defined in the PB. Several pathways of T cell exhaustion (i.e apoptosis, interferon signaling) were upregulated in SF-derived $CD8^+PD1^+$ T cells. Furthermore, Gene Set Enrichment Analysis (GSEA) showed the enrichment of the *exhaustion* gene set in the SF-derived $CD8^+PD1^+$ cell population.

Conclusion: $CD8^+PD1^+$ cells from the site of chronic autoimmune inflammation of JIA patients show genetic signature of *exhausted* cells. If confirmed at a functional level, those findings would lay the foundation for investigation on novel therapeutic strategies to induce T cell exhaustion in autoimmune diseases.

OR.32. Dependence of Autoreactivity in Lupus-associated Antiphospholipid Syndrome on Gram-positive Gut Microbiota

***Silvio Manfredo Vieira**, William Ruff, Michael Hiltensperger, Andrew Yu, Andrew Goodman and Martin Kriegel.*
★*Yale University School of Medicine, New Haven, CT*

Infectious triggers have been implicated in transient antiphospholipid antibody production but the cause of chronic antiphospholipid syndrome (APS) is unknown. We disrupted the gut microbiota composition in the $(NZWxBXSB)F_1$ model of lupus-associated APS using antibiotics after maturation of the immune system. Mice were treated orally with combined or single antibiotics (vancomycin, metronidazole, neomycin, ampicillin) targeting different microbial community structures starting at 6 weeks of age. Sera, urine and fecal samples were collected longitudinally and analysed for anti-β_2GPI titers and eubacterial 16S ribosomal DNA load by real-time PCR. Splenocyte proliferation was assessed by [^{3}H]-thymidine incorporation. Cytokines were measured by ELISA. H&E slides from kidneys and hearts were analyzed by microscopy. Not only combined antibiotics but also vancomycin or ampicillin alone lowered anti-β_2GPI antibodies at 4 months of age ($p=0.014$) and protected mice from deaths due to coronary microthrombi, pulmonary emboli or strokes ($p=0.005$). 16S rDNA sequencing on the MiSeq platform revealed preliminarily depletion of similar gram-positive anaerobic communities with vancomycin and ampicillin as were enriched in longitudinally collected human APS samples compared to controls. The regulatory cytokine IL-10 was increased in serum of protected mice. Proliferation of splenocytes to the autoantigen (using recombinant β_2GPI) was diminished compared to anti-CD3-induced proliferation ($p=0.0012$). Proteinuria due to lupus nephritis was also suppressed in microbiota-depleted mice ($p=0.026$). In summary, vancomycin-sensitive gut commensals are necessary for anti-β_2GPI-antibody-induced thrombembolic deaths and autoantigen-specific lymphocyte proliferation. These results support that gram-positive members of the gut microbiota are fundamentally involved in the pathogenesis of APS and systemic lupus.

OR.48. A Multidimensional Immunomics Approach Identifies an Immune Signature Possibly Relevant in Systemic Sclerosis Pathogenesis

***Hari Balaji Venkatanarayanan**[1,2], Andrea Hsiu Ling Low[3,4], Liyun Lai[1,2], Juntao Li[1,2], Raymond Ong[1,2], Jing Yao Leong[1,2], Camillus Chua[1,2], Agnieszka Maliszewska[1,2], Lakshmi Ramakrishna[1,2], Julian Thumboo[3,4] and Salvatore Albani[1]. [1]Duke-National University of Singapore Graduate Medical School, Singapore;[2] SingHealth, Singapore; [3]Singapore General Hospital, Singapore; [4]National University of Singapore, Singapore*

★ = member of a FOCIS Center of Excellence

Systemic sclerosis (SSc), is a chronic autoimmune disorder of the connective tissue. Limited knowledge regarding the immunological correlates in scleroderma pathophysiology impedes the development of therapeutic interventions. We have integrated in a cogent platform - Immunomics, highly multiplexed technologies with the objective of identifying unique immune signatures that cluster meaningfully at the single-cell level. 1) Mass spectrometry (CyTOF) analysis of peripheral blood using 35+ phenotypic and intracellular markers: both direct *ex vivo* and after stimulation with Topoisomerase I, an autoantigen relevant in SSc. 2) Gene expression studies of skin punch biopsies using NanoString. 3) Next-gen, RNA-seq analysis of the the whole transcriptome, including TCR usage of topoisomerase I specific T cells generated from the peripheral blood and skin microenvironment. Using this approach, we interrogated samples from SSc subjects (n=10) both from the blood and skin microenvironment with various high throughput experimental approaches. The dense data sets generated from these high throughput measurements were analyzed using both in-house generated multivariate analysis algorithms and computational tools such as PCA, ACCENSE and Random Forest. Our approach identified distinct clusters of T cells expressing either CXCR5, IL-17A or Granzyme B. This circumscribes a role for diverse T cell phenotypes such as follicular T helper (Tfh) cells that potentiate a B cell response or pathogenic T cells such as Th17 in SSc pathophysiology. Indeed, NanoString analysis of SSc skin revealed a set of highly expressed genes that were regulated by IL-17A. Finally, unique clonotypes of CXCR5 expressing topoisomerase I specific $CD4^+$ T cells could be identified.

OR.51. Epigenetic Traits Correlated to Clinical Activity in Juvenile Idiopathic Arthritis

Roberto Spreafico[1,2], Maura Rossetti[1,2], Carol Wallace[3], Daniel Lovell[4] and Salvatore Albani[2,5]. [1]Sanford-Burnham Medical Research Institute, San Diego, CA; [2]SingHealth Translational Immunology and Inflammation Centre, Singapore Health Services Pte Ltd, Singapore; [3]Seattle Children's Hospital and Research Institute, Seattle, WA; [4]★Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [5]Duke-NUS Graduate Medical School, Singapore, Singapore

Epigenetic regulation of gene expression is increasingly under scrutiny to understand the pathogenesis of multifactorial human diseases, such as juvenile idiopathic arthritis (JIA). Indeed, the low concordance rate between monozygotic twins (20-40%) underscores that, while this autoimmune disease has a genetic component, environmental triggers are fundamental in the disease pathogenesis. Epigenetic mechanisms are believed to integrate such non-genetic factors, and may underlie the dysregulation of the immune system. We interrogated the DNA methylome of patients before and after anti-TNF therapy withdrawal. Individual CpG sites were clustered in coherent modules without any a priori knowledge of their function. Strikingly, modules statistically associated to clinical activity were found to be enriched in CpG sites belonging to genes that mediate T cell activation. Conversely, modules linked to age or gender controlled fundamental, non-immune functions of the cell, such as metabolism. Of note, the DNA methylome was stable, showing little variation before and after therapy discontinuation. When a similar analysis was performed on matched transcriptomic data, we found that the correlation of mRNA abundances with clinical activity was much lower than that observed for DNA methylation. In summary, our work (i) demonstrates that medically relevant information is encoded in epigenetic traits; (ii) reveals biological functions tied to clinical activity; and (iii) establishes the superiority of epigenetic markers over gene expression-based markers.

W.1. Folate Receptor Beta in Giant Cell Arteritis Macrophages: A Pilot Study

***Shirley Aluquin**[1], Nancy Olsen[1], Vincent Aluquin[1], Manohar Ratnam[2] and Jozef Malysz[1]. [1]Penn State Hershey College of Medicine, Hershey, PA; [2]Wayne State School of Medicine, Detroit, MI*

Background: Giant cell arteritis (GCA) is an idiopathic vasculitis that affects older persons and presents with vision loss, stroke and tissue gangrene. Diagnosis is based on temporal artery biopsy (TAB) findings of activated macrophages (AMs) and lymphocytes infiltrating the vessel wall. The pathogenic role of AMs in GCA are related to their pro-inflammatory and destructive properties but studies aimed at macrophage-targeted mechanisms are minimal and empiric treatment with glucocorticoids and methotrexate have been disappointing. The membrane-

bound folate receptor beta (FRB) is a biomarker with potential for molecular-targeting in cancer. Recently, FRB was demonstrated to be expressed in AMs of autoimmune diseases like sarcoidosis and rheumatoid arthritis and found to transport folate conjugates/antifolate drugs into the macrophage cell, a novel mechanism that is unexplored in GCA.

Aim: To evaluate if FRB is selectively expressed in AMs in GCA.

Methods: Formalin-fixed paraffinized tissues were examined from 6 patients with GCA and 2 with normal TABs. Immunohistochemistry was performed using FRB, CD68 and CD3 antibodies for recognition of AMs, their distribution and composition of infiltrates.

Results: In GCA, inflammation was moderate-to-severe. AMs comprised 38.3 ± 4.1% of total infiltrate and majority was in the adventitia. CD3(+) lymphocytes accounted for 61.7 ± 4.1% of total infiltrate. FRB was selectively expressed in AMs and comprised 33.9 ±12.7% of the macrophage population in GCA. Controls showed no AMs or FRB expression.

Conclusion: We demonstrated for the first time that AMs in GCA selectively expressed FRB. Clinical outcome correlation and antifolate-binding properties of FRBs should be evaluated in future studies.

T.24. Anti-HMGCR Auto-antibodies in Necrotizing Autoimmune Myopathies: Diagnostic Value and New Insights into the Role of Complement in Pathogenesis

Olivier Boyer[1,2,3], Cecile Bergua[1,3], Helene Chiavelli[1,2,3] and Laurent Drouot[1,3]. [1]Inserm, U905, Rouen, France; [2]Rouen University Hospital, Rouen, France; [3]Normandy University, IRIB, Rouen, France

Background and objective: Necrotizing autoimmune myopathies (NAM) are a newly recognized group of severe acquired myopathies characterized by 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 a quantitative assay of anti-HMGCR aAbs and investigated their pathogenic role in an *in vivo* mouse model.

Methods: Recombinant human rhHMGCR was coupled to fluorescent beads and used to measure the levels of aAbs by Luminex. IgGs purified from anti-HMGCR aAb-positive patients were transferred to C57BL/6 or $Rag^{-/-}$ mice. Alternatively, mice were immunized with rhHMGCR plus adjuvant. The role of complement was studied by inactivation with cobra venom or using $C3^{-/-}$ mice.

Results: Anti-HMGCR positive patients were mostly females. Only 40% had been exposed to statins. Titers of IgG aAbs correlated with creatine kinase levels. In mice, injection of anti-HMGCR positive IgGs resulted in significant decrease in muscle strength. This was associated to moderate myofiber necrosis, and hIgG and C5b-9 deposits on myofibers. The effect was transient in immunocompetent mice (with production of anti-hIgG Abs) and prolonged in immunodeficient $Rag^{-/-}$ animals. Immunization with rhHMGCR also led to impaired muscle strength. In the absence of complement, anti-HMGCR positive IgGs did not transfer disease.

Conclusion: Anti-HMGCR aAb assays are helpful for NAM diagnosis. Experimentally, anti-HMGCR aAbs are directly pathogenic through activation of the classical complement pathway, supporting further evaluation of complement targeting therapies.

T.25. Bob1 Expression by B Cells is Required for Germinal Center Formation and Development of Collagen-Induced Arthritis

Maartje Levels[1,2], Melissa van Tok[1,2], Tineke Cantaert[1,2], Dominique Baeten[1,2] and Nataliya Yeremenko[1,2]. [1]★Academic Medical Center/University of Amsterdam, Amsterdam, Netherlands; [2]Amsterdam Rheumatology & Immunology Center, Amsterdam, Netherlands

Despite growing insights in the breach of immune tolerance, it remains currently unknown which molecules drive or control pathogenic B cells in rheumatoid arthritis (RA). Recently we have identified a marked increase in expression of the B cell-specific transcriptional co-activator Bob1 in RA synovitis. Additionally, we found that mice lacking Bob1 failed to produce pathogenic anti-collagen autoantibodies and were completely protected from collagen-induced arthritis (CIA), the animal model of RA.

In order to determine whether this remarkable resistance to CIA in the absence of Bob1 is related to failure of germinal center (GC) formation or to intrinsic B cells defects we transferred wild type (WT) B cells to Bob1-deficient animals, lacking functional GC, followed by CIA induction. After adoptive transfer, Bob1-deficient mice were still resistant to CIA, suggesting that this resistance is related to GC formation rather than to intrinsic B cells defects in the absence of Bob1. To prove that functional Bob1 exclusively in B cells is required for susceptibility to CIA, we adoptively transferred various combinations of WT and Bob1-deficient B and T cells to RAG-1-*null* mice followed by CIA induction. The results showed that only animals that received WT B cells displayed signs of arthritis and contained anti-collagen antibodies.

This data strongly suggest that expression of Bob1 in B cells is indispensable for GC formation and required for the development of CIA and the formation of anti-collagen antibodies. The mechanisms behind an aberrant Bob1 expression and the break of peripheral tolerance in RA are currently under investigation.

T.26. Cross-reactivity of the Human Gut Commensal *Roseburia Intestinalis* with the Autoantigen in Antiphospholipid Syndrome

William Ruff[1], Carina Dehner[1], Silvio Manfredo Vieira[1], Andrew Yu[1], Woojin Kim[1], Cassyanne Aguiar[2], Doruk Erkan[2], Andrew Goodman[1] and Martin Kriegel[1]. [1]★Yale University School of Medicine, New Haven, CT; [2]★Hospital for Special Surgery, New York, NY

Antiphospholipid syndrome (APS) is an autoimmune clotting disease characterized by autoantibodies interfering with coagulation. Given the vast antigenic potential of the gut microbiota, we hypothesized that adaptive immune responses to gut commensals may sustain chronic autoreactivity via cross-reactivity. In silico searches identified the common, gram-positive gut commensal *Roseburia intestinalis* as a prime candidate for molecular mimicry, containing peptide sequences highly homologous to both T and B cell immunodominant epitopes of the major autoantigen in APS, the plasma protein b_2-glycoprotein I (b_2GPI). PBMCs from APS patients proliferated significantly more to *R. intestinalis* protein extracts compared to phylogenetically closely related *Eubacterium rectale*, which lacks homologous peptides. We therefore cloned b_2GPI-specific CD4 memory T cells using a T cell library assay and tested cross-reactivity with peptides *in vitro*. Preliminary data supports that IL-2 but not IL-4, IL-10 or IL-17 secretion can be elicited from an autoantigen-specific Th1 clone in response to a *R. intestinalis* peptide that mimics a major T cell epitope in domain V of b_2GPI. Furthermore, Western blotting using patient plasma samples revealed specific bands for *R. intestinalis* including a potential cross-reactive protein at 24 kDa that contains 100% homology to the dominant B cell epitope in domain I of b_2GPI. Pre-adsorption using recombinant b_2GPI prior to Western blotting showed a decrease in this candidate band. These data support a working model whereby autoreactive T and B cell responses are sustained by cross-reactivity with a common gut commensal. Cross-reactivity with persistently colonizing microbiota could represent a general paradigm in chronic autoimmunity.

T.27. Aromatase Gene Expression is Upregulated in Diagnostic Muscle Biopsies from Girls with Untreated Juvenile Dermatomyositis

Dong Xu[1], Akadia Kachaochana[1,2], Adam Ostrower[1,2], John S. Coon, V[2], Gabrielle A. Morgan[1,2], Hong Zhao[2], Chiang-Ching Huang[3], Serdar E. Bulun[2] and **Lauren Pachman**[1,2]. [1]★*Ann & Robert H. Lurie Children's Hospital of Chicago Research Center, Chicago, IL; [2]Northwestern University, Chicago, IL; [3]University of Wisconsin-Milwaukee, Milwaukee, WI*

Background: We observed: WT-1 was massively hypomethylated in muscle biopsies (MBx) from untreated or treated active JDM. Others had shown: 1) WT-1 controls the proximal promoter II activity of aromatase, which regulates estrogen synthesis; 2) proinflammatory cytokines elicit aromatase production through its distal aromatase promoter I.4.

Hypothesis: Dysregulated estrogen homeostasis may play a role in JDM pathophysiology.

Methods: Ten girls (5 regular, 5 irregular menses) with JDM had MRI-directed IRB-consented MBx (mean age 9.0±3 yrs) were compared with MBx from 4 orthopedic control girls (16.0±1.0 yrs). Muscle total RNA was assayed for aromatase gene expression levels, qPCR (Taqman). Mesoscale measured plasma levels of proinflammatory cytokines (IL6, IL-1β and TNF-α, t test). The association of the level of aromatase gene expression with disease activity scores (DAS) for skin, muscle, and total score was determined.

Results: Aromatase mRNA levels were 9.78 fold higher in JDM MBx compared with healthy controls ($p=0.004$), but did not differ between JDM girls with either regular or irregular menses ($p=0.4$). Aromatase levels were not associated with any DAS. Promoter I.4 was the dominant aromatase promoter identified in JDM MBx. In JDM plasma, IL-6, IL-1β and TNF-α levels were elevated compared to controls ($p<0.05$).

Conclusion: Aromatase upregulation may be associated with JDM pathophysiology. We speculate: 1) elevated proinflammatory cytokines induce aromatase expression via the distal aromatase promoter I.4 in JDM; 2) high aromatase leading to increased local estrogen biosynthesis in muscle tissue may contribute to the targeted distribution of muscle involvement characteristic of JDM.

T.28. Different Sets of Proteins Correlate with Different Stages of Interstitial Lung Disease in Patients with Systemic Sclerosis

Isela Valera, Anagha Divekar, Dinesh Khanna, Fereidoun Abtin, David Elashoff, Paul Maranian, Rajan Saggar, Daniel Furst and Ram Singh. ★*University of California, Los Angeles, Los Angeles, CA*

Interstitial lung disease (ILD), a major manifestation of systemic sclerosis (SSc) progresses through inflammation, fibrosis and end organ damage, identified on high-resolution CT (HRCT) by ground-glass opacity (GGO), fibrosis and honeycombing, respectively. Finding biomarkers at the site of disease may open avenues for early detection of disease stages and potential targets of treatment.

We analyzed 96 proteins using a microplate-based multiplex array in 22 bronchoalveolar lavage (BAL) samples from the right middle (RML) and lower (RLL) lobes of 11 SSc-ILD patients. Levels of selected molecules were verified using ELISA. Correlation of protein levels with HRCT scores using mixed effects modeling revealed that while lipocalin-2, CCL21, VEGF-C and soluble gp130 correlated with both fibrosis (β=0.55-0.82,p=0.044-0.002) and GGO (β=0.49-0.82,p=0.048-0.002), ten additional proteins correlated only with fibrosis (β=0.39-0.76, p=0.048-0.004) and six additional proteins correlated only with GGO (β=0.5-0.73, p=0.049-0.006). On gene ontology analyses, proteins associated with GGO and fibrosis belonged to different coordinated biological processes. BAL proteins that associated with SSc-ILD exhibited a negative/no correlation with SSc-skin disease. A few proteins identified in BAL to

correlate with GGO/fibrosis were also detected in serum to associate with the presence of SSc-ILD (p<0.05-<0.001) in another cohort of 49 SSc patients (p<0.05-0.001).

In summary, we identified novel potential biomarkers for SSc-ILD including lipocalin-2, CCL21, Areg, CCL22, M-CSF and IL-16. Importantly, different protein markers that associate with different coordinated biological processes correlate with GGO and fibrosis. Further studies are needed to discern whether GGO and fibrosis indeed represent pathogenetically distinct stages of SSc-ILD.

T.29. Using the ACR and SLICC Criteria to Determine Disease Severity in Subacute Cutaneous Lupus Erythematosus

Janice Tiao[1] and Victoria Werth[2]. [1]★University of Pennsylvania, Philadelphia, PA; [2]United States Veteran Affairs Medical Center, Philadelphia, PA

Roughly 50% of patients with subacute cutaneous lupus erythematosus (SCLE) also meet criteria for systemic lupus erythematosus (SLE). However, previous studies have not characterized which SLE criteria such patients meet. This study is a case-control comparison of patients with SCLE/SLE against patients with only SCLE in order to identify which criteria SCLE/SLE patients are more likely to have using two sets of criteria, the 1997 ACR and 2012 SLICC criteria. The information was obtained from an ongoing database of lupus patients seen at the University of Pennsylvania and from their respective medical records. Using the ACR criteria, 28 patients (33%) were classified as SCLE/SLE and 57 (67%) as SCLE-only, compared with 23 (27%) SCLE/SLE and 62 (73%) SCLE-only patients using the SLICC criteria (p=0.5). Presence of the following was noted: alopecia, malar rash, discoid rash, photosensitivity, oral ulcers, arthritis, serositis, renal disorder, neurologic disorder, hematologic disorder, anemia, leukopenia, thrombocytopenia, $^{+}$ANA, $^{+}$anti-dsDNA, $^{+}$anti-Smith, $^{+}$anticardiolipin antibodies, $^{+}$lupus anticoagulant, $^{+}$beta-2-glycoprotein, and low complement. According to both sets of criteria, SCLE/SLE patients were more likely than SCLE-only patients to have oral ulcers (ACR 64% *vs.* 9%, p<0.0001; SLICC 61% *vs.* 15%, p<0.0001), $^{+}$anti-dsDNA (ACR 52% *vs.* 3%, p<0.0001; SLICC 65% *vs.* 3%, p<0.0001), and $^{+}$ANA (ACR 93% *vs.* 26%, p<0.0001; SLICC 92% *vs.* 32%, p<0.0001). These findings suggest that patients with SCLE/SLE are no more likely than their SCLE-only counterparts to exhibit systemic symptoms. This is consistent with the current line of thinking that considers SCLE as a marker of less severe lupus disease.

T.30. Tissue-resident Cells Maintain Local Immune Tolerance in Systemic Autoimmune Disease

Ram Singh, Jennifer King, Rachael Philips, Peter Kim and Anna Eriksson. ★University of California, Los Angeles, Los Angeles, CA

Systemic autoimmune diseases such as lupus affect multiple organs, usually in a diverse fashion where only certain organs are affected in individual patients. It is unclear whether the 'local' immune cells play a role in regulating tissue specificity in relation to disease heterogeneity in systemic autoimmune diseases. Here, we used skin as a model to determine the role of tissue-resident dendritic cells (DC) in local and systemic involvement within a systemic lupus disease model. Skin-resident DCs, namely Langerhans cells (LC), have been implicated in regulating tolerance or autoimmunity using elegant transgenic models, however, their role in local versus systemic immune regulation is unknown. We demonstrate that while lymphocytes from skin-draining lymph nodes of autoimmune-prone MRL/MpJ-*Fas*$^{lpr/lpr}$ mice react spontaneously to a physiological skin self-antigen desmoglein-3 (p<0.01), epicutaneous applications of desmoglein-3 induced FoxP3^{+} Tregs and T cell tolerance that is dependent on LCs. Inducible ablation of LCs in adult, preclinical MRL/MpJ-*Fas*$^{lpr/lpr}$ and MRL/MpJ-*Fas*$^{+/+}$ mice resulted in markedly accelerated lupus dermatitis (p<0.001), increased local macrophage infiltration (p=0.001), and increased autoantibodies against skin antigens (p=0.03-0.01), but had no effect on systemic autoantibodies such as anti-dsDNA antibodies or disease in other organs such as kidneys, lung, and liver. Furthermore, skin-draining lymph nodes of chronically LC-ablated MRL/MpJ-*Fas*$^{lpr/lpr}$ mice had significantly fewer CD4^{+} T cells producing anti-inflammatory cytokine IL-10 than LC-intact

controls (p=0.04). These results indicate that a skin-resident DC population regulates local tolerance in systemic lupus and emphasize the importance of the local immune milieu in preventing tissue-specific autoimmunity yet have no effect on systemic autoimmunity.

T.31. Generation and Characterization of Anti-citrullinated Protein Antibody-producing B Cells

Kristine Germar[1,2], Nathalie van Uden[1,2], Thais Rizzi[1,2], Sabrina Pollastro[1,2], Priscilla Kerkman[3], Ellen van der Voort[3], Evan Reed[4], Mark Kwakkenbos[5], Arjen Bakker[5], Karin Lundberg[4], Niek de Vries[1,2], Lars Klareskog[4], Hans Scherer[3], Rene Toes[3], Hergen Spits[5,2] and Dominique Baeten[1,2]. [1]Amsterdam Rheumatology and Immunology Center, Amsterdam, Netherlands; [2]★Academic Medical Center/University of Amsterdam, Amsterdam, Netherlands; [3]Leiden University Medical Center, Leiden, Netherlands; [4]★Karolinska Institutet/Karolinska University Hospital, Stockholm, Sweden; [5]AIMM Therapeutics, Amsterdam, Netherlands

Rheumatoid arthritis (RA) is the most frequent form of autoimmune arthritis with a prevalence of almost 1% worldwide. Anti-citrullinated protein antibodies (ACPA) are the most specific biomarker for rheumatoid arthritis (RA). Although therapies designed to deplete B cells, which are the precursor to antibody-secreting cells, are effective in the treatment of RA, they only modestly affect ACPA and have no effect on antibodies to recall antigens in serum of treated patients. This suggests that autoreactive B cells may contribute to RA pathophysiology by additional mechanisms independent of terminal differentiation toward ACPA-producing plasma cells. While much attention has been given to understand the role of the autoantibodies, the lack of tools has prevented the reliable identification of citrullinated protein (CP)-reactive B cells directly *ex vivo*. Here we describe the use of a novel B cell immortalization technique to establish CP-reactive B cell clones from the blood or synovial fluid of RA patients. These clones can secrete antibodies that specifically bind cyclic citrullinated peptide (CCP)2 yet show no reactivity to the control arginine variant. While clones generated through this method can secrete soluble antibody, they retain surface immunoglobulin expression and can internalize and present antigen to T cells. Finally, these clones have a unique surface profile of costimulatory molecules and can secrete both pro- and anti-inflammatory cytokines. Altogether, we propose that this novel tool can be utilized to characterize and model the function of autoreactive B cells in RA and potentially other autoimmune disorders with described autoantigens.

T.32. Analysis of Immune Cell Dysfunction in Systemic Sclerosis Patients

Michelle Fleury, Hans Dooms, Jennifer Snyder-Cappione, Cristina Vazquez Mateo, Justin Collins, Anna Belkina and Robert Lafyatis. Boston University, Boston, MA

Systemic Sclerosis (SSc) is an autoimmune connective tissue disorder associated with tissue fibrosis and has a ten year survival rate of less than fifty percent with few treatment options for patients. We hypothesized that immune dysfunction and failure of immunologic tolerance contribute to disease pathogenesis in these patients. We designed a novel fifteen color flow cytometry panel to characterize co-inhibitory receptor expression on five separate immune cell types from PBMCs of SSc patients and healthy controls. While we found no change in the relative frequencies of cells in the adaptive compartment of SSc patients, we found a general increase in the expression of the co-inhibitory receptors PD-1, LAG-3, CTLA-4, and Tim-3. Increase in the expression of these receptors has been previously associated with chronic T cell activation, supporting the presence of immune dysfunction. We also observed an increase in FoxP3$^+$ cells in SSc PBMCs, although the percentage of CD25^{hi}CD127$_{low}$ Tregs remained unchanged. In some patients, Foxp3$^+$ T cells expressed enhanced levels of CD127, which may be associated with altered Treg function. Follow up studies characterizing the role of up-regulation of co-inhibitory receptors in immune cells of SSc patients, as well as further examination of Treg suppressive capacity, will demonstrate if this dysfunction has a protective role or if it contributes to aggravated disease.

T.33. Longitudinal Microbial Community Profiling, IgA-Seq, and Gut Barrier Analysis in Antiphospholipid Syndrome

William Ruff[1]*, Woojin Kim*[1]*, Andrew Yu*[1]*, Silvio Manfredo Vieira*[1]*, Carina Dehner*[1]*, Cassyanne Aguiar*[2]*, Doruk Erkan*[2]*, Andrew Goodman*[1] *and Martin Kriegel*[1]*. [1]★Yale University School of Medicine, New Haven, CT; [2]★Hospital for Special Surgery, New York, NY*

The antiphospholipid syndrome (APS) is an autoimmune thrombophilic disorder with high mortality and unknown etiology. Based on work from our laboratory, APS is dependent on the gut microbiota in a spontaneous murine model. We therefore hypothesized that the gut microbiota represents a chronic trigger in human APS. We set out to characterize the fecal microbial community composition and gut barrier integrity longitudinally in APS patients at 3 monthly time points. 41 stool samples from 15 APS patients and 49 samples from 18 controls were collected to date. We also defined IgA-bound communities by IgA-Seq at one time point. The V4 region of the 16S rDNA gene was sequenced on the Illumina MiSeq platform and analyzed using QIIME and LEfSe. Initial community analysis indicated increased abundance of *Actinomyces, Coriobacteriaceae,* and *Desulfovibrionales* in APS patients relative to controls. Fecal calprotectin, a marker of intestinal inflammation, was significantly higher in APS patients ($p < 0.003$), as was fecal bacterial IgA coating by FACS ($p < 0.05$), supporting gut barrier dysfunction in APS. IgA-Seq revealed that the majority of *Actinomyces* and *Coriobacteriaceae* in APS patients is IgA coated, therefore recognized by the adaptive immune system. These commensals, in particular *Actinomyces* that are capable of synthesizing phospholipids, are attractive candidates we are studying in more depth using cultured strains, APS patient sera and PBMC. This study represents the first 16S rRNA community profiling of IgA-coated gut commensals in patients with non-gut autoimmunity and supports a role for dysbiosis and aberrant host-microbiota interactions in human APS.

T.34. Expression of Transcription Factors Associated with M1/M2 Activation is Modified During Blockade of IL-1 in Systemic Juvenile Idiopathic Arthritis

Claudia Macaubas[1]*, Justin Lee*[1]*, Yujuan Zhang*[1]*, Saloni Gupta*[1]*, Clarissa Klein*[1]*, M. Virginia Pascual*[2]*, Arielle Hay*[3]*, Susan Thompson*[4]*, Christy Sandborg*[1]*, Norman Ilowite*[3] *and Elizabeth Mellins*[1]*. [1]★Stanford University, Stanford, CA; [2]★Baylor Institute for Immunology Research, Dallas, TX; [3]Children's Hospital at Montefiore, New York, NY; [4]★Cincinnati Children's Hospital Medical Center,*Cincinnati, OH*

Systemic juvenile idiopathic arthritis (sJIA) is a chronic childhood disease, characterized by remitting fever, transient rash, and relapsing arthritis. Blockade of IL-1 has been shown to be effective as sJIA treatment, but the mechanisms underlying this effect are not well understood. Clinical and experimental data have implicated monocytes in sJIA pathogenesis. We investigated if monocyte phenotypes are affected by IL-1 blockade. Monocytes isolated from 22 patients treated with Rilonacept, an IL-1 trap, enrolled in the RAPPORT trial (RAndomized Placebo Phase study Of Rilonacept in the Treatment of sJIA), were tested by RT-PCR to determine level of genes associated with monocyte polarization and IL-1 expression. Blood was obtained at week 0, 2, 4, 14 and 24. Samples were grouped as baseline, early treatment (< 10 weeks) and later treatment (> 10 weeks); not all subjects were analyzed for every time point. Statistical analysis was performed using Kruskall-Wallis with Dunn's multiple comparisons test. Levels of IL-1beta and the P2X purinergic receptor 7 (P2RX7) decreased after 10 weeks of treatment. M2-associated transcription factors (TF) STAT6 and KLF4, and M1-associated TF IRF5 were also decreased in late treated samples. In contrast, expression of the TF PPARgamma is increased in both early and late samples. These results indicate that IL-1 blockade in sJIA is likely associated with changes in monocyte activation profile, with decrease in both M1- and M2-related TFs, and increase in PPARgamma, a TF that has been associated with inhibition of inflammatory response, and with driving immune cells towards anti-inflammatory profiles.

T.35. Increased Lymph Node $CD8^+$ T Cell Proliferation and Memory Cell Markers Upon Conditional Ablation of Langerhans Cells in Autoimmune Mice

Miguel-Angel Gutierrez, Ram Raj Singh, Isela Valera[3] and Ramesh Halder. ★University of California, Los Angeles, Los Angeles, CA

Systemic autoimmune diseases such as lupus are characterized by inflammation in many organs. The role of local tissue-resident immune cells in specific organ involvement in these diseases remains unclear. We evaluated the role of Langerhans cells (LC) as prototypic tissue-resident dendritic cells (DC) in eliciting local immune defects in the MRL model of lupus. Injection of diphtheria toxin (DT) in Lang-eGFP.DTR knock-in mice results in depletion of langerin$^+$ dermal DC (dDC) and LC. To assess LC's role in systemic autoimmunity, we introgressed the knock-in mutation from the stock B6 onto the MRL background. Serial DT injections every 7-10 days exacerbate inflammation in the skin (King et al) and cornea in MRL but not in B6 mice. To investigate underlying mechanisms, we assessed the effect of LC depletion on T cells in skin/eye draining cervical lymph nodes (cLN). There was a significantly higher proliferation of CD8$^+$, but not CD4$^+$, T cells in cLN of LC-ablated than control mice; such effect was not seen in spleen CD8$^+$ T cells. Also, LC-ablated MRL, but not B6 mice, exhibited reduced CD62L on cLN T cells. Further, freshly isolated dDC were more efficient than LC in promoting the proliferation of CD8$^+$, but not CD4$^+$, cNL T cells. Such local regulation of CD8$^+$ T cells may play a role in immune tolerance locally. A defect in this mechanism owing to reduced LC migration in MRL mice (Eriksson and Singh, 2008) may confer skin/eye inflammation, without affecting other organs.

T.36. Anti-**TIF1γ** Auto-antibodies and Dermatomyositis: A Link Between Cancer and Autoimmunity

*Audrey Aussy[1,2], Laurent Drouot[1,2], Romain Hardet[1,2] and **Olivier Boyer**[1,2,3]. [1]Inserm, U905, Rouen, France; [2]Normandy University, IRIB, Rouen, France; [3]Rouen University Hospital, Rouen, France*

Background and objective: The nature of the link between cancer and autoimmunity remains a mystery. Anti-TIF1γ auto-antibodies (aAbs) are associated to paraneoplastic dermatomyositis (DM). We aimed to characterize anti-TIF1γ aAbs, the clinical profile of anti-TIF1γ-positive DM patients and the consequence of breaking tolerance to TIF1γ.

Methods: 116 DM patients were analyzed. A recombinant rTIF1γ protein was coupled to fluorescent beads and used to measure aAb level by ALBIA/Luminex using class- or subclass-specific anti-human immunoglobulin G (IgG) antibodies. Avidity of anti-TIF1γ aAbs was evaluated by elution with increased concentrations of ammonium thiocyanate. Mice were immunized with rTIF1γ$^+$ adjuvant.

Results: ALBIA-TIF1γ immuno-assay detected Ab concentrations as low as 0.8 ng/mL, and had 100% sensitivity and 97% specificity. Nineteen patients were positive (10-535 AU/mL). Most aAbs were IgG1. Sera immuno-precipitated rTIF1γ. IFI on HEp2 cells revealed a finely granular nuclear fluorescence. The 16 anti-TIF1γ positive adults were 62±15 years old: 7 had amyopathic DM including 5 with neoplasia. aAbs levels were higher in case of associated cancer (170 vs 53 AU/mL). Highest aAb avidity was observed when neoplasia had preceded DM by several years. Immunized mice developed pathology of DM target tissues (muscle oedema, IgG deposits in muscle and skin).

Conclusion: High anti-TIF1γ levels is associated to cancer in DM. High aAb avidity in long term cancer-exposed subjects suggests affinity maturation, consistent with a primary anti-tumour response against TIF1γ. Results are compatible with DM being an 'adverse effect' of TIF1γ-specific antitumor response, uncovering new links between autoimmunity and cancer.

T.37. Two Soluble Forms of IL-6 Receptor, sIL-6R and sgp130, Regulate Physiological Status and Affect Inflammatory Status Pathologically

Kazuyuki Yoshizaki, Soken Nakazawa and Kazuko Uno. Osaka University, Suita, Japan

Interleukin 6 (IL-6) signal is mediated through unique two receptor system. One is an IL-6 binding molecule (IL-6R) and the other is a trans-signal molecule, gp130, which binds IL-6/IL-6R complex. Both receptors generate soluble

forms. Soluble IL-6R (sIL-6R) is secreted by cleaving membrane bound IL-6R by proteinase, Adam 10/17. The other soluble gp130 (sgp130) is spliced and produced from membrane gp130 gene. On the inflammatory status, Adam 10/17 activity is increased, and then sIL-6R secretion is elevated. On the other hand, sgp130 production is not changed both on physiological and inflammatory conditions, however, the fundamental production amount is different and dependent on the individual splicer activity in each person. Therefore, sIL-6R may accelerate the inflammation and sgp130 may be a natural IL-6 inhibitor by binding IL-6/sIL-6R complex *in vivo*.

Tocilizumab an IL-6 receptor antibody therapy showed the good efficacy, but not all for RA patients. Therefore, we would like to predict the clinical outcome to Tocilizumab therapy before therapy. Then we detected the amount and content of cytokine/chemokine/soluble receptor biomarkers in the pretreatment serum by Bio-Plex and Millipore assay systems. Multi linear and multi logistic statistical analysis were performed between biomarkers and clinical outcome of each patient.

In result, we found sgp130 in pretreatment serum was a most coincidable biomarker with outcome to Tocilizumab therapy, and serum level of sgp130 indicated the responsiveness of Tocilizumab therapy. Now we can decide the application of Tocilizumab to the individual RA patient before therapy to know the predictive outcome.

T.38. Optimizing the Use of ANCA Tests for Vasculitis in a High Throughout Immunology Diagnostic Laboratory

Ricardo Pujol Borrell[1,2,3], Jose Loureiro-Amigo[2], Ana Marin-Sánchez[2], Roser Solans-Laqué[1,2] and Maria Paz Bermejo López-Muñiz[2]. [1]★Universitat Autònoma de Barcelona, Barcelona, Spain; [2]Hospital Universitari Vall d'Hebron, Barcelona, Spain; [3]Vall d'Hebron Institut de Recerca, Barcelona, Spain

Antineutrophil Cytoplasm Antibodies (ANCA) are increasingly important in the diagnosis and treatment of vasculitis but immunology diagnostic laboratories still have difficulties to measure ANCA and invasive techniques are very often required to confirm diagnosis prior to initiate treatment. Here we report one year experience in applying a combination of IFL (two observers) with chemiluminescence based assays (CLIA) in the setting of a large academic hospital that is a referral center for vasculitis.

During the year 2014, of 1615 sera tested for ANCA, 298 (18.5%) were positive; atypical X-ANCA (50.3%), perinuclear P-ANCA (27.5%) and cytoplasmatic C-ANCA (22.1%). CLIA was positive for anti-PR3 in 36.4% of the C-ANCA+ve and for MPO in 35.4% of the P-ANCA+ve and negative for most (97.3%) of the X-ANCA+ve sera. Clinical records of ANCA positive patients were thoroughly reviewed. 53 (18.2%) of ANCA+ves had a diagnosis of ANCA-associated vasculitis (AAV). Others were: connective tissue diseases 26 (8.9%), non-AAV vasculitides 13 (4.5%), autoimmune liver diseases 13 (4.5%) and inflammatory bowel disease 11 (3.8%).

The combined use of IFL and CLIA tests had a satisfactory diagnostic accuracy. Positive P-ANCA combined with anti-MPO+ve had a PPV of 75% for ANCA AAV while of C-ANCA+ve with anti-PR3 had a positive predictive value (PPV) of 70.8% for granulomatosis with polyangeitis. In conclusion available methods for ANCA have a good PPV for AAV when IFL and CLIA techniques are combined. Yet more sensitive and specific tests are required to increase the proportion of cases of vasculitis that are accurately diagnosis using non-invasive techniques.

T.39. Innate Immunity and Vascular Injury Markers are Prognostic for Skin Deterioration in Systemic Sclerosis

Giuseppina Stifano, Lisa Rice and Robert Lafyatis. Boston University, Boston, MA

Patients affected by diffuse cutaneous systemic sclerosis (dcSSc) show variability in clinical presentation and in disease progression. The unpredictability of disease evolution presents particular challenges for clinicians.

Physicians have to decide without any reliable laboratory parameters, which patient does not need to be treated, and which is at high risk to develop more aggressive disease. Unfortunately, clinical disease markers (modified Rodnan skin score: MRSS) have done little to predict the trajectory of dcSSc. Therefore, identifying prognostic biomarkers of progressive disease is one of the highest priorities to aid clinical management in dcSSc patients. In our early studies we found in dcSSc skin biopsy an overexpression of TLR4, MD2 and CD14 genes, suggesting a role of innate immunity in the pathogenesis of the disease. Furthermore the gene expression of these markers correlates significantly with progressive skin fibrosis, as measured by the change in MRSS six months after the skin biopsy was performed (ΔMRSS). Based on these results we decided to analyze agnostically, microarray data on 26 dcSSc patients and correlate the gene expression with the ΔMRSS. We found that endothelial cell markers, PECAM-1 and CLND5, are prognostic of worsening skin fibrosis. This data was confirmed in a separated patient population by qPCR. These findings support a role of endothelium injury in dcSSc and particularly in the progression of skin fibrosis. We concluded that in early stages of dcSSc overexpression of innate immune activation and vascular injury in the skin are prognostic of clinical deterioration and fibrosis.

T.40. Novel Mutation of the TNRFSFA1 Gene Associated with TNF Receptor-associated Periodic Fever Syndrome (TRAPS)

Susanna Felsenstein and Andreas Reiff. Children's Hospital Los Angeles, Los Angeles, CA

Mutations in the extracellular domain of the tumor necrosis factor receptor (TNFRSFA1) define the phenotype of the TNF-receptor-associated periodic fever syndrome (TRAPS). Clinical presentation, risk of amyloidosis and agent-specific treatment response is determined by site-specific mutations and their effect on TNFR-protein structure. Understanding the genetic basis of TRAPS is critical in order to distinguish between benign polymorphisms, low-penetrance mutations and those resulting in a more severely affected phenotype. We report the case of a 12 year old girl of Russian/Romanian/Armenian and Azeri ethnicity presenting with 3-4 episodes of recurrent fevers annually lasting up to three weeks since the age of one year. Fever episodes were associated with increased inflammatory markers, macular and urticarial rashes, chills, severe arthralgia, myalgia, lymphadenopathy, abdominal pain, diarrhea and weight loss. An extensive infectious work up was negative. The patient initially responded to treatment with both colchicine and steroids, however therapeutic effects decreased over time. Her condition deteriorated with prolonged periods of fever, excessive weight loss and evidence of ongoing inflammation during symptom-free intervals and she became dependent on steroids for symptom control. Treatment with IL-1 or TNFα-blockade was initially refused by the parents. Sequencing of the TNFRSF1A gene revealed a novel mutation in exon 3 with the replacement of an Arginine- with a Glycine codon, resulting in a R53G substitution in the mature protein. This case describes a novel TNFRSFA1 missense mutation in the pathogenesis of the TRAPS phenotype and illustrates the role of molecular diagnostics for the improved understanding of the heterogeneity of autoinflammatory syndromes.

F.1. The Role of HLA-E Restricted $CD8^+$ T Cells in Regulation of Autoimmune Responses in Sjogren's Syndrome

Olga Petryna[1] and Leonard Chess[2]. [1]Mount Sinai Beth Israel Medical Center, New York, NY, United States; [2]★Columbia University, New York, NY

A subset Qa-1/HLA-E-restricted $CD8^+$ T cells (CD8(H), with functions in mediating self-nonself discrimination was found to serve a crucial immunoregulatory function in man. Its target was identified in humans as the heat shock protein leader sequence peptide Hsp60sp bound to HLA –E, expressed on effector $CD4^+$ T cells. Here we provide evidence that patients with Sjogrens syndrome display a functional defect in HLA-E restricted regulatory CD8 T cells

Methods: $CD8^+$ T cell inhibition assay: Target HLA-E–transfected cells B721/E were loaded with Hsp60sp and B7sp. CD8 (H) were added. We studied the CD8(H) inhibition of target B721/E cells loaded with Hsp60sp.

We tested the effect of the CD8(H) lines on the avidity of immune responses to self-antigen myelin basic protein MBP versus those to foreign antigen tetanus toxoid TT

CD8 cell booster: DCs were loaded with Hsp60sp and planted into $CD8^+$ T cells cultures from the test group. Hsp60sp-boosted CD8(H) cells were studied in assays as above.

Results: We compared 3 Sjogren's patients (SjS) to 4 healthy controls. The CD8(H) lines of healthy controls consistently suppressed B721/E cells loaded with Hsp60sp, while CD8(H) cells form SjS lacked the ability to suppress same target. CD8(H) cells of the control inhibited immune response to MBP self-antigen. Such regulatory function was impaired in tested SjS. DC booster restored the ability of CD8(H)T cells of the test group to regulate activated CD4 cells

Conclusions: We provide evidence that patients with Sjogrens syndrome display a defect in HLA-E restricted regulatory T cells important in self-nonself discrimination

F.2. Single Cell Interferon Signatures in Lupus Patient Monocytes Reveal a Differential Impact of Interferon Signaling between Monocyte Subtypes

Zhongbo Jin, Wei Fan, Mark Jensen, Jessica Dorschner, Danielle Vsetecka, Shreyasee Amin, Ashima Makol, Floranne Ernste, Thomas Osborn, Kevin Moder, Vaidehi Chowdhary and ***Timothy Niewold.*** *★Mayo Clinic, Rochester, MN*

Background: Type I interferon (IFN) is a primary pathogenic factor in human systemic lupus erythematosus (SLE). IFN signatures have been observed in immune cell populations. We examined gene expression in individual SLE patient monocytes in this study.

Methods: $CD14^{++}CD16^-$ classical monocytes (CLs) and $CD14dimCD16^+$ non classical monocytes (NCLs) from SLE patients were purified by magnetic separation. The Fluidigm C1 System was used for single cell capture and target gene pre-amplification. Rt-PCR was used to quantify expression of 87 monocyte-related genes. An individual cell IFN score was generated based upon the expression of 17 IFN-induced genes.

Results: Monocytes from the same SLE patient blood sample demonstrated varying levels of IFN-induced gene expression. In CLs, high IFN score correlated with CD32a, IL1B, and IL8 expression. In NCLs, high IFN score was correlated with inflammatory mediators, including cytokines such as IL12, IL23, and IL15; the immune receptors CD36, CD32a, CD80, and TLR7; and inflammatory signaling genes such as RELA, STAT2, IRAK1, and MyD88. CD16 transcripts were detected in a small group of classical monocytes, despite a lack of surface CD16 expression. In these cells, CD16 expression was positively correlated with IFN score ($p=0.019$).

Conclusion: This study reveals diverse IFN responses of individual monocytes, supports the idea that IFN signaling has distinct effects upon classical and non-classical monocytes. IFN may contribute to the transition from classical to non-classical subtype in SLE. Single cell studies can reveal effects of IFN on single immune cells which may be masked in whole blood or mixed cell populations.

F.3. Lupus-associated Functional Polymorphism in PNP Causes Cell Cycle Abnormalities in Human Immune Cells

Yogita Ghodke, Jessica Dorschner, Danielle Vsetecka, Shreyasee Amin, Ashima Makol, Floranne Ernste, Thomas Osborn, Kevin Moder, Vaidehi Chowdhary, Mark Jensen and ***Timothy Niewold.*** *★Mayo Clinic, Rochester, MN*

Systemic lupus erythematosus (SLE) is a multi-system, autoimmune disease characterized by autoantibodies to nucleic acids and nucleosomal proteins. The type I interferon pathway is dysregulated in SLE and IFN-α levels are high in patients. We performed a genome-wide association study and found that a missense SNP in the purine nucleoside phosphorylase (PNP) gene associates with high serum IFN levels in SLE (rs1049564, $P=1.24 \times 10^{-7}$). PNP is a key enzyme of purine metabolism. PNP deficiency leads to dysregulated levels of deoxynucleotides, a slowing or inhibition in DNA synthesis, and defective T cell and variable B cell immunity. We find that the rs1049564 variant of PNP induces an S phase block in lymphoblastoid cells. Cell lines with homozygous variant (TT) had ~2 fold increases in S phase block as compared to cells lines with homozygous non variant (CC). We further showed that rs1049564 variant induced S phase block can be pharmacologically reversed, and similar findings were observed in SLE patient cells. These results suggest that the rs1049564 PNP polymorphism is a loss of function variant, and the C to T substitution in PNP alters PNP function results in S-phase block in select cell subsets within the lymphocyte compartment. This may result in an increase in circulating apoptotic lymphocytes, and higher type I IFN in human SLE. These findings have pharmacogenomic implications, as the S-phase block can be rescued in our *in vitro* experiments, suggesting a potential for personalized therapeutics.

F.4. Study of KIR2DL2/2DL3/2DS2 Genes as Genetic Markers, and their Ligands HLA Genes, in Response to Disease Modifying Antirheumatic Drugs in Rheumatoid Arthritis

Erandi Velarde de la Cruz, Pedro Ernesto Sánchez Hernández, Claudia Azucena Palafox Sánchez, Jose Francisco Muñoz Valle, Omar Graciano Machuca, Trinidad García Iglesias, Margarita Montoya Buelna and María Guadalupe Ramírez Dueñas. Universidad de Guadalajara, Guadalajara, Mexico

Background: Disease Modifying Antirheumatic Drugs (DMARDs) are designed to interfere in progression of rheumatoid arthritis (RA) and reduce the progressive damage in joints in patients. However, patients do not respond similarly. KIR receptors and their ligands HLA have been associated with RA pathology; therefore, KIR and HLAgenes may influence the response to treatment. We evaluate the association of KIR and HLA-C genes with the response to treatment with DMARDs in patients with RA.

Methods: We included 67 patients diagnosed with RA and 100 clinically healthy subjects. KIR and HLA-C genes genotyping was performed using the PCR-SSP method. RA patients were classified as responders and non-responders to the treatment with DMARDs, we evaluate the association using statistical analysis by chi-square (χ^2) with Yates correction; results were regarded as statistically significant at $p<0.05$.

Results: Significant difference was observed in gene and genotypic frequencies of patients and controls, KIR2DL2 and KIR2DS4 were associated to RA as risk (p=0.007, OR=2.5) and protective factors (p=0.0232, OR=0.3429), respectively. Furthermore, AA genotype was associated as a protective factor (p=0.0028). We also observed association between KIR genes and response to treatment, KIR2DS2 was absent more in non-responders than in responders to methotrexate (p=0.005).

Conclusion: In most of non-responders patients to methotrexate, KIR2DS2 gene is absent. This suggests that KIR2DS2 receptor may be involved in the immunoregulation, in conjunction with methotrexate, which would be reflected in the response to the treatment.

F.5. Overexpression of RasGRP4 in Proliferative Synovitis Induces activation of Erk and p38-MAPK

Shinsuke Yasuda, Sanae Shimamura, Yuka Shimizu, Michihito Kono, Tomohiro Shimizu, Masahiko Takahata, Kenji Oku, Toshiyuki Bohgaki, Olga Amengual, Tetsuya Horita and Tatsuya Atsumi. Hokkaido University Graduate School of Medicine, Sapporo, Japan

Background: RasGRP4 is a guanine nucleotide exchange factor for small GTPase Ras and is expressed predominantly in the mast cells, monocytes and neutrophils. Ras activation as well as MAP kinase (MAPK) phosphorylation is known in the synovial tissues from patients with rheumatoid arthritis (RA). We recently identified overexpression of RasGRP4 in fibroblast-like synoviocytes (FLS) of some patients with RA and that RasGRP4 positively controls proliferation of FLS (Kono M et al. Arthritis Rheumatol. In press).

Objective: To clarify the mechanisms how RasGRP4 induces proliferation of FLS.

Methods: Type II collagen-induced arthritis (CIA) mice were intravenously given siRNA specific for RasGRP4. Synovial tissues from some RA patients were evaluated immunohistochemically for the expression of RasGRP4, Erk and p38MAPK. HEK293 cells originally lacking RasGRP4 were transfected with expression vector that encodes hRasGRP4. Phosphorylation of Erk and p38MAPK was evaluated in transfected cells using Western blotting.
Results: CIA mice treated with RasGRP4-specific siRNA showed reduced arthritis score as well as microCT findings. RasGRP4 as well as phosphorylated p38MAPK was preferentially expressed in synovial tissues in the hyperplastic synovial lining layer of the RA patients. Phosphorylation of Erk MAPK was also found, but at lesser extent. In HEK293 cells forced to express RasGRP4, Erk pathway as well as p38MAPK was readily activated at their steady state.

Conclusion: RasGRP4 expression in FLS from RA patients contributes to the activation of Erk and p38MAPK. Systemic knockdown of RasGRP4 improved arthritis in mice CIA. RasGRP4 is therefore a possible target for proliferative synovitis.

F.6. A Circulating Reservoir of Pathogenic-like CD4+ T Cells Shares a Genetic and Phenotypic Signature with the Inflamed Synovial Micro-environment

Roberto Spreafico[1,2], Maura Rossetti[1,2], Jorg van Loosdregt[1], Carol Wallace[3], Margherita Massa[4], Silvia Magni-Manzoni[5], Marco Gattorno[6], Alberto Martini[6], Daniel Lovell[7] and Salvatore Albani[2,8]. [1]Sanford-Burnham Medical Research Institute, San Diego, CA; [2]Singapore Health Services Pte Ltd, Singapore; [3]Seattle Children's Hospital and Research Institute, Seattle, WA; [4]Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; [5]IRCCS Ospedale Pediatrico Bambino Gesu`, Rome, Italy; [6]★University of Genoa and G-Gaslini Institute, Genoa, Italy; [7]★Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [81]Duke-National University of Singapore Graduate Medical School, Singapore

Systemic immunological processes are profoundly shaped by the micro-environments where antigen recognition occurs. Identifying molecular signatures distinctive of such processes is pivotal to understand pathogenic immune responses and manipulate them for therapeutic purposes. Unfortunately, direct investigation of peripheral tissues, enriched in pathogenic T cells, is often impossible or imposingly invasive in humans. Conversely, blood is easily accessible, but pathogenic signatures are diluted systemically as a result of the strict compartmentalization of immune responses. We identified a small subset of circulating CD4+ T cells replicating the phenotypic signature of lymphocytes infiltrating the inflamed synovium. These circulating pathogenic-like lymphocytes (CPLs) were enriched in synovial clonotypes and exhibited strong production of pro-inflammatory cytokines. Importantly, CPLs were expanded in JIA patients who did not respond to therapy, and also correlated with disease activity in RA patients.

F.7. A Comparison of ANA Positive and ANA Negative Gene Expression Profiles from Rheumatoid Arthritis Patients Before and After Treatment with TNF Inhibitors

Laurie Davis and Andreas Reimold. ★UT Southwestern Medical Center, Dallas, TX

Peripheral blood mononuclear cells (PBMC) and serum were obtained from rheumatoid arthritis (RA) patients (n = 11) before and after treatment with anti-TNF-α therapy. PBMC total cellular RNA was purified and gene expression

profiles assessed on Illumina arrays. Initially, 8 RA patients were Rheumatoid factor-positive and 8 were anti-cyclic citrullinated peptide antibody-positive. After 3 months of anti-TNF-α therapy, 6 patients converted to antinuclear antibody (ANA)-positive. Transcript analysis of RA patient samples converting to ANA-positive on anti-TNF-α therapy identified 64 transcripts that were differentially regulated by at least 1.5 fold comparing pre versus post treatment samples by t-test and Benjamini-Hochberg correction. These gene expression profiles were complex with two RA patients demonstrating remarkably increased gene expression for a set of transcripts that encoded proteins involved in cell adhesion, cell stress and lipid metabolism which were reduced after treatment. Interferon-inducible transcripts were elevated in an ANA negative subset after anti-TNF-α therapy but not in the ANA positive group. Future studies will examine the protein expression and cell types that harbor the novel cell adhesion transcripts and investigate the clinical phenotypes of different array-based subsets in a larger study population. Gene expression predictors for response to therapy previously identified were inconclusive at this early timepoint. In summary, we observed unique transcriptional profiles in PBMC from RA patients after anti-TNF-α therapy. This pilot study suggests that transcriptional profiling is a precise way to measure the impact of anti-TNF-α therapies and reveals novel pathways that likely influence the immune response.

F.8. Differential Cytokine and Regulatory Cells in Discoid and Subacute Cutaneous Lupus

Janette Furuzawa-Carballeda, Silvia Mendez-Flores, David Faz, Yeraldin Esquivel and Gabriela Hernandez-Molina.
★*Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico City, Mexico*

The aim of this study was to characterize and compare the presence of diverse cytokines and regulatory T and B cells in skin biopsies of two subsets of cutaneous lupus: discoid lupus (LED) and subacute lupus (SCLE). We included 20 patients with active LED, 15 patients with active SCLE, 16 healthy skin controls (paired by age ±5 years and site of biopsy) and 5 patients with hypertrophic scares (HSc). Tissues were examined by an indirect immunoperoxidase technique for IL-22. To determine the subpopulation of $CD4^+$/IL-$17A^+$, $CD25^+$/IL-4^+,$CD4^+$/IFN-gamma$^+$ expressing T cells, $CD123^+$/IDO^+ pDCs, $CD25^+$/$Foxp3^+$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 5.5 years, 93.8% were women. Seventy-two percent of the patients had received prednisone and 84% immunosuppresors at biopsy assessment. Our results showed that the main cellular participation in both group of cutaneous lupus were Th17and Th22 responses. These subpopulations were elevated in both SS varieties, however prevailed in LED. Only IL-22 was increased in HSc. We also observed an increased Th1 and Th2 response in both cutaneous lupus groups and HSc versus controls, however of a lower magnitude. $CD25^+$/$Foxp3^+$Treg cells and $CD123^+$/IDO^+ pDCs but not $CD20^+$/IL-10^+- producing B cell were also elevated in both groups of cutaneous lupus and HSc. The explanation of these differences may be related to disease activity and treatment.

3967. Magnetic Resonance Imaging in the Diagnosis of Meniscus Damage of the Knee Joint

B. Bakiev. Tashkent Medical Academy, Urgench, Uzbekistan

The aim was to improve the diagnosis of patients with injuries of the lateral meniscus of the knee joint using magnetic resonance imaging. We studied 326 patients with injuries of the knee joint (IKJ) is constantly involved in sports. All surveyed performed conventional orthopedic examination and magnetic resonance imaging (MRI). MRI T1 and T2 used modes, studies were performed in the coronary and sagittal planes apparatus Siemens MAGNETOM Avanto 1,5 T Images (IKJ) in the frontal projection mainly were necessary to determine the status of the collateral ligaments, the femoral condyles, menisci, the intercondylar eminences large tibia. The lateral collateral ligament is identified on the back of the front sections in the form of a thin strip of low-intensity signal on the MP-T1 and T2 images. Bundle separated from the lateral meniscus (IKJ) layer of adipose tissue, which gives MP-high signal intensity in both images. (IKJ) meniscus looks like a bundle of fibers with low signal intensity extending from the medial femoral condyle. The images passing through the posterior edge of the femoral condyles, meniscus (IKJ) visualized on two -

three 5mm sections adjacent to each other. Traced the state of the anterior and posterior horns of the lateral and medial meniscus. Thus, MRI is very important for diagnosis of injuries of the knee joint. When preoperative preparation MRI improves diagnostics and increases the positive outcome of the surgery.

Bone Marrow or Stem Cell Transplantation

OR.06. IL-10-engineered Human CD4$^+$ Tr1 Cells Kill Leukemic Cells and Protect from Graft-versus-Host Disease

***Silvia Gregori**[1], Grazia Locafaro[1,2], Grazia Andolfi[1], Fabio Russo[1], Barbara Camisa[3], Fabio Ciceri[1], Attilio Bondanza[1] and Maria-Grazia Roncarolo[1,4]. [1]San Raffaele Scientific Institute, Milan, Italy; [2]University of Milan-Bicocca, Milan, Italy; [3]San Raffaele Scientific Institute, Milan, Italy; [4]★Stanford University School of Medicine, Stanford, CA*

T regulatory type 1 (Tr1) cells are characterized by the co-expression of CD49b and LAG-3 and the ability to secrete high amounts of IL-10. Tr1 cells control immune responses by IL-10 and TGF-beta production and by killing myeloid cells *via* a granzyme B-dependent mechanism. Tr1 cells are induced *in vitro* upon activation in the presence of recombinant human IL-10 or tolerogenic dendritic cells secreting high amounts of IL-10 (DC-10). Proof-of-principle clinical trials showed that these *in vitro* generated Tr1 cells are safe and can modulate Graft-versus-Host Disease (GvHD) after allogeneic hematopoietic stem cell transplantation (allo-HSCT). However, the effect of Tr1 cells on Graft versus Leukemia (GvL) has not been investigated yet.

We recently developed an alternative method to generate a homogeneous population of Tr1 cells, using lentiviral vector (LV) encoding for human IL-10. Enforced IL-10 expression converts human CD4$^+$ T cells into Tr1 (CD4$^{IL\text{-}10}$) cells that suppress xenogeneic GvHD in humanized models. In the present study, we demonstrate that these CD4$^{IL\text{-}10}$ cells selectively kill myeloid cell lines and myeloid primary blasts in a HLA-class I- and granzyme B-dependent manner *in vitro*. CD4$^{IL\text{-}10}$ cell-mediated cytotoxicity is specific for CD13$^+$ cells and requires CD54 and CD112 expression on target cells, including primary leukemic blasts. Importantly, adoptive transfer of CD4$^{IL\text{-}10}$ cells mediates anti-leukemia effects and prevents xenogeneic GvHD *in vivo*, in a clinically relevant humanized model of immunotherapy for leukemia.

These findings pave the way for designing personalized immunotherapy approaches using CD4$^{IL\text{-}10}$ cells to prevent GvHD while preserving GvL after allo-HSCT to cure myeloid malignancies.

T.41. Coaxing MSCs Towards a More Tolerogenic Phenotype via Hypoxia and Cytokine Priming

***Holly Wobma**, Stephen Ma, Mariko Kanai, Kenneth Nakazawa, Sarindr Bhumiratana and Gordana Vunjak-Novakovic. ★Columbia University, New York, NY*

Over the last decade, great progress has been made towards better understanding the immunomodulatory properties of mesenchymal stem cells (MSCs): immunoprotection (enabling the use of allogeneic MSCs) and immunosuppression (inhibiting lymphoid cell activity and promoting tolerogenic phenotypes). It has been established that for MSCs to express their immunomodulatory functions, they must first be "licensed" by the right environmental milieu. IFN-γ is one molecule that has been shown to license MSCs and upregulate their expression of immunosuppressive proteins. However, IFN-γ only minimally upregulates the highly tolerogenic protein HLA-G. We hypothesized that dual priming of MSCs via IFN-γ and hypoxia would promote a highly immunomodulatory MSC phenotype. To test this hypothesis, we monitored the changes in expression of important immunosuppressive proteins and the onset/offset of these changes. Dual stimulation led to the upregulation of a number of immunosuppressive molecules, with IFN-γ leading to a 10^3-10^4 fold increase in Indoleamine 2,3-dioxygenase (IDO), and hypoxia (1% oxygen) contributing a 10^2 fold increase in HLA-G production, amongst other improvements. These

effects were seen after two days of stimulation, were sustained during four days of stimulation, and lingered several days following the removal of the stimulus. These data demonstrate the benefit of priming MSCs for therapeutic use.

T.42. Regulatory T Cell (Treg) Therapy Delays Renewal of the T Cell/ Treg Compartment After Autologous Bone Marrow Transplantation in Experimental Arthritis

Eveline M. Delemarre[1], Theo van den Broek[1], Jenny Meerding[1], Sarah T.A. Teklenburg-Roord[1], Ellen J.M. Wehrens[1], Sven Olek[2], Marianne L. Boes[1], Martijn J.C. van Herwijnen[1], Femke Broere[1], Berent J. Prakken[1] and Femke van Wijk[1]. [1]★UMC Utrecht, Utrecht, Netherlands; [2]Epiontis GmbH, Berlin, Germany

Since their discovery 20 years ago, regulatory T cells (Treg) have been intensively studied for therapeutic strategies. One of the most promising Treg-based applications is in transplantation settings. Autologous stem cell transplantation (aSCT) is a last resort treatment for patients with refractory autoimmune diseases. Treg are thought to play an important role in inducing disease remission after aSCT. Eliciting the role of donor and host Treg in aSCT is not possible in humans due to autologous nature of the intervention. We therefore investigated their role during immune reconstitution and re-establishment of immune tolerance and their therapeutic potential following autologous bone marrow transplantation (aBMT) in a proteoglycan (PG)-induced arthritis mouse model.

A congenic marker was used to differentiate the origin of Treg. After an initial predominance of host Treg, graft-derived Treg started dominating and displayed a more naïve and stable phenotype with superior suppressive capacity. A therapeutic approach was initiated by infusing extra Foxp3GFP$^+$Treg with the graft to suppress the early pro-inflammatory phase of effector T cells. Infusion of Foxp3GFP$^+$Treg in two different doses did not elicit additional clinical improvement; however both groups demonstrated a delayed reconstitution of the graft-derived T cell compartment.

T.43. Allogeneic Bone Marrow Transplantation in the Treatment of Human C1q Deficiency

Richard Olsson[1], Stefan Hagelberg[1], Bodil Schiller[1], Olle Ringden[1], Lennart Truedsson[2] and Anders Åhlin[1]. [1]★Karolinska Institutet, Stockholm, Sweden; [2]Lund University, Lund, Sweden

Objectives: Human C1q deficiency is a rare disorder associated with systemic lupus erythematosus (SLE) and increased susceptibility to severe bacterial infections. In patients with C1q deficiency there is great variation in the severity of symptoms, but most of them require medical therapy and some develop treatment-resistant disease. Since C1q is produced by monocytes, it has been suggested that allogeneic hematopoietic bone marrow transplantation may cure these patients.

Patients and Methods: We have so far treated five patients with C1q deficiency. In three cases, SLE symptoms remained relatively mild after the start of medical therapy, but two patients developed treatment-resistant SLE and we decided to pursue treatment with allogeneic bone marrow transplantations.

Results: Here, we report a 9-year-old boy and a 12-year-old girl with refractory SLE who restored C1q production after allogeneic bone marrow transplantation. This resulted in normal functional properties of the classical complement pathway followed by reduced severity of SLE symptoms. The boy developed post-transplant lymphoproliferative disease (PTLD), which resolved after treatment with rituximab and donor lymphocyte infusions. Unfortunately, the donor lymphocyte infusion induced severe cortisone-resistant gastrointestinal graft-versus-host disease and the patient died from multiple organ failure four months after transplantation. Despite the absence of any risk factors for PTLD also the girl developed PTLD, which was successfully treated with rituximab. She is doing well 27 months after transplantation, and clinically all signs of SLE have resolved.

Conclusion: Allogeneic bone marrow transplantation can cure SLE in human C1q deficiency and should be considered early in subjects resistant to medical therapy.

T.44. *CXCR4* Haploinsufficiency Enhances Hematopoietic Stem Cell Engraftment

David McDermott[1], Jiliang Gao[1], Qian Liu[1], Craig Martens[2], Marie Siwicki[1], Paejonette Jacobs[1], Erin Yim[1] and Philip Murphy[1]. [1]National Institute of Allergy and Infectious Diseases, Bethesda, MD; [2]National Institute of Allergy and Infectious Diseases, Hamilton, MT

Warts, Hypogammaglobulinemia, Infections, and Myelokathexis Syndrome (WHIMS) is a rare primary immunodeficiency caused by hyperfunctional mutations in the chemokine receptor, CXCR4. We recently discovered an index patient who had clinically reverted about 20 years prior to presentation but who had two daughters who had the typical syndrome. The index patient was found to be a somatic mosaic in the hematopoietic system. Her myeloid derived cells had an abnormal deleted chromosome 2 copy (missing 164 genes including the disease allele *CXCR4*^{R334X}), while her lymphoid derived cells remained WHIM. Whole genome sequencing demonstrated that a single hematopoietic stem cell (HSC) had undergone chromothripsis (chromosome shattering) which then engrafted and spontaneously repopulated the bone marrow myeloid lineages. Murine competive bone marrow repopulation experiments using *Cxcr4*$^{+/0}$, *Cxcr4*$^{+/WHIM}$, and *Cxcr4*$^{+/+}$ mice have demonstrated that partially inactivating this gene could provide a general strategy to enhance HSC engraftment. Genome editing techniques are currently being developed to selectively delete the WHIM allele in HSC with the goal of permanently curing the disease in other WHIM patients. If successful, this strategy may empower bone marrow transplantation for many hematopoietic diseases without toxic bone marrow conditioning.

T.45. The Survival Mechanism of Amniotic Fluid-derived Stem Cells in an Inflamed Microenvironment After Allogeneic Hematopoietic Stem Cell Transplantation is Mediated by the Concerted Action of IL-6 and IFN-γ

Shiu-Huey Chou, Cian-Ru Yang, Emily Ting and Yu-Chieh Lo. Fu-Jen Catholic University, New Taipei City, Taiwan

With immunosuppressive character, mesenchymal stem cells (MSCs) become an attractive candidate used for allogeneic cell transplantation. The tissue environment of recipients is inflamed and inappropriate for MSCs to live, Interestingly, we observed that MSCs did survive and expand without losing their function. To maximize the clinical potential of MSCs, the underlying mechanisms by which MSC functionality is controlled must be understood. To this end, we have examined the MSC functional characters by using mouse amniotic fluid-derived stem cells (AFSCs) and allogeneic stimulated T cells co-culture in a series of functional studies. Our data showed that proliferate rate of AFSCs were increase in inflamed culture. Moreover, AFSCs showed highly resistance to irradiation, CsA, and apoptotic inducer, also exhibit anti-apoptotic genes included survivin, MCl-1, XIAP etc. The cytokine array data suggested that up-regulated of IL-6 and IFN-γ may contribute to "the survival mechanisms" of AFSCs in the inflamed environment. Our data showed that IL-6 alone is necessary for AFSC proliferation, anti-apoptosis, and increase resistance to immunosuppressive drugs. Conversely, IFN-γ alone involved in AFSC-mediated T cell suppression through NO-mediated pathway when anti-IFN-γ Abs or iNOS inhibitor was added to the co-culture for blocking. However, IFN-γ and IL-6 appeared to have synergy action in maintain AFSCs survive in inflamed environment when anti-IFN-γ and anti-IL-6 moAbs were added to the co-culture for blocking, simultaneously. Taken together, AFSCs can survive in inflamed environment via the concerted action of IFN-γ and IL-6.

Cytokines/Chemokines

OR.17. Neuroprotective Effects of IL-22 During CNS Inflammation

Rachel Caspi, Mary Mattapallil, Rachael C. Rigden, Carlos R. Zárate-Bladés, Phyllis B. Silver, Dror Luger and Chi-Chao Chan. NEI, NIH, Bethesda, MD

IL-22 has opposing effects in different tissues, from pro-inflammatory (skin, joints) to protective (liver, intestine) but little is known about its effects on neuronal tissue. We examined this using the induced models of experimental autoimmune uveitis (EAU) and experimental autoimmune encephalomyelitis (EAE). During EAU, IL-22 was produced by $CD4^+$ eye-infiltrating cells, most of which co-produced IL-17. IL-$22^{-/-}$ mice immunized for EAU or for EAE had exacerbated disease, as did wild type mice treated with anti-IL-22 systemically or intraocularly during the expression phase of EAU. Retinal glial Müller cells in uveitic eyes had upregulated IL-22rα1 expression. Furthermore, IL-22 upregulated IL-22rα1 expression on Müller cells in culture and enhanced their inherent ability to suppress effector T cells. Finally, IL-22 injected into the eye concurrently with IL-1β inhibited the IL-1-induced expression of multiple proinflammatory and proapoptotic genes in retinal tissue. Our results suggest that IL-22 can function locally within the CNS to ameliorate inflammatory damage and provide neuroprotection by affecting multiple molecular and cellular pathways.

OR.44. Regulation of IL-10 Expression in Human Pro-inflammatory $CD4^+$ T Cells in Response to TNF Blockade

Ceri A Roberts[1], Veerle Fleskens[1], Megha Rajasekhar[1], Klaus S. Frederiksen[2], Hayley G. Evans[1,3] and Leonie S. Taams[1]. [1]★King's College London, London, United Kingdom; [2]Novo Nordisk A/S, Biopharmaceuticals Research Unit, Inflammation Biology, Måløv, Denmark; [3]★University of Oxford, Oxford, United Kingdom

It is well established that $CD4^+$ effector T cell subpopulations can acquire regulatory potential characterised by interleukin-10 (IL-10) expression, but the underlying cellular and molecular mechanisms are yet to be fully elucidated. We recently showed that TNF blocking drugs increase IL-10 expression in human $IL\text{-}17^+$ $CD4^+$ T cells and identified IKZF3/Aiolos as a putative regulator of IL-10. Here we examined whether other biologics or cytokine inhibitors also induce IL-10, explored additional functional effects of TNF blockade on $CD4^+$ T cells and further investigated the role of Aiolos in IL-10 induction. Addition of the TNF blocking drug adalimumab to $CD4^+$ T cell/monocyte co-cultures induced IL-10 expression in pro-inflammatory $IL\text{-}17^+$, $IFN\text{-}\gamma^+$ and $TNF\text{-}\alpha^+$ $CD4^+$ T cell subpopulations. Conversely, exogenous TNF-α suppressed IL-10 expression in these cells. IL-1R1 blockade, but not IL-6R blockade (using tocilizumab) or costimulation blockade (using abatacept), significantly increased IL-10 expression in $CD4^+$ T cell subsets. Gene expression profiling revealed 220 genes commonly regulated by TNF blockade in $IFN\text{-}\gamma^+$ and $IL\text{-}17^+$ cells, which were enriched for cell cycle-associated functional annotations. Addition of TNF blocking drugs impaired $CD4^+$ T cell proliferative responses; however IL-10 induction and reduced $CD4^+$ T cell proliferation did not appear to be interdependent. We found that TNF blockade also induced IL-10 in $CD4^+$ T cells activated in the absence of monocytes. Finally, pharmacological knockdown of IKZF3 reduced IL-10 expression by $CD4^+$ T cells and impaired TNF-blockade-mediated induction of IL-10 expression. Current experiments are aimed at knockdown of IKZF3 using RNAi to further investigate its potential role in IL-10 expression by human $CD4^+$ T cell subpopulations.

T.46. Identification of CXCR8, the New Chemokine Receptor for the Critical Macrophage Chemotactic Factor CXCL17

Amanda Burkhardt[1], Jose Luis Maravillas-Montero[1], Peter A. Hevezi[1], Christina D. Carnevale[1], Martine Smit[2] and Albert Zlotnik[1]. [1]University of California, Irvine, Irvine, CA; [2]★VU University Amsterdam, Amsterdam, Netherlands

Chemokines are a superfamily of chemotactic cytokines that direct the movement of cells throughout the body under homeostatic and inflammatory conditions. Chemokines bind to G protein-coupled receptors (GPCRs) expressed on

the surface of target cells to initiate intracellular signaling cascades and induce chemotaxis. Although the cognate receptors of most chemokines have been identified, the receptor for the mucosal chemokine CXCL17, the most recent chemokine ligand reported, is still undefined. Using a multifaceted approach we have successfully identified the receptor for CXCL17, and in accordance with established chemokine receptor nomenclature have named it chemokine (C-X-C motif) receptor 8 (CXCR8). CXCR8 is expressed in CXCL17-responsive human monocytes, dendritic cells (DCs) and monocytoid cell lines. Additionally, transfection of CXCR8 into Ba/F3 cells rendered them responsive to CXCL17 as measured by calcium mobilization assays. CXCL17 is a chemokine preferentially expressed in mucosal tissues, and, accordingly, the expression of CXCR8 mirrors this preferential mucosal expression pattern. Importantly, Cxcl17$^{-/-}$ mice, exhibit defective recruitment of mucosal macrophage populations, as evidenced by reduced expression of CXCR8^{+} cells in mucosal tissues and by defective chemotaxis of mucosal mononuclear cells to CXCL17 *in vitro*. Furthermore, CXCR8 exhibits structural and physiological features of other chemokine receptors. We conclude that CXCR8 is a novel chemokine receptor and represents the only new member to be added to this superfamily in the last 10 years. Taken together, these observations strongly suggest that the mucosal CXCL17-CXCR8 axis represents a new target for therapeutic intervention in pathophysiological or inflammatory processes of the respiratory or digestive systems.

T.47. Incidence of Measles in Vaccinated Nigerian Children: Role of Pro-inflammatory Cytokines

Adesina Adeiga[1], Adedayo Faneye[2] and Oluwatoyin Awoderu[1]. [1]Nigerian Institute of Medical Research Yaba, Lagos, Nigeria; [2]University of Ibadan, Ibadan, Nigeria

Vaccination remains the most cost-effective intervention to avert measles outbreak in developing countries of the world including Nigeria. However measles outbreak among previously vaccinated children has become a public health concern. Hence this study investigated the role of pro-inflammatory cytokines in measles susceptibility during an outbreak in the sited rural and semi-urban communities of Ogun State Nigeria.

A descriptive cross-sectional study was conducted on 91 sick children presented at health facilities during measles outbreak in four local government of Ogun State Nigeria and these were Odogbolu, Ewekoro, Ado-odo and Abeokuta North local government areas. Enzyme Immunoassay of measles-specific IgM and IgG were performed to confirm measles infection and protection in clinically suspected children. The enzyme immunoassay was also performed to measure the serum cytokines and the clinical presentations were evaluated.

Sixty-five (71.43%) of the 91 children screened had IgM antibody confirming measles infection. Thirty-one (47.7%) of the infected children were vaccinated with 10 (32.3%) of them developing IgG, while 19 (55.9%)of the 34 unvaccinated children had IgG that probably developed from subclinical infection. Twenty-seven(45.5%) children developed severe infection with high values of IL-12 above normal range of 3-90ng/l . Seventeen (63%) of these were vaccinated and 8 (47.1%) of these 17 children were about 2 years of age who developed very high values of IL-1beta and TNF-alfa above normal values of 1-20ng/l and 20-40ng/l respectively.

Findings from this study revealed association between persistent elevation of pro-inflammatory cytokines and measles susceptibility in previously vaccinated Nigerian children.

T.48. Analyses of Serum IL-6 and CRP in Patients with Type 2 Diabetes Mellitus Treated with Hyperbaric Oxygenotherapy

Vladimir Jurisic[1], Sinisa Ristic[2], Dragana Puhalo Sladoje[2] and Veljko Maric[2]. [1]University of Kragujevac, Kragujevac, Serbia; [2]University of East Sarajevo, Foca, Herzegovina

Type 2 diabetes mellitus (T2DM) represents over 80% of all diabetics and is increasing in incidence as a result of changes in human behavior and increased body mass index with many clinical complications. Previous studies

indicate a favorable effect on the hyperbaric oxygen therapy (HBOT) application on glicoregulation, but not so much data exist about the effects this therapy on inflammatory markers. Based on this, we have analyzed a relationship between the serum inflammation markers including IL-6 and CRP in 41 patients with T2DM patients. The classical laboratory analysis for disease monitoring; include determination of glucose, cholesterol and triglycerides plasma level before the first HBOT treatment, and after the tenth treatment of HBOT. All patients underwent HBOT in 10 sessions, with 100% oxygen to 2.4 atmospheres (ATA) for two weeks, five days a week for a period of one hour. The Wilcoxon Rank Sum test, indicated significant decrease glucose level ($p< 0.022$), CRP ($p<0.05$) and IL-6 ($p<0.05$) in our 41 patients after 10 cycles of HBOT in comparison to values before therapy. However, HBOT has not led to a statistically significant decrease in serum levels of triglycerides and cholesterol ($p> 0.05$). In summary, our results indicated that HBOT is expressed favorable effects on glucoregulation parameters in patients with T2DM, as well as on inflammation markers including IL-6 and CRP.

T.49. METRNL (Interleukin 39) is a Novel Cytokine Associated with Barrier Tissues and Alternatively Activated Macrophages

Irina Ushach[1], Amanda Burkhardt[1], Cynthia Reyes[2], Monica Vazquez[1], Peter Hevezi[1] and Albert Zlotnik[1]. [1]University of California, Irvine, Irvine, CA; [2]University of Baja California, Mexicali, Mexico

Cytokines are involved in many functions of the immune system including the onset, amplification and resolution of immune responses. Through bioinformatics analysis of a comprehensive database of gene expression (BIGE: Body Index of Gene Expression) we identified a small secreted protein (~27kDa) encoded by a poorly characterized gene called meteorin-like (*Metrnl*), that is highly expressed in mucosal tissues, skin and activated macrophages. Further studies revealed that *Metrnl* is produced by Alternatively Activated Macrophages (AAM) and M-CSF cultured bone marrow macrophages (M2-like macrophages) upon activation. In the skin, *Metrnl* is expressed by resting fibroblasts and IFNγ-treated keratinocytes. Given its strong expression in human skin, we performed a screen of human skin-associated diseases to investigate whether Metrnl could play a role in pathogenic conditions. The results indicate that *Metrnl* is significantly over-expressed in several human skin diseases including psoriasis, prurigo nodularis, actinic keratosis and atopic dermatitis. Furthermore, *Metrnl* is also up-regulated in synovial membranes of human rheumatoid arthritis. Taken together, these results indicate that Metrnl represents a novel cytokine, which is likely involved in both homeostatic and inflammatory processes. Its expression pattern and disease association strongly suggests that it likely participates in both innate and acquired immune responses, and that it may play a role in certain human inflammatory diseases. Because of these characteristics, we suggest it should be renamed Interleukin 39 (IL-39).

T.50. IL-21R Blockade Inhibits Secondary Humoral Responses and Halts the Progression of Pre-established Disease in NZB/W F1 Systemic Lupus Erythematosus

Ming Zhang[1], Anna Knize[1], Gang Yu[1], Stephanie Grauer[2], Vicki Wilson[2], Kelly Berry[2], Brian Chan[2], Lydia Green[2], Rutilio Clark[3], Martin Wolfson[3], Mei Han[3], Joshua Pearson[3], Palaniswami Rathanaswami[2], Ai Ching Lim[3], John Delaney[3], John Babcook[2], Hailing Hsu[1] and Marc Gavin[3]. [1]Amgen, Thousand Oaks, CA; [2]Amgen, Burnaby, BC, Canada; [3]Amgen, Seattle, WA

Systemic lupus erythematosus (SLE) is a complex autoimmune disease driven in part by chronic B and T lymphocyte hyper-responsiveness to self antigens. IL-21 or IL-21R deficiency in mice dramatically reduces inflammation and B and T cell activation in models of autoimmunity, including SLE; however, whether IL-21 is essential for the maintenance and amplification of pre-established inflammation has not been widely examined in various animal models. Here, we have examined the impact of novel mouse IL-21R neutralizing antibodies on recall responses to antigen challenge and on disease progression in the NZB x NZW F1 (BWF1) SLE model. IL-21R blockade specifically inhibited secondary IgG responses to sheep red blood cell immunization. In BWF1 mice, IL-21R blockade completely inhibited onset of nephritis, which was associated with dramatic reductions in splenomegaly and B and T

cell activation. When administered to mice with preexisting disease, the anti-IL-21R antibody halted disease progression and mortality, and reversed nephritis in a subset of mice. Furthermore, treatment cessation was not followed by rapid re-emergence of disease. Our results highlight the importance of IL-21 in promoting humoral recall responses and in sustaining autoimmune inflammation.

T.51. The Generation of Multimeric and Highly Active Cytokine-Fc Fusion Proteins

Akikazu Murakami and Hidehiro Kishimoto. University of the Ryukyus, Okinawa, Japan

Cytokines are a large family of more than 100 small proteins that involved in essentially immune system. Cytokine therapy has emerged as a significant class of highly effective pharmaceuticals. Cytokines have a short half-life, so that they need to be administered at high doses to achieve their therapeutic effects. But, high-dose therapy sometimes induces adverse effects of cytokines-associated toxicity. Therefore, strategies for cytokine engineering have become important to improve the pharmacokinetic and pharmacodynamics properties of cytokine therapy. Recently, it has been reported that cytokine combined with anti-cytokine antibody extends the half-lives, and enhances biological activities *in vivo*. Cytokine-antibody complex is large enough not to be cleared by renal filtration. In this study, we generated cytokine-Fc (γ and μ) fusion proteins and evaluate their cytokine activity for cytokine-dependent cell lines. IgG type, dimeric cytokine-Fc fusion proteins, showed 4~19 times higher biological activities than free cytokine, moreover, IgM type, decameric or dodecameric cytokine-Fc fusion proteins, showed 9~13 times higher biological activities than IgG type. We hypothesized the reasons why multimeric cytokine-Fc fusion proteins have strong biological activities are avidity effect that enhance cell signaling by cross-linking receptor and half-life extension in cell culture medium. We will inspect these hypotheses and the function of cytokine-Fc fusion proteins *in vivo.*

T.52. Stabilizing Platelet Degranulation in EDTA-anticoagulated Blood Provides Improved Sample Quality for Biomarker Assays

Frank Sinquett, Daniel Marchiarullo, Justyna Dudaronek and David Craft. BD Life Sciences - Preanalytical Systems, Franklin Lakes, NJ

Objective: Anticoagulation with EDTA inhibits matrix-metalloproteases and is often the anticoagulant of choice for the detection/quantitation of peptide/protein biomarkers. Exposure to EDTA results in platelet release of mitogens, cytokines, adhesion proteins, hemostatic factors, and other signaling molecules leading to non-physiological elevations of molecular targets for clinical research and diagnostic applications. Platelet degranulation can be directly interrogated via flow cytometric (FC) detection of CD62P (α-granules) and CD63 (dense granules/lysosomes). Here, we examined the use of an EDTA-based platelet stabilizer (EPS) chemistry in blood collection tubes for the suppression of platelet α-granule release.

Methods: Blood from 10 subjects was collected into evacuated blood collection tubes containing EDTA, EPS, or citrate theophylline adenosine dipyridamole (CTAD). Platelet CD62P/CD63 expression was measured by FC across a 24hr period. A subset of specimens was stimulated with ADP or TRAP to evaluate the strength of the EPS chemistry. PDGF, TGFβ, VEGF, and RANTES(CCL5) levels were evaluated from matched plasma samples via ELISA. Results: Within 2 hours, 47% of EDTA-treated platelets were found positive for CD62P as opposed to 5% in EPS or CTAD. The addition of ADP or TRAP to EDTA specimens increased CD62P expression to 85% and 99%, respectively, but only 17% and 33% in EPS specimens. Additionally, we observed significant reductions in CD62P surface expression across a 24hr hour period of WB sample dwell in both EPS and CTAD compared to EDTA. Matched plasma analysis revealed significant reductions in PDGF (60%-96%), TGFβ (61%-83%), VEGF (28%-47%), and RANTES (58%- 96%) levels.

Conclusions: These findings suggest that platelet stabilizers are necessary at the time of collection for the detection and quantitation of circulating platelet derived biomarkers while retaining the benefits of EDTA-based anticoagulation.

T.53. STAT3 is a Critical Cell Intrinsic Regulator of Human Innate-like T Cell Numbers and Function

Elissa Deenick[1,2], Robert Wilson[1], Megan Ives[1,2], Steve Holland[3], Jean-Laurent Casanova[4,5], Gulbu Uzel[3] and Stuart Tangye[1,2]. [1]Garvan Institute, Darlinghurst, Australia; [2]University of New South Wales, Darlinghurst, Australia; [3] National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD; [4]The Rockefeller University, New York, NY; [5]Necker Hospital for Sick Children, Paris, France

Innate-like T cells such as γδ T cells, natural killer T (NKT) cells and mucosal-associated invariant T (MAIT) cells are a major component of the immune system, however the cytokine signalling pathways that control their development and function in humans are unknown. Primary immunodeficiencies caused by single gene mutations provide a unique opportunity to investigate the role of specific molecules in regulating human lymphocyte development and function. Autosomal dominant-hyper IgE syndrome (AD-HIES) is caused by heterozygous loss-of function mutations in *STAT3*. We used lymphocytes from these patients to assess the roles of STAT3 in NKT, MAIT and γδ T cell differentiation *in vivo* and *in vitro*. Patients with *STAT3* mutations had reduced numbers of peripheral blood MAIT and NKT, but not γδ T, cells. Analysis of *STAT3* mosaic individuals revealed that this effect was cell intrinsic. Surprisingly, the residual STAT3-deficient MAIT cells expressed normal levels of the transcription factor RORγt. Despite this they displayed a reduced ability to secrete IL-17A and IL-17F, but were able to secrete normal levels of cytokines such as IFNγ and TNFα. To determine which cytokine receptors were required upstream of STAT3 we examined patients with loss-of function mutations in *IL12RB1* and *IL21R* and found decreases in MAIT and NKT cells, respectively. Thus, this reveals for the first time the essential role of STAT3 signalling downstream of IL-23R and IL-21R in controlling human MAIT and NKT cell numbers.

T.54. New Multiplex Assay Panels for Quantification of Cytokines, Interferons and Chemokines Involved in Innate and Adaptive Immune Responses

Binggang Sun, Jingjing Yao, Patricia Lopes and Shaoquan Ji. BioLegend, Inc., San Diego, CA

Pathogens, such as bacteria and viruses, trigger immune responses upon infection. Innate immune response is mediated by innate immune cells (e.g., macrophages, neutrophils, and dendritic cells) via the production of cytokines such as interferons (e.g., IFN-α, β, g, λ), interleukins (e.g., IL-1, 6, 8, 10, 12, 18, 27, 33), TNF-α, and chemokines (e.g., MCP-1, IP-10, RANTES, MIP-1). Adaptive immune response is mediated by T cells and B cells. T helper cells secret Interleukins (IL-2, 4, 5, 9, 10, 13, 17, 21, 22), IFN-g and TNF-α, and play pivotal roles in immune regulations. In addition, these cytokines and chemokines are also critically involved in inflammatory diseases. Therefore, expression profiling of these cytokines and chemokines is important in achieving in-depth understanding of the immune responses and various disease processes.

We have developed multiplex assay panels, using fluorescence–encoded beads that are suitable for various flow cytometers. Each panel allows simultaneous quantification of 13 related analytes. For example, the T Helper Cytokine Panel includes Interleukins (IL-2, 4, 5, 6, 9, 10, 13, 17A, 17F, 21, 22), IFN-g and TNF-α. The Interferon Panel includes interferons (IFN-α, β, g, λ1, λ2), interleukins (IL-1β, IL-6, IL-8, IL-10, IL-12), TNF-α, GM-CSF and IP-10. Each antibody pair was carefully optimized for assay specificity, sensitivity, accuracy and reproducibility. These panels have been validated by detecting expected changes in biological samples. These panels are of high quality, low cost and ease of use, providing an alternative multiplex solution to the biomedical and drug discovery community.

T.55. Characterization of Immune Regulatory Mechanisms Elicited by PPD and ESAT-6 Tuberculosis Protein in BCG-vaccinated Volunteers

Cecilia Huaman, Philip Seegren, Stephanie Phelan, Mustafa Rasheed, Aarti Vadalia, Dennis Liu and Anthony Cristillo. Advanced Bioscience Laboratories, Rockville, MD

Mycobacterium bovis Bacille Calmette-Guerin (BCG), the only vaccine available at present for tuberculosis, is mostly effective at preventing disseminated TB in children, but shows variable protection in adults. *Mycobacterium tuberculosis* infection can result in two clinical outcomes: active and latent infection. We hypothesize that suppressive and regulatory mechanisms, resulting from bacteria-host interaction determine the outcome of TB infection. To characterize possible regulatory mechanisms elicited by Protein Purified Derivative, PPD, and the Mtb protein 6 kDa Early secreted antigenic target (ESAT-6) may play a role on the efficacy of BCG vaccine in young adults, we investigated T cells with regulatory/exhaustion phenotype and the cytokine milieu. PBMC from BCG-vaccinated and non-BCG vaccinated human subjects were examined for the percentage of T cells with regulatory and exhaustion phenotype by using flow cytometry markers such as PD-1/CTLA-4/ FOXP3. Characterization of IFNg, IL-10 and IL-17 responses were assessed by ELISpot. In addition, supernatants were assayed for pro-inflammatory and anti-inflammatory cytokines using Luminex system. We observed increased CTLA-4 expression in BCG-vaccinated donors along with decreased IL-17A and IFNg when stimulated with CD3/CD28 in combination with ESAT6 as compared to CD3/CD28 alone. No difference in PD-1 and TIM-3 expression between BCG-vaccinated and non-vaccinated were observed upon stimulation with Ag85B, ESAT6, or PPD. The putative role of immune regulatory mechanisms in the TB clinical outcome will be discussed.

Diabetes and Other Autoimmune Endocrine Diseases

OR.14. The Frequency of Follicular Helper and Regulatory T Cells Correlates with Progression to Type 1 Diabetes in Humans and Non-obese Diabetic Mice

Georgia Fousteri[1], Tatiana Jofra[1], Roberta Di Fonte[1], Angela Stablini[1], Michela Locci[2] and Manuela Battaglia[1]. [1]San Raffaele Diabetes Research Institute, Milan, Italy; [2]La Jolla Institute for Allergy and Immunology, La Jolla, CA

Type 1 diabetes (T1D) is an autoimmune disease where several immunological checkpoints are thought to have been bypassed. Dysregulated number and behaviour of T follicular helper (Tfh) and regulatory (Tfr) cells -a specialized subset of FOXP3$^+$ Treg cells- are thought to contribute in disease pathogenesis and particularly in the development of islet-specific autoantibodies in humans and non-obese diabetic (NOD) mice. Here we analyzed the frequency of circulating CD4$^+$CD45RA$^-$CXCR5$^+$ Tfh and CD4$^+$ CXCR5$^+$FOXP3$^+$ Tfr-cells in pediatric and adult donors at-risk (i.e. in healthy autoantibody-positive subjects) and in donors at more advanced stages of the disease (i.e. at disease onset and long-term). We found a significant increase in circulating Tfh and Tfr cell-frequency that correlated with T1D onset in pediatric but not in adult donors. Interestingly, the frequency of circulating Tfh cells also correlated with the number of autoantibodies in healthy autoantibody-positive pediatric donors. This data suggests the presence of a differential clinical phenotype between pediatric and adult subjects that might reflect diverse pathogenic pathways leading to T1D. Murine studies revealed that Tfh and Tfr-cell frequency is also increased in the peripheral blood of prediabetic NOD mice when compared to BALB/c mice. Preliminary data from spleen and pancreatic lymph nodes of diabetic donors showed that Tfh cells are present at normal levels, whereas Tfr cell frequency is reduced. Together, our findings identify Tfh and Tfr cell alterations as important biomarker of T1D in pediatric subjects and suggest that treatments specifically targeted at these populations could potentially increase the pipeline of T1D interventions.

T.56. Autoantibodies to Deamidated Proinsulin in Type 1 Diabetes

Jing Jin, Pan Liu, Lisa Wren, Hee-Kap Kang, James Rosati, Natalie Monson, Grazia Aleppo and Xunrong Luo.
★*Northwestern University Feinberg School of Medicine, Chicago, IL*

Type 1 diabetes (T1D) and celiac disease (CD) are frequently concomitant conditions that also share common HLA isotypes such as HLA-DQ2 and DQ8 in high-risk populations. The main autoantigen in CD is deamidated alpha-gliadin, a high affinity ligand for DQ2/8 that generally favor deamidated epitopes. We speculated that deamidated autoantigens might also be present in islets and contribute the pathogenesis of T1D. We examined healthy human pancreatic tissue sections and observed strong expression of tissue transglutaminase (TG2 - the main deaminase for CD) in discrete islet cell populations. Proteomic studies revealed prominent glutamine deamidation of proinsulin. We next studied T1D subjects for serum antibodies to either native or deamidated proinsulin. While we detected antibody to native proinsulin in ~20% patients, up to 50% patients were positive for anti-deamidated proinsulin antibody. In an NOD cohort, we conducted a longitudinal study and found that anti-deamidated proinsulin antibody could be detected prior to the onset of clinical diabetes, and when present, predicted diabetes onset in 100% of the cases. T cell ELISPOT assay of the antibody-positive mice revealed stronger interferon gamma responses when stimulated by deamidated proinsulin compared to native proinsulin. Further mapping of the antigenic segment(s) pinpointed an epitope located across the B-chain and the C-peptide. Taken together, we conclude that deamidation of proinsulin may be initiated by TG2 expressed in islets, and that humoral and cellular responses to the deamidated proinsulin are predictive of diabetes onset and may serve as a useful biomarker for disease prediction.

T.57. A Reduction in Mda5 Signals a Virus-specific Type 1 Interferon Response that Leads to a Regulatory Phenotype and Protects from Type 1 Diabetes in NOD Mice

Pamela Lincez and Marc Horwitz. ★*University of British Columbia, Vancouver, BC, Canada*

Type 1 interferon (IFN-I) responses are critical in antiviral immunity. In children at risk of type 1 diabetes (T1D), a unique IFN-I transcriptional signature precedes islet autoimmunity. IFN-I induced by RNA viruses like coxsackievirus are strongly linked to recent onset T1D. Genetic variants in the T1D risk locus, IFIH1, have been linked to protection from T1D and result in reduced expression of RNA virus sensor melanoma differentiation-associated protein 5 (MDA5). We translated this reduction in MDA5 expression on to the non-obese diabetic (NOD) mouse to establish its importance in T1D. We observed protection from T1D and a specific IFN-I signature that we believe is a result of reduced (not eliminated) MDA5 signaling. We hypothesize that IFN-I induced after an environmental stimulus, correlates with a strong autoreactive T cell response. We observe tissue and cell-specific IFN-I responses that are limited to MDA5 and not another RNA sensor TLR3. We demonstrate that the cells responsible for the sustained presence of the IFN-I signature are $CD11b^{+}CD11c^{+}$ cells and disease pathogenesis is specific to the virus-induced IFN-I response. These results suggest that MDA5 signaling is essential in regulating the IFN-I signature that mediates autoreactive T cell responses and determines disease fate. This work implicates MDA5 signaling as a critical factor in T1D susceptibility and in protection against T1D-inducing agents like coxsackievirus.

T.58. Subpopulations of **β Cells** Indicate **β Cells Dedifferentiation in Type I Diabetes**

Jinxiu Rui. ★*Yale School of Medicine, New Haven, CT*

Clinical data suggests that there are changes in b cells during the immunologic attack that causes Type 1 diabetes (T1D) leading to functional impairment followed by partial recovery after diagnosis. Here, during the progression of autoimmune diabetes in NOD mice, we identified a novel subpopulation of β cells that differs from B cells in non-autoimmune mice in terms of granularity and the expression of genes of differentiation. The novel population appeared after 6 weeks of age and increased in proportion of total insulin+ cells during disease progression. Treatment with anti-CD3 mAb prevented the development of the new subpopulation. Lineage tracing in transgenic MIP-GFP mice confirmed they were β cells. These cells showed reduced expression of genes either associated with β cell function and differentiation (e.g. Ins1, Ins2, Pdx1,Nkx6.1, FoxO1 and MafA) but increased expression of genes associated with early developmental stages (e.g. MycL1 and Neurog3) and regeneration (e.g. Reg1). In addition, these cells showed increased expression of immune modulatory molecules as PDL1, 41BBL, HLA-E and Qa-2. These studies suggest that in response to autoimmunity, B cells reduce production of insulin, express immunologic

inhibitory molecules, and genes associated with dedifferentiation. The rapid recovery of B cells after anti-CD3 treatment suggests that these cellular changes may avert immunologic destruction and create a population that may recover function.

T.59. 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 Rituximab

Tania Habib[1], Archana Brahmandam[2], Andrew Funk[1], Yolanda Ng[2], Carla Greenbaum[1], Elizabeth Whalen[1], Jane H. Buckner[1] and David J. Rawlings[2]. [1]★Benaroya Research Institute, Seattle, WA; [2]★Seattle Children's Research Institute, Seattle, WA

Autoreactive B cells 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 B cells 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 whether they can be modulated by rituximab. We found that BCR-triggered proximal signaling in total and naïve B cells was modestly *increased* in Ab+ non-progressors as compared to age- and gender-matched Abneg FDR controls; but was significantly *reduced* in Ab+ progressors when compared to Ab+ non-progressors. Longitudinal analysis revealed that diminished BCR signaling was present in Ab+ progressors prior to diagnosis and at disease onset. Parallel analyses of the B cell compartment revealed significant expansion of transitional B cells in Ab+ progressors as compared to Abneg FDR controls. In our preliminary assessment of participants in the TrialNet rituximab trial, we observed significantly increased BCR signaling in total B cells from subjects who had relatively sustained C-peptide at 1 year whether in treatment or placebo groups. Importantly, BCR responses progressively declined in subjects with a dramatic fall in C-peptide levels. Collectively, these data suggest that individuals at risk for T1D may exhibit increased BCR responsiveness and that diminished BCR signaling and altered transitional B cell homeostasis are key features of new-onset T1D subjects.

T.60. Qualitative Changes in the Clonal iNKT Repertoire of People with Recent Onset Type 1 Diabetes Mellitus

Anna Tocheva[1], Salah Mansour[1], Richard Holt[1] and Stephan Gadola[1,2]. [1]University of Southampton, Southampton, United Kingdom; [2]Novartis Institutes of Biomedical Research, Basel, Switzerland

Invariant natural killer T cells (iNKT) are a conserved class of regulatory T cells that recognize lipid antigens presented by CD1d. The human iNKT repertoire is heterogeneous with regard to the iNKT T cell receptor (iNKT-TCR) affinity for CD1d, which may translate into functional differences between iNKTs expressing *high*- versus *low*-affinity iNKT-TCR. Dysregulated iNKT function has been reported in human autoimmune diseases. However, the role of iNKTs with different TCR affinity has not been examined. To address this, we determined whether clonal and functional differences exist in the iNKT repertoire between people with type 1 diabetes (T1D) and healthy controls that may contribute to the loss of immune tolerance in T1D. By using different recombinant alpha-galactosylceramide-loaded/CD1d-tetramers, we determined the clonal composition of the iNKT repertoire in peripheral blood of people with T1D (duration ≤4 years) and age- and gender-matched healthy controls. While iNKT frequency was similar between controls and people with T1D, the *ex vivo* clonal iNKT repertoire in T1D was significantly skewed toward iNKT clones expressing lower-affinity iNKT-TCR. Furthermore, iNKTs from people with T1D bearing *high*-affinity iNKT-TCR expanded significantly less in response to iNKT agonists and produced less regulatory cytokines compared to *high*-affinity iNKTs from healthy controls. Here, we report for the first time that the quality of the iNKT repertoire is disrupted in human T1D and that the loss of iNKT clones with *high*-affinity TCR may be involved in the immune dysregulation observed in autoimmune diseases, which should inform future therapies aiming to mobilize the tolerance-inducing functions of iNKT cells.

T.61. RAGE Augments the Inflammatory Response in T1D T Cells

Sean Durning, Paula Preston-Hulburt and Kevan Herold. ★Yale School of Medicine, New Haven, CT

The receptor for advanced glycation end products (RAGE) is part of the immunoglobulin super family of receptors exhibiting pro-inflammatory responses. It interacts with a variety of damage-associated ligands and plays a major role in hyperglycemia-induced complications and autoimmune disorders. We have recently shown T lymphocytes from patients with type 1 diabetes (T1D) express elevated amounts of intracellular RAGE compared to healthy controls (HC) that directly correlates with higher *Ager* gene expression levels. These cells show evidence of a pro-inflammatory profile including increased expression of IL-18, TLR2 and other proinflammatory ligands and signaling pathways. In addition, we found higher RAGE expression in T cells from at-risk non-diabetes subjects that progress to T1D versus non-progressors, suggesting intracellular RAGE expression in T cells may serve as a biomarker of T1D progression and have a functional role in disease pathogenesis at an early stage. Diabetes antigen specific $CD8^+$ T cells express increased levels of RAGE compared to other antigen reactive $CD8^+$ cells in patients with T1D. Nanostring gene expression-analysis of $RAGE^+$ vs RAGE- $CD4^+$ T cells from patients with T1D show increased expression of IL-6R, suggesting RAGE may in part activate T cells through STAT3 signaling. Based on these findings, we hypothesize that RAGE plays an important role in the progression of autoimmune T1D. Activation of diabetes antigen specific T cells by RAGE ligands associated with cell damage may lead to B cell killing.

T.62. Validation of Shared T Cell Receptor ß-Chain Clonotypes in Type 1 Diabetes

Karen Cerosaletti[1], Chester Ni[2], Janice Chen[1], Vivian Gersuk[1], Carla Greenbaum[1] and Damien Chaussabel[1,3]. [1]★Benaroya Research Institute, Seattle, WA; [2]Volometrix, Inc., Seattle, WA; [3]Sidra Medical and Research Center, Doha, Qatar

Autoreactive T cells play an important role in the destruction of pancreatic beta cells in type 1 diabetes (T1D). We hypothesized that shared disease-related T cell receptors (TCRs) that are expanded in the blood of T1D and at-risk subjects may serve as useful biomarkers. High throughput sequences (HTS) from peripheral T cells of 10 T1D, 7 at-risk subjects, and 11 HLA matched controls were analyzed to identify TCR ß-chains that were significantly enriched in T1D and at-risk subjects versus controls. The highest scoring clonotypes from the HTS analysis (n=11), as well as six clonotypes from islet-specific T cell clones, were validated in 100 T1D, 50 type 2 diabetics (T2D), and 100 matched controls. Clonotype-specific Taqman assays were developed, validated, and used to screen RNA from flow-sorted naïve and memory CD4 and CD8 T cells on a Fluidigm real-time PCR platform. We found six of 17 clonotypes were present in ≥10% subjects in the validation cohorts. Of these, two clonotypes from the HTS analysis and one GAD-specific TCR were detected significantly more often in T1D subjects compared to controls or T2D. The GAD clonotype was shared amongst the most T1D subjects (38%) while a CD8 clonotype had the highest discrimination between T1D and controls, 27% vs. 9% respectively. Clonotypes were detected most frequently in the CD8 memory subset. These findings indicate that individual TCR clonotypes are shared in up to 40% of T1D and may be useful as personal biomarkers to monitor disease progression or response to therapy.

T.63. Mechanisms of Enhanced T Cell Responses to IL-6 in Type 1 Diabetes

Christian Hundhausen, Alena Roth, Karen Cerosaletti, Carla Greenbaum and Jane H. Buckner. ★Benaroya Research Institute, Seattle, WA

Due to its unique role in balancing effector with regulatory T cell function, IL-6 has emerged as key cytokine in the etiology of multiple autoimmune diseases including rheumatoid arthritis, multiple sclerosis and psoriasis. However, the role of IL-6 in type 1 diabetes (T1D) has not been determined. Using phospho-flow cytometry on human PBMC we show that IL-6-mediated STAT3 phosphorylation (pSTAT3) is significantly increased in CD4 and CD8 T cells of

T1D patients compared to healthy controls. The effect 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 was IL-6 receptor (IL-6R) surface expression, which positively correlated with pSTAT3 levels. Reduced surface expression of TACE and ADAM10, major sheddases of the IL-6R, in resting T cells of type 1 diabetic subjects provided a mechanistic explanation for the elevated IL-6R surface levels in T1D. Analysis of other factors potentially responsible for increased IL-6/pSTAT3 signals in T1D, such as differential expression or genetic variation of IL-6 signaling components gp130, JAK1, TYK2, STAT3 or SOCS3, did not reveal differences between controls and patients. In conclusion, our study demonstrates the presence of enhanced IL-6-mediated STAT3 responses in T cells from patients with T1D and suggests that expression of surface metalloproteinases may contribute to the increased IL-6R surface levels. The functional relevance of increased IL-6/pSTAT3 in T1D with respect to the development of Th17 cells and T effector cell resistance is currently under study.

T.64. Inhibition of Hyaluronan Synthesis Restores Immune Tolerance During Autoimmune Insulitis

*Nadine Nagy[1], Gernot Kaber[1], Pamela Johnson[2], John Gebe[2], Ben Falk[2], Marika Bogdani[2], **Hedwich Kuipers**[1], Michel Gooden[2], Robert Vernon[2], Anthony Day[3], Daniel J. Campbell[2], Thomas Wight[2] and Paul Bollyky[2]. [1]★Stanford University, Palo Alto, CA; [2]Benaroya Research Institute, Seattle, WA; [3]University of Manchester, Manchester, England*

Abundant deposits of the extracellular matrix polysaccharide hyaluronan (HA) characterize autoimmune insulitis in human type 1 diabetes (T1D) but the significance of these deposits is unclear. We find that HA is critical for the pathogenesis of autoimmune diabetes. Using the DO11.10xRIPmOVA (DORmO) mouse model of T1D, we show that HA deposits are temporally and anatomically associated with the development of insulitis, in patterns similar to those seen in human TID. Moreover, treatment with an inhibitor of HA synthesis, 4-methylumbelliferone (4-MU), halted progression to diabetes even after the onset of insulitis. 4-MU reduced HA accumulation, constrained effector T cells to non-destructive insulitis, and increased numbers of intra-islet Foxp3$^+$ regulatory T cells (Treg). Consistent with this, Treg differentiation was inhibited by HA and anti-CD44 antibodies and rescued by 4-MU in an ERK1/2-dependent manner. These data may explain how peripheral immune tolerance is impaired in tissues under autoimmune attack, including islets in T1D. We propose that 4-MU, already an approved drug used to treat biliary spasm, could be repurposed to prevent, and possibly treat, T1D in at-risk individuals.

T.65. Gene Expression Profiling of the Pancreas to Identify Candidate Genes Involved in the Pathogenesis of Type 1 Diabetes

*Rebecca Fuhlbrigge, C. Garrison Fathman and **Linda Yip**. ★Stanford University, Stanford, CA*

The pancreata of auto-antibody positive (AA$^+$) and early onset Type 1 diabetes (T1D) patients have been shown to differ from those of healthy individuals. We performed microarray analyses on pancreas samples of healthy control, AA$^+$, and T1D subjects to identify candidate genes and pathways that may contribute to the pathogenesis of T1D. One-color microarray analyses were performed in individual pancreata of 8 non-diabetic AA$^-$ controls, 6 AA$^+$ and 10 late-stage T1D subjects. Data were analyzed using GeneSpring GX 11.5, and QPCR was performed to confirm changes in gene expression. 75 and 334 genes were significantly changed by ≥2 fold in the pancreata of AA$^+$ and T1D patients compared to controls. Only a third of genes that were changed in AA$^+$ patients were similarly changed in T1D patients, suggesting that the majority of changes that occur during the pre-diabetic stage of disease are transient and do not persist in late-stage T1D patients. Six of the genes that were differentially expressed in AA$^+$ patients have previously been associated with T1D, T2D, obesity and/or metabolism by GWAS or SNP analysis, and several dysregulated genes are of biological relevance. The expression of *FCGR2B,* which encodes the low affinity immunoglobulin gamma Fc region receptor II-b, was significantly reduced in AA$^+$ subjects. Reduced expression of *Fcgr2b* has previously been linked to autoimmune susceptibility in various rodent models of disease including the non-obese diabetic mouse model. These data suggest that gene expression profiling may be used to identify

candidate genes that contribute to the pathogenesis of T1D.

T.66. Correlational Analysis of Gene Expression in the Pancreata of At-risk Auto-antibody Positive Individuals and Non-obese Diabetic Mice to Predict Stage of Disease Progression in Pre-diabetic Individuals

Keiichi Kodama, Rebecca FuhIbrigge, C. Garrison Fathman and Linda Yip. ★Stanford University School of Medicine, Stanford, CA

Currently, there is no reliable method to determine the risk of progression of disease in auto-antibody positive (AA^+) pre-diabetic patients. We asked whether correlational analysis of gene expression in the pancreata of AA^+ individuals and pre-diabetic non-obese diabetic (NOD) mice could be used to predict and stage disease progression. Microarrays were performed on the pancreata of human non-diabetic controls (n=8) and at-risk AA^+ subjects (n=6); and groups of 6 NOD and NOD.B10 mice at 10 days of age (early-stage; T cell initiation), 4 weeks of age (mid-stage; peri-insulitis) and 12 weeks of age (late-stage; destructive insulitis). Pearson's correlation analysis was performed to compare gene expression profiles between each human AA^+ subject and pre-diabetic NOD mice during the early, mid or late stages of disease [log (each AA/mean control) vs. mean log (NOD/NOD.B10)]. The gene expression profiles of 5 of the 6 AA^+ subject were significantly correlated with the early, mid, and/or late-stage of disease in NOD mice. Interestingly, the single AA^+ subject that did not correlate with the NOD profiles was the oldest individual studied (47 year old), suggesting the possibility that this subject may have been a non-progressor. By principal component analysis, control subjects and subjects in the early and mid/late stage of disease clustered into 3 distinct groups. Time-course analysis showed that ~700 genes were changed from early to mid/late stage, including several secreted proteins. It is possible that these secreted proteins can be measured in the serum and used as potential biomarkers of disease progression.

T.67. Direct (Cyclic Dinucleotides) and Indirect (DNA Nanoparticles) Activation of the Adaptor Stimulator of Interferon Genes (STING) Attenuate Type I Diabetes Progression in NOD Female Mice

Henrique Lemos[1], Eslam Mohamed[1], Lei Huang[1], Phillip R. Chandler[1], Yoshihiro Hayakawa[2], Rafal Pacholczyk[1], David Munn[1] and Andrew L. Mellor[1]. [1]Georgia Regents University, Augusta, GA; [2]Aichi Institute of Technology, Toyota, Japan

DNA sensing to activate Stimulator of Interferon Genes (STING) in dendritic cells (DCs) drives T cell responses against tumors in mice. Paradoxically, we recently reported that STING activation following DNA nanoparticle (DNP) treatment attenuated Experimental Autoimmune Encephalomyelitis (EAE) and antigen-induced arthritis by activating STING, to produced IFN-αβ and indoleamine 2, 3 dioxygenase. These findings prompted us to evaluate if DNPs could be effective in slowing spontaneous Type I Diabetes (T1D) progression in Non-Obese Diabetic (NOD) mice. DNPs restrained T1D onset in NOD mice, a treatment that was optimized by combining DNPs with antigen therapy (Insulin B chain). DNPs activate STING after its DNA content is catabolized into cyclic dinucleotides (CDNs). Previously, we verified that direct STING activation by CDNs also ameliorated EAE. Surprisingly, c-diGMP promoted no effect in reducing T1D progression. Microbial diguanylate monophosphate (c-diGMP) and mammalian cyclic guanyl-adenyl monophosphate (2'3'-cGAMP) have unique phosphodiester linkages between saccharide rings, c[G2'5'pG(2'5')p for cdiGMP and c[G(2'5')pA(3'5')p] for 2'3'-GAMP. Polymorphisms of STING may affect the immune responses to different isoforms of CDNs. As NOD mice responded to DNP we hypothesized that they should respond to its product, 2'3'-cGAMP. In fact, 2'3'-cGAMP slowed T1D progression in NOD mice. Interestingly, both isoforms could induce the production of pro-inflammatory cytokines, but only 2'3'-cGAMP could induce significant amount of IFNαβ. These results show that either direct or indirect STING activation by DNPs or CDNs, respectively, promote anti-diabetogenic responses in NOD mice. Moreover, these results reveal a previously unidentified defect in NOD mice in responding to bacterial CDN.

T.68. The Immunoregulatory Roles of CD226 and TIGIT During Type 1 Diabetes

Wen-I Yeh, Christopher Fuhrman, Howard Seay, Mark Atkinson and Todd Brusko. ★University of Florida, Gainesville, FL

Genetic studies in type 1 diabetes (T1D) implicate critical roles of antigenic priming (signal 1) and co-stimulation (signal 2) in controlling T cell activity. Two recently discovered co-stimulatory molecules, CD226 and T cell immunoreceptor with Ig and ITIM domains (TIGIT) are crucial in $CD4^+$ T cell function, where a CD226 non-synonymous polymorphism is associated with several autoimmune diseases including T1D. CD226 and TIGIT compete for ligand CD155 and are functionally opposing. CD226 is thought to facilitate pro-inflammation in T conventional (Tconv) cells, whereas TIGIT potentiates regulatory T cell (Treg) activity. However, the cellular distribution and mechanisms by which TIGIT and CD226 mediate human Treg/Tconv cell function are ill-defined. Here, we report that CD226 and TIGIT expression delineates unique $CD4^+$ T subsets. Natural Treg cells primarily express TIGIT, whereas naive Tconv cells are $CD226^{neg/low}$, and fully differentiated cytokine producing effector T cells are $CD226^{high}$. To further understand their function, we modulated gene expression using lentiviral vectors. CD226 over-expression in Tconv cells resulted in increased IFNY production by 2-fold whereas TIGIT promoted Treg cell suppressive capacity without impacting FOXP3 expression. Next, we assessed if an imbalance of CD226/TIGIT expression occurs in T1D. Treg cells derived from the pancreatic lymph nodes of T1D donors obtained through the Network for Pancreatic Organ donors with Diabetes (nPOD) program showed increased ratio of CD226/TIGIT expression (4-fold). These data suggest this co-stimulatory axis may be dysregulated during diabetogenesis and is a potential therapeutic pathway for tolerance induction by up-regulation TIGIT on Treg cells or blocking CD226 on Tconv cells for T1D treatment.

T.69. GAD65 Vaccination Probably Reduces Insulin Loss in Recent Onset Type 1 Diabetes

Craig Beam[1], Colleen MacCallum[1], Kevan Herold[2], Diane Wherrett[3], Jerry Palmer[4] and Johnny Ludvigsson[5]
[1]Western Michigan University, Kalamazoo, MI, [2]★Yale School of Medicine, New Haven, CT; [3]★University of Toronto, Toronto, ON, Canada; [4]★University of Washington, Seattle, WA; [5]Linköping University, Linköping, Sweden

With mixed results, the GAD65 vaccine has been studied in several randomized controlled trials (rct)[1-3] as an effective intervention for insulin preservation in Type 1 diabetes: The initial rct [1] to appear in the literature was positive. Yet follow-up studies[2,3]had statistically negative results.

We conducted a reevaluation of these three studies using Bayesian methods. Our approach added uninformative prior distributions (i.e. equipoise) to the same statistical models used in each study. Our analyses were conducted separately for each rct. Bayesian methods are also available to synthesize data from independent studies. However, methods for synthesis require greater homogeneity in study populations, treatments and endpoints than currently exists

From the initial study[1], Bayesian analysis estimated a probability of a reduction in insulin loss (measured by stimulated C-peptide) from GAD to be 99.38%. Bayesian analysis of the first follow-up study[2] lowered the probability of reduced insulin loss from GAD to 68.82%, yet this value is above equipoise. The second follow-up study[3] increased the probability of insulin-loss reduction from GAD to over 97.00%.

In sum, evidence from published randomized controlled trials consistently suggest that the GAD vaccine probably acts to lessen the loss of insulin production in the new onset Type 1 diabetic patient. However, further examination of the Bayesian analyses and published evidence suggests that the reduction in loss might be small with the dosing protocols considered thus far. We conclude that the entirety of published evidence points toward continued investigation of the GAD vaccine and, specifically, into ways to increase its effectiveness.

T.70. Development of Nanoparticle-coupled Regulatory T Cell Vaccine for Treatment of Type 1 Diabetes

***Judit Cserny**, Jamal Lewis, Benjamin Keselowsky, Howard Seay, Michael Haller, Daniel Perry and Todd Brusko. University of Florida, Gainesville, FL*

Regulatory T cells (Treg) play a critical role in maintaining peripheral tolerance. As such, their use as a potential cellular therapeutic has been proposed for the treatment of Type 1 Diabetes and other autoimmune and inflammatory diseases where they are believed to be defective in number and/or in function. One potential caveat to this approach is the need for exogenous IL-2 to support adequate Treg engraftment and functional stability. Systemic delivery of IL-2, however, entails significant risk of off-target side-effects and toxicity.

Here we present a novel method for local delivery of IL-2 where we encapsulated this growth factor into biodegradable nanoparticles (NP) and covalently conjugated the IL-2 loaded NP to the cell surface of Treg. The particles were fabricated from poly(lactide-co-glycolide) (PLGA), an FDA-approved material by the double-emulsion method, using PLGA-polyethylene glycol maleimide (PLGA-PEG-Mal). This produced IL-2 loaded PLGA-PEG-maleimide NPs that reacted with cell surface sulfhydryl groups, resulting in a stable thioether bond. We show that the IL-2 released from the NPs is able to induce its signaling pathway resulting in STAT5 phosphorylation and support cell viability *in vitro*. Next, we tested the function of IL-2 NP conjugated Treg (Treg-IL-2NP) in an *in vivo* xeno-GvHD model. Treg-IL-2NP treatment resulted in less inflammation in the skin, evidenced by less thickening of both the epidermis and dermis. We also observed less immune infiltration in the subdermal region than in the BSA-NP treated control.

This study is funded by the Diabetes Research Connection.

T.71. Standardized Whole Blood Immune Monitoring for a Clinical Trial of Ustekinumab in Patients with New-onset T1D

***Ashish Marwaha**, Jan Dutz, Megan Levings and Thomas Elliott. ★University of British Columbia, Vancouver, BC, Canada*

Type 1 diabetes (T1D) results from the autoimmune destruction of insulin-producing pancreatic beta-cells. There is now mounting evidence that pro-inflammatory pathways, which are mediated by T cells that secrete IL-17 and IFN-γ, play a critical role in the loss of beta cells. These data suggest that blockade of T cells that secrete IL-17 and IFN-γ may halt or reverse disease in subjects with recent-onset T1D. Agents to facilitate this approach are currently in clinical use. Ustekinumab, a humanized monoclonal antibody that targets the shared p40 subunit of IL-12 and IL-23, has been used for the treatment of psoriasis, an indication for which it has proven to be safe and effective. We have initiated a phase II open-label clinical trial of ustekinumab for the prevention and treatment of new-onset T1D (NCT#02117765).

Five autoantibody positive adult T1D subjects within 3 months of diagnosis have been enrolled in the study so far. Peripheral blood samples have been obtained before administration of ustekinumab and one month after the initial dose. Six standardized whole blood flow cytometry leukocyte profiling panels have been developed by Beckman Coulter and previously validated by the ONE Study clinical trial consortium. We used these panels to monitor changes in the major leukocyte subsets as well as characteristics of T cell, B cell and dendritic cell subsets after administration of ustekinumab. The results of these preliminary analyses will be presented. This method can be used to establish a standardized method for immune monitoring of patients undergoing immune therapy in T1D clinical trials.

T.72. TSHR Stimulating Antibodies (TSAbs) from Graves' Disease Patients Stimulate Thymocytes and may Contribute to the TSAb Response Maturation

Ricardo Pujol Borrell[1,2,3], Roger Colobran[1,3], Ana Marin-Sánchez[2], Anna Casteras[3], Gabriel Obiols[2], Raul Abella[2], Fernández-Doblas Joaquín[2], Massimo Tonacchera[4], Anna Lucas[5, 1] and Mireia Gimenez-Barcons[3].
[1]★Universitat Autònoma de Barcelona, Barcelona, Spain; [2]Hospital Universitari Vall d'Hebron, Barcelona, Spain; [3]Vall d'Hebron Institut de Recerca, Barcelona, Spain; [4]Universita di Pisa, Pisa, Italy; [5]Hospital Univ Germans Trias i Pujol, Badalona, Spain

Graves disease (GD) is an autoimmune disease in which autoantibodies stimulate the TSHR (TSAbs). We have reported that, *TSHR*, which is a GD susceptibility gene, may predispose to disease through the effect of the associated alleles on TSHR expression in the thymus, this implying central tolerance in pathogenesis. Beside and intriguingly, a frequent feature of GD is unexplained thymic hyperplasia. In this report, we expand previous reports on the expression of TSHR in thymocytes demonstrating it by protein an molecular methods, and also that TSHR expression is confined to immature thymocytes, this suggesting a role in thymocyte maturation. *In vitro* experiments on freshly isolated human thymocytes and measurement of cAMP production, demonstrated that human and bovine TSH can stimulate thymocytes through TSHR. More importantly, a human monoclonal antibody (M22) that originated from a GD patient, GD TSAb$^+$ sera and TSAb$^+$ purified IgGs also induced the production of cAMP. TSHR blocking human monoclonal antibody K1-70 and irrelevant IgGs showed no activity. Thymocytes stimulation by TSAbs, may contribute to explain 1) the thymic hyperplasia observed in GD and 2) the initial expansion of the autoimmune response to TSHR by facilitating the eggress of autoreactive T cells. These results suggest a scenario in which the interaction between peripheral lymphoid tissue and the thymus through anti-TSHR antibodies leads to an iterative cycle of stimulation that favors the selection of progressively higher affinity TSAb-producing B lymphocytes. This may be a clue to understand why TSHR autoantibodies are so often stimulating rather than blocking.

General Autoimmunity

OR.31. Gut Flora Regulates T Cell Immune Responses in Humanized Mice

Nalini Vudattu[1], Songyan Deng[1], Paula Preston-Hurlburt[1], James Reed[1], Richard Torres[1], Silvio Manfredo Vieira[1], Bentley Lim[2], Martin Kriegel[1], Andrew Goodman[1] and Kevan Herold[1]. [1]★Yale University, New Haven, CT; [2]Microbial Sciences Institute, West Haven, CT

The gut microbiome can modulate immune tolerance. In our earlier studies, we showed that, in humanized mice, immune tolerance to a xenogeneic skin graft could be induced by teplizumab (a FcR non-binding anti-CD3). Teplizumab induced migration of T cells to the lamina propria via a CCR6/CCL20 gradient where the cells acquired production of IL-10 and expression of Foxp3. We asked whether the gut microflora was necessary for this mechanism of induced tolerance. NOD.scidγc$^{-/-}$ mice were reconstituted with human CD34$^+$ stem cells at birth. After 12 weeks, half of the mice received a cocktail of 4 broad spectrum antibiotics and all mice received a xenogeneic (B6) skin graft with or without teplizumab. There was a significant prolongation of graft rejection in mice treated with teplizumab but not when they received antibiotics ($p=0.026$). A similar number of cells infiltrated the gut in the teplizumab treated mice with or without antibiotic treatment. In the presence of antibiotics, the levels of IL-6 ($p=0.05$) and RORγt ($p=0.04$) were elevated in gut infiltrating T cells even without anti-CD3 mAb treatment. There was an increase in gene expression of IFNg ($p=0.05$), IL-10 ($p=0.02$), TGFb ($p=0.04$), RORγt ($p=0.02$), GATA3 (0.03), and Foxp3 ($p=0.03$) in response to teplizumab treatment but there was not a further increase to teplizumab when antibiotics were given. We conclude that the gut microflora can modify response to immunologics and affect their ability to induce tolerance. Further studies of the microflora may enable targeting of drugs to individuals most likely to show biologic responses.

OR.37. Creation and Function of Engineered Antigen-specific Human Regulatory T Cells for Autoimmunity: A Lesson from CAR Therapy for Cancer

David Scott[1], Yong Chan Kim[1], Aihong Zhang[1] and Kai Wucherpfennig[2]. [1]Uniformed Services University, Bethesda, MD; [2]Dana-Farber Cancer Institute, Boston, MA

Application of expanded polyclonal T regulatory cells (Tregs) offers great promise for the treatment of undesirable immune responses: To increase efficacy and reduce possible non-specific side effects, we elected to create *antigen-specific Tregs,* based on chimeric antigen receptor (CAR) therapy in leukemia. Therefore, we have engineered a myelin basic protein-specific TCR in a retroviral vector and transduced it to human naïve $CD4^+$ T cells and Tregs. The TCR V genes came from an MBP-specific T cell clone derived from an MS patient (Ob2F3, Ota et al., *Nature* 346, 183-7, 1990). We observed that transduced Tregs were activated *in vitro* in response to MBP peptide on DR2 APC and upregulated Foxp3, LAP and Helios expression. Importantly, these engineered MBP-specific Tregs suppressed proliferation and cytokine production by MBP-specific T effector cells in the presence of MBP peptide. "Bystander" suppression of MBP responses *in vitro* could be achieved with Tregs specific for an unrelated epitope (e.g., coagulation factor VIII) when both peptides were together and presented by the appropriate MHC. Mechanistic studies demonstrated that suppression may involve transfer of soluble mediators as well as cell contact. We propose that MBP-specific Tregs may be able to control pathologic inflammation *in vivo*. [Supported by NIH grants RO1 AI035622 and R56 HL061883 (DWS) and PO1 AI045757(KW)]

OR.43. Inhibition of Naive and Memory $CD4^+$ Helper T Cells by Localized Skin Heme Oxygenase-1 Induction and Antigen Delivery in Mice and Non-human Primate

***Julien Pogu**[1], Séverine Remy[1], Philippe Blancou[2], Ignacio Anegon[1] and Thomas Simon[3]. [1]★INSERM UMR 1064-CRTI, Nantes, France; [2]Université de Nice Sophia Antipolis, Sophia Antipolis, Nice, France; [3]University of Maryland School of Medicine, Baltimore, MD*

One barrier to prevention and treatment of autoimmune diseases is the difficulty of antigen-specific tolerization of ongoing T cell responses. We developed an innovative protocol of antigen specific tolerization based on intradermal administration of a heme oxygenase-1 (HO-1) inducer along with auto-antigen. We first evaluated the tolerogenic effect against $CD4^+$ T cell responses in EAE mouse model induced by immunization with MOG(35-55). We demonstrated that prophylactic administration of HO-1 inducer along with MOG(35-55) inhibits EAE development. Indeed, 90% of treated mice are EAE free compare to none for control. Moreover, when treatment is administrated after EAE onset (i.e., mean clinical score, 0.72 ± 0.1) we observed significant reduction of EAE severity. This protection is associated with a decrease in absolute number of infiltrating cell in CNS compared to control (i.e two fold decrease of T cell and tenfold decrease of monocyte derived DCs). Interestingly, no $HO\text{-}1^+$ cells were found in the CNS after treatment. However, we observed 24 hours after treatment a strong recruitment of $HO\text{-}1^+$ DC localized in the draining lymph node of the injection site. In perspective to clinical application, we showed that intradermal injection of a clinically approved HO-1 inducer in baboons lead to $HO\text{-}1^+$ MoDC appearance in draining lymph nodes. Moreover, in baboons previously immunized against tuberculin, intradermal injection of HO-1 inducer and tuberculin led to a complete inhibition of DTH responses further months after treatment. Overall, clinically approved $HO\text{-}1^-$ inducers represent a promising approach for induction of antigen-specific tolerance in patients suffering from autoimmune diseases.

F.9. Tumor Suppressor Death-associated Protein Kinase Negatively Regulates Th17 Differentiation and EAE Development in Mouse

***Ting-Fang Chou**[1,2], Ya-Ting Chuang[3], Ruey-Hwa Chen[2,3], Adi Kimchi[4] and Ming-Zong Lai[1,2,3].*

[1]National Defense Medical College, Taipei, Taiwan; [2]Academia Sinica, Taipei, Taiwan; [3]National Taiwan University, Taipei, Taiwan; [4]Weizmann Institute of Science, Rehovot, Israel

The tumor suppressor death-associated protein kinase (DAPK), which contains a calcium/calmodulin-regulated serine/threonine kinase domain, is a T cell activation negatively regulator. We found that some autoantibodies were significantly increased in *Dapk*$^{-/-}$ mice, while the role of DAPK in T lymphocyte is still unclear. Th17 contributes to the development of experimental autoimmune encephalomyelitis (EAE), a model to mimic multiple sclerosis (MS). Here we showed that DAPK negatively regulates EAE. EAE was exacerbated in *Dapk*$^{-/-}$ mice. T cell-specific transgenic expression of active DAPK suppressed EAE induction, illustrating the suppressive effect of DAPK at the T cell stage. We further found that that loss of DAPK led to profound increase in Th17. Th1 cell differentiation and T-bet expression, in contrast, was not significantly altered. DAPK-deficiency led to a significant upregulation of RORγt and RORα, the transcription factors dictating IL-17 expression. Our results reveal a negative signaling cascade from DAPK to IL-17 expression and EAE generation, and suggest that activation of tumor suppressor DAPK could be used to target inflammatory autoimmune diseases.

F.10. CD8 T Cell-mediated Bone Marrow Failure

Katrina Hoyer, David Gravano, Dan Davini, Philip Sanders and Jennifer Manilay. University of California Merced, Merced, CA

Acquired aplastic anemia is a bone marrow failure disorder characterized by bone marrow hypoplasia and blood pancytopenia and in a majority of patients is presumed to be autoimmune in nature. Clinical evidence implicates a detrimental role for CD8$^+$ T cells in this disease and a beneficial role of Foxp3$^+$ regulatory T cells (Tregs) in maintaining immune tolerance in the bone marrow. Using the IL-2-deficeint autoimmune mouse model, with a deficit in functional Tregs, we demonstrate a critical role for CD8$^+$ T cells in the development of Th1-mediated aplastic anemia. CD8$^+$ T cells promote hematopoietic stem cell dysfunction and depletion of myeloid lineage progenitor cells, resulting in anemia. Adoptive transfer experiments demonstrate that CD4 T cells are required to provide CD8 T cells help to accumulate in the bone marrow and expedite disease progression. We have further noted a specific accumulation of TCR Vβ6 CD8$^+$ T cells in the bone marrow, raising the possibility that this disease may be due to an antigen-specific immune response.

F.11. Evidence for Autophagy as a Possible Mechanism that Contributes to the Antiproliferative Effects of Testosterone in the Autoreactive T Cells

Ting Jia, Annandurai Anandhan, Chandirasegaran Massilamany, Rajkumar Rajasekaran, Rodrigo Franco Cruz and Jay Reddy. University of Nebraska-Lincoln, Lincoln, NE

Gender-disparity is well-documented in the mouse model of experimental autoimmune encephalomyelitis (EAE) induced with proteolipid protein (PLP) 139-151 in that, female but not male SJL mice, show a chronic relapsing-remitting paralysis. Therapeutically, testosterone has been used successfully to ameliorate the severity of EAE. However, the underlying mechanisms mediating the protective effects of testosterone are unclear. Since EAE is typically mediated by CD4 T cells, we tested the hypothesis that testosterone selectively modulates the expansion and functionalities of antigen-specific T cells. Using various tools such as, proliferation assay, major histocompatibility complex class II dextramers for PLP 139-151, and cell-viability assay, we noted that testosterone affected the viability of naïve T cells treated with or without anti-CD3, and also when antigen-sensitized T cells were stimulated with PLP 139-151 leading us to evaluate the effects of testosterone on cell death pathway. Testosterone-induced cell death was found to be associated with increased activation/cleavage of caspase-3 and poly (ADP-ribose) polymerase-1. We next evaluated whether autophagy contributes to the antiproliferative responses of testosterone by examining the expression of the mammalian target of rapamycin (mTOR), p62, beclin-1 and microtubule-associated protein 1A/1B-light chain 3 (LC3) I and LC3 II by western blotting analysis. We observed that mTOR-expression was significantly decreased with a corresponding increase in the expression of LC3 II including the ratio between LC3 II/LC3 I. Our data suggest that testosterone exerts its effects on both proliferating and non-proliferating cells similarly, and that autophagy may contribute to the immunomodulatory effects of testosterone in T cells.

F.12. Essential Role of IL-4 on Treg Mediated Immune Suppression

Wei Cheng Yang, Ying Yu Chen, Wei Hsin Hong and Chia-Rui Shen. Chang Gung University, Taoyuan, Taiwan

IL-4 was reported to be able to not only tuning Foxp3 expression in T cells but also dampening Treg mediated immune suppression. However, we found that supplement of IL-4 alleviated AIHA progression in NZB mice may through accumulating $CD4^+CD25^+Foxp3^+$ Tregs, in addition to the induction of FcγRIIb2 on splenic macrophages. It implies the beneficial role of IL-4 in peripheral tolerance. In this study, we focused on deciphering the effects of IL-4 on Tregs, particularly on their suppressive capabilities. Both *in vitro* and *in vivo* models were applied for Treg functional assessment. Although IL-4 knockout (IL-4 KO) mice and congenic C57BL/6 (B6) mice demonstrate no significant differences in their Treg numbers, the expression of Foxp3 in Tregs from IL-4 KO mice is less than it is in Tregs from B6 mice. To verify whether the Foxp3 expression is representative for Treg function, we had performed *in vitro* suppression assay. It appeared that IL-4 KO Tregs were inferior to those from B6 mice in suppressing their autologous effector T cells *in vitro*. Supplement of IL-4 rescued the function of IL-4 KO Tregs whereas neutralization of IL-4 weakened Tregs from B6 mice. Moreover, Tregs from OVA sensitized IL-4 KO mice demonstrated poor suppression against the peptide-primed OTII effectors. Finally, it presented a severe disease activity and prolonged recovery when experimental autoimmune uveitis (EAU) was induced in IL-4 KO mice. In conclusion, these results indicate IL-4 as one of the crucial cytokines in Treg mediated immune suppression *in vivo* and *in vitro*.

F.13. A New FAS/FASL/CASP10-independent Mechanism for the Generation of Autoimmune Lymphoproliferative Syndrome in Humans

Eduardo Finger. SalomaoZoppi Diagnosticos, São Paulo, Brazil

Autoimmune lymphoproliferative syndrome (ALPS) is a rare proinflammatory condition caused by impairment in immune homeostasis, due to an inability of the immune system to eliminate autoreactive or activated lymphocytes via apoptosis. In two thirds of the cases where the disease could be traced to an identifiable genetic defect, it presented itself as a monogenic disease involving one of three genes: FAS, FASL or CASP10. In approximately 30% of the cases, no genetic defect could be identified.

This abstract presents the case of a 26 year old male who exhibited an ALPS phenotype from birth, but was not diagnosed until it progressed to a life threatening disease involving disseminated encephalomyelitis. Interestingly, genetic investigation of the disease revealed not the monogenic pattern involving one of the 3 genes mentioned above but a polygenic combination of mutations involving genes not previously related to this particular phenotype: NOD2, MEFV and CASP8AP2, the latter not previously connected to any pathological condition in humans.

Our investigation extended to the immediate relatives of the patient and revealed that the father carries the NOD2 mutation, the sister carries the MEFV mutation and the mother, both, the MEFV and the CASP8AP2 mutations, all without any detectable consequences.

Together, these results represent the 1st instance of ALPS deriving not from a monogenic defect, but instead, from a polygenic combination of 3 genes previously unrelated with this phenotype and the 1st instance where a mutation in CASP8AP2 is implicated in human disease.

F.14. Stress is Detrimental and Moderate Exercise is Beneficial in an Animal Model of Lupus Nephritis: Extra-medicinal Influences Patients May Have in Autoimmune Disease

Nicholas Young, Sudha Agarwal, Saba Aqel, Jeffrey Hampton, Kendra Jones, Lai-Chu Wu, Nicole Powell, John Sheridan, Michael Bruss and Wael Jarjour. The Ohio State University, Columbus, OH

Chronic inflammation is the hallmark of autoimmune disease and both stress and exercise have been shown to elicit immuno-modulatory effects. We have previously demonstrated that daily, moderate exercise can suppress inflammation systemically by inhibiting NFκB in an acute model of inflammation. Conversely, repeated social stress has been shown to stimulate proinflammatory immune cell egress and trafficking, which exacerbates autoimmunity. To examine the effects of both moderate exercise and stress on chronic autoimmune-mediated inflammation, we used the NZM2410 animal model, which develops lupus nephritis spontaneously at 22-40 weeks of age. Early removal criteria was defined by a threshold of 20% weight loss and a blood urea nitrogen (BUN) level above 50 mg/dL. To determine the influence of exercise in this system, mice were exercised daily at moderate intensity. All control mice met early removal criteria by 34 weeks of age. In contrast, only 50% of the exercising mice met this criteria at the 39 week time point. Similarly, using a well-established social disruption stress model, BUN levels and weights were monitored and kidney tissue was collected when a mouse undergoing the stress protocol met the early removal criteria. Each experimental mouse was paired with an age-matched control counterpart for comparative analysis by histopathological scoring. Repeated social stress significantly induced more severe inflammatory pathology, as characterized by glomerular hypercellularity and hyperplasia of the Bowman's capsule. Collectively, our data suggests that stress reduction and moderate exercise could be used as potent therapeutic modalities to control the inflammation associated with lupus nephritis.

F.15. Immunomodulation Induced by Mycobacterial Heat Shock Proteins on Non-obese Diabetic (NOD) Mice Cells *In Vitro*

Gecilmara Pileggi, *Aline Clemencio, Sonir Antonni and Celio Silva. Universidade de São Paulo, Ribeirão Preto, Brazil*

Despite substantial efforts in recent years towards a better understanding of the role of heat shock proteins (HSPs) in inducing and/or controlling autoimmune diseases such as diabetes, success has remained elusive. Although immunotherapy with mycobacterial hsp65 as a DNA vaccine (DNAhsp65) can protect non-obese diabetic (NOD) mice against diabetes and can therefore be considered in the development of new immunotherapeutic strategies, we do not know the basic immune mechanisms involved in the process of immunomodulation. We searched for the potential of DNAhsp65 as well as the mycobacterial hsp65 and hsp70 proteins to modulate immune response of NOD mice cells by evaluating the pattern of cytokines released in a co-culture of antigen presenting cells (APCs) and T cells in *vitro*. Based in the knologde of the importance of cytokines produced by the Th1, Th2 and Th17 in controlling diabetes immune response in NOD mice, this may be considered a valid way to evaluate induced immunomodulation. Indeed, our results have showed that NOD mice cells treated with DNAhsp65 or with the recombinant proteins hsp65 or hsp70, having the effect of reducing the production of cytokines of the Th1 and Th17 pattern of immune response and present in parallel the activation of IL-10 release. Evaluations at other time points of co-cultures with longer follow-up as well as in NOD animals treated with DNAhsp65 or with the recombinant proteins could be valuable to further investigate the potentially beneficial effects of DNAhsp65 immunotherapy of diabetes.

F.16. Innate Immunity Drives the Initiation of Murine Model of Primary Biliary Cirrhosis

Ya-Hui Chuang. *National Taiwan University, Taipei, Taiwan*

Invariant natural killer T (iNKT) cells play complex roles in bridging innate and adaptive immunity by engaging with glycolipid antigens presented by CD1d. The influence and modulation mechanism of iNKT cells in autoimmunity has not been fully defined. We have suggested previously that iNKT cells are involved in the initiation of PDC-E2-specific autoimmunity and immunopathology in primary biliary cirrhosis (PBC). We have taken advantage of our well established xenobiotic-induced murine autoimmune cholangitis model in an effort to address whether iNKT cell activated by a Th2-biasing agonist, (2s,3s,4r)-1-*O*-(a-$_D$-galactopyranosyl)-*N*-tetracosanoyl-2-amino-1,3,4-nonanetriol (OCH), can inhibit the development of autoimmune cholangitis. Similar to a-GalCer treated 2-OA-BSA immunized mice, although OCH treatment led to a weaker IFN-g production than a-GalCer, OCH enhanced antigen-specific

antibody production, augmented $CD8^+$ T cell activation, lymphocyte infiltrates, portal inflammation, bile duct damage as well as liver fibrosis. Genetic depletion of CD1d resulted in decreased anti-mitochondrial antibodies, cell infiltrates, and IFN-g production of liver mononuclear cells in 2-OA-BSA immunized mice. Our data reinforce the concept that iNKT cells has a critical influence of in the pathogenesis of autoimmune cholangitis and also suggest that the Th2-biased cytokines induced by OCH may also impact disease initiation in a cascade of immune reactions primed by activated iNKT cells, including T cell activation, and emphasizing therefore the critical roles of innate immunity in this murine model of PBC.

F.17. Suppresion of Experimental Autoimmune Uveitis by Targeting PD-1 T Cells

Chia-Rui Shen[1], Yihshiou Hwang[2], Tun-Ywin Hung[1], Wei Hsin Hong[1] and Chaolin Liu[3]. [1]Chang Gung University, Taoyuan, Taiwan; [2]Chang Gung Memorial Hospital, Taiwan; [3]Ming Chi University of Technology, New Taipei, Taiwan

Autoimmune uveitis is the category of inflammatory eye diseases that affects uvea-retinal tissue and results in visual loss. The inflammation appears to be mainly associated with activation of autoreactive T cells. It is known that PD-1 negatively regulates T cell activation, and interaction between PD-1 and its ligand (programmed death ligand-1, PD-L1) plays a protective role in developing autoimmune diseases. Here we sought to develop a PD1-targeting approach to suppress experimental autoimmune uveitis (EAU), which is the animal model of human autoimmune uveitis. Successful construction of recombinant adeno-associated virus (AAV) delivering PD-L1 (rAAV.PD-L1) was confirmed by *in vitro* transfection and infection studies, in which the expression of PD-L1 was significantly increased and assayed by both flow cytometric and western blot analysis. EAU mice were induced with the interphotoreceptor retinal binding protein ($IRBP_{1-20}$) synthetic peptide, and treated intraocularly with the recombinant AAV expressing PD-L1. The results demonstrated that rAAV.PD-L1 treatment significantly ameliorated the severity of EAU by reducing the retinochoroidal infiltration of lymphocytes. Splenic T cells derived from the treated group produced significantly lower levels of proinflammatory cytokines, including IFN-γ and IL-17, indicating the suppression of autoreactive T cell activation. Taken together, EAU mice receiving rAAV.PD-L1 therapy showed a significant improvement, including clinical and histopathological intraocular inflammation, inhibition of T cell activation and reduction of proinflammatory cytokine production. Our data suggest the gene delivery of PD-L1 can ameliorate the EAU intraocular inflammation through suppressing the proinflammatory cytokine produced by IRBP-specific T cells, which may be a promising therapeutic strategy in uveitis management.

F.18. Cytokine Production by the Invariant Natural Killer T Cell Lineage is Regulated by the Transmembrane **Protein Kinase IRE1α**

Srinath Govindarajan[1], Eveline Verheugen[1], Takao Iwawaki[2], Dirk Elewaut[1] and Michael Drennan[1]. [1]★Ghent University Hospital, Ghent, Belgium; [2]Gunma University, Gunma, Japan

The inositol-requiring enzyme 1a (IRE1a) is a type I endoplasmic reticulum (ER) transmembrane protein containing both kinase and sequence-specific endoribonuclease (RNase) activities. Upon ER stress, activation of IRE1a results in the cleavage of an intron from mRNA coding for X box binding protein 1 (*Xbp-1*), thereby generating a transcription factor that regulates the expression of genes important for restoring cellular homeostasis. In this study, we show that T cell receptor (TCR)-dependent activation of the invariant natural killer T (iNKT) cell lineage results in splicing of *Xbp-1* mRNA as well as the upregulation of genes associated with induction of the unfolded protein response (UPR). A subsequent analysis of iNKT cell development in T cell-specific IRE1a knock-out mice revealed comparable tissue distribution and maturation profiles when compared to controls, however, TCR-dependent cytokine production by the iNKT cell lineage was severely impaired in the absence of IRE1a. In this context, impaired cytokine production by IRE1a-deficient iNKT cells was not due to the altered expression of transcription factors such as T-bet or GATA-3, but was consequence reduced mRNA stability for cytokines such as IFNg and IL-4. Furthermore, we show that T cell-specific IRE1a knock-out mice are protected from oxazolone-induced ulcerative colitis, suggesting that IRE1a activity within the iNKT cell lineage may be a therapeutic target for treatment of the disease.

★ = member of a FOCIS Center of Excellence

F.19. Early Autoimmune Pathogenesis in the Islet of NOD Mice is Inhibited After Neonatal Immunization with Insulinoma Exosomes

M. Jubayer Rahman[1], Danielle Regn[2], Roman Bashratyan[2] and Yang Dai[2]. [1]NIDDK/NIH, Bethesda, MD, United States; [2]The Scripps Research Institute, San Diego, CA

Exosomes (EXOs) are nano-sized microvesicles released by cells encountering physiological and pathological stimuli. We have recently found that an insulinoma cell line, MIN6, released immunostimulatory exosomes that, surprisingly, activated autoreactive B and T cells in prediabetic non-obese diabetic (NOD) mice. Thus, EXOs may be a novel, endogenous target of autoimmunity. To study humoral immune response to EXOs, NOD neonates were chosen due to their limited number of mature T cells, and immunization was performed three times. At the age of 4 weeks, EXO-induced B cells are recruited specifically in the pancreas, resulting in a delay of early T cell expansion in the pancreatic draining lymph nodes. EXO-specific Igs persist in periphery through the age of 8 weeks; clearly, there is an Ig isotype-switching from Th1 to Th2 profiles as compared with age/gender-matched, non-immunized NOD mice. Most importantly, at this age of life, NOD mice normally develop insulitis, however, almost all the islets from the EXO-immunized mice are free from lymphocyte infiltration. This indicates an early protection of the islets from the T cell-mediated autoimmune pathogenesis. The effect is partially explained by increased levels of immunosuppressive autoantibodies in the immunized sera, which inhibited APC function and subsequent activation of autoreactive T cells *in vitro*. Intriguingly, adoptive transferring the EXO-induce lymphocytes into NOD.*scid* mice resulted in accelerated development of T1D, suggesting that EXO-immunization might also induce autoreactive T cells. Thus, autoreactive B and T cells specific for EXOs may function in regulation and/or pathogenesis at different stages of T1D development in NOD mice.

F.20. OX40 Ligand Contributes to Human Lupus Pathogenesis by Promoting T Follicular Helper Response

Nathalie Schmitt[1], Clement Jacquemin[2], Cecile Contin-Bordes[2], Yang Liu[1], Priya Narayanan[1], Jean-Francois Moreau[2], Robert L. Coffman[3], Patrick Blanco[2] and Hideki Ueno[1]. [1]Baylor Institute for Immunology Research, Dallas, TX; [2]University of Bordeaux, Bordeaux, France; [3]Dynavax Technologies Corporation, Berkeley, CA

Increased activity of T follicular helper (Tfh) cells plays a major pathogenic role in systemic lupus erythematosus (SLE). However, the mechanisms that cause aberrant Tfh responses in SLE remain elusive. Here we provide evidence that the OX40 ligand (OX40L)-OX40 axis contributes to the aberrant Tfh response in SLE. OX40L was expressed by myeloid antigen-presenting cells (APCs), but not B cells, in blood and in inflamed tissues in adult and pediatric patients with SLE. The frequency of circulating OX40L-expressing myeloid APCs positively correlated with disease activity and the frequency of $ICOS^+$ blood Tfh cells in SLE. OX40 signals promoted naïve and memory $CD4^+$ T cells to express multiple Tfh molecules, and were sufficient to induce them to become functional B cell helpers. Mechanistically we found that immune complexes containing RNA induce OX40L expression on myeloid APCs via TLR7 activation. Our study provides a rationale to target the OX40L-OX40 axis as a therapeutic modality for SLE.

F.21. Protein Kinase C (PKC)-θ-selective Inhibitor R687 Blocks T Cell Activation and Protects in Experimental Models of Immune-mediated Disease

Alexander Owyang, Matthew Duncton, Florentino San Pablo, Art Bagos, Ernest Tai, Meagan Chan, Roy Frances, Stacey Siu, Sothy Yi, Chi Young, Rajinder Singh, Donald Payan, Esteban Masuda and Sacha Holland. Rigel Pharmaceuticals, South San Francisco, CA

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 several potent and specific compounds. We have previously shown that PKC-θ inhibitors are effective in blocking T cell activation in acute and chronic disease models. In experimental autoimmune encephalomyelitis (EAE), we showed that PKC-θ inhibitor R683 reduced the influx of $CD4^+$ T cells into the CNS, and also reduced the number and percentage of TH17 cells. Lead compound R687 has improved pharmaceutical properties over R683, while maintaining potent inhibition of PKC-θ in biochemical assays (IC50 < 2nM). R687 is inactive in a panel of tyrosine and serine-threonine kinase signaling assays in cells and is not cytotoxic. R687 exhibits excellent selectivity for inhibition of PKC-θ over other PKC family members in biochemical assays. This correlates with specific inhibition of PKC-θ-dependent pathways in cells. When administered orally, R687 is highly efficacious in adjuvant-induced arthritis (AIA), transplant rejection, and EAE, highlighting the central role of PKC-θ in T cell-dependent pathogenic processes. Histological and molecular analyses of these *in vivo* studies show that inhibition of PKC-θ with R687 leads to reduction of inflammatory cytokines and pathways.

F.22. Defects in the Lyn and Aire Pathways Cooperate to Promote Autoimmune Uveitis

***Irina Proekt**, Corey Miller, Marion Jeanne, Kayla Fasano, Douglas Gould, Mark Anderson and Anthony DeFranco.*
★*University of California, San Francisco, San Francisco, CA*

Studies of genetic factors associated with human autoimmunity suggest a multigenic origin of autoimmune susceptibility. To address how defects in different immune tolerance pathways cooperate to result in disease, we investigated whether genetic alterations in two such pathways might synergize to produce autoimmunity in mice. The intracellular protein tyrosine kinase Lyn is critical for inhibitory receptor function in innate immune cells and B cells. Lyn-deficient mice develop systemic but not organ-specific autoimmunity. In contrast, Autoimmune Regulator (Aire) promotes expression of tissue-specific antigens by thymic epithelial cells and T cell negative selection. Aire-deficient mice develop multiorgan autoimmunity, including autoimmune uveitis, due to lack of thymic expression of the retina-specific interphotoreceptor retinoid binding protein (IRBP). $Aire^{GW/+}$ knockin mice express a dominant negative allele of Aire, have low but detectable thymic expression of IRBP and do not develop uveitis. To determine whether lack of Lyn inhibitory pathways could cooperate with a partial defect in T cell central tolerance, we generated $Aire^{GW/+}$ $Lyn^{-/-}$ double mutants. Remarkably, over 50% of these mice developed severe uveitis that was accompanied by a rise in anti-IRBP antibodies and an expansion of IRBP-specific CD4 T cells. To determine the contribution of different Lyn-expressing cell subsets to uveitis, we crossed $Aire^{GW/+}$ mice to a panel of Cre lines with an inducible Lyn knockout allele. Absence of Lyn in DCs alone was sufficient to drive disease, suggesting that Lyn-deficient DCs may have increased ability to prime IRBP-specific T cells that have escaped negative selection in the $Aire^{GW/+}$ thymus.

F.23. Itolizumab (Anti-CD6) Long-term Immunomodulation in Autoimmune Diseases

***Enrique Montero**. Center of Molecular Immunology, Havana, Cuba; Biocon Limited, Bangalore, India*

CD6 is a membrane glycoprotein with three scavenger receptor cysteine-rich domains (SRCR), contributing to lymphocyte activation and adhesion. Therapeutic interventions aiming $CD6^+$ T cells depletion or blocking migration to target organs were earlier explored, using monoclonal antibodies (mAb) and recombinant receptor/ligand interfering fusion proteins. The dynamic peripheral repopulation skewed towards an immunocompetent CD6- cell subpopulation, and the limited impact on $CD8^+$ cells trafficking via SRCR3-ALCAM (Activated Leukocyte Cell Adhesion Molecule) interaction respectively, may explain the limited success of those approaches. Subsequent evidences on CD6 mediated costimulation leading to lymphocytes pro-inflammatory commitment, the CD6 overexpression on T cells in the inflammation sites along with the enrichment in $CD6^+$ B cells and other lineages, stimulates further options in this therapeutic model. Itolizumab (non-agonistic non-depleting humanized IgG1) and its predecessor ior t1 (mouse IgG2a mAb) recognize a distinct epitope on human CD6 SRCR1, with dominant expression in autoimmune inflamed tissues. Itolizumab inhibits T cell proliferation, reduces the IL-17, IFNγ, IL-6, TNFα secretion and molecules associated with cell activation and adhesion. Retaining this action despite the CD6-ALCAM binding, warranty such effect during the immunopathological process with abundant Th1 and Th17 $CD6^+$ cells infiltrating the target organs.

Itolizumab immunotherapeutic effect in Psoriasis led to CD6-targeted therapy validation, promoting a significant clinical response persisting after the last administration, without immunosuppression. Further pre-clinical and clinical evidences in Rheumatoid Arthritis, Multiple Sclerosis, Type 1 Diabetes among others, encourages additional exploration on CD6 as a unique multi-cell types therapeutic target preventing the induction and perpetuation of inflammation leading to an autoimmune long-term immunomodulation.

F.24. Small Wonders: Carbon Nanotechnology to Treat Autoimmune Diseases

Redwan Huq[1], E. Loic Samuel[2], Thomas Lee[1], Mark R. Tanner[1], Fatima S. Khan[1], Rajeev Tajhya[1], Taeko Inoue[1], Robia Pautler[1], James M. Tour[2] and Christine Beeton[1]. [1]Baylor College of Medicine, Houston, TX; [2]Rice University, Houston, TX

Autoimmune diseases, such as multiple sclerosis and rheumatoid arthritis, are mediated by a type of white blood cell—T lymphocytes. Current treatments for these diseases are broad immunosuppressants associated with life-threatening side effects, necessitating the development of new therapeutic strategies. The inflammatory microenvironment in these diseases generates large quantities of harmful reactive oxygen species (ROS). However, low levels of intracellular ROS act as signaling molecules necessary for T lymphocyte activation. Therefore, intracellular ROS represent attractive targets for modulating T lymphocyte activity and for treating autoimmune diseases.

Carbon nanoparticles can scavenge ROS with higher efficacy than dietary and endogenous antioxidants. The affinity of carbon nanoparticles for specific cell types represents an emerging tactic for targeted therapy. Here, we show that nontoxic poly(ethylene)-glycol-functionalized hydrophilic carbon clusters (PEG-HCCs) are the first carbon nanoparticles to be preferentially internalized by T lymphocytes over other splenic immune cells. We use this selectivity to attenuate T lymphocyte function *in vitro* without affecting major functions of macrophages, an immune cell subset crucial for physiological activation of T lymphocytes. We demonstrate the effectiveness of PEG-HCCs in reducing T lymphocyte-mediated inflammation in delayed-type hypersensitivity and in ameliorating experimental autoimmune encephalomyelitis and pristane-induced arthritis, animal models of multiple sclerosis and rheumatoid arthritis, respectively. Our results suggest that the remarkable selectivity of PEG-HCCs for T lymphocytes is a novel and promising route for treating T lymphocyte-mediated autoimmune diseases without inducing broad immunosuppression.

F.25. Viral Myocarditis Induced with Coxsackievirus B3 Involves the Mediation of Mono Antigen-specific Autoreactive CD4 and CD8 T Cell Responses

Chandirasegaran Massilamany, Rajkumar Rajasekaran, Rakesh Basavalingappa, David Steffen and Jay Reddy. University of Nebraska-Lincoln, Lincoln, NE

Dilated cardiomyopathy (DCM) can develop in individuals affected with myocarditis, and enteroviruses like coxsackievirus B3 (CVB) are commonly suspected in patients with myocarditis/DCM, but the clinical significance of CVB infection remains elusive. Using the mouse model, we recently demonstrated that A/J mice infected with CVB show the generation of pathogenic CD4 T cells specific to cardiac myosin (Myhc) 334-352 that can transfer disease to naïve animals. We now report that Myhc 334-352 also encompasses two determinants namely, Myhc 338-348 and Myhc 338-347, and presented here are four key findings. (i) Both epitopes induce myocarditis by generating CD4 and CD8 T cell responses as evaluated by histology and magnetic resonance microscopy imaging. (ii) Major histocompatibility complex (MHC) class I-stabilization assay revealed that Myhc 338-347 binds H-2D^{d}, but not H-2K^{k} or H-2L^{d} alleles, whereas Myhc 338-348 could bind H-2D^{d} but with only marginal affinity. (iii) The CD8 T cell responses induced by both Myhc 338-348 and Myhc 338-347 were specific as evaluated by MHC class I/H-2D^{d} dextramer staining and the antigen-sensitized CD8 T cells produced predominantly, interferon-gamma and tumor necrosis factor-alpha, the signature cytokines of cytotoxic T cells, and (iv) CVB-infected animals showed the

generation of CD8 T cells responding to both Myhc 338-348 and Myhc 338-347, in addition to the appearance of Myhc 334-352-specific CD4 T cells. Taken together, the data suggest that the postinfectious myocarditis induced by CVB may involve the generation of diverse autoreactive T cell types that target the same antigens, but they inflict damage through different mechanisms.

F.26. A Dendritic Cell-stromal Cell Axis Maintains Immune Responses in Lymph Nodes

Varsha Kumar. ★Hospital For Special Surgery, New York, NY, United States

Within secondary lymphoid tissues, stromal reticular cells support lymphocyte function, and targeting reticular cells is a potential strategy for controlling pathogenic lymphocytes in disease. However, mechanisms that regulate reticular cell functions are not well understood. Here we show that during an immune response in lymph nodes, dendritic cells (DCs) maintain reticular cell survival in multiple compartments. DC-derived lymphotoxin beta receptor (LTβR) ligands are critical mediators, and LTβR signaling on reticular cells mediates cell survival by modulating podoplanin (PDPN). We show that PDPN modulates integrin-mediated cell adhesion, which maintains cell survival. This DC-stromal axis maintains lymphocyte survival and the ongoing immune response. Our findings identify new functions for DCs and PDPN in regulating reticular cell survival and delineate a novel DC-stromal axis that can potentially be targeted in autoimmune or lymphoproliferative diseases.

3958. Some Parameters of Immune System in Patients with Primary Antiphospholipid Syndrome Nephropathy

Z. Kamalov and D. Akramkhodjaeva. Academy of Sciences of the Republic of Uzbekistan, Tashkent, Uzbekistan

Currently, there is a steady increase in the number of patients requiring dialysis treatment or a kidney transplant. One of the causes of vascular disease of the kidneys is the antiphospholipid syndrome. The problem of APS nephropathy (APSN) is regarded as a priority immunonefrology. The aim: to study some parameters of immune system in patients with primary kidney disease, coupled with the APS. The study included 76 patients with primary nephropathy. Of these, 31.6% of patients were diagnosed with APS. Verification of the diagnosis was performed by serological method, identifying key serological markers APSN (BA, β2-GP I) in patients with this disease. Immunological study included the level of CD3, CD4, CD8, CD16, CD19, CD25 and SD95 lymphocytes. The level of IgA, IgM, IgG was determined by Mancini. The level of IL-1, IL-4 and γ-IFN was measured in serum by ELISA. The results showed that the immune system of the patients characterized by reduced levels of CD3, CD4, CD8 $^{+}$ lymphocytes and the activation of CD25 and CD95. While there is a polyclonal activation of humoral immunity, increased content of B lymphocytes, the level of IgA, IgG, IgM and circulating immune complexes. The study of cytokine levels showed that in patients primary nephropathy associated antiphospholipid syndrome observed increase in the concentration of pro-inflammatory (IL-1β, IFN-γ) and anti-inflammatory cytokines (IL-4). Increased synthesis of the studied cytokine plays a pathogenic role in the maintenance of the autoimmune process in primary renal disease on the background of antiphospholipid syndrome.

Genetics

F.27. Geneset Signatures Associated with Adaptive Immunity Following Seasonal Influenza A/H1N1 Vaccination

Inna Ovsyannikova, Richard Kennedy, Iana Haralambieva, Krista Goergen, Diane Grill, Ann Oberg and Gregory Poland. ★Mayo Clinic, Rochester, MN

A systems vaccinology approach offers understanding of molecular signatures associated with immunological protection and provides a new approach to evaluate vaccine-induced immune response variations.

To evaluate gene signatures associated with humoral response among healthy individuals after seasonal influenza vaccine, we enrolled 159 healthy subjects (50-74 years-old) who received one dose of trivalent influenza vaccine containing the A/California/7/2009/H1N1-like virus. Influenza-specific serum hemagglutination-inhibition (HAI) and virus-neutralization antibody (VNA) titers, and mRNA-sequencing on PBMCs, were performed using samples obtained at Days 0 and 28 after vaccination. Externally defined genesets significant with the mRNA expression changes over time ($p<0.005$, n=112) were used in cross-validated penalized regression models to predict HAI or VNA change from Day 0 to Day 28.

The median (IQR) age of the study subjects was 59.5 (55.3, 66.3) years (98.7% Caucasians). Day 0 influenza-specific median HAI and VNA titers (1/80; 1/40-1/320) showed the presence of pre-existing antibodies. Both HAI and VNA titers increased by Day 28 (1/320; 1/160-1/640, $p<0.001$). Several genesets had genes remain in the predictive models for both HAI (n=7) and VNA (n=36). The genesets/genes associated with HAI are RNA-transcription factor (TTF2); chemokine/cytokine/receptors (CCR9, IFNG, IL10RA); cytochromes (CYB5R2,3, CYB561); and carbonic-anhydrases (CA2,6,8,11,14). The genesets associated with VNA include TNF ligand TNFSF11, cytokines/receptors IFNG, IL7, IL27, IL12A, and interferon-inducible transcription factors (IRF7,9). Six genesets/genes (IFNG; CA2,6,8,11,14; TTF2; DOPEY2; GSTM1,2) were in common for both HAI and VNA responses.

The identification of gene signatures associated with HAI and VNA responses may provide a better understanding of the genetic markers of immune response.

F.28. HLA-DRB1 *13:02, *09:01 and *04:01 Classical Alleles are Associated with Rheumatoid Arthritis Disease Severity in African Americans

Richard Reynolds and S. Louis Bridges, Jr. ★University of Alabama at Birmingham, Birmingham, AL

The largest contributor of rheumatoid arthritis (RA) genetic risk is *HLA-DRB1*, but its role in disease severity is less clear and only reported for Caucasians. Here we tested the hypothesis that *HLA-DRB1* alleles are associated with total Sharp-van der Heijde score in 525 seropositive African American RA patients. A zero inflated negative binomial model of Sharp scores was fit conditional on disease duration, smoking, sex, body mass index (BMI), and local ancestry (model 1). Model two was model one plus 21 variables representing counts {0, 1, 2} of *HLA-DRB1* alleles (frequency > 1%). Model three was model two plus 21 variables indicating the interaction between local ancestry and *HLA-DRB1* alleles. The median (IQR) Sharpe score was 3 (0, 21). Disease duration was associated negatively with zero-inflated radiographic damage ($P = 5.5E-12$) and positively with Sharp score ($P < 2.0E-16$). Likelihood ratio tests of model 1 vs 2 demonstrated that *HLA-DRB1* classical alleles were significantly associated with Sharp scores ($P = 0.026$). Tests of model 2 vs 3 demonstrated this effect was not ancestry specific ($P = 0.33$). False discovery rate corrected p-values indicated *HLA-DRB1* *09:01 ($P = 0.027$), *13:03 ($P = 0.027$), and *04:01 ($P = 0.049$) were significantly associated with only the count part of model 2. Therefore *HLA-DRB1* risk alleles are a source of variability in RA severity in African Americans. Disease duration is strongly associated with both the absence of erosions and the non-zero inflated counts of Sharp scores, but the genetic association is confined entirely to the latter.

F.29. Genome Editing in T Cells

Kathrin Schumann. ★University of California, San Fransisco, San Francisco, CA

T cell genome engineering holds great promise for cancer immunotherapies and cell- based therapies for HIV and autoimmune disease. CRISPR/Cas9 is a robust technology facilitating genome engineering in many cell types, but its

applications in primary human T cells have been limited by poor efficiency. Here we report improved efficiency of genome engineering in both pro-inflammatory and regulatory human $CD4^+$ T cell subsets using Cas9:single-guide RNA ribonucleoproteins (Cas9 RNPs). Cas9 RNP delivery allowed ablation of CXCR4, a co-receptor for HIV entry. Cas9 RNP electroporation could reduce by up to 70% the number of cells with high cell surface expression of CXCR4. Importantly, Cas9 RNPs promoted a high frequency of targeted replacement of genome sequences in primary T cells by homology-directed repair (HDR), which was previously unattainable. HDR-mediated 'knock-in' could be achieved with ~15% efficiency. Finally, Cas9 RNPs also enabled a human *in vitro* model of the multi-organ autoimmune disease Immunodysregulation Polyendocrinopathy Enteropathy X-linked Syndrome (IPEX), where FOXP3 mutations impair regulatory T cell differentiation. These studies establish Cas9 RNP technology for diverse experimental and therapeutic genome engineering applications, including efficient DNA sequence replacement with HDR, in primary human T cells.

F.30. Assessment of the *L-selectin* rs2205849 (-642 C>T) and rs2229569 (725 C>T) Polymorphisms in Acute Coronary Syndrome

Maria Sandoval Pinto, Jorge Ramón Padilla-Gutiérrez, Ilian Janet García González, Angélica Valdez Haro, Emmanuel Valdés Alvarado, Jose Francisco Muñoz Valle and Yeminia Valle. Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Mexico

Introduction: The acute coronary syndrome (ACS) is a complex disease where genetic and environmental factors are involved. *L-selectin* is a candidate gene for ACS progression due to its participation in the adhesion of monocytes and lymphocytes to endothelial cells. The rs2205849 polymorphism in the *L-selectin* gene consist of a C>T change at the -642 promoter region. The C allele has been associated with changes in gene expression thus associated with an increase in the risk of cardiovascular disease. The rs2229569 polymorphism consists of a C>T change at the 725 base and it has been associated with sL-selectin level variations.

Objective: To associate the *L-selectin* rs2205849 and rs2229569 polymorphisms with ACS.

Methods: Were recruited 210 patients with ACS classified according to American College of Cardiology and 210 healthy subjects (HS) age-matched from Western Mexico. All individuals accepted and signed an informed written consent. The polymorphisms were identified by PCR-RFLP. The genotype and allele differences were estimated by Fisher's exact test. The association was evaluated by OR and 95% of confidence intervals. The significance level was $p<0.05$.

Results: Genetic distributions were in accordance with Hardy-Weinberg expectations. The genotype and allele frequencies of the rs2205849 and rs2229569 polymorphisms showed statistically differences between groups [(OR=0.558, p=0.006; (OR=0.578, p=0.0001), (OR=0.371, p<0.0001; OR=0.473, p=0.008)]. These results suggest that the T allele in both polymorphisms confers protection for the development of ACS.

Conclusion: The *L-selectin* polymorphism variants rs2205849 and rs2229569 could confer protection against Acute Coronary Syndrome development in Western Mexico population.

F.31. Analysis of the Polymorphisms rs1800470 and rs1800471 of *TGFB1* and rs3087465 of *TGFBR2* in HPV Infected Women

Stephanie Badaro Garcia, Kleber Paiva Trugilo, Guilherme Cesar Martelossi Cebinelli, Nathália Tatakihara, Ana Paula Lombardi Pereira, Michelle Mota Sena, Érica Romão Pereira, Nádia Calvo Martins Okuyama, Fernanda Costa Brandão Berti, Luis Fernando Lasaro Mangieri, Maria Angelica Ehara Watanabe and Karen Brajão de Oliveira. Londrina State University, Londrina, Brazil

Cervical cancer is the third most common type of cancer among Brazilian women, caused mainly by HPV persistent infection. The TGFB, a cytokine regulator of cell growth and differentiation, appears to be involved in certain cancers. TGFB action depends on its binding to their receptors (TGFBR). Polymorphisms in TGFB ligand-receptor axis can change the tumor microenvironment favoring or hindering tumor evolution. Therefore, the presence of the TGB1 polymorphisms rs1800470 and rs1800471, and rs3087465 of TGFBR2 in HPV positive (n=119) and negative (n=94) women were evaluated by PCR-RFLP. The genotypes of the two polymorphisms TGFB1 were combined into nine haplotypes according to the *in vitro* production levels: High Producer (HP), Intermediate Producer (IP) and Low Producer (LP). When HPV negative women were compared to positive ones regarding TGFB haplotypes, it was possible to notice that the low and intermediate haplotypes conferred protection to HPV infection OR=0.59 IC95%(0.3 – 0.99), but there was no difference when the haplotypes were analyzed related with TGFBR2 genotype (p>0.05). However, when the high production haplotypes were compared to the low production added the intermediate production haplotypes, in accordance to the TGFBR2 genotype among the different diagnosis groups, it was observed that HSIL and cervical cancer patients presented predominantly GG genotype of TGFBR2 associated to a high producer haplotype (p=0.004). Thereby, the GG genotype as known to be not associated with cancer protection, when correlated with allele A, could be in synergism to the TGFB high producers' haplotypes promoting a stronger immunosuppression that could favor lesions progression.

Immune Monitoring

OR.10. Development of a Plasma Biomarker of Germinal Center Activity for Human Vaccine Trials

Colin Havenar-Daughton[1], Sudhir Kasturi[2,3], Elise Landais[4], Jennifer Wu[1], Samantha Reiss[1], Ata Ur-Rasheed[3], Jens Wrammert[3], Francois Villinger[2,3], Iavi Protocol C. Team[5], Rafi Ahmed[3], Pascal Poignard[4], Bali Pulendran[2,3], Shane Crotty[1] and Simon Belanger[1]. [1]La Jolla Institute for Allergy and Immunology, La Jolla, CA; [2]Yerkes National Primate Research Center, Atlanta, GA; [3]★Emory University School of Medicine, Atlanta, GA; [4]The Scripps Research Institute, La Jolla, CA; [5]International AIDS Vaccine Initiative, New York, NY

Germinal centers (GC) perform the remarkable task of optimizing B cell antibody responses by processes such as antibody affinity maturation and differentiation of memory B and plasma cells. Extensive affinity maturation, required for the generation of broadly neutralizing antibodies (bnAb), will be a critical parameter to monitor if HIV bnAb are to be induced via vaccination. However, lymphoid tissue is rarely available from immunized humans. This makes monitoring germinal center activity, via enumeration of GC B cells and GC $CD4^+$ T follicular helper (GC Tfh) cells, problematic. The CXCL13-CXCR5 chemokine axis plays a major role in organizing both B cell follicles and GCs. As GC Tfh can produce CXCL13, we explored its use as a blood biomarker to report tissue GC activity.

Plasma CXCL13 levels were elevated two fold (p=0.015) in top HIV bnAb producing versus non-bnAb producing individuals in the IAVI Protocol C cohort. In animal studies, elevated plasma CXCL13 levels were detected 7 days post immunization. CXCL13 levels correlated with germinal center activity as measured by the percentage of GC Tfh cells in draining lymphoid tissues in both mice (r=0.822, p=0.002) and macaques (r=0.866, p=0.001). Plasma CXCL13 levels after immunization correlated with binding antibody titers at early (r=0.645, p<0.0001) and memory (r=0.693, p<0.0001) time points, as well as bone marrow plasma cell numbers (r=0.724, p<0.0001). Plasma CXCL13 levels in immunized humans are now under study. We highlight the potential use of CXCL13 as a plasma biomarker of germinal center activity in human vaccine trials and other clinical settings.

OR.35. PD-1 Downstream Signaling Pathway in Activated CD8 T Cells Linked to Mitochondria Apoptosis Molecules as a Result of PD-1 and B7-H1 Interaction

Roxana Dronca, Xin Liu, Lisa Kottschade, Rob McWilliams, Mike Thompson, Svetomir Markovic and Haidong Dong. ★Mayo Clinic, Rochester, MN

★ = member of a FOCIS Center of Excellence

Programmed death 1 (PD-1) blockade therapy aims to restore antitumor immunity. Since PD-1 is expressed by activated CD8 T cells shortly after antigen stimulation, merely detection of PD-1 expression by T cells may not be able to predict the engagement of PD-1 with its ligands. We demonstrated that Bim (a pro-apoptosis molecule) up-regulation is dramatically reduced in primed CD8 T cells in mice lacking B7-H1, a ligand of PD-1, and in tumor-reactive CD8 T cells in mice lacking PD-1. *In vitro*, B7-H1 fusion protein induced Bim up-regulation in both murine and human pre-activated CD8 T cells, and that Bim up-regulation was abolished with anti-PD-1 or anti-B7-H1 blocking antibodies. In metastatic melanoma patients, the frequency of Bim^+ $PD\text{-}1^+$ CD8 T cells was significantly increased in the peripheral blood compared to healthy donors ($p<0.01$). Importantly, the baseline frequency of$Bim^+PD\text{-}1^+$ per CD8 T cells in the peripheral blood of melanoma patients seemed to be lower in responders to anti-PD-1 therapy than in non-responders. In responders, the levels of Bim were reduced by at least 2-fold in $PD\text{-}1^+$ CD8 T cells after the first 3 months of treatment. Our results indicate that Bim is a downstream signaling molecule of PD-1 and that Bim up-regulation reflects the engagement of PD-1 with its ligand (B7-H1). Measurements of Bim frequency and levels in tumor-reactive $PD\text{-}1^+$ CD8 T cells may help to select cancer patients most likely to benefit from anti-PD-1 therapy.

OR.45. Autoantibody Profiling Using Autoantigen Microarrays Revealed Autoantibody Patterns Associated with Breach in Immune Tolerence and Disease Activity

Quan-Zhen Li[1], Mei Yan[1], Prithvi Raj[1], Honglin Zhu[1,2], Xiaoxia Zu[2] and Edward Wakeland[1] [1]★University of Texas Southwestern Medical Center, Dallas, TX; [2]Xiangya Hospital, Central South University, Changsha, China

Autoantigen microarrays bearing 95 autoantigens were used for profiling 4 types of autoantibodies (IgG, IgM, IgA and IgE) in 240 well defined SLE patients and matched healthy controls (HCs). Using mean plus 2 standard deviation of normalized signal intensity (NSI) as positive cut-off value, our analysis revealed that the average number of positive IgG autoantibodies in SLE is 28.5 with over 95% of SLE patients harbor 5 or more autoantibodies, comparing with 3 positive IgG Autoantibodies in HCs with 25% have 5 or more autoantibodies ($p<0.05$). A group of 19 IgG autoantibodies targeting to various nuclear antigens (dsDNA, chromatin, histone, Sm/RNP, PCNA, CENP-B, etc.) were significantly enriched in SLE by clustering analysis. The presence of IgG autoantibodies against DNA-associated antigens were most significantly associated with disease activity (SLEDAI) and lupus nephritis ($r=0.56$, $p<0.001$) in SLE. The IgG autoantibodies revealed in HCs were mostly those targeting to non-nuclear antigens, such as collagens, alpha-actinin, heparin and cardiolipin. However, some ANA positive HCs exhibited higher IgM specificities targeting to both non-nuclear and nuclear antigens whereas in SLE the IgM autoAb reaction were relatively lower. IgA autoantibodies were detected in over 50% of SLE patients, preferentially targeting to DNA- and RNA-associated antigens indicating the high prevalence of IgA autoantibodies may play a role in SLE. IgE autoantibodies were not detectable for most autantigens except about 15% of SLE patients showed positive IgE autoantibodies to a small subset of DNA antigens. Further analysis is underway to reveal correlation between the autoantibody patterns with disease phenotypes.

OR.57. Single-cell, 42-plex Cytokine Profiling Reveals Deep Functional Heterogeneity in Normal Immune Defense and Autoimmune Pathogenesis

Fan Rong. ★Yale University, New Haven, CT

Despite recent advances in single-cell genomic, transcriptional and mass cytometric profiling, it remains a challenge to collect highly multiplexed measurements of secreted proteins from single cells for comprehensive analysis of immune functional states. Herein we combine spatial and spectral encoding with PDMS microchambers for co-detection of 42 immune effector proteins secreted from single cells, representing the highest multiplexing recorded to date for a single-cell secretion assay. Using this platform to profile differentiated macrophages stimulated with lipopolysaccharide (LPS), the ligand of Toll-like receptor (TLR)-4, reveals previously unobserved deep functional heterogeneity and varying levels of pathogenic activation. Advanced viSNE clustering analysis identified multiple

distinct dynamic functional subsets and this subpopulation architecture is conserved throughout the cell activation process and prevails as it is extended to other TLR ligands and to primary macrophages derived from a healthy donor. The results demonstrate that the phenotypically similar cell population still exhibits large degree of intrinsic heterogeneity at the functional and cell behavior level, which account in part for the varying degree of innate and adaptive immune defense. On the other hand, applying this technology to both memory and effect T helper cells from autoimmune disease (Lupus) patients indicates the correlation between polyfunctional subpopulations and the pathogenesis. The results underscore the complexity and multifunctionality of immune cell repertoire implicated in normal immune defense and autoimmune pathology. Our technology enables full-spectrum dissection of immune functional states at the single-cell level and opens new opportunities to quantify deep functional heterogeneity for more comprehensive and accurate immune monitoring.

F.32. The Effects of CTLA-4-CD28 Gene Variants on the Development of Colorectal Cancer

Bayram Kiran[1], Ozlem Kuçukhuseyin[2], Saime Turan[2], Soykan Arikan[3], Yigit Duzkoylu[3], Ezgi Nurdan Yenilmez, Arzu Ergen[2], Umit Zeybek[2], Gulbu Isitmangil[4], Huseyin Kiran[5] and Ilhan Yaylim[2]. [1]Kastamonu University, Kastamonu, Turkey; [2]The Institute of Experimental Medicine, Istanbul, Turkey; [3]Istanbul Educating and Training Hospital, Istanbul, Turkey; [4]Haydarpasa Numune Education and Research Hospital, Istanbul, Turkey; [5]Anadolu University, Eskisehir, Turkey

Background: Colorectal cancer (CRC) which has an incidence of 10% in men and 9.2% in women is the third most frequent cancer in worldwide. The aim of the present study was to determine the distribution of CTLA4 318 C>T (rs5742909), CTLA4 49 A>G (rs231775) and CD28 C>T (rs3116496) variants and the plasma levels of CTLA4 and CD28 in CRC patients to evaluate the risk and the progression of the disease. Material and Method: 80 CRC patients and 115 healthy volunteers as controls were included in the present study. The CTLA4 318 C>T (rs5742909), CTLA4 49 A>G (rs231775) and CD28 C>T (rs3116496) genotypes were determined by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, and sCTLA4 and sCD28 serum levels were determined by using commercially available enzyme linked immunosorbent assay (ELISA) kits according to manufacturer's instructions. Results: The serum levels of sCTLA4 in CRC patients vs healty control was 0.326±0.034ng/ml→0.372±0.039ng/ml, and it was 2.63±0.473ng/ml→2.10±0.220ng/ml for sCD28 levels. The genotype distributions in CRC patients are 92.5% CC, 7.5%CT, 0.0% TT for CTLA4 318 C>T (rs5742909); 47.5% AA, 45.0% AG, 7.5% GG for CTLA4 49 A>G (rs231775); and 6.2% CC, 33.8% CT, 40.0% TT for CD28 C>T (rs3116496), and in control subjects those were 77.8%, 20.4%, 1.8%; 46.9%, 45.1%, 8.0%; and 12.4%, 32.7%, 54.9%, respectively. Conclusion: The present study was a preliminary study to establish the link between sCTLA4 or sCD28 levels or CTLA4 -318 C>T, CTLA4 -49 A>G, CD28 C>T polymorphisms and pathogenesis of CRC among Turkish population.

F.33. Comprehensive Study of 3 Nomenclatures to Discriminate CD8 Subsets in Healthy Volunteers and in Kidney Transplant Recipients

Michelle Yap[1,2], Gaelle Tilly[1,2], Magali Giral[1,2,3], Sophie Brouard[1,2,3], and Nicolas Degauque[1,2]. [1]INSERM UMR 1064, Nantes, France; [2]★Université de Nantes, Nantes, France; [3]Centre Hospitalier Universitaire Hôtel-Dieu, Nantes, France

Historical classification to distinguish central memory (CM), naïve, effector memory (EM), and terminally-differentiated effector memory (TEMRA) CD8 T cells is based on CD45RA and CCR7. Alternative classifications rely on CD45RA in combination with CD27 or CD28. We aimed to provide a comprehensive understanding of the benefits of each nomenclature in normal and in immune-challenged individuals.

Multicolor flow cytometry was performed on PBMC from 16 healthy volunteers (HV) and 133 kidney transplant recipients (TX) with a stable graft function to characterize CD3, CD8, CD27, CD28, CD45RA, CCR7, CD57, TBET, Perforin, Granzyme B and IL7R expression.

Naïve and TEMRA CD8 had similar characteristics when identified using the three nomenclatures. On the other hand, while there is a higher frequency of CD45RA-CD27$^+$ CM (HV 34.96±11.63; TX 20.78±12.87%) as compared to CD45RA-CCR7$^+$ CM (HV 3.19±1.98; TX 7.76±6.76%), CCR7 and CD127 expression was lower in CM identified using CD27 as opposed to those identified using CCR7 in both HV and TX. CD28 can identify two types of EM cells: CD45RA-CD28$^+$ (EM Early) and CD45RA-CD28- (EM Late). When compared to CD45RA-CD27$^-$ and CD45RA$^-$ CCR7- EM, EM Late showed a higher CD57, Tbet, and GZMB expression. Conversely, EM Early displayed similar or lower levels of these markers when compared to CD45RA-CD27$^-$ and CD45RA-CCR7$^-$ EM cells in HV and TX.

We illustrate the strength of each set of markers in HV and in TX. The CD45RA$^+$CD28 combination allows for extra insight into 2 types of EM cells with different characteristics; alternatively CM nomenclature differed in specific markers.

F.34. Assessment of Innate and Adoptive Immunity for the Characterization of Multiorgan Dysfunction Syndrome (MOD) After Mechanical Circulatory Supp

Yael Korin, Joanna Schaenman, Nicholas Nicholas Wisniewski, Martin Cadeiras, Murray Kwon, Tiffany Sidwell, Gemalene Sunga, Galyna Bondar, Mario Deng and Elaine Reed. ★ University of California, Los Angeles, Los Angeles, CA

Clinical outcomes vary after MCSD with some patients developing MOD and death, mediated by a systemic and aberrant immune response. We hypothesized that using multiparameter flow cytometry to assess the activation and inhibitory status of innate and adaptive immune cells would shed light into the mechanism of MOD and be predictive of clinical status and risk of death.

Peripheral blood mononuclear cells were isolated from 24 consented patients pre- and post-MCSD. Immune phenotyping was performed by flow cytometry. Clinical risk was calculated using the MELDI (Model for End-stage Liver Disease excluding INR) score. Statistical analyses were performed using Mann-Whitney t-test and Kruskal Wallis or linear regression.

Patients with higher MELDI score exhibited significantly higher percentage of CD14$^+$ monocytes and CD56$^+$ NK cells ($p<0.0001$ and $p=0.006$). NK cells expressed significantly higher percent of the cytotoxic marker CD16 ($p=0.0008$). Expression of TLR4 on monocytes one day post MCSD was inversely correlated with higher MELDI ($p=0.014$), suggesting the possibility of exposure to bacterial antigens. Assessment of T cells showed that increased expression of the exhaustion marker KLRG-1 as well as KLRG-1$^+$/PD-1$^+$ was strongly associated with MELDI score ($p<0.001$) and death ($p=0.022$). Similarly, increased frequency of T cells expressing the exhaustion markers CD57 and PD-1 was associated with higher MELDI score ($p=0.004$) and death ($p=0.008$).

These data suggest that immune dysfunction is part of the mechanism leading to MOD. Thus monitoring the peripheral blood can be utilized to improve patient outcomes by improving MCSD candidate selection and post-implant surveillance.

F.35. Systematic Whole Blood Stimulation and Cryopreservation for Improved Data Consistency in Immune Monitoring

Henrik Mei, Cariad Chester and Holden Maecker. ★Stanford University, Stanford, CA

State of the art flow and mass cytometry combined with bioinformatics permit high throughput, highly multiparametric assays for systematic analyses of blood immune cells. As measurements and analyses become more sophisticated, results are increasingly vulnerable to unwanted technical sample-to-sample variation that may arise from differences in retrieval, resting, shipping, handling, stimulation and (cryo-)preservation of blood samples.

To this end, we have validated the Smart Tube platform (Smart Tube Inc., Palo Alto, CA) for use with whole blood samples for cytometric analyses. The platform allows for machine-controlled stimulation, incubation, and fixation of whole blood, followed by cryopreservation at -80ºC and later batch-wise sample thawing, erythrocyte lysis, and cell analysis.

We present a workflow for a representative assay and confirm compatibility with mass-tag cell barcoding, provide a list of antibody clones compatible with Smart Tube reagents, and address the recovery of cells after repeated sample freeze-thaw cycles. We tested for correlation of immunophenotyping data derived from Smart Tube-derived cells to that of leukocytes or PBMC from conventional preparation protocols.

Finally, we provide an analysis of cell signaling responses to *in vitro* whole blood stimulation with cytokines and other cell activators across major leukocyte subsets.

The Smart Tube platform enables identical sample preparation, stimulation, and fixation conditions in a multi-center sample collection setting with minimal on-site work as well as joint sample processing and analyses in central laboratories. Automated sample handling platforms may contribute to reducing technical variability in blood samples collected for immunomonitoring in clinical and biomarker discovery studies.

F.36. Defining Differences of EBV-specific Immune Response Between Transplant Controls and PTLD Patients Using High-dimensional Mass Cytometry

Dongxia Lin[1,2], Steven Schaffert[2], Heiner Zimmermann[3], Raif Trappe[3], Olivia Martinez[2] and Holden Maecker[2]. [1]Fluidigm Corporation, South San Francisco, CA; [2]★Stanford University, Stanford, CA; [3]Universitatsklinikum Schleswig-Holstein, Kiel, Germany

Post-transplant lymphoproliferative disease (PTLD) is a life-threatening complication of solid organ transplantation often associated with Epstein-Barr virus infection and an impaired-host T cell response. However, there is no proven consensus on what constitutes a protective T cell response to EBV infection in transplant recipients. In this study, we simultaneously analyzed virus-specific T cell phenotypes, functions, and antigen specificity in 37 parameters using high-dimensional mass cytometry (CyTOF® system). PBMCs were obtained prior to treatment from 10 patients with PTLD (average age = 60, time to PTLD = 13.7 years post-transplant). We compared T cell responses to peptide pools derived from lytic and latent EBV antigens in both adult transplant controls and PTLD patients. The results suggest that adult transplant controls can mount $CD8^+$ T cell responses to both lytic and latent EBV antigens, including some multiple-cytokine-producing cells. In contrast, PTLD patients have much weaker responses to both lytic and latent antigens. Sparse k-means clustering analysis of cytokine-producing $CD8^+$ T cells in these two groups identified three major clusters with different features. One of these clusters, which had CD56 expression and cytotoxic markers, was significantly reduced in PTLD patients in both lytic and latent antigen responses ($P < 0.02$). Automated gating and principal components analysis of total PBMC also revealed global decreases in $CD56^+$ NKT^- like cells in PTLD patients compared to transplant controls.

F.37. Long-term Follow-up of Minoritary B and T Cell Subsets During Fingolimod Treatment In Relapsing-remitting Multiple Sclerosis Patients

Aina Teniente-Serra, Jose Vicente Hervás, Bibiana Quirant-Sanchez, Maria Jose Mansilla, Laia Grau-Lopez, Cristina

Ramo-Tello and Eva Maria Martinez-Caceres. Hospital Universitari Germans Trias i Pujol, ★Universitat Autònoma de Barcelona, Badalona, Spain

Fingolimod is an oral treatment for multiple sclerosis (MS) that interferes with the sphingosine-1-phosphate receptor-1 leading to the entrapment of lymphocytes in lymph nodes.

Aim: To determine the long-term effect of fingolimod on minor lymphocyte subpopulations.

Material and methods: Longitudinal study. Phenotypic analysis of whole blood of 13 relapsing-remitting MS patients before and after (+1,+3,+6,+9,+12) months of treatment, using 8-color multiparametric flow-cytometry (FACSCantoII, BD Biosciences). Fingolimod treatment reduced lymphocyte counts from month +1 until end of follow-up (basal:1728±520 *vs* +1m:511±224 cells/µl, p<0.001) with an important reduction in the percentage of naïve T cells ($CD4^+$: basal:33.34±12.05 *vs* +1m:11.78±8.42%; $CD8^+$: basal:38.21±16.63 *vs* +1m:4.72±4.82%, p<0.001). No significant changes in the percentage of central memory, early and late effector memory and effector (TEMRA) T cells were found. Interestingly, a parallel increase of Th17 (basal:5.76±1.94 *vs* +1m:12.30±4.79,p=0.012) and Tregs (basal:3.78±0.94 *vs*+1m:8.01±4.06,p=0.040) was observed already in month +1 of treatment. These changes remained stable until month +12.

Although the percentage of total B cells decreased (basal:10.86±8.43 *vs* +1m:4.62±1.81%,p<0.001), no changes were found in percentages of naïve, unswitched and switched memory B cells. An increase in the percentage of $CD24^{hi}CD38^{hi}$ transitional B cells, from month +3 until end of follow-up (basal:7.02±3.99 *vs*+3m:22.68±11.35%,p=0.006) was observed.

In conclusion, fingolimod treatment induces already in the first months an important decrease of lymphocyte counts as well as changes in percentages of minor lymphocyte subpopulations that remain until month +12. The control of Th17 by Treg might partly explain the beneficial effect of fingolimod in MS, deserving further investigation.

F.38. Optimization of $CD4^+$ CliniMACS Isolation for Human *in vivo* Imaging

***Philippa J P Lait**[1,2], Daiju Iwata[1], Emily L Williams[1,2], Andrew D Dick[1,2,3], David Shima[1] and Richard W J Lee[1,2,3]. [1]Moorfields Eye Hospital and University College London Institute of Ophthalmology, London, United Kingdom; [2]University of Bristol, Bristol, United Kingdom; [3]University Hospitals Bristol NHS Foundation Trust, Bristol, United Kingdom*

Animal models have informed our current understanding of leukocyte dynamics in the eye. These studies have implicated leukostasis, the process of leukocyte adhesion and rolling, as an important early step leading to altered vascular permeability with the potential to subsequently contribute to vision loss. In humans, little is known about how these cellular processes contribute to disease progression or the therapeutic potential of understanding and thus modulating them. Intravenous fluorescein infusion is routinely used to visualise vascular permeability *in vivo* in patients with ophthalmic inflammation. Here, we propose a system for using fluorescein to stain isolated lymphoid cells *ex vivo* and then track them *in vivo* after autologous cell transfer. We describe optimisation of the protocol for isolation of $CD4^+$ T cells (Miltenyi cliniMACS system, purity = 92.03% of live cells) from human peripheral blood apheresis products to reduce the level of contaminating cells, in particular $CD4^{lo}$ cells. We have also optimised the *ex vivo* fluorescein labelling conditions and assessed cell viability and persistence of fluorescein labelling in culture conditions that broadly mimic the environment *in vivo*. We see that cells remain viable and fluorescein staining persists for at least 24 hours in these conditions, but then dramatically reduces by 96 hours post labelling. In addition, cell surface expression of CD25, CD62L and CD69, and intracellular expression of IL-17, IFNγ, IL-4 and IL-10, are not altered under optimum fluorescein labelling conditions, indicating that this will not induce $CD4^+$ cell activation prior to autologous transfer.

F.39. Differential Gene Expression Patterns Following Whole Blood Stimulation to a Range of Microbial Product

Alejandra Urrutia[1,2], Céline Possémé[1], Raouf Djebali[1], Vincent Rouilly[1,3], Benoit Albaud[3], David Gentien[3], Darragh Duffy[1,2], Lluis Quintana-Murci[1,4] and Matthew Albert[1,2]. [1]★Institut Pasteur, Paris, France; [2]INSERM U818, Paris, France; [3]★Institut Curie, Centre de Recherche, Paris, France; [4]CNRS URA3012, Paris, France

Identification of genetic and environmental determinants of immunological variance requires the use of standardized immunophenotyping technologies. With this goal in mind we developed and applied a suite of whole blood, syringe-based assay systems that minimize pre-analytical errors, to reproducibly assess induced innate or adaptive immune responses. In a specific collection of healthy individuals with a homogeneous ethnic background, we have defined the transcriptomic and proteomic signatures induced by complex microbes, MAMPs, cytokines and T cell activators, providing healthy donor reference values for induced immunological mediators. Using an optimized single-step extraction method we quantified 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 gene expression patterns unique for each immune stimulation systems, including up to a 5000 fold increase for certain genes to viral stimulation. We are currently extending this analysis across 1000 healthy donors for selected stimuli, permitting the identification of genetic and environmental determinants of naturally occurring immune response variation. A better understanding of this variation may help to explain differential susceptibility to disease or response to therapeutic intervention.

F.40. Using Phase 0 Biomarker Discovery to Inform Phase 2 Biomarker Strategy: Novel Cell Subsets in Idiopathic Pulmonary Fibrosis (IPF)

Janet Staats[1], Anne Minnich[2], Cliburn Chan[1], Michael Hedrick[2], Kelly Plonk[1], Laurie Snyder[1], Jennifer Enzor[1] and Kent Weinhold[1]. [1]★Duke University, Durham, NC; [2]Bristol-Myers Squibb, Princeton, NJ

The use of polychromatic flow cytometry (PFC) to measure cellular phenotype and function of immune cells is rapidly increasing as a tool for identifying treatment-induced effects. The common method used to develop a PFC panel requires identifying markers of interest *a priori* and using these selected markers to build a single panel. In the absence of preliminary data, the *a priori* list used for panel development is normally based on existing literature and, therefore, is biased towards known populations. Also, where little is known about the populations associated with a specific disease, creating an *a priori* list of markers to include in a panel can be challenging. An alternative approach is to use multiple broad-based PFC panels to minimize bias in Phase 2 studies; however, this method increases labor, costs, and decreases statistical power. We propose a hybrid strategy that complements a knowledge-based search with automated discovery for novel cell subsets. Five PFC discovery panels were used in a Phase 0 comparison between IPFs and normals, three for peripheral blood mononuclear cells (PBMC's) and two for bronchoalveolar lavage fluid (BALF) cells. Panels included literature-defined subsets plus broad classes of immune cell subsets. PFC data were analyzed using automated clustering to identify cell subsets that were differentially expressed in the IPF cohort. This approach revealed several novel cell subsets not previously described in the literature. The most informative markers were combined into a single PFC panel to be used in the Phase 2 study of BMS-986020, an LPA1 antagonist, in IPF.

F.41. CytoFLEX: 13-color Parametric Analysis for TBNK-subsets on the CytoFLEX™ Instrument

Jonel Lawson, Karen Fischer and James Tung. Beckman Coulter Inc., Miami, FL

CytoFLEX is the newest addition to the platform of flow cytometers offered by Beckman Coulter, Life Sciences Division. This compact system provides up to 3 lasers and 13 colors for fluorescence detection. The instrument incorporates the latest technologies, including fiber array photodiode, FAPD, which provides high resolution, dynamic

range and sensitivity in signal detection. To demonstrate the ability of the CytoFLEX's multicolor detection, the standard multicolor panel design principles were followed to develop a 14 marker, 13-color experiment. The cocktail design process also took into account the desired immunophenotypic markers, the available fluorochromes, and the use of well-defined control populations to identify the classic TBNK sub-populations. Data was collected from normal, whole blood stained with single color reagents and lysed with VersaLyse Lysing Solution. The data was then analyzed using the CytExpert software and is presented here with special emphasis on the optimization of flow analysis and compensation visualization. Results show that all TBNK populations were well resolved, notably the $CD25^+$ subset in $CD4^+$ T cells, which ordinarily requires a fluorescence minus one control to accurately distinguish the $CD25^+$ population. The data presented here demonstrates the ability of the CytoFLEX to provide sound detection and resolution for even the most dim versus negative populations.

F.42. Development and Validation of a Flow Cytometric Whole Blood Assay for Broad Leukocyte Immunophenotyping: An Application of the Human Immunophenotyping Consortium (HIPC) Proposal

William McAuliffe, Soma Ray, Lakshmi Amaravadi and Devangi Mehta. Biogen Idec, Cambridge, MA

Introduction: The understanding of the immunomodulatory potential of relevant therapies can be a vital component to their strategic clinical development and commercialization. Flow cytometric analysis can provide a reconnaissance of the immune cell repertoire, and therefore provide insights into a clinical subject's immune status. There is a need, however, for the creation of methods that are robust enough for global longitudinal multi-site clinical studies. Described here is the development and analytical validation of immunophenotyping panels modeled after the HIPC standardization proposal[1].

Methods: HIPC proposals were used to design three core panels to identify major T cell, B cell, and myeloid subsets. A companion panel was created to assess Th1/2/17 subsets by intracellular cytokine staining. Panels were optimized for whole blood and tested against five normal donors where the applicability of the chosen markers and gating strategies were assessed. Panels were validated to assess sample stability and precision.

Results: Panels identified the major subsets in whole blood, with ambiguity only occurring for some low frequency subsets (<5% of total leukocytes). HIPC proposed markers were generally confirmed in our assessments, with the exception of T-regulatory and T-helper cell subsets where alternative gating/phenotypes are recommended. Sample stability of a few days and precision (≤30%CV) was generally demonstrated for all panels.

Conclusions: Development and validation of whole blood immunophenotyping methods, modeled after HIPC recommendations, has been accomplished. The methods have been implemented in ongoing global clinical trials with the goal of aiding in the standardization and accessibility of immunophenotyping data from these types of studies.

1. Maecker, HT; McCoy, JP; Nussenblatt, R. (2012). NatRevImmunl.12(3):191-200.

F.43. Persistent Expression of Specific Inflammasome Genes Stratifies Older Adults

David Furman[1], Christopher Bolen[1], Francois Haddad[1], Vladimir Jojic[2], Jean-Francois Moreau[3], Julie Dechanet-Merville[4], Corry Decker[1], Mark Davis[1] and Benjamin Faustin[4.1]★Stanford University, Stanford, CA; [2]★University of North Carolina, Chapel Hill, NC; [3]University of Bordeaux, CIRID, UMR/CNRS 5164, Bordeaux, France; [4]University of Bordeaux II, Bordeaux, France;

Chronic inflammation is a common feature of many age-associated diseases but the underlying mechanisms remain unknown. The inflammasomes are molecular complexes that trigger the maturation of interleukin-1 family cytokines (IL1FC), which increase during aging and are involved in the pathogenesis of cardiovascular disease. Here we show

that the expression of specific inflammasome gene modules stratifies older individuals into two extreme immunological states, those with persistent elevated expression or inflammasome high (IH), which show constitutive production of IL1FC, high rates of hypertension, increased arterial stiffness and poor familial longevity versus those with the opposite phenotype. IH individuals also exhibit increased circulating metabolites from nucleotide metabolism, which up-regulate *NLRC4* expression and production of IL1FC in primary monocytes. Intake of caffeine, an adenosine inhibitor, negatively correlated with inflammasome gene module expression and coffee-derived compounds dampened the production of IL1FC. Thus, targeting particular inflammasome components may ameliorate inflammation and limit disease progression.

F.44. Biomarkers on Patient T Cells Diagnose Active Tuberculosis and Monitor Treatment Response

***Toidi Adekambi**, Chris Ibegbu, Jyothi Rengarajan, Susan Ray, Stephanie Cagle, Ameeta Kalokhe, Yun F Wang, Yijuan Hu and Cheryl Day. ★Emory University, Atlanta, GA*

Background: The identification and treatment of individuals with tuberculosis (TB) is a global public health priority. Accurate diagnosis of pulmonary active TB (ATB) disease remains challenging and relies on extensive medical evaluations and detection of Mycobacterium tuberculosis (Mtb) in the patients' sputum. Further, the response to treatment is monitored by sputum culture conversion, which takes several weeks for results. Here, we sought to identify blood-based host biomarkers associated with ATB and hypothesized that immune activation markers on Mtb-specific $CD4^+$ T cells would be associated with Mtb load *in vivo* and could thus provide a gauge of Mtb infection.

Methods: Using polychromatic flow cytometry, we evaluated the expression of immune activation markers on Mtb-specific $CD4^+$ T cells from individuals with asymptomatic latent Mtb infection (LTBI), ATB, and from ATB patients undergoing anti-TB treatment.

Results: Frequencies of Mtb-specific IFN-g^+CD4^+ T cells that expressed immune activation markers CD38 and HLA-DR as well as intracellular proliferation marker Ki-67 were substantially higher in subjects with ATB compared to those with LTBI. These markers accurately classified ATB and LTBI status with cutoff values of 18%, 60%, and 5% for $CD38^+$IFN-g^+, HLA-DR^+IFN-g^+ and Ki-67^+IFN-g^+, respectively, with 100% specificity and greater than 96% sensitivity. These markers also distinguished individuals with untreated ATB from those who had successfully completed anti-TB treatment and correlated with decreasing mycobacterial loads during treatment.

Conclusion: We have identified host blood-based biomarkers on Mtb-specific $CD4^+$ T cells that discriminate between ATB and LTBI and provide a set of tools for monitoring treatment response and cure.

F.45. Multidimensional Data Reveal Increased LAIR1 Expression in Heterogeneous Populations of Memory T Cells of T1D

*Ian Frank, Duangchan Suwannasaen, Jerill Thorpe, Scott Presnell, Carla Greenbaum, Jane H. Buckner and S. **Alice Long**. ★Benaroya Research Institute, Seattle, WA*

Immune signatures can be used as biomarkers and often imply underlying mechanisms of disease. Some immune phenotypes are present in controls carrying genetic risk but are most accentuated in patients carrying risk, suggesting genetics and disease combine to determine immune status. Here, we use high-dimensional, exploratory techniques to discover immune phenotypes associated with both genetics and disease state. We found increased transcription of the inhibitory molecule LAIR1 in memory CD4 T cells of T1D subjects carrying the *PTPN2*rs1893217 risk allele by microarray, which was confirmed by qPCR. Protein expression of LAIR1 on different cell subsets was tested using mass and flow cytometry. For mass cytometry, we optimized our panel using 3 "metal minus many" panels obtaining similar results by flow cytometry (R^2=0.879). SPADE analysis of control and T1D subjects stratified by *PTPN2*rs1893217 genotype showed LAIR1 expression to be constitutively high on monocytes and variably

expressed on lymphocytes. Within the CD4 T cells, LAIR1 was expressed most highly on naïve and long-term memory cells with the LAIR1$^+$ memory cells clustering as multiple smaller nodes in the periphery suggesting greater heterogeneity. Using both mass and flow cytometry, we found a slight but significant increase in LAIR1 expression associated with the *PTPN2*rs1839217 risk allele and expansion of LAIR1$^+$ memory CD4 T cells in T1D. Future studies will explore the hypothesis that increased expression of the ITIM LAIR1 with combined genetic risk and disease is due to an altered phosphotome and compensatory mechanisms induced to control disease associated inflammation.

F.46. Implementation of State-of-the-art Diagnostic Tests for Primary Immunodeficiency

Maura Rossetti, Ping Rao and Elaine F. Reed. ★University of California, Los Angeles, Los Angeles, CA

Primary immunodeficiency encompasses a wide range of inherited or acquired diseases of the immune system, whose primary manifestations are recurrent infections. Timely diagnosis is critical to ensure that patients get immediate, life-saving treatment. The Immune Assessment Core (IAC), a component of the UCLA Immunogenetics Center, is a CLIA-certified facility providing expertise and cutting-edge technologies for immune assessment in the field of transplantation, autoimmunity, hematopoiesis and beyond. The IAC is implementing clinical-grade assays to probe the functions of various immune cell subsets involved in pediatric immunodeficiency, including chronic granulomatous disease (CGD), common variable immunodeficiency (CVID) and T cell/combined immunodeficiency. Some of these assays are also fundamental for proper immune monitoring upon hematopoietic bone marrow transplantation, the optimal therapy currently available for most of these genetic diseases. We report here the most current developments in basic immunophenotyping (memory T cell panel), in-depth assessment of B cellsubsets (CVID), evaluation of oxidative burst in granulocytes and monocytes (CGD), and assessment of T- and B cellfunctionality by CFSE-based mitogen/antigen lymphocyte proliferation assay (Mit LPA and Ag LPA), with focus on assay standardization and validation. The IAC believes that these standardization efforts fit well with the goals of Human Immunophenotyping Consortium (HIP-C) initiative. As such, the IAC aims at working closely with the HIP-C disseminate these standardized assays for the benefit of both clinical and research communities.

Immunity and Infection

OR.07. Induction of Blood ICOS$^+$PD1$^+$CXCR3$^+$ Memory Tfh Cells is Associated with Antibody Affinity Maturation in Influenza Vaccination

Salah-Eddine Bentebibel[1], Surender Khurana[2], Nathalie Schmitt[1], A. Karolina Palucka[1], Hana Golding[2] and Hideki Ueno[1]. [1]Baylor Institute for Immunolgy Research, Dallas, TX; [2]Food and Drug Administration, Bethesda, MD

Administration of seasonal trivalent influenza (TIV) vaccines provides protection from influenza in approximately 60-90% of healthy young adults. The protection is mainly conferred by the generation of high-affinity antibodies against hemagglutinin that prevent virus entry. Yet, the immunological mechanism that leads to the induction of protective antibody responses remains largely unknown. We have recently shown that influenza TIV vaccination induces the emergence of activated ICOS$^+$PD-1$^+$CXCR3$^+$ memory Tfh cells at day 7 post-vaccination in blood, which correlates with an increase of antibody titers at day 28. Mechanistically, we found that ICOS$^+$PD-1$^+$CXCR3$^+$ blood memory Tfh cells efficiently help memory B cells, but not naïve B cells, to differentiate into plasma cells that produce influenza specific antibodies. This raises a possibility that ICOS$^+$PD-1$^+$CXCR3$^+$ blood memory Tfh cells confer protection only by boosting pre-existing antibodies but not by inducing affinity maturation. In this study, we determined whether the emergence of ICOS$^+$PD-1$^+$CXCR3$^+$ blood memory Tfh cells in blood at day 7 post-vaccination is associated with affinity maturation. We obtained sera samples at day 0, 7, and 28 post-vaccination, and measured polyclonal antibody affinity against pandemic H1N1 with surface plasmon resonance. We found that antibody affinity against pandemic H1N1 rapidly increased and reached a plateau at day 7 post-vaccination. Importantly, we found that the

increase of ICOS^{+}PD-1^{+}CXCR3^{+} memory Tfh cells positively correlated with the affinity maturation of antibodies against pandemic H1N1 only in subjects with pre-existing cross-reactive antibodies. We will discuss how ICOS^{+}PD-1^{+}CXCR3^{+} memory Tfh cells induce affinity maturation in influenza vaccination.

OR.25. Oral Challenge with Wild-type *Salmonella* Typhi Induces Activation of Circulating Monocytes and Dendritic Cells in Individuals who Develop Typhoid Disease

***Franklin Toapanta**[1], Paula Bernal[1], Stephanie Fresnay[1], Thomas Darton[2], Claire Jones[2], Claire Waddington[2], Christoph J Blohmke[2], Gordon Dougan[3], Brian Angus[2], Myron Levine[1], Andrew J Pollard[2] and Marcelo Sztein[1]. [1]University of Maryland School of Medicine, Baltimore, MD; [2]★University of Oxford, Oxford, United Kingdom; [3]Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

A new human challenge model with wild-type *Salmonella* Typhi (*S*. Typhi) was recently developed. In this model, 10^4 CFU resulted in 65% of subjects developing typhoid fever (TD) 5-10 days post-challenge. TD included meeting clinical (≥38°C for ≥12h) and/or microbiological (*S*. Typhi bacteremia) endpoints. One of the first lines of defense against pathogens are the cells of the innate immune system (e.g., monocytes, dendritic cells -DCs-). Various changes in circulating monocytes and DCs have been described in the murine *S*. Typhimurium model; however, whether similar changes are present in humans remains unknown. To address these questions, a subset of volunteers (5 TD and 3 who did not develop TD -NoTD-) were evaluated for changes in circulating monocytes and DCs. Expression of CD38 and CD40 were upregulated in monocytes and DCs in TD volunteers during the disease days. Moreover, integrin α4β7, a gut homing molecule, was upregulated in monocytes but not in DCs. CD21 upregulation was only identified in DCs. These changes were not observed among NoTD volunteers despite oral challenge. Moreover, monocytes and DCs from NoTD volunteers showed increased binding to *S.* Typhi one day after challenge. These monocytes showed phosphorylation of p38MAPK, NFkB and Erk1/2. In contrast, monocytes from TD volunteers showed only a moderate increase in *S.* Typhi binding 48h and 96h post-TD, and only Erk1/2 phosphorylation. This is the first study to describe different activation and migration profiles, as well as differential signaling patterns, which relate directly to the clinical outcome following oral challenge with *S.* Typhi.

OR.28. IL-7 Enhances NK Cell Activation and Effector Function and NK Cell IL7 Receptor Alpha Chain (CD127) Expression is Negatively Associated with HCV Level During Chronic HCV Infection

***Chelsey Judge**[1], Lenche Kostadinova[1], Yngve Falck-Ytter[1,2] and Donald Anthony[1,2]. [1]Case Western Reserve University, Cleveland, OH; [2]Veterans Administration Medical Center, Cleveland, OH*

Hepatitis C (HCV) affects approximately 170 million worldwide, with over 60 percent of cases resulting in chronic infection. New improved therapies will likely be capable of removing the virus. However, eliminating HCV altogether will require a preventative vaccine. To create vaccines against HCV, the role of the innate immune system in clearance and containment of HCV during natural infection needs to be further elucidated. Natural killer (NK) cells have been shown to directly mediate control of HCV *in vitro*, via cytokine secretion and cytotoxicity. The IL-7 receptor, α chain (CD127) is expressed on NK cells, with greatest expression on the CD56^{bright}16^{-} NK subset. We characterized NK cells within PBMC as CD7^{+}3^{-}14^{-}19^{-} and CD56^{bright}16^{-}, CD56^{dim}16^{+} or CD56^{-}16^{+} by flow cytometry, and measured CD127 expression on these NK subsets of 25 uninfected controls and 34 HCV-infected treatment naïve subjects. We observed CD56^{bright}16^{-} NK CD127 expression negatively correlates with HCV level (R= -0.40, p=0.02). Furthermore, our data shows that IL-7 (10ηg/mL) can enhance lysis of JFH-1 clone HCV infected Huh7.5 target cells. Additionally, we demonstrate IL-7 mediates activation of NK cells, by upregulation of CD69 expression on the CD56^{bright}16^{-} NK subset of control subjects (n=11,p=0.049). Furthermore, we provide evidence IL-7 induces NK IFNγ production in samples from control subjects (n =11, p=0.001) and HCV-infected subjects (n=11, p=0.001), implying that IL-7/CD127 signaling facilitates HCV-directed NK effector functions. Identifying CD127 as a potential target for improved NK effector function within chronic viral infection may have implications for more effective vaccine or therapeutic strategy design.

★ = member of a FOCIS Center of Excellence

OR.46. Complex Associations of T Cell Antigen Specificity and T Cell Exhaustion in Chronic HBV Infection

Yang Cheng[1], Mei-Ling Leong[1], Jinmiao Chen[1], Paola De Sessions[2], Martin Lloyd Hibberd[2], Michael Poidinger[1], Antonio Bertoletti[3], Seng Gee Lim[4] and Evan Newell[1]. [1]Singapore Immunology Network, Singapore; [2]Genome Institute of Singapore, Singapore; [3]Duke-NUS, Singapore; [4]National University Health System, Singapore,

Chronic hepatitis B virus (CHB) infection is associated with T cell dysfunction and persistent yet highly variable viral burden. However, inconsistent definition of T cell exhaustion raised questions of generality of this cellular phenotype in a natural persistent infection. How such antigen-driven disease persistently reshapes the state of antigen-specific $CD8^+$ T cells by overexpressed exhaustion markers remains unclear. Here, coupling mass cytometry and combinatorial tetramer strategy, we probed 563 different HLA-A*1101-restricted T cell specificities spanning the entire HBV viral genome on peripheral $CD8^+$ T cells from 42 CHB patients across four clinical stages. In conjunction, antigen-specific T cells were simultaneously profiled using 26 differentiation and coinhibitory markers (PD-1, TIM-3, LAG-3, CTLA-4, 2B4, CD160, BTLA, and HVEM). Dimensional reduction analysis (t-SNE) highlights the remarkable heterogeneity of $CD8^+$ T cells and suggested the diverse usages of coinhibitory markers in different memory-effector subsets influenced by the development of CHB. T cells specific for numerous novel HBV epitopes also displayed variable profiles of coinhibitory markers. For instance, pol_{106}-specific T cells had significantly higher 2B4 and lower BTLA expression compared to other HBV and control viral epitopes. In addition, the heterogeneous composition of pol_{106}-specific T cells linked to the progression of disease. Taking further, HBV viral load showed inverse correlation with the frequency of $core_{178}$-specific T cells expressing high levels of PD-1 and 2B4, and their distinct memory profiles were associated with improved clinical outcome. Thus, frequencies and differentiation profiles of HBV-specific $CD8^+$ T cells may be useful for stratifying CHB patients, perhaps with predictive value.

OR.47. *Ex Vivo* Characterization of CD4 Responses Against the Seasonal Influenza Vaccine Using a Novel Multiplexed HLA Class II Tetramer Staining

Hannes Uchtenhagen[1,2], Cliff Rims[1], Gabriele Blahnik[1], Jane H. Buckner[1] and Eddie James[1]. [1]★Benaroya Research Institute, Seattle, WA; [2]★Karolinska University Hospital, Stockholm, Sweden;

The study of human T cell responses is an important frontier in areas such as allergy, autoimmunity, infectious disease, and vaccine research. However, our ability to characterize antigen-specific $CD4^+$ T cell responses directly *ex vivo* is limited, typically requiring large sample volumes. We have developed a multiplexed *ex vivo* staining protocol for parallel characterization of $CD4^+$ T cell responses against multiple epitopes in conjunction with extensive surface marker phenotyping from a single vial of frozen PBMC. This approach was applied to study $CD4^+$ T cell responses in healthy volunteers receiving the 2014 seasonal flu vaccine, which includes the recent influenza A/Texas/50/2012 strain. Despite prevalent use of this vaccine, our understanding of the elicited responses remains limited, particularly with respect to the relative boosting of naïve and memory T cells by strains included in the current vaccine. Utilizing a multiplex tetramer panel that includes epitopes from both current and previous vaccine strains, we identified epitope-specific differences in T cell frequency and degree of boosting by the seasonal vaccine that were dependent on their degree of conservation between strains. Distinct patterns of surface phenotype marker expression, including CXCR3, CXCR5 and CCR7 were identified, suggesting heterogeneity between subjects and epitope-specific effects. Our results demonstrate stronger boosting of responses specific to current vaccine strains, albeit at lower total frequencies than for established immunodominant responses. The developed protocol facilitated direct enumeration and characterization of multiple $CD4^+$ T cell responses *ex vivo* from limited samples and can be readily applied to study T cell responses in other settings.

OR.50. Effect of Mass Drug Administration with Praziquantel on Anti-schistosome Immune Responses in School Children from Western Kenya

Eric Ndombi[1,2], Diana Riner[3], Bernard Abudho[2], Nupur Kittur[3], Diana Karanja[2] and Dan Colley[3]. [1]Kenyatta University, Kisumu, Kenya; [2]Kenya Medical Research Institute, Kisumu, Kenya; [3]University of Georgia, Athens, GA

Prevalence and intensity of schistosomiasis infection in children living in endemic areas is greater than in adults. Most countries where schistosomiasis is prevalent have intensified efforts to control the disease, utilizing annual mass drug administration (MDA) using praziquantel. The main target of MDA programs are school-age children as recommended by the World Health Organization (WHO). The effect of annual MDA on schistosome-specific immune responses has not been investigated. Our study seeks to determine the age children develop immune correlates of protection against re-infection and whether this age is altered by MDA. About thirty children per class were recruited in a cross-sectional study in neighboring primary and secondary schools in Rarieda, Siaya County, Western Kenya. Venous blood was collected, and whole blood cultured with schistosome-antigens SEA and SWAP. Culture supernatants were assayed for cytokines by ELISA. Anti-schistosome IgE, IgG4 and total IgG were also assayed by ELISA. Data was analyzed using Graph pad Prism 4. Baseline infection prevalence increased with age as did the percentage of responders producing the regulatory cytokine IL-10 to parasite antigens. There was no significant change in anti-schistosome IgE, IgG4 and total IgG one year post-treatment. However, after a similar period since first MDA, there was an increase in the number of responders producing Th1 and Th2 cytokines to parasite antigens leading us to conclude that one year of MDA boosts cytokine responses to parasite antigens.

OR.54. Human T Cell Subset Compartmentalization and Repertoire Maintenance Over Life

Joseph Thome, Boris Grinshpun, Yufeng Shen[2] and Donna Farber. ★Columbia University, New York, NY

Knowledge of human T cell responses and the maintenance of the T cell repertoire is largely derived from studies in peripheral blood, which only represents 2-3% of the total T cells in the body. Through novel analyses of TCR sequences we have determined how naïve and effector memory (TEM) T cells are distributed and maintained in tissue sites over decades. Using TCR deep-sequencing of tissues acquired from organ donors, we are able to assess the clonality and overlap of TCR clones in lymphoid sites. When examining TEM populations in the inguinal lymph node, lung-draining lymph node, and spleen of five donors, there is increased clonality within CD8 TEM compared to CD4 TEM whereas all naïve cells show similar diversity. Additionally when assessing the extent of clonal overlap between tissues, it is shown that CD4 TEM are largely site-specific, even in lymphoid organs whereas CD8 TEM share the same clones between diverse tissues. Interestingly, naïve T cells exhibit an increase in clonality with age. In donors where thymic output is negligible (>40 years), there is a large decrease in the diversity of the naïve T cell repertoire indicating a lack of new naïve T cell clones in peripheral sites and a reliance on homeostatic proliferation. Together these results suggest a novel dynamic mechanism for T cell subset compartmentalization and maintenance highlighting potentially different roles for CD4 and CD8 TEM. CD4 TEM maintain resident in the tissue responding to acute infections, while CD8 TEM indicate activation due to chronic viruses.

OR.55. Contributors to Decreased Vaccine Response in the Elderly

Bonnie B. Blomberg, Alain Diaz, Maria Romero, Ana Marie Landin and Daniela Frasca. ★University of Miami, Miami, FL

Generation of both memory B cells and plasma cells is required for a protective influenza vaccine. We previously showed that serum antibodies and the *in vitro* B cell (AID, activation-induced cytidine deaminase) response to a new influenza vaccine are decreased in elderly individuals and the AID response to CpG before vaccination predicts the level of vaccine response.

Surprisingly, we found that young and elderly individuals who had been immunized in at least two consecutive influenza vaccine seasons with the same H1N1 strain showed similar frequencies of influenza vaccine-specific memory B cells but the levels of serum antibodies were lower in the elderly. These findings suggest that multiple re-exposures to the vaccine cause a divergence between memory B cell frequencies and antibody levels with age, which may be partially due to impaired differentiation of memory B cell to plasma cells in the elderly.

To test the hypothesis of defective plasma cell development, initially in old mice, we measured the percentage of plasma cells and Blimp-1 in LPS-stimulated B cells and found them significantly lower in the old. Also, the average fold reduction in the secreted amount of IgM and IgG3 was higher than the average reduction in $CD138^+$ plasma cells (10-fold vs 2-fold) in the old mice suggesting qualitative and quantitative plasma cell defects in aging. Ongoing studies in mice and humans of different ages will identify the molecular regulators of the defects leading to reduced antibody quantity and quality in the elderly.

OR.60. Sustained Glycolytic Switch Required for Rapid Effector Function of TEMRA CD8 T Cells in Healthy Volunteers and in Immune-stimulated Patients

Michelle Yap[1,2], Gaelle Tilly[1,2], Magali Giral[1,2,3], David-Axel Laplaud[1,2,3], Sophie Brouard[2,3,1], Claire Pecqueur[4] and Nicolas Degauque[1,2]. [1]INSERM UMR 1064, Nantes, France; [2]★Université de Nantes, Nantes, France; [3]Centre Hospitalier Universitaire Hôtel-Dieu, Nantes, France; [4]INSERM UMR 892, Nantes, France

TEMRA CD8 T cells have been shown to accumulate with age or chronic antigen stimulation, leading to senescence of the immune system. However, they are also involved in many pathogenic processes, including kidney transplant rejection and bone regeneration. We assessed TEMRA CD8 functionality and metabolic profile in healthy volunteers and in kidney transplant recipients and multiple sclerosis patients.

Cytokine secretion and proliferation of naïve, and TEMRA CD8 cells were assessed after 2 and 5 days anti-CD3 stimulation that was supplemented with IL-2, IL-7 or IL-15. A Seahorse XF Analyser characterized mitochondrial respiration and glycolysis at basal and upon PMA/Ionomycin activation. Finally, glycolysis and mitochondrial respiration were inhibited, and pro-inflammatory cytokines secretion was assessed.

We found that TCR stimulation is sufficient to induce proliferation and pro-inflammatory cytokine secretion in TEMRA CD8 but not in contrast to naïve CD8 cells. IL-2, IL-7 and IL-15 stimulation results in rapid STAT5 phosphorylation and enhanced proliferation in TEMRA CD8. The immuno-metabolic profile of TEMRA CD8 showed that resting TEMRA cells exhibit a greater amount of ATP that is rapidly mobilized upon stimulation. TEMRA cells exhibit polarized and functional mitochondria, and switch rapidly to glycolysis upon activation. Finally, glycolysis and not mitochondrial respiration is necessary for pro-inflammatory cytokine secretion by TEMRA cells in healthy volunteers and in patients with low or high immune stimulation.

In contrast to their depiction as senescent cells, we show that TEMRA CD8 T cells shares similar immuno-metabolic features with effector memory and strengthen the need to re-assess their pathogenic role.

F.47. Smith-Lemli-Opitz Syndrome Fibroblasts Reveal the Importance of Endogenous Cholesterol Biosynthesis to the Innate Immune Response

Kristin Gabor[1], Michael Fessler[1], Christopher Wassif[2] and Forbes Porter[2]. [1]National Institute of Environmental Health Sciences, Research Triangle Park, NC; [2]National Institutes of Health, Bethesda, MD

Smith-Lemli-Opitz Syndrome (SLOS) is a rare disorder caused by a defect in cholesterol biosynthesis that presents in infancy with multiple developmental abnormalities. SLOS results from mutation of 7-dehydrocholesterol reductase (DHCR7), resulting in reduced cholesterol and a coordinate increase in its precursor, 7-dehydrocholesterol.

Lipid rafts are cholesterol-enriched membrane microdomains that serve as signaling platforms in multiple prototypical pathways, including the Toll like Receptors (TLRs). Raft-dependent signaling is sensitive to cholesterol levels. We hypothesized that SLOS cells would display abnormal TLR signaling, thus offering a unique opportunity to define the importance of endogenous cholesterol biosynthesis to the innate immune response.

Primary dermal fibroblast lines from SLOS patients were confirmed for the SLOS metabolic phenotype by GC/MS. SLOS fibroblasts produced significantly lower IL-6 and IL-8 upon lipopolysaccharide (LPS) stimulation. Intriguingly, an inverse correlation was observed between clinical severity scores of SLOS patients and IL-6 response to LPS, and a direct correlation between residual DHCR7 enzyme activity and IL-6 production. Further, SLOS cells displayed a decrease in NFkB p65 activation upon LPS stimulation compared to WT. Using DHCR7 inhibitors on RAW264.7 macrophages to recapitulate the SLOS phenotype revealed that LPS-dependent signaling events are inhibited in SLOS macrophages.

Taken together, the SLOS mutation confers abnormal innate immune responses. The correlation between clinical severity and LPS responsiveness of SLOS cells may suggest a clinically relevant role for altered innate immunity in SLOS. Understanding mechanisms by which cholesterol impacts innate immune function may provide novel insight into pathogenesis and therapy of a wide range of immune-mediated diseases.

F.48. Absence of ERAP Partially Rescues the Flu-**specific Vβ8.1**$^{+}$ CTL which are Normally Deleted in B7/B27 Coexpressing HLA Transgenic Mice

Ali Akram[1] and Robert Inman[2]. [1]★University of Toronto, Ajax, Ontario, Canada; [2]Toronto Western Hospital, Toronto, Ontario, Canada

Introduction: The role of HLA-B27 in modulating host response to infection is undefined, yet B27 confers susceptibility to arthritis. Despite co-dominant expression of class I MHC alleles, immune response to viral infections is characterized by immunodominance (ImDc). Defining factors contributing to ImDc has proved difficult due to multiple MHC-I allele co-expression in humans and normal mice. Materials and Methods: To overcome this limitation, we generated human MHC-I transgenic (Tg) mice deficient for endogenous mouse MHC-I molecules and express only one or two human MHC-I allele(s) in the presence or absence of ERAP expression. Results: Studies with flu-infected B27/ERAP$^{-/-}$ Tg mice revealed a reduced B27/NP383-391 influenza response compared to B27/ERAP$^{+/+}$ mice. Studies with flu-infected B7/ERAP$^{-/-}$ Tg mice revealed no change in B7/NP418-426 influenza response compared to B7/ERAP$^{+/+}$ Tg mice. Subsequent flu-specific studies revealed that ERAP deficiency in B27/ERAP$^{-/-}$ is associated i) with a partial deletion of Vβ8.1^{+} B27/NP383-restricted CD8^{+} T cells, and ii) the generation of B27-restricted NP383-391 flu epitope is ERAP dependent. Similar to CTL responses seen with B27/ERAP$^{-/-}$ Tg mice, in flu-infected B7/B27/ERAP$^{-/-}$ Tg mice a significantly reduced B27/NP383-restricted CTL response was detected while there was no change in the response level of B7/NP418-restricted CTL. The reduction in B27/NP383-391 CTL response, to our surprise, was not due to the complete absence of flu specific B27/NP383-391 Vβ8.1^{+}CD8^{+} T cells. These T cells were partially rescued. Conclusion: The selective deletion of B27-restricted T cells has important implications for models defining the role that HLA-B27 plays in susceptibility to ankylosing spondylitis.

F.49. Functional Heterogeneity of Human Memory CD4^{+} T Cells Specific for *C. difficile* Toxins in Patients with Active Disease

Laura Cook, May Wong, Megan Levings and Theodore Steiner. ★University of British Columbia, Vancouver, BC, Canada

Clostridium difficile infection (CDI) is a frequent cause of bacterial diarrhea, with approximately 25% of patients relapsing after treatment. The pathogenicity of CDI is known to require the activities of its toxins, TcdA and TcdB, but the T cell-mediated response to these toxins remains uncharacterized. We collected blood from patients experiencing

relapsing CDI, and from volunteers with no history of CDI. $CD4^+$ T cell responses to the toxins were measured using a flow cytometry assay that identifies antigen-specific $CD4^+$ T cells by co-expression of CD25 and OX40 following 44h incubation with antigen. On average 2% and 0.2% of all circulating $CD4^+$ T cells in CDI patients responded to TcdB and TcdA respectively whereas TcdA and TcdB-specific $CD4^+$ T cells were not detected in healthy controls. To define whether CDI infection polarized $CD4^+$ T cells into specific Th cell subsets, expression of CCR4, CXCR3, CCR6, and CD39 was measured on antigen-specific cells. The TcdB-specific $CD4^+$ T cells were functionally heterogeneous, with an approximate 1:1 ratio of Tregs to T effectors, which contained Th1, Th2 and Th17 cells in roughly equivalent proportions. We also measured anti-TcdA/TcdB IgG antibodies but found they were not significantly different between patients and controls, indicating that anti-TcdA/TcdB $CD4^+$ T cells are a more specific marker of disease. Tracking how toxin-specific $CD4^+$ T cell responses change following treatment and/or vaccination not only has the potential to predict relapse, it will also deliver insight into how human $CD4^+$ T cell memory develops in response to this prevalent bacterial pathogen.

F.50. Human Natural Killer Cell Repertoire Diversity Predicts HIV-1 Acquisition

Dara Strauss-Albee[1], Julia Fukuyama[1], Emily Liang[1], Justin Jarrell[1], Alison Drake[2], John Kinuthia[3], Grace John-Stewart[2], Susan Holmes[1] and Catherine Blish[1]. [1]★Stanford University, Stanford, CA; [2]★University of Washington, Seattle, WA; [3]Kenyatta National Hospital, Nairobi, Kenya

The immunological basis of protection from HIV-1 has remained elusive, suggesting that, like the genetic basis of many complex inherited conditions, its identification may require a systems-based approach. Fast-acting natural killer (NK) cells participate in early recognition of HIV-1-infected cells using a spectrum of activating and inhibitory receptors whose combinatorial assortment grants extraordinary phenotypic and functional diversity. However, the functional significance of this diversity has never been established. Here, we investigated how the human NK cell repertoire shapes HIV-1 susceptibility. We obtained banked pre-infection samples from thirteen women who acquired HIV-1 and 24 matched controls. Within this cohort, we show via mass cytometry and non-parametric species estimation that the diversity of the NK cell repertoire predicts HIV-1 acquisition. A 100-point increase in NK diversity is associated with 2.5-fold increased risk of HIV-1 acquisition (95% CI 1.2, 6.2). In a second cohort, we find that NK repertoire diversity is a stable characteristic over 6 months *in vivo*, demonstrating its utility as a clinical predictor. NK cell diversity does not correlate with *in vitro* viral suppression capacity, explaining the requirement for a systemic approach. High NK cell diversity is, however, associated with a terminally differentiated repertoire skewed toward cytokine production and away from division and cytotoxicity. Further, individuals become progressively dissimilar as their NK repertoires diversify. These results show that NK repertoire differentiation is an idiosyncratic process shaped by an individual's unique encounters. Thus, a highly diverse, ramified innate immune repertoire may actually be counterproductive to *de novo* antiviral responses.

F.51. TNF-**α Plays a Pivotal Role in Streptococcal Pyrogenic Exotoxin A**-induced $CD4^+CD25^+FOXP3^+$ Regulatory T Cells

Chun-Hao Lu[1], Ming-Chieh Lin[2] and Ming-Ling Kuo[1]. [1]Chang Gung University, Taoyuan, Taiwan,. [2]National Taiwan University, Taipei, Taiwan

Bacterial superantigens are potent stimulator of T cells. They cause toxic shock, and are also implicated in atopy and sepsis in humans. Several studies have described that superantigen exotoxins of *Staphylococcus aureus* and *Streptococcus pyogenes* can induce $CD4^+CD25^+FOXP3^+$ Treg cells. However, the underlying mechanism still remains unknown. We found that stimulation of PBMC with streptococcal pyrogenic exotoxin A (SPEA) resulted in dose-dependent and time-dependent FOXP3 expression in $CD4^+$ T cell population. Characterization of this response confirmed that $CD4^+CD25^+FOXP3^+$ Treg cells arose from $CD4^+CD25^-$ T cells and required antigen presenting cells (APC). The $CD4^+CD25^h$ T cells sorted from the culture of PBMC performed the suppressive ability. Moreover, the production of TNF-α was also dose-dependent. TNFR2, one of the TNF-α receptors, was indicated to be important

for Treg cells. In addition, TNF-α was proposed to induce $p27^{kip1}$ expression which was considered to be critical for Treg cell differentiation. The increased TNFR2 expression was detected in SPEA-induced $CD4^+CD25^+FOXP3^+$ Treg cells. Neutralizing TNF-α with specific antibody showed the decreased induction of $CD4^+CD25^+FOXP3^+$ Treg cells. We further found that $p27^{kip1}$ expression in $CD4^+CD25^h$ T cells decreased in the early days of stimulation but dramatically reversed in later days, which may show its importance in increased FOXP3 expression. Taken together, our data provide a mechanism for the superantigen induction of Treg cells, which helps bacterial evasion of the immune response.

F.52. Epstein-barr Virus (EBV) Encoded dUTPase Exacerbates the Immune Pathology of Lupus Nephritis *in vivo* by Up-regulation of TLR2 and IL-17

Nicholas Young, Marshall Willaims, Michael Bruss, Jeffrey Hampton, Maria Ariza and Wael Jarjour. The Ohio State University Wexner Medical Center, Columbus, OH

Systemic lupus erythematosus (SLE) pathogenesis is influenced by environmental, hormonal, and genetic factors alike. Viral infection is an environmental contributor previously shown to be associated with SLE. In particular, Epstein-Barr virus (EBV) has a strong link with SLE etiology and there is an increasing body of evidence to support a potential role for EBV in SLE. Accordingly, serum of SLE patients contains elevated levels of antibodies to several EBV antigens and increased EBV infection rates of renal tubular epithelial cells are observed in patients. In this study, we explored the mechanism by which EBV may contribute to SLE pathogenesis. Previously, we have demonstrated that EBV-deoxyuridine triphosphate nucleotidohydrolase (dUTPase) can activate the innate immune receptor TLR2 and lead to the production of type 1 interferon and other proinflammatory cytokines. To study the role of dUTPase in SLE, we used the NZM2410 mouse model, which spontaneously develops severe, early-onset lupus nephritis. Mice were injected with either recombinant EBV-dUTPase or PBS and kidney tissue was harvested for histological analysis when blood urea nitrogen levels were greater than 50 mg/dL. Our results indicate that dUTPase injection significantly increased the severity of glomerulonephritis and the inflammatory infiltrates into the interstitium and tubules. Furthermore, immunohistochemical staining for TLR2 and IL-17 was significantly enhanced in dUTPase-injected mice relative to PBS controls. Collectively, these data show that EBV-dUTPase can exacerbate renal pathology in lupus nephritis through the induction of both TLR2 and IL-17 and suggest that EBV may be a trigger for innate immune system inflammation in SLE.

F.53. Progressive Loss of CD4 Lymphocytes in Chronic Granulomatous Disease

Robert Roberts and Kevin Yehsheng Wang. ★University of California, Los Angeles, Los Angeles, CA

Patients with chronic granulomatous disease (CGD) are at risk for bacterial and fungal infections due to inability of their neutrophils to generate oxygen radicals used in killing micro-organisms. We have observed the progressive loss of CD4 lymphocytes in 3 patients with the X-linked form of the disease

The first patient is a 28 year old male whose CD4 count was 284/mm3 in 2006 with a CD3 count total of 560/mm3. The numbers varied but in 2014 his CD4 was down to 173/mm3 and CD3 total was 490/mm3. There has also been a trend of a decreased CD4/CD8 ratio of 0.68. The patient also has a lesion in his lung thought to be fungal.

The second patient is now 34 years old. His CD4 count was 534 /mm3 in 2006 and CD3 count was 1593/mm3 which was within normal for age. His most recent CD4 count in 2014 was 169/mm3 and CD3 was 611/mm3. His CD4/CD8 ratio was low at 0.36.

The third patient, now deceased, had a CD4 count of 144/mm3 and CD3 total count of 306/mm3 in 2007 with a ratio CD4/CD8 of 0.88. He had a CD4 count of 59/mm3 and CD3 count of 162/mm3 with a ratio of 0.54 in 2008.

These patients usually have normal immunoglobulin levels, negative for HIV infection, and negative for antibody for anti-nuclear antibody. The decrease in CD4 in CGD has been noted before but unclear if it makes these patients more susceptible to opportunistic infections. Most are taking prophylactic antibiotics anyway.

F.54. CD44 Controls T Cell Exhaustion and Viral Persistence During Chronic Viral Infection

Florent Carrette, Roberto Tinoco, Monique Barraza and Linda Bradley. Sanford Burnham Medical Research Institute, La Jolla, CA

During a chronic viral infection, inhibitory receptors play a crucial role in controlling viral persistence and T cell exhaustion. However, the role of homing molecules in this process has been poorly investigated. Using the chronic LCMV virus model, Clone 13, we found that expression of CD44, a cell surface glycoprotein broadly used to identify activated T cells, dampens antigen specific T cell responses. In CD44-deficient hosts, we observed a significant increase in antigen specific CD4 and CD8 T cells functions with decreased PD-1 expression and a striking increase in multiple cytokine production. T cell accumulation was not due to increased proliferation based on BrdU incorporation, and the increased CD8 T cell response required CD4 T cell help because CD8 T cell exhaustion was maintained in CD4 depleted CD44-deficient mice. Using a bone marrow chimera approach, we found that restricting the CD44 deficiency in the non-hematopoietic compartment was sufficient to reproduce the observations made in the complete CD44 deficient hosts. Finally CD44-deficiency resulted in viral clearance by d15pi. Importantly, treatment of WT mice with a CD44-blocking antibody increased antigen specific CD4 and CD8 T cell recovery and some aspects of T cell function as early as d9pi. Taken together, these results indicate that CD44 is a novel inhibitory receptor that can be targeted to improve T cell response during chronic viral infections.

F.55. Cytomegalovirus Encoded Homologs of SLAM Receptors, a New Class of Immune Modulators

Ana Angulo[1,2], Natalia Pérez-Carmona[2], Domenec Ferrë[2], Pablo Martínez-Vicente[2] and Pablo Engel[1,2]. [1]★University of Barcelona, Barcelona, Spain; [2] Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain

Viruses have evolved a number of mechanisms to circumvent host immune defenses. Cytomegaloviruses (CMVs) are ubiquitous, species-specific members of the herpesvirus family known to contain a rich arsenal of gene products with immunomodulatory roles, including homologs of cellular immune proteins captured during co-evolution with their hosts. We have discovered a number of homologs of signalling lymphocytic activation molecule (SLAM) family receptors in CMVs that infect human and other primates. SLAM family receptors are involved in the regulation of both innate and adaptive immunity, which occurs upon engagement with their ligands via homotypic or heterotypic interactions through their N-terminal immunoglobulin domains. We report that the cellular genes were acquired by retrotranscription at different moments of the virus-host co-evolution. Three of these viral SLAM homologs exhibit an exceptional amino acid identity in their immunoglobulin ectodomain respect to the corresponding host parental protein (SLAMF6, CD48 or Ly9). Accordingly, we found that these viral proteins, which get expressed either at the cell surface or as soluble molecules, interact with their host counterpart ligands. Lowest conserved viral SLAM homologs do not hold the ability to recognize their corresponding cellular counterpart ligands, and we show for two of these molecules that instead have diverged to perform additional immunomodulatory activities. Common features of these viral SLAMs, comparing to their host homologs, are higher N- and O-glycosylations in their ectodomains and lack of ITSM signalling motifs in their cytoplasmic tails. Taken together, our findings indicate novel immune evasion mechanisms based on the exploitation of virally encoded SLAM homologs.

F.56. Characterization of Group A Streptococcus Specific CD4$^+$ T Cell Responses in Pediatric Patients

Jennifer Dan[1,2] Colin Havenar-Daughton[2] Shane Crotty[2]. [1]★University of California, San Diego, San Diego, CA; [2]La Jolla Institute for Allergy and Immunology, La Jolla, CA

★ = member of a FOCIS Center of Excellence

CD4+ T cells play important roles in human immune responses to infections. However, it can be difficult to measure pathogen-specific CD4+ T cell responses in peripheral blood, as antigen-specific CD4+ cells are usually quite rare. Furthermore, T follicular helper cells (Tfh) comprise a distinct CD4+ subset that are essential providers of help to B cells, but germinal center Tfh cells (GC Tfh) are absent in blood. In contrast, GC Tfh cells can be abundant in lymphoid tissue. Using pediatric tonsils, we are able to assess Group A Streptococcus (GAS)-specific CD4+ T cells responses both quantitatively and qualitatively. We use GAS protein preparations to identify GAS-specific Tfh populations within tonsils, after subtracting for a negative control antigen (antibiotic-killed Lactococcus, a non-pathogenic Gram positive bacteria). Using a range of flow cytometry approaches, we are now able to characterize GAS-specific CD4+ T cell phenotypic characteristics, proliferation capacity, and cytokine profiles. Tonsils provide a readily available source to study GAS-specific CD4+ T cell responses and their potential biological relevance, in particular GAS-specific Tfh responses.

F.57. Pre-BCR Selection does not Interfere with B-1 Cell Development but Shapes its Repertoire

Mohamed Khass, Tessa Blackburn, Peter Burrows and Harry Schroeder. ★University of Albama at Birmingham, Birmingham, AL

The peritoneal cavity (PEC) compartment contains both self-renewing B-1 cells, which first develop in the fetal liver (FL), and conventional B cells (B-2) that develop in the postnatal bone marrow (BM). B cell development in BM requires passage through the pre-BCR checkpoint, where the surrogate light chain (SLC) proteins (λ5 and VpreB) test Mu HC integrity. The role of the pre-BCR in development of B-1 cells has not been fully investigated, thus we used λ5 KO/BALB/c mice, which are unable to form the pre-BCR, to address this issue. We first assessed the frequency of cells undergoing proliferation or apoptosis in the PEC. Since the third complementary determining region heavy chain (CDR-H3) dictates the composition of the B cell repertoire, we also performed an in depth analysis of the CDR-H3 repertoire in the B-1 and B-2 PEC subsets. Compared to WT, B-1 cells lacking pre-BCR selection proliferated normally, however the λ5 KO B-1a cells had a decreased frequency of apoptosis. The λ5 KO B-1a cells had a decrease in the use of DFL gene segments, an increase in DSP gene segments, enhanced use of RF2 and RF3, and an increase in J_H3 usage. The CDR-H3 repertoire of λ5 KO B-1a cells was enriched for hydrophobic and charged amino acids, and depleted of tyrosine and serine. These data suggest that while the numerical population of the PEC by B-1 cells is unaffected by the absence of pre-BCR selection, the content of their repertoire, and thus the range of epitopes recognized, is changed.

F.58. Interleukin-17A is Associated with Increased Alveolar Neutrophilia in Acute Respiratory Distress Syndrome

Carmen Mikacenic[1], Elizabeth Hansen[1], Frank Radella[1], Renee Stapleton[2], Sina Gharib[1] and Mark Wurfel[1]. [1]★University of Washington, Seattle, WA; [2]University of Vermont, Burlington, VT

Acute Respiratory Distress Syndrome (ARDS) is an inflammatory condition of the lung that occurs in response to many forms of critical illness, including sepsis and severe trauma. Acute lung injury occurs with disruption of the alveolar-capillary barrier leading to protein-rich edema fluid accumulating in the airways. Neutrophilic inflammation predominates and plays a central role in ARDS pathogenesis. IL-17A is known to participate in neutrophil recruitment particularly in mouse models of pneumonia and lung injury. In this study, we hypothesized that IL-17A is associated with alveolar neutrophilia in ARDS. We measured IL-17A, using an antibody-based immunoassay, in paired serum and bronchoalveolar lavage (BAL) samples from 86 subjects with ARDS enrolled in a negative clinical trial. In the BAL fluid, we quantified the percentage of alveolar neutrophils, total alveolar protein concentration, and expression of the IL-17A receptor, *IL17RA*, in isolated alveolar macrophages. We tested for associations between IL-17A concentration organized by tertiles and the percentage of alveolar neutrophils as well as total alveolar protein content using linear regression. We found that increasing BAL IL-17A and serum IL-17A were highly associated with an increased percentage of alveolar neutrophils ($\beta=22$; $p<0.0001$ and $\beta=13$; $p<0.002$, respectively). Higher BAL IL-17A

was also associated with increased alveolar protein content ($p<0.01$) and increased expression of *IL17RA* by alveolar macrophages ($p<0.05$). Taken together, these data show that IL-17A is associated with alveolar inflammation and damage in ARDS and that alveolar macrophages are permissive to IL-17A signaling. This study supports a novel role for IL-17A in ARDS pathogenesis.

F.59. Development and Qualification of a Dual-purpose, Multiplex Serology Assay for the Diagnosis of Recent Respiratory Syncytial Virus Infection and Assessment of Vaccine Immunogenicity

Sarah Maifeld[1], Bodrey Ro[2], Hoyin Mok[3], Marla Chum[1], Li Yu[1], Tansy Leonardson[1], Vera Chio[1], Bandita Parhy[3], Samuel Park[2], Ann Falsey[4,5], Edward Walsh[4,5], C. Kathy Wang[1], Mark Esser[6], Xavier Paliard[1] and Fengrong Zuo[7]. [1]MedImmune, Mountain View, CA; [2]Genentech, South San Francisco, CA; [3]Gilead Sciences, Foster City, CA; [4]★University of Rochester, Rochester, BY [5]Rochester General Hospital, Rochester, NY; [6]MedImmune, Gaithersburg, MD; [7]Medivation, San Francisco, CA

Respiratory syncytial virus (RSV) is a leading cause of lower respiratory illness in children, older adults and individuals with compromised immune systems. To facilitate the clinical evaluation of vaccine candidates, we developed and qualified a dual-purpose multiplex serology assay to simultaneously diagnose recent RSV infection and measure vaccine immunogenicity.

We show that a customized, multiplexed IgG serology assay using four RSV antigens (F, N, Ga and Gb) and Meso Scale Discovery (MSD)'s electrochemiluminescence (ECL) technology provides a sensitive, specific and precise method to measure the humoral response to RSV antigens following natural infection and vaccination. In a proof-of-concept study, the diagnostic sensitivity and specificity of the F, N, Ga and Gb readouts were evaluated using a "gold standard" panel of acute and convalescent phase serum samples from elderly subjects. The combined results demonstrated concordance to the "gold standard" diagnosis, reaching 98% diagnostic sensitivity and 100% specificity. Additionally, the combination of readouts provided higher diagnostic sensitivity than an FDA exempt *in vitro* diagnostic ELISA or a microneutralization assay.

The linear dilutability, relative accuracy and precision of the RSV 4-plex assay were qualified prior to use in MedImmune's Phase 1 trials of MEDI7510, an adjuvanted RSV sF subunit vaccine. The qualified method allowed for the simultaneous measurement of vaccine immunogenicity using the F readout and detection of recent RSV infection using the N, Ga and Gb readouts. In summary, the RSV 4-plex assay provides a sensitive, specific and high-throughput approach for simultaneously measuring RSV-specific antibodies elicited by natural infection or vaccination.

F.60. T Cell Responses to Respiratory Syncytial Virus (RSV) are Reduced in 50-64 Year Olds Compared to 20-40 Year Olds

Stacie Lambert[1], Xavier Paliard[1], Mark Esser[2], Shahin Aslam[1], Sarah Maifeld[1] and Cindy Shambaugh[1]. [1]MedImmune, Mountain View, CA; [2]MedImmune, Gaithersburg, MD

Respiratory syncytial virus (RSV) is a major cause of lower respiratory tract infections in older adults. While both humoral and cellular immunity participate in host responses to RSV, advancing age results in declines in cellular immunity. In particular, functional RSV F-specific T cells are reduced in elderly (65+) adults compared to healthy younger adults. To address whether differences in RSV immunity could be observed in older adults (aged 50-64 years), we evaluated their RSV-specific humoral and cellular immune responses in comparison to 20-40 year olds.

43 donors aged 50-64 and 19 donors aged 20-40 were recruited at clinical sites that had been recruited to participate in the Phase 1 trials of Medimmune's adjuvanted RSV vaccine candidate, MEDI7510. Whole blood was processed to serum (10ml) and cryopreserved PBMCs (50ml). Serum was evaluated for RSV A2 virus neutralization titers and

binding titers to RSV fusion (F) antigen. PBMC were first assessed for quality (viability and functionality against a superantigen). PBMCs that passed acceptance criteria were evaluated by RSV F-specific IFNgamma ELISPOT and multiplexed cytokine release using an RSV sF peptide pool as the stimulating agent.

Our data show that individuals aged 50-64 have decreased RSV F-specific IFNgamma responses compared to individuals aged 20-40 despite strong superantigen responses. Notably, CD8 cell numbers were decreased in the 50-64 year old cohort. However, no significant differences between F-specific binding titers or functional antibody titers were observed. This suggests that cellular immune responses to RSV may become impaired by as early as 50 years of age.

F.61. Characterization of Antibody Responses After BG505 SOSIP.664 Trimer Immunizations in Mice

Joyce Hu[1], Jordan Crampton[1], Thomas Ketas[2], Albert Cupo[2], Marit van Gils[3], Kwinten Sliepen[3], Steven de Taeye[3], Rogier Sanders[3], John Moore[2] and Shane Crotty[1]. [1]La Jolla Institute of Allergy and Immunology, La Jolla, CA; [2]Weill Cornell Medical College, New York, NY; [3]★University of Amsterdam, Amsterdam, Netherlands

Soluble BG505 SOSIP.664 trimers were designed to mimic the native conformation of the HIV-1 spike, which has been validated by structural studies. These trimers are efficiently recognized by most broadly neutralizing antibodies (bNAbs) but by only a few non-neutralizing antibodies. A critical goal towards which we are working is to learn how to use the trimers to induce Tier 2 NAbs after protein vaccination. Tfh cells are central to the effectiveness of protein vaccines given their role in germinal center (GC) responses and in the induction of long-term humoral immunity. Importantly, Tfh cells control somatic hypermutation by selectively providing signals to higher affinity B cells in the GC. Because bNAbs emerge over time via a complex affinity maturation pathway, understanding and manipulating GC responses is a key component of an HIV-1 vaccine program. Here, we immunized mice (Balb/cJ or 129S1/Sv1mJ) with the BG505 SOSIP.664 trimers over a multi-month period to assess the impact of various parameters on germinal center responses and to identify immunological obstacles that will need to be overcome to induce Tier 2 NAbs. We measured antibody titers by ELISA, antibody quality by Octet, neutralization of Tier 1 and Tier 2 viruses with the TZM-bl cell assay, and Tfh cell and antigen-specific GC B cellresponses by flow cytometry. These experiments provide insight into the impact of different parameters on trimer-specific antibody responses, including variation of antigen doses, adjuvants and prolonged antigen release.

F.62. Identification of an Immune Biosignature Correlated with Pulmonary Tuberculosis Stages

Fabiana Zambuzi, Milena Espindola, Luana Soares, Leonardo Lima, Thaisa Araujo, Caroline Fontanari, Margarida Nascimento, Valdes Bolella and Fabiani Frantz. Universidade de Sao Paulo, Ribeirao Preto, Brazil

It is estimated that about one third of the world population is infected with Mtb, showing that despite the well established treatment and cure, Tuberculosis still represents an important public health problem worldwide. Our study aimed to correlate changes in the immune response developed from patients at different stages of pulmonary tuberculosis with clinical aspects of each stage. Thus, the study included 17 patients with active disease, 14 individuals with latent tuberculosis and 16 uninfected controls. The blood was collected for immune mediators quantification in the plasma. We showed that patients with active tuberculosis have increased plasma levels of cytokines IL-6, IP-10, TNF-α, IL-12 and IL-5. We also showed that patients with active disease established higher number of correlations between plasma cytokines and presented higher frequencies of “high producers” for the majority of the analyzed mediators, indicating an activated state of the immune system. Finally, once tuberculosis patients presented increased levels of some mediators, we correlated these with the degree of lung injury, and consequently tuberculosis severity. We have showed that TNF-α was correlated with disease progression in tuberculosis. In conclusion, we demonstrated that patients with active tuberculosis are more activated than the other groups and among the cytokines/chemokines differentially expressed, TNF-α, IL-6, IP-10, IL-5 could discriminate between active disease from others, featuring a biosignature to tuberculosis. From these mediators, TNF-α might act

as a prognostic tool.

F.63. Cellular Dynamics of an Immune Response to Norovirus Infection

Antony Cutler, Sarah Caddy, Joao Oliveira, Ricardo Ferreira, Ian Goodfellow, Linda Wicker, John Todd and Frank Waldron-Lynch. ★University of Cambridge, Cambridge, United Kingdom

In DILT1D (NCT01827735), a mechanistic study to determine the effects of single doses of interleukin-2 (IL-2) in type 1 diabetes, a participant experienced an unrelated self-limiting gastrointestinal norovirus infection a day after IL-2 administration. The longitudinal design and intense sampling protocol proximal to the IL-2 dose allowed an unprecedented insight into the largely uncharacterized human immune response to this common virus. Norovirus infection induced a burst of endogenous IL-2 release and a coincident secondary peak of STAT5 phosphorylation in peripheral lymphocytes. A proportion of the immune events induced by exogenous IL-2 alone were mirrored in the norovirus response, however substantial activation of circulating adaptive and innate immune cells and antigen-presenting cell populations over, above and broader than those induced by IL-2 alone occurred. Large transient pulses of IFN-gamma, IP-10 and CRP and lower levels of TNF-alpha and IL-6 were observed in the serum with no increase in IL-1 beta. Concomitant with the large interferon response, Siglec-1 expression was induced on classical monocytes along with a sustained increase of soluble Siglec-1 in the plasma. Infection driven increases in neutrophil and monocyte numbers were quickly resolved. NK, NKT, emCD4$^+$, cmCD8+ and emCD8$^+$ T cells transiently increased CD69 and dendritic cells and monocytes upregulated CD40 expression 48-72h after first symptoms. At day 6 post-infection the frequency of CD103$^+$ CD8$^+$ memory T cells transiently increased in the periphery. Anti-norovirus GII.4 VLP antibody titres peaked at day 27 post-infection and declined thereafter. Infections give perspective to IL-2 induced perturbations of the peripheral immune system.

F.64. Whole Transcriptome of Liver and Lung Reveals Differential Immune Response Pattern that Correlates to Bacilli Containment in Experimental Tuberculosis

Rogerio Rosada[1], Fabiano Pais[2], Rodrigo Rodrigues[1], Priscilla Silva[1], Wendy Rios[1], Izaira Brandao[1], Ana Masson[1], Anna Salim[2], Flavio Araujo[2], Fabiani Frantz[1], Guilherme Oliveira[2], Celio Silva[1] and Lucia Faccioli[1]. [1]Universidade de Sao Paulo, Ribeirao Preto, Brazil; [2]Centro de Pesquisas Rene Rachou/MG, Belo Horizonte, Brazil

Prophylactic and therapeutic tools for tuberculosis (TB) control are still needed. In humans, extrapulmonary tuberculosis occurs at low rates and the liver represents one of the last internal organs to be infected, a phenomenon that has not been entirely elucidated. On the other hand, it is well known that the immune response on liver is very efficient against a variety of pathogen infections. Thus, the aim of this work was to investigate the components related to the hepatic environment that could be associated to the mycobacteria elimination in this organ (presenting 10000 less bacilli recovery than lung in mice). We compared the lung and liver whole transcriptome (by RNAseq) from mice infected by *Mycobacterium tuberculosis* and applied functional and enrichment analysis using Metacore platform. In liver environment it was detected a profile of the immune response activation and fibrosis mediators, acute phase proteins and cytokines related to the control of disease. On the other hand, in lung there is a prevalence of pro-inflammatory mediators that are not related to protection, amyloid proteins accumulation and lower levels of NOTCH2 (an important factor linked to bacillus containment process); furthermore we detected increase expression in mucin and cytokines (TGF-β and IL-13) that are related to disease progression and the regulation of STATs activation. Those results highlighted some relevant molecules and patterns of activation/deactivation that could be used to target the pulmonary TB through the fine tunning of immune response, improving the protective role against the infection.

F.65. Phenotype and Functional Attributes of Herpes Simplex Virus Specific CD8$^+$ T Cells in HSV-1

Seropositive Asymptomatic Individuals

Arif Khan, Ruchi Srivastava and Lbachir BenMohamed. University of California, Irvine, Irvine, CA

A staggering number of individuals carry herpes simplex virus type (HSV) 1 and/or type 2. The majority of HSV infected individuals are asymptomatic (ASYMP) without any recurrent disease (cold sore, ocular and genital herpes) even though spontaneously reactivated virus shed in the body fluids (saliva, tears and vaginal secretions). However, small proportions of HSV-seropositive individuals are symptomatic (SYMP) and experience endless recurrences of herpes disease and often require continuous antiviral therapy. Objective of our study was to address why ASYMP individuals do not show any symptoms in-spite of similar virus shedding among ASYMP and SYMP individuals. We used HSV-1 specific gB tetramer to determine the nature and frequency of memory $CD8^+$ T cell subsets in the peripheral blood of HSV-seropositive ASYMP and SYMP individuals. We analyzed phenotype and functions of gB-specific $CD8^+$ T cells from HLA-A*02:01 positive, HSV-1 seropositive individuals. We did not observe any difference in the frequency of gB-specific $CD8^+$ T cells. However, ASYMP individuals maintained a significantly higher frequency of effector memory $CD8^+$ T cells (T_{EM}) with multifunctional effector phenotype in terms of IFN-gamma, $CD107^{a/b}$, granzyme B, and perforin production; and increased expression of KLRG-1 (terminal effectors) as compared to SYMP individuals. We observed increased proliferation of gB-specific $CD8^+$ T cells in ASYMP individuals. SYMP individuals expressed higher level of gB-specific PD-1 and Tim-3 than ASYMP individuals, which indicates an exhaustion phenotype of $CD8^+$ T cells. Our findings provide an insight into the role of effector memory $CD8^+$ T cells in mounting protection against reactivating herpes virus.

F.66. Functional and Phenotypic Analysis of HLA-A02:01-restricted Epitopes Identified from the Herpes Simplex Virus Tegument Protein VP13/14 Specific $CD8^+$T Cells

Ruchi Srivastava, Arif Khan and Lbachir BenMohamed. University of California, Irvine, Irvine, CA

$CD8^+$ T cells have the potential to control HSV-1 infection. However, very limited information has been available on $CD8^+$ T cell epitopes or the functionality of antigen specific T cells during infection. In this study, we have used various predictive computer-assisted algorithms to identify 10 potential HLA-A*02:01-restricted $CD8^+$ T cell epitopes from the VP13/14 protein. VP13/14, a major tegument protein of herpes simplex virus type 1, targeted by $CD8^+$ T cells from HSV individuals. However, whether and which VP13/14-epitope-specific $CD8^+$ T cells play a role in the protection seen in seropositive healthy asymptomatic (ASYMP) individuals (who never had clinical herpes disease) remain to be determined. Three out of ten epitopes exhibited high to moderate binding affinity to HLA-A*02:01 molecules. Effector $CD8^+$ T cell responses specific to each peptide epitope, were studied in ten HLA-A*0201 positive HSV-seropositive ASYMP and SYMP individuals, assessed by a combination of tetramer frequency, $CD107^{a/b}$ cytotoxic degranulation, and IFN-g production. High frequency of multi-functional effector $CD8^+$ T cells was directed against three epitopes: $VP13/14_{286-294}$, $VP13/14_{504-512}$ and $VP13/14_{544-552}$ in ASYMP individuals. We also found that ASYMP individuals had significantly higher proportion of $CD8^+$ effector memory T cells compared to SYMP individuals. Moreover, immunization of HLA-A*02:01 transgenic mice with the three Immuno-dominant $CD8^+$ T cell epitopes induced robust and poly-functional epitope-specific $CD8^+$ T_{EM} cells that were associated with a strong protective immunity against ocular herpes infection and disease. This study describes three novel VP13/14 epitopes eliciting strong $CD8^+$ T cell responses that may facilitate epitope based vaccine design.

Immunodefiency- Primary or Acquired

OR.09. Novel Roles for STAT3 in Follicular Helper T Cell Function

Hao Wu[1], Paulla Teuscher[1,2], Linlin Xu[1], Hong Liu[1], Mark Kaplan[1] and Alexander Dent[1]. [1]Indiana University School of Medicine, Indianapolis, IN; [2]Purdue University Indianapolis, Indianapolis, IN

★ = member of a FOCIS Center of Excellence

STAT3 is required for T follicular helper cell (Tfh) development. In our study, compared to WT mice, CD4 T cell specific STAT3 knockout (STAT3KO) mice had severely impaired Tfh cell differentiation at early stage (day 3) after sheep red blood cells (SRBC) immunization, however recovered to comparable level at day 7. Also, Tfh cell percentage in STAT3KO intestinal Peyer's patches (PP) was only slightly decreased. To better understand the role of STAT3 in Tfh cell differentiation and function, we examined the effect of loss of STAT3 on Tfh cells in two different tissues, spleen and PP. Unexpectedly, STAT3KO Tfh cells had higher proliferation capacity and decreased apoptosis. Both spleen and PP STAT3KO Tfh cells had significantly increased Bcl6 expression and cells producing IL-4, important B cell helper cytokine. STAT3KO mice had severe decrease of germinal center B (GCB) cells and lower anti-SRBC IgG and IgG1 titers after SRBC systemic immunization. Interestingly, in STAT3KO PP, the percentage of IgG1 class switched GCB cells induced by gut microbiota was dramatically increased. Furthermore, PP STAT3KO Tfh cells expressed much higher Tbet, Gata3 and Blimp1. Mechanistically, we found the increase of IL-4 production by STAT3KO CD4 T cells was due to failure of Bcl6 suppression. In conclusion, these data indicate that STAT3 can repress the expression of Bcl6 in Tfh cells and also regulate Bcl6 activity. Moreover, we reveal that STAT3 function in Tfh cells is greatly affected by the precise immune environment.

OR.29. Emerging Role of Innate Lymphocytes (ILCS) in Allergic Inflammation in Murine Model of Leaky SCID/OMENN Syndrome

***Krisztian Csomos**[1,2], Sanhong Yu[3], Boglarka Ujhazi[1,2], Benjamin Causton[2], Carin Dahlberg[4], Lisa Westerberg[4], Benjamin Medoff[2], Marco Colonna[5], Dale Umetsu[6], Luigi D. Notarangelo[7] and Jolan Walter[1,2,7].*
[1]★Massachusetts General Hospital for Children, Boston, MA; [2]Massachusetts General Hospital, Harvard Medical School, Boston, MA; [3]Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA; [4]★Karolinska Institute, Stockholm, Sweden; [5]★Washington University School of Medicine, Saint Louis, MO; [6]Genentech, San Francisco, CA; [7]★Boston Children's Hospital, Harvard Medical School, Boston, MA

Rationale: Hyperinflammation is the hallmark of Omenn syndrome (OS). Patients with OS have highly restricted T and B cell repertoire secondary to impaired V(D)J recombination with low RAG activity. Hyperinflammation was solely contributed to this oligoclonal autoreactive T cell compartment, mainly identified in the skin and colon of patients in Omenn syndrome, whereas the lung as a site of inflammation has not been previously examined. In addition, the role of the innate system and environmental triggers has not been fully investigated. Homozygous *rag1*$^{S723C/S723C}$ (*mut/mut*) mouse model of leaky SCID/OS provides a unique opportunity to investigate lung pathology, altered function of innate lymphoid cells (ILCs) and the role of environmental triggers, such as viral infections.

Methods: Lung ILCs and their allergic cytokine production were assessed in *mut/mut* mice by flow cytometry at steady state and after high and low-dose TLR3 stimulation mimicking acute and chronic viral infections, respectively. Airway hyperresponsiveness after methacholine challenge was evaluated.
Results: Increased presence of ILC2s was noted in the lung of *mut/mut* mice that was further enhanced after acute high dose and chronic low dose TLR3 stimulation. Both type of TLR3 stimulation increased the capacity of ILCs to produce type-2 cytokines (IL-5 and IL-13) accompanied by increased eosinophil and neutrophil infiltration. A fraction of *mut/mut* mice showed airway hyperreactivity.

Conclusions: We propose the lung as a previously unrecognized site for allergic hyperinflammation in OS. Our data suggest that ILCs may contribute to the pathogenesis of allergic inflammation in our murine model of Leaky SCID/OS.

OR.39. Characterization of Polyclonal and Antigen-specific CD4^{FOXP3} T Cells for Treg Cell-based Immunotherapy of Autoimmune Diseases

***Laura Passerini**[1], Eva Rossi Mel[1,2], Claudia Sartirana[1], Brian Donald Piening[3], Michael Snyder[3], Maria-Grazia*

Roncarolo[1,3] and Rosa Bacchetta[1,3]. [1]San Raffaele Scientific Institute, Milan, Italy; [2]University of Rome Tor Vergata, Rome, Italy; [3]★Stanford University School of Medicine, Stanford, CA

Forkhead-box-P3 (FOXP3) is the transcription factor guiding the function of thymus-derived (t) T regulatory cells (Tregs), key players in the maintenance of peripheral tolerance, as demonstrated by the wasting syndrome developing in patients with FOXP3 mutations (IPEX). Treg-based therapies to modulate immune response and restore tolerance in autoimmunity have been explored. We showed that lentivirus-mediated gene transfer of FOXP3 in IPEX $CD4^+$ T cells, converts conventional T (Tconv) cells into tTreg-like cells ($CD4^{FOXP3}$) endowed with potent *in vitro/vivo* suppressive activity. To better characterize the $CD4^{FOXP3}$ T cell product we assessed the gene expression profile of $CD4^{FOXP3}$ T cells vs tTregs. RNA-sequencing showed that upon ectopic FOXP3 expression minor alteration of gene expression occurred, with a total of 167 differentially expressed genes ($CD4^{FOXP3}$ vs control transduced T cells, q value<0,25). Only a minority of these genes mirrored those differentially expressed by tTregs vs Tconv cells, including Treg-associated genes involved in Treg suppressive function, such as CTLA4 and TIGIT, and genes involved in the regulation of cell proliferation/activation. These findings confirm that minimal transcriptional perturbation is sufficient to endow Tconv cells with regulatory function, as long as FOXP3 is stably expressed. We further tested the use of our platform for the generation of Ag-specific Treg-like cells. We found that Ag-specific $CD4^{FOXP3}$ T cells stably express FOXP3 and specifically respond to their cognate Ag. These results pave the way for cell-based therapies with autologous Treg-converted lymphocytes for the treatment of T cell mediated disorders of genetic or unknown origin.

OR.53. Human Naïve T Cell Compartment Revisited: Neonatal Thymectomy Alters Phenotype and Function of Peripheral Naïve T Cells

Theo van den Broek, Eveline Delemarre, Willemijn J.M. Janssen, Jasper C.A. Broen, Kiki Tesselaar, Jose A.M. Borghans, Berent J. Prakken, Koos J.G. Jansen and Femke van Wijk. ★UMC Utrecht, Utrecht, Netherlands

Naïve T cell homeostasis in adulthood is maintained by peripheral proliferation and not by thymic output. Recently, neonatal immunity has been characterized by the high expression of the signatory effector cytokine IL-8 in naïve T cells. In children, the role of the thymus is more apparent as premature immune aging of the T cell compartment is seen after neonatal thymectomy. Here we determined the role of the thymus in the phenotypical and functional maintenance of the naïve T cell compartment in children and adolescents.

A unique homogenous cohort of children (1-5 years, n=11) and adolescents (12-25 years, n=26) all thymectomized in the first month of life, and age-matched controls were included. T cell phenotyping, cytokine and aging analysis, and calcium-flux assays were performed and compared to thymic output and presence (CD31 expression and MRI-scan).

T cell phenotyping indicated premature immune aging of the total T cell compartment after neonatal thymectomy as shown by increased expression of CD57, TIM3, FAS, Ki-67 and cytokine expression profile. Interestingly, the signatory IL-8 production and calcium flux capacity of naïve T cells were greatly diminished in thymectomized children (16.8% vs 1.4% IL-8+,$p<0.000$) within the naïve T cell compartment. IL-8 production correlated with $CD31^+$ T cells independent of naïve/memory status. Thymic tissue regeneration resulted in restoration of calcium flux capacity and IL-8 production of naïve T cells, but was still impaired when thymic regeneration did not occur.

Neonatal thymectomy results in altered naïve T cell immunity, suggesting a previously unidentified functional differentiation within the naïve T cell compartment. Functional regeneration of thymic tissue, induces rejuvenation and restoration of the naïve T cell compartment.

OR.56. Noncoding RNAs Regulate Thymic T Cell Output Under Normal and Pathophysiological Settings

Nicolai Van Oers[1], Ashley Hoover[1], Shaheen Khan[1], Ondine Cleaver[1] and M.Teresa de la Morena[1,2]. [1]★University of Texas Southwestern Medical Center, Dallas, TX; [2]Children's Health, Dallas, TX

Patients with 22q11.2 deletion syndrome often present with a T cell lymphopenia. This is caused by defects in the thymic epithelial cells (TECs), following the impaired specification of the 3rd pharyngeal pouch from which the thymus and parathyroid organs develop. Profiling of 4 hypoplastic thymii from 22q11.2ΔS patients revealed a deficiency of a microRNA (miR-205) and its surrounding long noncoding RNA, MIR205HG. Both noncoding RNAs are expressed in normal epithelial cells, with the murine homolog of MIR205HG (MIR205.001) spatiotemporally expressed in the pharyngeal pouches, telencephalon, and the thymus. We developed several mouse models to conditionally ablate each noncoding RNA. Mice lacking miR-205 selectively in TECs exhibited a significant decline in thymopoiesis over time. When stressed with a dsRNA mimic (polyI:C), causing a strong type I interferon response, these mice exhibited a much more severe thymic atrophy compared to littermate controls. These thymic phenotypes were more severe when the lncRNA was conditionally ablated in TECs. Moreover, the targeted elimination of the lncRNA in mice and the conditional ablation of this RNA in the pharyngeal apparatus caused some embryonic lethality, and the pups that were born were small in size and had a reduced fat mass. The molecular mechanisms contributing to these phenotypes are currently being explored. Interestingly, some of the findings are similar to the diverse clinical presentations of 22q11.2 deletion syndrome patients, suggesting that miR-205 and its surrounding lncRNA could contribute to disease pathology.

W.2. Evaluation of a Flow Cytometry-based Lymphocyte Proliferation Assay as an Alternative to Tritiated Thymidine Uptake for Clinical Use

Eszter Lazar-Molnar[1], Karin Chen[2], Thomas B. Martins[2], Attila Kumanovics[1,2] and Harry Hill[1,2]. [1]University of Utah, Salt Lake City, UT; [2]Associated and Regional University Pathologists Institute for Clinical and Experimental Pathology, Salt Lake City, UT

Measuring lymphocyte proliferation *in vitro* is critical in evaluating patients with suspected cellular immune dysfunction. Routinely and historically the ^{3}H-thymidine incorporation assay has been used in clinical laboratories to measure lymphocyte proliferation after mitogenic activation. However, this method requires the use of radioactive material, and it has the limitation that it does not permit simultaneous, lineage-specific identification of the proliferating cells. The aim of this study was to evaluate the performance of a recently developed, non-radioactive assay, which is based on 5-ethynyl-2' deoxyuridine (EdU) uptake, followed by small-molecule based fluorescent labeling and subsequent multi-color flow cytometry detection. After optimizing lymphocyte stimulation conditions and assay parameters, the EdU assay showed very good reproducibility, with low intra-and inter-assay variations. Analysis of healthy controls showed consistent, reproducible proliferative responses and the assay was robust and flexible enough to fit clinical laboratory workflow (delayed stimulation, delayed labeling, or analysis after staining). Analysis of primary immunodeficiency patients showed that the EdU assay is a sensitive method to detect reduced functional responses. Importantly, the data showed very good correlation with the ^{3}H-thymidine incorporation method in both patients and healthy volunteers. Following confirmation in a larger cohort of patients, the Click-iT EdU flow cytometry-based proliferation assay can be a viable alternative for the ^{3}H-thymidine incorporation assay. In combination with various mitogen, antigen and antibody-based stimulation methods it will provide a powerful mechanistic approach to evaluate lymphocyte dysfunction in immunodeficient patients.

W.3. Role of DOCK8 in Thymic Treg Development

Katrina Randall[1], Cindy Ma[2,3], Hsei Di Law[1], Stuart Tangye[3,4], Chris Goodnow[1,2] and Stephen Daley[1,4].

[1]Australian National University, Canberra, Australia; [2]Garvan Institute of Medical Research, Darlinghurst, Australia; [3]University of New South Wales, Darlinghurst, Australia; [4]Monash University, Clayton, Australia

★ = member of a FOCIS Center of Excellence

DOCK8 immunodeficiency is a rare and devastating primary immunodeficiency characterised by susceptibility to a limited range of cutaneous viral infections, recurrent sinopulmonary infections, eczema and allergic disease[1,2]. DOCK8 has been found to play a role in the production or function of a number of cells in the immune system in both mouse and man – including T cells, B cells and NKT cells[1,2,3,4]. The human disease differs from the mouse models in the marked Th2 polarisation seen in the patients with many having concurrent high levels of IgE[1,2].

Conflicting reports have been published about whether there is a numerical deficit in circulating T_{REG} in patients with DOCK8 immunodeficiency but there appears to be a functional defect in *in vitro* suppressive assays of these cells[5]. By contrast, we show that in mice, DOCK8 does not affect the percentage of CD4 T cells that are FoxP3 positive in either the blood or spleen, and that these peripheral Treg appear to have relatively normal suppressor activity in *in vitro* assays. In mice, the predominant defect is a cell intrinsic decrease in the production of FoxP3 positive Treg in the thymus and this defect becomes apparent in the CD4 single positive population prior to the expression of the FoxP3 transcription factor, using novel markers to track Treg thymic maturation.

1. Zhang et al. NEJM, 361:2046, 2009
2. Engelhardt et al. JACI 124:1289, 2009
3. Randall et al. JEM, 208:2305, 2011
4. Crawford at al, Blood, 122:2052, 2013
5. Janssen et al, JACI, 134:1365, 2014

W.4. Lymphocyte Compartment Development in Patients with DiGeorge Syndrome

Adam Klocperk, Anna Sediva, Tomáš Kalina, Eva Froňková, Jana Kayserová, Kristýna Sismová and Ester Mejstříková. ★ 2nd Medical School, Charles University and University Hospital Motol, Prague, Czech Republic;

Purpose: Syndrome diGeorge is associated amongst other clinical signs with various degrees of thymic dysplasia, immunodeficiency and autoimmune disorders. Development of T-lymphocytes is severely impaired, as is T-B lymphocyte interaction. This could be the cause for increased prevalence of autoimmune disorders in these patients. Purpose of this study is to measure the degree of lymphocyte compartment disturbance in patients with molecularly verified diGeorge syndrome, in particular B-lymphocyte subpopulations and Tregs.

Methods: Total T cells, $FoxP3^+$ Tregs, $Helios^+$ $FoxP3^+$ Tregs, total B cells and B-lymphocyte subsets were measured in 46 samples from 37 patients, 21 samples from males, 25 from females, age 3 months to 19 years, using multicolor flow-cytometry.

Results: Total absolute Tregs were significantly lower in diGeorge patients as compared to controls in all age groups (0-20 years) ($p=0.0016$). The percentage of Tregs in $CD4^+$ T cells, however, was not different in patients and controls in all age groups ($p=0.661$) and neither could we find any significant difference in the percentage of $Helios^+$ Tregs between patients and controls ($p=0.238$). Number of switched memory B cells was below healthy reference median in 91% of samples, below healthy reference 5th percentile in 70%. Transitional B cells were above healthy reference median in 91%, above healthy reference 95th percentile in 43%.

Conclusion: Our findings show that B-lymphocyte maturation is indeed impaired in diGeorge syndrome, and that low absolute Tregs are a reflection of typical T cell lymphopenia rather than a specific Treg deficiency. Helios expression was not affected in diGeorge syndrome.

W.5. HLA-DO Expression Correlates with Autoimmunity in Common Variable Immunodeficiency

Raffaele De Palma, Giusi Barra, Giuseppe Spadaro and Giuseppe Pasquale. Second University of Naples, Napoli, Italy

Common Variable Immunodeficiency (CVI) is a heterogeneous group of diseases. A subset of ICV patients develop Autoimmune Diseases (AD) and/or Cancer. Here, we studied the expression of non classical HLA-Class II in patients affected by CVI with and without AD and/or Cancer. 27 patients affected by ICV were studied. Eleven patients had clinical manifestations of AD, as Vitiligo, Thyroiditis, Thrombocytopenia, six had AD and Cancer. After informed consensus, we obtained blood and prepared mRNA to check the expression of "classical HLA-Class II" genes (HLA-DP, HLA-DQ, HLA-DR) and "non classical HLA-Class II" genes (HLA-DM and HLA-DO). All the single chains forming each protein codified by these genes were measured by REAL-TIME PCR. We found a significant increase of the expression of HLA-DOA and HLA-DOB, the two chains forming HLA-DO, only in patients affected by CVI and Autoimmune Disease. Moreover, HLA-DO was upregulated in all six patients with Cancer. No differences were found in the expression of other classical and non classical HLA-Class II genes. HLA-DO has been shown to be regulated differently from other HLA-Class II. In particular, we previously have shown that HLA-DOA is regulated in a peculiar way due to epigenetic mechanisms. HLA-DO has been proposed to have a role in editing the self/non self peptides to be presented to start the immune response. The increased expression of HLA-DO may be a marker of Autoimmunity in CVI and could help to explain how these patients develop autoimmunity and, in many cases, Cancer.

W.6. Increased Small Bowel Permeability Correlates with Autoantibody Levels in RAG1-deficient Murine Model Of Leaky SCID

Boglarka Ujhazi[1,2], Krisztian Csomos[2], Craig Sturgeon[3], Francisco De Beca[4], Zsofia Simon-Vecsei[1], Alessio Fasano[3], Laszlo Fesus[1], Luigi D. Notarangelo[5], Ilma R. Korponay-Szabo[6] and Jolan E. Walter[2,5]. [1]University of Debrecen, Debrecen, Hungary; [2]★Massachusetts General Hospital; Harvard Medical School, Boston, MA; [3]★Massachusetts General Hospital for Children, Boston, MA; [4]Dana-Farber Cancer Institute, Boston, MA; [5]★Children's Hospital Boston, Harvard Medical School, Boston, MA; [6]Heim Pál Children's Hospital, Budapest, Hungary

Rationale: A broad repertoire of autoantibodies has been identified among RAG-deficient patients and *rag1*$^{S723C/S723C}$ (*mut/mut*) mouse model of Leaky SCID (LS). Among others, anti-tissue transglutaminase autoantibody (anti-tTG autoAb) was detected in a fraction of *mut/mut* mice and increased in titers after low dose TLR3 stimulation mimicking chronic viral infection. As the anti-tTG antibodies were similar to those detected in celiac disease we elected to investigate small bowel pathology and permeability in *mut/mut* mice with and without TLR3 stimulation and correlate this to the levels of specific (anti-tTG) and non-specific (ssDNA, dsDNA) autoantibodies.

Methods: *Mut/mut* and wild type mice were injected weekly with low dose TLR3 agonist intraperitoneally (PolyI:C, 50µg). After 5 weeks of treatment, sera were collected and gut permeability was monitored with micro-snapwells by measuring transepithelial electrical resistance (TEER). Small bowel inflammation was determined by Marsh score on histology specimen.

Results: *Mut/mut* mice showed increased small bowel permeability that further worsened upon chronic low dose TLR3 stimulation. Increased small bowel permeability correlated with both specific and non-specific autoantibody levels. *Mut/mut* mice displayed intraepithelial and lamina propria inflammatory infiltration, crypt hyperplasia and villus atrophy with increased Marsh scores compared to *wt/wt* mice.

Conclusions: Previous studies mainly focused on colonic inflammation in human and mice of partial rag deficiency. Our findings shed light that small bowel inflammation and increased permeability is present and can be further induced by TLR stimulation mimicking viral infection in mouse model of Leaky SCID. This process may contribute to the generation of wide array of autoantibodies.

★ = member of a FOCIS Center of Excellence

W.7. TH17 Predominance in Murine Model Of RAG-deficient Leaky SCID: A Novel Aspect of Immune Dysregulation

Jolan E. Walter[1,2,3], Krisztian Csomos[1,2], Katalin Kis-Toth[4], Boglarka Ujhazi[1,2], Divij Matthew[5], Kelly Capuder[3], Stefano Volpi[3], Francesca Rucci[3], Attila Szanto[6], Otto Walter[7], Eva Csizmadia[4], Frederick Alt[8], George Tsokos[4], Marco Colonna[9], Mike Recher[10] and Luigi D. Notarangelo[3]. [1]★Massachusetts General Hospital, Boston, MA; [2]Massachusetts General Hospital for Children, Boston, MA; [3]★Boston Children's Hospital, Boston, MA; [4]Beth Israel Deaconess Medical Center Boston, MA;[5]★University of Colorado at Denver/ National Jewish Health, Denver, CO; [7]University of Massachusetts, Worchester, MA; [8]Harvard Medical School, Boston, MA; [9]★Washington University School of Medicine, Saint Louis, MO; [10]University Hospital, Boston, MA

Introduction: Hypomorphic mutations in recombination activating genes (RAGs) result in severe restriction of T and B cell repertoire and broad clinical features of autoimmune manifestations and hyperinflammation beyond infections. Prior reports suggest viral infections as triggers of hyperinflammation and expansion of autoreactive clones. Although the profile of these autoreactive oligoclonal T cells has not been extensively studied.

Methods: Utilizing a homozygous rag1$^{S723C/S723C}$ (*mut/mut*) mouse model of leaky SCID, T cell subsets and their cytokine profiles (IL-17, TNFα, IFNγ) were assessed by flow cytometry in multiple organs at baseline and after high dose TLR3 stimulation mimicking acute viral infection.

Results: At baseline, despite T cell lymphopenia, accumulation of CD4 T cells were noted in multiple organs (lung, spleen, intestine) and produced more inflammatory cytokines (IL-17, TNFα, IFNγ) after *in vitro* restimulation in *mut/mut* v.s. *wt/wt* mice. In *mut/mut* mice a T cell population co-expressing IL-17 and TNFα cytokines was detected. Mimicking acute viral infection with high dose i.v. poly(I:C) treatment, sublethal to *wt/wt* mice, was fatal within 10 hours in 100% of *mut/mut* mice and induced an inflammatory cytokine surge. Transfer of *wt/wt* regulatory T cell did not prevent the cytokine surge and fatal outcome in *mut/mut* mice, whereas crossing with either MDA5 or TLR3 knock-out mice resulted in increased survival.

Conclusions: In our murine model of leaky SCID with *rag1* mutation, besides TH2 skewing, T cell compartment is also polarized toward a previously undetected TH17 phenotype. This compartment may contribute to hyperinflammation and fatal cytokine surge after acute viral infections.

W.8. Selective IgA Deficiency is Associated with *PVT1*/*MIR1208*, *DGKZ*/*ATG13*/*AMBRA1*, *AHI1* and *CLEC16A*

Paola Bronson[1], Tushar Bhangale[1], Michael Seldin[2], Ward Ortmann[1], Ricardo Ferreira[3], Javier Martin[4], Alessandro Plebani[5], Vassilios Lougaris[5], Tomas Freiberger[6,7], Jiri Litzman[8], Vojtech Thon[8], Qiang Pan-Hammarström[9], Lennart Hammarström[9], Robert Graham[1] and Timothy Behrens[1].[1]Genentech, Inc., South San Francisco, CA; [2]University of California, Davis, CA; [3]Cambridge Institute for Medical Research, Cambridge, United Kingdom; [4]Consejo Superior de Investigaciones Científicas, Granada, Spain; [5]University of Brescia, Spedali Civili di Brescia, Brescia, Italy; [6]Centre for Cardiovascular Surgery and Transplantation, Brno, Czech Republic; [7]Masaryk University, Brno, Czech Republic; [8]St. Anne's University Hospital, Brno, Czech Republic; [9]★Karolinska Institutet, Stockholm, Sweden

IgAD is the most common primary immune deficiency. Association of IgAD with *HLA-DQB1*02:01* is well established, and a previous GWAS identified *IFIH1* Ala946 as the first non-MHC locus for IgAD. The current study tested ~9.5M variants in 1,635 IgAD patients and 4,852 controls. The strongest association was 2.7kb upstream of *HLA-DQA1* (rs9272226**C*, OR=0.31, *P*=3.3x10^{-92}). Association with *IFIH1* Ala946 was confirmed (OR=0.7, P=3.7x10^{-15}). There was an additional protective association of a rare *IFIH1* missense variant rs35667974**C* (Val923; OR=0.34, P=2.6x10^{-8}). Four novel non-MHC loci showed significant association (P<5x10^{-8}) with IgAD: *PVT1/MIR1208* (rs11299600*TA/T; OR=0.73, 4.31x10^{-11}), *DGKZ/ATG13/AMBRA1* (rs4565870*C, OR=1.38, P=6.7x10^{-10}), *AHI1* (OR=1.3, P=8.4x10^{-10}), and *CLEC16A* (rs34069391**GT*; OR=0.71, P=1.4x10^{-9}). 25 additional loci had suggestive

association ($5x10^{-8}$<P<$1x10^{-5}$), including *FAS* (rs12257092*T, OR=1.27, P=$6.7x10^{-8}$) and *GATA3* (rs1244181*A, OR=0.73, P=$5.3x10^{-8}$). A GWAS-based pathway analysis supports a shared genetic etiology between IgAD and autoimmunity, and enrichment for association in *FOXP3*$^+$ T regulatory cell (T reg) signature genes. 13 genes intersect multiple significant pathways: *FAS*, *CD86*, *CD28*, *IL2*, *IL2RA*, *CD80, TPO*, *IL10*, *CD40*, *IL1A*, *IL1B*, *IL6*, and *FASLG*. Interrogation of peak IgAD variants, and variants correlated with peak IgAD variants, for regulatory function (eQTLs, histone marks and open chromatin in immune cells, transcription factor binding sites) identified 45 regulatory variants, including risk eQTLs for lower *AHI1* expression. GWAS loci for IgAD and autoimmune disease were enriched for regions bound to *FOXP3* (ChIP-Seq peaks) and transcribed in T regs (active histone marks). These data identify additional genes contributing to IgAD risk, and support the hypothesis that autoimmune dysregulation contributes to IgAD pathogenesis.

W.9. Hemophagocytic Lymphohistiocytosis and Subcutaneous Panniculitis T Cell Lymphoma Associated to a Heterozygous Mutation in STXPB2

Ricardo Pujol Borrell[1], Monica Martinez-Gallo[2], Roger Colobran[3], Laura Vinas[2], Laura Alonso-Garcia[2], Ingrid Lopez-Lerma[2], Joan Carles Ferreres[2], Cristina Diaz de Heredia[2] and Jose Luis Dapena[2]. [1]★Universitat Autònoma de Barcelona, Barcelona, Spain; [2]Hospital Universitari Vall d'Hebron, Barcelona, Spain; [3]Vall d'Hebron Institut de Recerca, Barcelona, Spain

Hemophagocytic lymphohistiocytosis (HLH) is a life threatening disorder whose main features are prolonged fever, cytopenia, hypercytokinemia, hemophagocytosis and hepatosplenomegaly. Mutations in the genes involved in the cytotoxic mechanism cause the familial form of HLH (FHL). Secondary forms are triggered by infections, sepsis, malignant diseases, autoimmune disease, macrophage activation syndrome and rare conditions.

Here, we report the case of an 11-year-old boy from non-consanguineous parents who developed a HLH associated with a parvovirus-*B19* infection. The patient fulfilled more than 5 criteria to establish the diagnosis of HLH (prolonged fever, pancytopenia, esplenomegaly, bone-marrow hemophagocytosis, hyperferritinemia and impaired cytotoxic activity). The genetic analysis identified a new heterozygous mutation in *UNC13D gene* (p.Arg1075Gln) and a heterozygous missense SNP in *STXBP2* gene (Val205Ile). The rest of genes involved in FHLH were studied and mutations were discarded. He was successfully treated according to HLH2004-protocol with a complete remission. However, three years later the patient was presented with a subcutaneous panniculitis T cell lymphoma associated with HLH and laboratory test consistent with HLH reactivation. Histopathologic findings from skin biopsy revealed a mixed-pattern of panniculitis and lipophage macrophages. Finally, the patient was treated with hematopoietic stem-cell transplantation.

Similar presentation have been reported by Pasqualini et al. in an 11-year-old boy who developed a relapsing HLH associated to cytophagic-histiocytic panniculitis carrying also a monoallelic missense mutation in *STX11* gene. Together with the recent first demonstration of the pathogenic role of dominant-negative *STXBP2* mutations leave an open the question of how interpret heterozygous mutations in genes involved in the pathogenesis of HLH.

W.10. First Human Correlate of *RUVBL2* Defect: Interface of Adaptive and Innate Immunity

Yuliya Kleschenko[1], Shawn Lipinski[2], Ozlem Goker-Alpan[2], Mattew Plassmeyer[1], Denise Loizou[2], Oral Alpan[2] and Michelle Tseng[1]. [1]Amerimmune, Fairfax, VA; [2]O&O ALPAN, Fairfax, VA

A 47-year-old female was referred for genetic evaluation for a decade long recurrent infections and muscle weakness. She had reductions in all of her antibody levels; IgG 541mg/dl, IgA 28mg/dl and IgM of 32mg/dl, absent S. *pneumonia* and H. *influenza* titers post vaccination, normal T, B and NK cell numbers, but low IgM expressing B cells. She had a normal CD40L up-regulation on T cells with PMA/ionomycin stimulation but CD40 expression on B cells showed a dim pattern compared to age matched control. CD69 did not upregulate on IgG$^+$ and IgM$^+$CD20$^+$ cells

after CD79b stimulation. Whole Exome Sequencing identified a variant in the *RUVBL2* gene, IVAS8+5G>A (c.633+5G>A). The patient was also found to be a compound heterozygote for mutations in the *SCN10A*, possibly explaining her other phenotypic findings of muscle weakness. *RUVBL2* encodes a DNA helicase involved in recombination and double strand-break repair and is an ATP-binding protein that belongs to the AAA^+ family of ATPases. In mice, it is required for T cell development and T-dependent antibody responses. Intracellular staining for *RUVBL2* protein showed no apparent difference in the patient lymphocytes compared to control, however, monocytes showed significant up-regulation. This is the first description of a human with *RUVBL2* variant with a clinical phenotype of antibody deficiency where it seems to have impact on T-independent B cell response as well as raising the possibility of impact of an innate immune cell, such as the monocyte, on adaptive immune response.

Immunodermatology

OR.19. Pro-growth/Survival Effect of IL9 Breaks Peripheral Immune Tolerance in Psoriasis and Psoriatic Arthritis

Siba Raychaudhuri[1,2] and Smriti Raychaudhuri[1,2]. [1]Veterans Affairs Medical Center, Sacramento, CA; [2]University of California, Davis, Mather, CA.

IL-9 is secreted by naive $CD4^+$T cells in response to TGF-β and IL-4. IL-9 is a growth factor for T cells and also induces Th17 cells differentiation, thus regulate T cell mediated immune response. Regulatory role of the Th9 cells in human immune system and its aberrant activation in autoimmune diseases is currently being investigated. We evaluated sorted $CD3^+$T cells from peripheral blood (PB) and synovial fluid (SF) of psoriatic arthritis (PsA) (n=10), rheumatoid arthritis (RA) (n=10) and osteoarthritis (OA, n=10) to determine its functional significance in the pathogenesis of PsA. IL-9 levels were significantly elevated in SF (689.8±47.81pg/ml) and serum (501.3±37.13pg/ml) of PsA and RA patients (serum:1065±90.01pg/ml and SF:773±82.9pg/ml) compared to SF (369.1±36.84pg/ml) and serum of OA (307.3±50.39pg/ml). Activated T cells of PsA-SF had higher levels of IL-9R (IL-9 receptor). PsA-SF were enriched with IL-9 producing $CD3^+$T cells compared to OA-SF. Further we demonstrated that activated synovial T cells of PsA and RA produced significantly more IL-9 than OA ($p<.001$, t-test). IL-9 induced significant proliferation of $CD3^+$T cells of PB and SF from RA and PsA compared to media ($p<.001$, t-test). IL-9R-ab inhibits IL-9 induced proliferation of the PsA- and RA-SF $CD3^+$T cell. Important observation is that IL-9 is functionally active, and a pro-growth/survival factor for the localized pathologic T cells in the inflammatory SF. The pro-growth/survival function of IL-9 may be a contributing factor for alteration of peripheral immune tolerance. To our knowledge, this is the first report on functional role of IL-9 in human autoimmune arthritis.

OR.34. Cellular Stress Caused by Disturbed Lipid Metabolism in Fetal Skin Predisposes for Development of Allergic Disorders

Denis Khnykin[1,3], Meei-Hua Lin[2], Louise Bjerkan[3], Naoko Goto-Inoue[4], Yoshikazu Uchida[5], Ingrid Hausser[6], Jeffrey H. Miner[2] and Frode Jahnsen[1,3]. [1]★Oslo University Hospital-Rikshospitalet, Oslo, Norway; [2]★Washington University School of Medicine, St.Louis, MO; [3]University of Oslo, Oslo, Norway; [4]Tokyo Metropolitan University, Tokyo, Japan; [5]★University of California San Francisco, San Francisco, CA; [6]★University Clinic Heidelberg, Heidelberg, Germany

The prevailing hypothesis that a compromised skin barrier is a major factor predisposing to development of atopic diseases is inconsistent with the fact that most skin barrier diseases are not associated with atopy. Here we show that the rare skin disorder Ichthyosis Prematurity Syndrome (IPS), caused by mutations in the SLC27A4 gene encoding fatty acid transport protein 4 (FATP4), is strongly associated with atopic dermatitis-like skin inflammation, IgE-mediated atopic manifestations beyond the skin, and a peripheral blood eosinophilia. Importantly, skin inflammation and eosinophilia were already present in utero, indicating the inherent propensity of affected keratinocytes to generate proinflammatory signals. Intrinsically activated keratinocytes were also found in mouse

fetuses lacking FATP4 and in grafted *Fatp4* mutant skin. Finally, we show that FATP4 mutations lead to lipid misbalance in affected keratinocytes resulting in oxidative and tissue stress. These results suggest that cellular stress caused by altered lipid metabolism in the epidermis is a key factor triggering the deregulated activation of keratinocytes and creating a proallergic microenvironment independent of the external milieu and the adaptive immune system. A combination of cutaneous epidermal stress and increased exposure to allergens due to a compromised skin barrier may be a general mechanism predisposing to systemic allergic responses.

OR.42. Interacting Psoriasis Cytokine Pathways Differentially Modulated by Four Biological Therapeutics or Candidates

*Chris Russell[1], Jeannette Bigler[1], James Chung[2], Paul Klekotka[2], David Martin[1] and **Ajay Nirula**[2]. [1]Amgen, Seattle, WA; [2]Amgen, Thousand Oaks, CA*

Autoimmune inflammatory diseases, including psoriasis, result from interactions of underlying genetic susceptibility, environmental inputs, and molecular responses that lead to altered regulatory feedback loops. Using transcriptional profiling and cellular analysis of biopsy samples obtained from psoriasis and other dermatological clinical trials, we have established a system to identify coordinated gene sets indicative of specific cell types and/or of active cytokine signaling pathways. Psoriasis lesional skin consistently demonstrates multiple elevated cytokine pathways, including IL-17, TNF, IL-23 and interferon(s). Relative activation of these pathways varies between individuals, and can affect response to therapeutics. We examined pathway specific temporal responses to targeted biological therapeutics against IL-23p19, TNF, IFN-gamma, and IL-17RA in baseline and post-treatment biopsies at time points up to week 52 where each therapeutic exerted distinct effects.

Inhibition of IL-17, IL-23, or TNF pathways each led to reduced activity of the other two pathways along with therapeutic benefit, but with different timescales. IL-17RA blockade by brodalumab generated the most rapid molecular change across the broadest range of genes that compose the psoriasis transcriptome. Changes included normalization of downstream IL-17 and TNF signature genes within two weeks, tightly coupled to patient response, and slower normalization of genes downstream of IL-23 or IFN-gamma. Targeting of TNF or IL-23 rapidly reduced the specific mRNAs immediately downstream of each cytokine, with slower effects on other pathways, in responding patients. The data demonstrate that the TNF, IL-23, and IL-17 pathways all contribute to an interdependent positive feedback loop necessary to drive the inflammatory state.

W.11. Analysis and Modulation of the Interplay Between T Cells and Keratinocytes in Allergic Contact Dermatitis

***Daniel Deibel**, Isabel John, Guido Wabnitz and Yvonne Samstag. ★Institute of Immunology, Heidelberg, Germany*

Allergic contact dermatitis (ACD) is a very frequent occupational skin disease, which results in a T cell mediated damage of skin. As the most common cell type in the skin, keratinocytes (KC) exhibit a variety of important immune functions in ACD. Already in the sensitization phase KCs release many pro-inflammatory cytokines and chemokines, which can attract T cells to the epidermis. We are currently investigating the interplay between T cells and KCs during the initiation and elicitation phase of ACD. In this regard we could demonstrate, that primary human KCs can be induced to express important antigen presenting cell relevant surface molecules (HLA-DR, ICAM-1, CD80) when treated with IFN-γ. Furthermore, loading with superantigen and co-culturing with human peripheral blood T cells led to an upregulation of activation molecules on T cells and subsequently to a strong T cell proliferation. To characterize the importance of KCs as non-professional antigen presenting cells and their interaction with T cells in ACD in detail, we have investigated the formation of immune synapses between both cells as well as the functional consequences of this interaction (T cell activation and proliferation) .

W.12. Study of *KIR/HLA* Genotypes in Mestizos from Western Mexico with Psoriasis Vulgaris

Omar Graciano Machuca[1], Anabell Alvarado-Navarro[1], Erandi Velarde de la Cruz[1], Guadalupe Villanueva-Quintero[2], María Guadalupe Ramírez Dueñas[1] and Pedro Ernesto Sánchez Hernández[1]. [1]Universidad de Guadalajara, Guadalajara, Mexico; [2]Instituto Dermatológico de Jalisco, Guadalajara, Mexico

Background: Psoriasis vulgaris is a chronic inflammatory skin disease. Part of this inflammatory infiltrate consists of $CD4^+$ and $CD8^+$T lymphocytes, and NK cells. The function of NK cells and some populations of T lymphocytes are regulated by interaction between KIR and HLA molecules. Certain *KIR* genes and *HLA* alleles have shown associations with development of autoimmune diseases, including psoriasis.

Objective: Determine the association between KIR/HLA genotypes and PsV in mestizos from western Mexico.

Materials and methods: Ninety-nine patients diagnosed with PsV and eighty healthy subjects (HS) were typed for 14 *KIR* genes and *HLA-C1* and *C2* groups by PCR-SSP Comparisons of gene and genotype frequencies were performed by Chi-square test and presented with odds ratio, 95% confidence intervals.

Results: No significant differences in gene frequencies of *KIR* and *HLA-C* groups were found. However, a higher frequency of *KIR2DL2* and *2DS2*in PsV group compared with HS was observed.

Conclusions: Our results show no association between *KIR/HLA* genes and PSV. In addition, other genetic and environmental factors may be playing a leadership role in the development of this pathology in the mestizo population of western Mexico. A high-resolution typing is necessary to determine if the presence of specific alleles may have a susceptibility role for PsV.

W.13. Conditioning of Dendritic Cells with Fibroblasts Causes Polarization of Dendritic Cells Toward a Tolerogenic State

Mohsen Khosravi-Maharlooei, Reza Jalili, Sanam Salimi-Elizeh, Jacqueline Lai, Mohammadreza Pakyari, Malihesadat Poormasjedi-Meibod, Ruhangiz Kilani, Jan Dutz and Aziz Ghahary. ★University of British Columbia, Vancouver, BC, Canada

There is a controversy about the effect of fibroblasts on dendritic cells (DCs). We checked different features of fibroblast primed DCs including their ability to express co-inhibitory and co-stimulatory molecules, pro- and anti-inflammatory cytokines and ability to induce T cell proliferation. Also, we checked their ability to migrate to lymphatic tissues and present fibroblast derived antigens after encountering fibroblasts. Our results showed that both co-inhibitory (PD-L1, PD-L2 and B7H4) and co-stimulatory (CD86) molecules were up regulated in *in vitro* cultured DCs after encountering fibroblasts. After intra peritoneal injection of fibroblasts, both syngeneic and allogeneic fibroblasts caused significant increase in total DC count and the percentage of DCs having co-inhibitory and co-stimulatory molecules. Priming of DCs with syngeneic and allogeneic fibroblasts caused reduced proliferation of CD4 and CD8 T cells. Even activation of fibroblast primed DCs couldn't retrieve their ability to induce T cell proliferation. Likewise, priming of DCs with fibroblasts blocked the ability of ovalbumin-pulsed DCs to induce proliferation of ovalbumin-specific CD4 T cells. Compared to non-activated DCs, fibroblast-primed DCs had significantly higher expression levels of IL-10 and IDO. Fibroblast-primed DCs had significantly reduced IL-12 expression level compared to activated DCs. After priming with fibroblasts, DCs could migrate to lymphatic tissues and present fibroblast-derived antigens (ovalbumin). As we only checked presentation of ovalbumin on MHC-I, it can either show that fibroblast membrane is integrated into DC membrane or fibroblast derived antigens are cross-presented on MHC-I of DCs. In conclusion, after priming with fibroblasts, DCs gain tolerogenic features.

W.14. Immune Signature of Psoriasis: Gene Expression Profiles in the Blood

Dario Kivelevitch[1], Caitrona Ryan[2], Xuan Wang[1], Mamta Sharma[1], Nandhini Raman[1], Bobbak Mansouri[2], Alan Menter[2] and ***Gerlinde Obermoser****[1]. [1]Baylor Institute for Immunology Research, Dallas, TX; [2]Menter Dermatology Research Institute, Dallas, TX*

Psoriasis is a chronic, inflammatory skin disease that affects 2-3% of the population worldwide. Dysregulation of the immune system is considered a major contributor to disease pathogenesis. A total of 143 untreated adult patients with moderate to severe plaque psoriasis were enrolled in two cohorts in addition to 71 matched healthy controls and clinical data and blood specimen were collected. Patients were off topical drugs for 2 weeks, systemic medication for 4 weeks, TNFα inhibitors for 12 weeks, and anti-IL-12/IL-23 treatment (ustekinumab) for at least 24 weeks. In a subgroup of 35 patients effect of treatment with anti-TNFa (adalimumab) or anti-IL-12/IL-23 was studied at a week 16 follow-up visit. 65% of patients were male with a mean age of 46 years, disease duration 18 ys, PASI 14. Using a modular analysis framework to study gene expression in the blood, we observed a distinct transcriptional profile in untreated psoriasis patients compared to healthy controls with over-expression of inflammation related modules (M4.2, M4.13, M5.1), and myeloid lineage (M3.2, M4.6), while cytotoxicity-related modules (M3.6, M4.15) and protein synthesis annotated modules (M4.3, M5.9) were under-expressed. These changes reversed upon treatment in the patients studied at week 16. While some of the observed changes may be interpreted with an efflux of immune cells from the blood to the skin (for example NK cells), others may be related to the systemic comorbidity in psoriasis. Our study provides framework and tools for systems studies of psoriasis as a systemic disease, treatment response, and future personalized medicine applications.

Immunology of the Eye

W.15. Frequency and Function of Regulatory T Cells in Intraocular Tuberculosis

Ravi Kumar*, Naresh Sachdeva, Reema Bansal, Sonia Virk, Surya Prakash Sharma and Amod Gupta. Post Graduate Institute of Medical Education and Research, Chandigarh, India*

Intraocular tuberculosis (TB) is an ocular inflammatory disease prevalent in India and other tropical countries. The role of T cells and their subsets in pathogenesis of intraocular TB is not completely understood. We hypothesize that intraocular TB involves an imbalance between regulatory T cells (Tregs) and proinflammatory (Th1 and Th17) cells leading to ocular inflammation.

We recruited 15 subjects with PCR confirmed intraocular TB along with 15 disease controls (patients with macular hole or epiretinal membrane). After obtaining informed consent, 12 ml of heparinised peripheral blood was collected. Peripheral blood mononuclear cells were isolated for immunophenotyping of T cells including Tregs, Th1 and Th17 cells, stimulation assays and intracellular cytokine analysis.

The frequency of $CD4+CD25^{hi}FoxP3^{+}$ Tregs was lower in intraocular TB (0.69±0.57%) as compared to disease control group (3.13±2.55%) (p=0.002). Tregs in intraocular TB also showed lesser expression of IL-2 receptor (p=0.0005) and intracellular TGF-β (p=0.003). There was no significant difference in CD152 expression on Tregs in the two subject groups. Following *in-vitro* stimulation, the frequency of Th1 cells increased in intraocular TB subjects (19.70±13.57) as compared to controls (6.0±5.48) (p=0.015), whereas no significant difference was observed in the frequency of Th17 cells between the two groups. Our results indicate that a decreased frequency and function of Tregs is associated with an elevated Th1 response in patients with intraocular TB. Our findings further suggest that lesser number and hypo-suppressive phenotype of Tregs may have pathogenic implications in situations where an autoimmune response is worsened by tubercle bacilli or its antigens.

W.16. Early B Cell Depletion Attenuates Experimental Autoimmune Uveitis by Limiting Pathogenic T Cell

Priming

Sohyun Jeon, Yingyos Jittayasothorn and Rachel Caspi. National Eye Institute, National Institutes of Health, Bethesda, MD

B cell depletion using Rituximab (anti-CD20) is beneficial in treating autoimmune diseases such as systemic lupus erythematosus and multiple sclerosis, but there are few studies on the role of B cells in ocular autoimmunity. We used a mouse model of experimental autoimmune uveitis (EAU) induced in B10.RIII mice by immunization with Interphotoreceptor Retinoid-Binding Protein (IRBP) and/or its pathogenic epitope $IRBP_{161-180}$. Mice depleted of B cells by anti-CD20 treatment before immunization with $IRBP_{161-180}$ showed a significant reduction in disease severity and associated T cell responses, as measured by the number of IFN-γ^+ and IL-17A$^+$ CD4 cells and Bcl6$^+$ Tfh cells. Interestingly, Th17:Th1 ratio was increased in response to B cell depletion suggesting that B cells may preferentially help Th1 differentiation. In contrast, there was no effect on disease severity when B cells were depleted 7 days after immunization, compatible with a role for B cells in antigen presentation rather than in effector or regulatory mechanisms in this model. Furthermore, early B cell depletion had minimal or no effect when B10.RIII mice were immunized with native IRBP protein rather than peptide, suggesting that B cells might preferentially present antigen(s) that do not require extensive processing. The data suggest that rituximab may hold promise as treatment for chronic uveitis by reducing the priming and recruitment of new antigen-specific T cells into the effector pool.

W.17. Anterior Segment Optical Coherence Tomography (OCT) in Rodent Models of Uveitis

Kathryn Pepple, Woo Jun Choi and Ruikang Wang. ★University of Washington, Seattle, WA

Uveitis, or ocular inflammation, is a blinding disease. Animal models of uveitis have identified immune mechanisms underlying uveitis pathogenesis and have been used to test new therapies. However, due to the small size of rodent eyes, features of inflammation such as anterior chamber (AC) cell are difficult to assess without sacrificing the animal for tissue analysis. Optical Coherence Tomography (OCT) is a non-invasive imaging modality that can detect and automatically quantify AC cell in human patients with uveitis. We describe here methods to adapt these techniques for use in animal models of uveitis, and development of an image based algorithm for objective assessment of the level of AC inflammation in rodent eyes. This system is a non-lethal assay that allows for repeat analysis of a single animal over the natural course of the disease or in response to treatment. For this study, acute inflammation was induced in 13 Lewis rats and 7 C57Bl6 mice. Eyes were imaged using either a spectral domain (SD) OCT system or a Swept Source (SS) OCT system at serial time points over the course of inflammation. Images were segmented and AC cell counts were performed with novel software. AC cell counting performed by the automated software correlated well with results obtained by human graders, and histology confirmed the inflammation visualized by OCT. In conclusion, OCT imaging of the rodent AC provides for longitudinal *in vivo* detection of ocular inflammation that correlates well with post mortem histologic evidence of inflammation and can be quantified automatically.

Immunoncology

OR.36. Photon Irradiation Enhances the Efficacy of Antigen-specific Vaccination in Experimental Glioma

Martina Ott[1,2], Katharina Ochs[1,2,4], Sara Chiblak[1,3], Michael Breckwoldt[1,4], Amir Abdollahi[1,3], Wolfgang Wick[2,1] and Michael Platten[1,2]. [1]German Cancer Research Center (DKFZ), Heidelberg, Germany; [2]Center for Tumor Diseases, Heidelberg, Germany; [3]★University of Heidelberg Medical School, Heidelberg, Germany; [4]University Hospital Heidelberg, Heidelberg, Germany

Glioblastoma is the most common and aggressive human brain tumor. Despite multimodal therapy the prognosis for Glioblastoma patients is poor with a median survival of 14 month after diagnosis. Antigen-specific vaccination targeting tumor antigens such as EGFRvIII or IDH1R132H are in clinical development in gliomas. However,

microenvironmental factors promote local immunosuppression and-evasion. In addition, there is uncertainty, how to integrate active immunization in standard of care, which is radiochemotherapy. We investigated the effect of the combinatorial treatment of peptide vaccination and low dose irradiation in a syngeneic experimental malignant gliomas model. To this end mice bearing intracranial gliomas, which overexpress the highly immunogenic gp100, were either vaccinated, irradiated using 5x2Gy or received the combinatorial treatment. While peptide vaccination showed no effect on tumor growth and irradiation delayed glioma growth, the combination of both led to complete tumor regression in approximately 70% of the mice. Analysis of tumor infiltrating lymphocytes (TIL) revealed an increase of gp100-specific TILs in the combination group. Additionally, there was high PD1 expression on gp100-specific TILs in the vaccinated group, but the expression was almost abolished in the combination group. An adoptive transfer of gp100-specific $CD8^+$T cells from TCR transgenic (Pmel-1) mice in combination with irradiation also increased the number of gp100-specific TILs, however tumor growth was not influenced suggesting that merely increasing the number of tumor-specific CTLs is not sufficient to induce tumor regression. Our data suggest that low-dose irradiation alters the gliomas microenvironment to promote the infiltration of glioma-specific T cells and enhance the efficacy of antigen-specific peptide vaccination.

W.18. Detection of Metastatic Breast Cancer Cells in the Sentinel Lymph Node by Flow Cytometry

Ciputra Hartana, Laszlo Szekely and Ola Winqvist. ★Karolinska Institutet, Stockholm, Sweden

Background: The Sentinel node, as the first tumor draining lymph node, is frequently the first site of metastasis and an ideal site to harvest T cells for immunotherapy. In order to use the sentinel node for immunotherapy, we need to secure a single cell detection method of metastasis.

Aim: To investigate the use of FACS for detection of sentinel node metastasis in breast cancer patients.

Methods and Results: PBMCs were spiked with MCF-7 breast cancer cell lines in concentrations from 9% to 0.012%. Cells were stained intracellularly for Pancytokeratin and epithelial cell adhesion molecule (EpCAM). Pancytokeratin positive breast cancer cells were reliably detected down to as few as 0.012% events. However, 0.005% of background staining was detected when only PBMC was stained with Pancytokeratin. Staining for EpCAM resulted in an almost complete recovery of tumor cells, and as few as 0.012% added cells were detected with no EpCAM positive cells were found in PBMC only samples. The use of FACS for detection of metastatic breast cancer cells will be verified in 100 sentinel nodes from patients with breast cancer after comparing with conventional H&E staining and Immunohistochemistry. Expanded sentinel node T cells will be cultured under optimal growth conditions with immunophenotyping and cancer detection by FACS of the cultured cells will in turn be used as release criteria for transfusion.

Conclusion: FACS is a fast, reliable, and sensitive method for detection of lymphatic metastasis allowing for a sentinel node based immunotherapy clinical trial of breast cancer patients.

W.19. Comparison of Immunity in Mice Cured of Primary/Metastatic Growth of EMT6 or 4THM Breast Cancer by Chemotherapy or Immunotherapy

Reginald Gorczynski[1] Nuray Erin[2] and Anna Podnos[1]. [1]University Health Network, Toronto, ON, Canada; [2]Akdeniz University College of Medicine, Antalya, Turkey

EMT6 tumors are cured in CD200R1KO mice following surgical resection and immunization with irradiated EMT6 cells and CpG. Wild-type (WT) animals developed pulmonary and liver metastases within 30 days of surgery. We describe growth and metastasis of both EMT6 and a highly metastatic 4THM tumor in WT mice receiving Fab anti-CD200R1 along with CpG/tumor cell immunization. Metastasis was followed macroscopically (lung/liver nodules) and microscopically by cloning tumor cells *in vitro* from draining lymph nodes (DLN) harvested at surgery. We compared

these results with local/metastatic tumor growth in mice receiving 4 courses of combination treatment with anti-VEGF and paclitaxel. In WT mice receiving Fab anti-CD200R, no tumor cells were detectable following immunotherapy, and $CD4^+$ cells produced increased TNFa/IL-2/IFNg on stimulation with EMT6 *in vitro*. No long-term cure was seen following surgery/immunotherapy of 4THM, with both microscopic (tumors in DLN at limiting dilution) and macroscopic metastases present within 14d of surgery. Chemotherapy attenuated growth/metastases in 4THM tumor-bearers and produced a decline in lung/liver metastases, with no detectable DLN metastases in EMT6 tumor-bearing mice-these latter mice nevertheless showed no significantly increased cytokine production after restimulation with EMT6 *in vitro*. EMT6 mice receiving immunotherapy were resistant to subsequent re-challenge with EMT6 tumor cells, but not those receiving curative chemotherapy. Anti-CD4 treatment caused tumor recurrence after immunotherapy, but produced no apparent effect in either EMT6 or 4THM tumor bearers after chemotherapy treatment.

Conclusion: Immunotherapy, but not chemotherapy, enhances $CD4^+$ immunity and affords long-term control of breast cancer growth and resistance to new tumor foci.

W.20. Tumor Infiltrating Regulatory T Cells in Urinary Bladder Cancer are Highly Activated, Correlate with Tumor Stage and Display an Induced Regulatory Phenotype

***Malin Winerdal**[1], David Krantz[1], Robert Rosenblatt[2], Ali Zirakzadeh[1], Emma Ahlén Bergman[1], Max Winerdal[1], Johan Hansson[3], Benny Holmström[4], Markus Johansson[5], Per Marits[1], Amir Sherif[2] and Ola Winqvist[1]. [1]★Karolinska Institutet, Stockholm, Sweden; [2]Umeå University, Umeå, Sweden; [3]Uppsala University, Uppsala, Sweden; [4]Akademiska University Hospital, Uppsala, Sweden; [5]Sundsvall-Härnösand County Hospital, Sundsvall, Sweden*

Muscle invasive bladder cancer has poor prognosis despite modern treatments. As immunotherapeutic regimens are promising, there is a need to better understand the cross-talk between the tumor and the immune system. Tumor infiltrating lymphocytes (TIL) expressing the Treg transcription factor FOXP3 has been linked to both good and poor prognostic claims in different forms of cancer. Here, we investigate the Treg population in urinary bladder cancer patients, where we have previously shown that $FOXP3^+$ TIL are associated to better prognosis.
Patients with suspected muscle invasive urinary bladder cancer were included in the study. Samples from tumor, macroscopically healthy bladder tissue, sentinel and non-sentinel lymph nodes and peripheral blood were collected at TUR-B and/or cystectomy for analysis by multicolor flow cytometry. Stable commitment to the Treg lineage was investigated by epigenetic analysis of sorted $CD4^+$ T-lymphocyte populations. The $CD4^+FOXP3^+$ Treg fraction was significantly higher in the tumor than all other sited investigated including non-malignant bladder. These Treg TILs were $CD45RO^+$ and expressed high levels of activation and Treg effector markers such as CD69, HLA-DR, CD39 and CTLA-4. Interestingly, muscle invasive tumors had lower TIL $CD4^+FOXP3^+$ fraction at the invasive front of the tumor compared to non-invasive tumors. Also, $CD4^+$ TIL displayed a highly methylated *FOXP3* promoter, suggesting that these cells were not committed Treg but rather $CD4^+$ T-lymphocytes with induced FOXP3 expression. Speculatively, differences between induced FOXP3 expression and stably committed Tregs may account for the spread of prognostic impact of $FOXP3^+$ TILs in different forms of cancer.

W.21. B Cells in Tumor Draining Lymph Nodes are Good Antigen Presenting Cells in Patients with Urinary Bladder Cancer

***Ali Zirakzadeh**[1], David Krantz[1], Malin Winerdal[1], Christian Lundgren[1], Emma Ahlén Bergman[1], Ciputra Adijaya[1], Johan Hansson[2], Benny Holmström[3], Markus Johansson[4], Per Marits[1], Amir Sherif[5] and Ola Winqvist[1]. [1]★Karolinska Institutet, Stockholm, Sweden; [2]Uppsala University, Uppsala, Sweden; [3]Akademiska University Hospital, Uppsala, Sweden; [4]Sundsvall-Härnösand County Hospital, Sundsvall, Sweden; [5]Umeå University, Umeå, Sweden;*

Cancer is one of the leading causes of death with seven million deaths world wide, each year. Previous work by our group has demonstrated that the *in vitro* expansions of sentinel node-acquired, tumor specific $CD4^+$ T cells are

promising to be used in adoptive immunotherapy. In order for naive T helper cells to get activated, they need APCs, presenting the antigen for them using MHC-II. In this study, we have used FASCIA (Flowcytometric Assay of Specific Cell-mediated Immune response in Activated cells) to study B cells and their role as APCs in patients with urinary bladder cancer. We isolated lymphocytes from blood, sentinel node and tumor of the patients and stimulated them with autologous tumor extract. After cultivation for 6-7 days, the cells were analyzed by multi-color flow cytometry, using FASCIA. In our preliminary data patients displayed a higher B cell activation in sentinel node compared to non-sentinel node and blood, after stimulation with autologous tumor extract for 6-7 days. Furthermore, $CD4^+$ T cells from sentinel node could form blasts and get activated after being in co-culture with sentinel node acquired B cells in presence of tumor antigen. However the $CD4^+$ T cells could not form blasts and get activated when co-cultured with B cells treated with HLA-DR-blocking antibody. This indicates antigen presenting ability of sentinel node acquired B cells. our results demonstrate a $CD4^+$ T cell dependent tumoral B cell response upon stimulation with autologous tumor antigen which may be exploited for immunotherapy.

W.22. Evaluation of Biochemical Balance in Selected Interleukin Profiles Among Patients with Other than Cancer Types of Gastric Neoplasms

Wojciech Blogowski[1], Anna Madej-Michniewicz[2], Marta Budkowska[2], Daria Salata[2], Barbara Dolegowska[2] and Teresa Starzynska[2]. [1]University of Zielona Gora, Zielona Gora, Poland; [2]Pomeranian Medical University, Szczecin, Poland

Gastric neoplasms form a group of heterogenic malignancies that are associated with a high percentage of mortality among affected individuals. The latest results demonstrated that an abnormal network of interactions between multiple cytokines is an important molecular mechanism that supports the development of gastric cancer and its systemic spread. Nevertheless, to date no comprehensive analysis of potential associations between cytokine balance and development of other types of gastric malignancies (that in comparison to cancer have much more favorable prognosis) can be found. Therefore, in this study we i) compared biochemical balance in selected interleukin profiles (IL-1, IL-6, IL-8, IL-10, IL-12, IL-17 and IL-23) among 25 patients with gastric malignancies (gastrointestinal stromal tumors, neuroendocrine neoplasms, lymphomas) and 40 healthy volunteers, as well as, ii) verified the potential associations between systemic levels of examined interleukins and TNM staging of these neoplasms. The results demonstrated that patients with other than cancer type of gastric malignancy have no statistically significant differences in systemic levels of examined interleukins (in all cases at least $p>0.15$). Moreover, similar values of calculated interleukin ratios were also observed (in all cases at least $p>0.09$). Finally, no significant associations were found between systemic interleukins (ratios) values and TNM staging of these neoplasms. Therefore, our study demonstrated that in humans presence of other than cancer type of gastric malignancy is not accompanied by disturbed systemic biochemical balance in multiple interleukins. This phenomenon may be, at least partially, responsible for much more favorable prognosis associated with these neoplasms in comparison to gastric cancer. (IP2014003273)

W.23**. Sensor Function for Butyrophilin 3A1 in Prenyl Pyrophosphate Stimulation of Human Vγ2Vδ2 T Cells**

Craig Morita and Hong Wang. ★University of Iowa, Iowa City, IA, Iowa City, IA and Veterans Affairs Health Care System, Iowa City, IA

Vγ2Vδ2 T cells play important roles in human immunity to pathogens and in cancer immunotherapy by responding to isoprenoid metabolites such as (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate and isopentenyl pyrophosphate. The immunoglobulin superfamily protein, butyrophilin 3A1 (BTN3A1), has been shown to be required for prenyl pyrophosphate stimulation. We proposed that the intracellular B30.2 domain of BTN3A1 binds prenyl pyrophosphates resulting in a change in the extracellular BTN3A1 dimer that is detected by Vγ2Vδ2 TCRs. Such B30.2 binding has been recently demonstrated. However, others reported that the extracellular BTN3A1 immunoglobulin V-like (IgV) domain binds prenyl pyrophosphates leading to the proposal that the Vγ2Vδ2 TCR recognizes the complex. To

distinguish between these mechanisms, we mutagenized residues in the two binding sites and tested the mutant BTN3A1 proteins for their ability to mediate prenyl pyrophosphate stimulation of Vγ2Vδ2 T cells to proliferate and secrete TNF-α. Mutagenesis of residues in the IgV site had no effect on Vγ2Vδ2 T cell proliferation or secretion of TNF-α. In contrast, mutagenesis of residues within the basic pocket and surrounding V regions of the B30.2 domain abrogated prenyl pyrophosphate-induced proliferation. Mutations of residues making ionic bonds to the pyrophosphate moiety also abrogated TNF-α release whereas residues making contact to the alkenyl chain were somewhat more tolerant of alanine mutations. Some mutations further from the B30.2 binding site also diminished stimulation suggesting that the B30.2 domain may interact with a second protein. These findings support intracellular sensing of prenyl pyrophosphates by BTN3A1 rather than extracellular presentation.

W.24. Hyperthermia and Chemotherapy Mediated Effects on Tumor Cell Proliferation and Heat Shock Protein Expression in Human Colon Cancer

Martin Gasser, Tanja Grimmig, Rebecca Thum, Romana Moench, Christoph Germer and Ana Maria Waaga Gasser. University of Wuerzburg, Wuerzburg, Germany

In patients with peritoneal carcinomatosis (PC) of gastrointestinal cancer hyperthermic intraperitoneal chemotherapy (HIPEC) represents a promising treatment option integrated into multimodal concepts. Heat shock proteins (HSP) play a major role in cellular stress response conferring increased resistance in tumor cells. Therefore we analyzed HSP expression profiles in tumor cells exposed to hyperthermic and chemotherapeutic stress. To mimic HIPEC-like conditions HT29 colon cancer cells were exposed to varying hyperthermic conditions for 60 min with additional 5-fluorouracil (5-FU) treatment. HSP expression was analyzed 30 min, 24h, 48h, and 72h after treatment using Western Blot and RT-qPCR. Untreated cells cultivated at 37°C served as controls. Additionally, effects on tumor cell proliferation were determined 24h, 48h and 72h after treatment by MTS-assay. Hyperthermia caused temperature dependent upregulation of HSP27, HSP70, HSP72, and HSP90 gene and protein expression that was further increased by additional cytotoxic 5-FU treatment. 5-FU initiated progressive rise in HSP gene expression up to 72h after 1h exposure at normothermia as well as under hyperthermia. After isolated hyperthermia tumor cell proliferation was recovered at 72h. Antiproliferative effects of 5-FU could not be enhanced by further increased hyperthermia. Antiproliferative effects of hyperthermia induced during HIPEC therapy seem to be negatively influenced by highly conserved HSP mechanisms. Our findings suggest that HSP27, HSP70/72, and HSP90 are significantly involved in hyperthermia and chemotoxicity mediated cell stress repair mechanisms. While initial increase in HSP expression can counteract cytotoxic effects during HIPEC therapy long-term elevated expression levels may cause lasting resistance to cellular stress.

W.25. PDGF Induces Cell Growth and Glycolysis in Colon Cancer

Martin Gasser, Romana Moench, Vinicius Kannen, Tanja Grimmig, Christoph Germer and Ana Maria Waaga Gasser. University of Wuerzburg, Wuerzburg, Germany

The platelet derived growth factor (PDGF) plays an important role in several solid tumors. Involved in cell migration and proliferation primarily of stromal cells in cancers PDGF represents a key target in cancer therapy. The aim of this study was to analyze the specific role of PDGF on tumor cell proliferation and metabolism in colorectal cancer (CRC). The human colon cancer cell line HT-29 was cultured and stimulated time-dependently with PDGF. Additionally, inhibition of the PI3k/Akt/mTOR-pathway was performed simultaneously to PDGF stimulation. 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 investigate the effects of PDGF on proliferation MTS assays were performed. Additionally, mRNA levels of PDGF and metabolic factors in tumors from patients with CRC were analyzed by RT-qPCR. PDGF stimulation resulted in increased HT29 cell proliferation compared to untreated controls. Blocking of Akt resulted in inhibition of pS6, activation of Retinoblastoma (Rb) and reduced tumor cell growth. Additionally, under stimulation a higher glycolytic rate was observed while oxygen consumption remained unaltered. Investigated tumors from patients

with CRC showed a stage-dependent increase in PDGF expression and a boosted glycolytic rate. The cytokine PDGF induced proliferation accompanied with an altered glycolysis in HT-29 colon cancer. An increased glycolysis and tumor cell proliferation support accelerated cell proliferation and tumor growth. The growth reducing effect of Akt inhibition indicates the PI3K/Akt/mTOR-pathway to play a crucial role in CRC progression and therefore could be an important target in cancer therapy.

W.26. Human Glandular Kallikrein (hK2): A Negative Regulator in Prostate Cancer Immunotherapy

Seema Dubey and Dev Karan. ★University of South Carolina School of Medicine, Columbia, SC

To develop an improved therapeutic efficacy of immunotherapy targeting prostate cancer, we used a panel of three genes (prostate-specific antigen: PSA; prostate stem cell antigen: PSCA, and human kallikrein: hK2) to construct the adenovirus-based vaccine. First, we engineered an adenovirus based bivalent vaccine for simultaneous targeting of PSA and PSCA antigens on prostate cancer cells, and demonstrated improved therapeutic efficacy where up to 80% of the mice became tumor free. Using PSA-transgenic mouse, we further demonstrated that administration of Ad-virus mixing with Gelfoam enhanced the cytotoxic T cell activity in the presence of self-antigen, and allowed multiple boosting vaccinations to maintain a long term immune response. To further augment the efficacy of immunotherapy, we developed an ad-vector co-expressing PSA, PSCA, and hK2 antigens (Ad5-PPK2) for simultaneous targeting of three antigens on prostate cancer cells. Mouse prostate tumor cells co-expressing human PSA, PSCA and hK2 antigens were used as tumor targets. Surprisingly, the presence of hK2 antigen in the vaccination regimen abrogates the antitumor immunity of bivalent approach targeting PSA and PSCA antigens. Further analysis of immune responses revealed that hK2 predominantly induces antigen-specific $CD4^+$ T cells which may contribute towards immunosuppression as T regulatory cells, and help tumor cells to escape. While we continue to understand the mechanism of hK2-induced immune evasion, these studies provide a 'clinical proof of concept' and a strong rationale to support the second generation of an Ad-PSA/PSCA targeted immunotherapy for prostate cancer.

W.27. Exosomes Secreted by Multiple Myeloma (MM) Cells and Bone Marrow (BM) Stromal Cells (BMSCs) Reciprocally Modulate Cell Adhesion and Impact on MM Cell Migration and Bortezomib-induced Apoptosis

Josefina Udi[1,2], Reinhard Voll[1], Daniela Ureta[2], Margarita López[2], Ralph Wäsch[1], Monika Engelhardt[1] and Élida Álvarez[2]. [1]★Freiburg University Medical Center, Freiburg, Germany; [2] University of Buenos Aires, Buenos Aires, Argentina

Introduction: In MM pathogenesis, the interaction of plasma cells and the BM microenvironment plays a crucial role. Exosomes are secreted by most cell types and are implicated in intercellular communication, drug resistance and tumor progression.

Objectives: We purified and studied the influence of exosomes secreted by MM cell lines (MMCLs) and BMSCs on cell adhesion, migration and bortezomib-induced apoptosis.

Methods: Exosomes were purified from the supernatants of MMCLs and BMSCs by ultracentrifugation. Purity was confirmed by electron microscopy and western blot for CD63. Exosomal protein concentration was quantified by Bradford. CD44 and ICAM1 were evaluated by flow cytometry. Apoptosis was assessed via annexinV/7-AAD-staining. Chemotaxis to M2-10B4 exosomes was studied with chemotaxis chambers. Migrated cells were counted by flow cytometry.

Results: IM-9 exosomes showed a characteristic saucer-like morphology, ranging in diameter between 30-120nm. MM cell and M2-10B4 exosomes expressed CD63. Exosomal protein concentration from MMCLs varied between 1.2-1.7µg/µl and was 1.4µg/µl for M2-10B4. M2-10B4 cells incubated with MMCL exosomes expressed higher levels of CD44 and ICAM-1. M2-10B4 exosomes induced a significant increase in CD44 on IM-9 cells. IM-9 apoptotic cells

after 24h with 10nM bortezomib decreased from 72% to 61% with M2-10B4 exosomes and from 74% to 62% with IM-9 exosomes, suggesting exosome-induced bortezomib resistance. Migration of IM-9 cells increased with 200µg/ml M2-10B4 exosomes.

Conclusions: Our findings underline the role of M2-10B4 exosomes on adhesion and migration of MM cells, and the protection of exosomes on bortezomib-induced cytotoxicity, suggesting their involvement in a paracrine/autocrine loop modulating treatment response and MM cell survival.

W.28. Identification of a Caveolin-1-associated Signature in CD4+ T Cell Malignancies

Tyler Herek[1], Timothy Shew[1], Heather Spurgin[1], Brittany Conroy[1], Tomas Helikar[2] and Christine Cutucache[1]. [1]University of Nebraska at Omaha, Omaha, NE; [2]University of Nebraska at Lincoln, Lincoln, NE

Caveolin-1 (CAV1) is a scaffolding protein involved in cellular processes including immune synapse formation, signal transduction, oncogenesis, and tumor suppression. This heterogeneously expressed molecule was previously identified as a member of a tolerogenic signature driving tumor-induced immunosuppression within Chronic Lymphocytic Leukemia (CLL). Upregulation of CAV1 is correlated with aggressive CLL cases, wherein an expansion of T-regulatory cells and impairment of T cell immune synapse formation is observed. Given the established role of CAV1 in the B cellpopulation of CLL, we sought to investigate the role of CAV1 in another lymphoid cell, $CD4^+$ T cells. We constructed a computational model illustrating the role of CAV1 in $CD4^+$ T cells utilizing the Cell Collective, an open-source modeling platform. Through the Cell Collective, the $CD4^+$ T-lymphocyte model was constructed using primary literature sources, and verified via immunohistochemical staining of $Cav1^{+/+}$ and $Cav1^{-/-}$ tissue. We have translated our computational findings into a meta-analysis of T cell malignancies, to identify a CAV1-associated signature. From publically available gene expression data, we compiled 198 T cell malignancy samples and 67 healthy controls to analyze. Creating a gene list based on *CAV1* expression, we identified 225 proteins positively correlated ($R > 0.5$, $p < 0.01$) across all samples. These associated molecules were enriched for extracellular matrix organization, vasculature development, growth factor binding, and cell adhesion. Expression analyses showed a predominant overexpression of the CAV1-associated gene signature among mature T cell malignancies. These results demonstrate the role of CAV1 in $CD4^+$ T cell malignancies and its influence on immune function in the tumor microenvironment.

W.29. Development of Patient-specific Double-donor Humanized (DDH) Mice Model

Hyojun Park[1], Sung-Yeon Joo[2], Yeongbeen Kwon[2], Jae Berm Park[1], Miyoung Kim[2] and Sung Joo Kim[3,1]. [1]Samsung Medical Center, Seoul, South Korea; [2]Samsung Biomedical Research Institute, Seoul, South Korea; [3]Sungkyunkwan University School of Medicine, Seoul, Korea

The research of humanized mice modeling for cell therapy are attempting Human fetal thymic tissue and fetal bone fragment were implanted to 6 weeks aged NSG mice. 2 weeks later, mice were preconditioned with 30mg/kg busulfan intraperitoneally. After 24 hours, BM-CD34 cells and FL-CD34 cells injected intravenously. Both DDH mice and SDH mice showed over 20 percent of $CD45^+$ human cells in their periphery from 8 weeks after the infusions of hematopoietic stem cells and maintained human cell engraftments until the termination of this experiments. Human $CD3^+$ T cells and human $CD19^+$ B cells were also reconstituted in their periphery. 24 weeks after transplantation, we investigated their tolerance induction between patient donor and fetal donor in our DDH mice. Each splenocyte obtained from DDH mice and SDH mice were stimulated with irradiated mononuclear cells from human bone marrow donor, fetal donor and 3rd party donor. As we expected, the splenocytes were proliferated explosively against only 3rd party sitmulator. Besides, the splenocytes were proliferated against patient and third party stimulators. Also, we observed their tolerance induction through skin graft assay. DDH mice were retained grafted patient' skin and fetal skin. SDH mice were retained only fetal skin.For the chimerism study, we transplanted HLA-A mistmatched human bone-marrow derived CD34+ hematopoietic stem cells and human fetal liver derived hematopoietic stem cells into

DDH mice. In their periphery, mixed chimerism was observed through flow cytomeric analysis.We investigated the cytotoxicity of their T cells against patient's tumor cells using suvivin peptide-pulsed DCs from patients and fetal donor.

W.30. STAT3 Activation is Required for the Antiapoptotic Effects of Prolactin in Cervical Cancer Cells

Ana Pereira-Suarez, Adrian Ramirez de Arellano, Edgar López Pulido and Jose Francisco Muñoz Valle. Universidad de Guadalajara, Guadalajara, Mexico

Prolactin (PRL) has been implicated in the development of different types of cancer. However, signaling pathways might be activated by prolactin receptor (PRLR). JAK/STAT is an important pathway associated with PRL effects, and might activate antiapoptotic genes that could lead to progression of tumorigenesis. PRL is associated with cell survival by inhibition of apoptosis and the precise activated signaling pathways for this process are still questioned. The purpose of this study was to evaluate the activation of different signaling pathways in response to PRL as well as to identify the expression of antiapoptotic genes.

Cervical cancer cell lines HeLa, SiHa and C-33A were stimulated with PRL and non stimulated cells were used to measure basal protein expression. Inhibition assays were performed by using Jak2 specific inhibitor AG490, either alone or in combination with PRL. Western blot were carried out to evaluate protein expression of the different signaling pathways and antiapoptotic proteins. Significant effects were determined by using ANOVA test.

STAT3 was significantly activated in cervical cancer lines in comparison with non-tumorigenic keratinocytes HaCaT. No significant differences were found when analyzing MAPK and PI3K signaling pathways. An increase of antiapoptotic genes Bcl-xl, Bcl-2 and survivin was observed after stimulus with PRL; however, after inhibition with AG490, the expression of antiapoptotic genes was decreased.

Our data suggests that STAT3 is an important signaling pathway activated by PRL in cervical cancer cells and it modulates the expression of antiapoptotic genes. Blocking STAT3 could represent a possible therapeutic strategy in cervical cancer.

W.31. Genetic Drivers of Immune Recognition in Human Breast Cancer

Ines Simeone[1], Wouter Hendrickx[2], Samreem Anjum[1], Halima Bensmail[1], Ena Wang[2], Francesco Marincola[2], Michele Ceccarelli[1] and Davide Bedognetti[2,3]. [1]Qatar Computing Research Institute, Doha, Qatar; [2]Sidra Medical and Research Center, Doha, Qatar; [3]National Institutes of Health, Bethesda, MD

Recent studies have described intratumoral immune-gene signatures associated with better responsiveness to immunotherapy and prolonged survival. In general, these gene signatures reflect the activation of interferon stimulated genes, the recruitment of lymphocytes through specific chemokines, and the activation of immune-effector functions. We refer to these as the Immunologic-Constant-of-Rejection (ICR) (Galon, Angell, Bedognetti, Marincola, Immunity, 2013). Representative ICR genes include IRF1, STAT1, TBX21, IL12, IFNG, GZMs, PRF1, GNLY, CXCL9, CXCL10 and CCL5. Immunotherapy is beginning to be explored in breast cancer but the immune biology of this aggressive disease is poorly understood. Whether specific genomic alterations of tumor cells can influence or prevent immune recognition is unclear. To answer this critical question we mined the genomic data of the TCGA breast cancer dataset. By using consensus clustering analysis based on RNA-seq data of >1000 breast cancer samples, we defined 4 immunophenotypes with progressive expression of the ICR genes (ICR1<ICR2<ICR3<ICR4). Notably, expression of ICR genes correlated with that of targetable immuno-suppressive molecules such as PDL1, CTLA4 and IDO. The survival rate progressively decreased from ICR4 to ICR1. Copy number aberration analysis identified deletions and amplifications typifying the ICR groups. Segment 4q21, which includes several chemokines genes such as CXCL9, CXCL10, CXCL11 and CXCL13, was significantly amplified only in the immunofavorable

group (ICR4). By processing sequencing data, we found that the number of somatic missense mutations progressively decreased from ICR4 to ICR1 groups. In addition, we described specific somatic mutations preventing or promoting the development of immunofavorable phenotype, therefore linking cancer genomic instability with immune recognition.

W.32. Loss of CD160 Binding in Lymphoma-associated Mutant Herpesvirus Entry Mediator Promotes Inhibitory Signaling Through B and T Lymphocyte Attenuator

***John Sedy**, Brian Ware, William Schleimer, Paula Norris and Carl Ware. Sanford-Burnham Medical Research Institute, La Jolla, CA*

The TNF receptor superfamily member, herpesvirus entry mediator (HVEM, *TNFRSF14*) and its multi component ligand-receptor network play a central role in maintaining immune homeostasis. Persistent viruses specifically target HVEM, while tumor cells frequently harbor mutations in *TNFRSF14* that may alter its homeostatic functions. The impact of pathogen and tumor-associated variants on HVEM-dependent signaling are unclear because HVEM engages multiple ligands that promote both proinflammatory and inhibitory signaling. Here we show that HVEM mutations associated with human follicular and diffuse large B cell lymphoma uniformly disrupt binding to CD160, while subsets of mutations retain binding to B and T lymphocyte attenuator (BTLA) and LIGHT (TNFSF14). CD160 expression competes with BTLA for HVEM binding in lymphocytes, limiting the capacity for HVEM to engage BTLA inhibitory signals. However, selectivity for BTLA by mutated HVEM or a viral paralog results in unhindered inhibition of extracellular signal-regulated kinase-1/2 (ERK-1/2) signaling and Interleukin-2 transcription that correlates with activation of Src homology 2 domain-containing phosphatase-1/2 (SHP-1/2). The evolutionary acquisition of ligand selectivity for the inhibitory receptor BTLA and loss of binding to CD160 by viruses and tumor mutations reveals a common mechanism of immune modulation to thwart inflammatory signaling.

W.33. Controlling CAR-T Cell Therapy Using Antibody Based Switches

***David Rodgers** and Travis Young. Calibr, San Diego, CA*

Autologous cell therapy using patient derived T cells engineered to express chimeric antigen receptors (CAR) is a revolutionary method of treating hematological cancer. Through this approach, T cells are redirected to target antigens expressed on cancer cells. Despite the success of CAR-T cell therapy in the clinic, patients often suffer from serious side-effects such as the excessive release of pro-inflammatory cytokines and off-target cytotoxicity; the latter restricting the development of conventional CAR-T cells to antigens with highly tumor-restricted expression. The inherent toxicity and long term persistence of conventional CAR-T cells may deter wide spread uptake of this technology. To address this problem we have developed a two component platform that combines the control of monoclonal antibody therapy with the potency of cell therapy. This platform provides a safer and more dynamic therapy by enabling the CAR-T cell to be switched “on or off” using an engineered immunological synapses between the CAR-T and cancer cells. This avoids dangerous off-target activation and provides an additional safety mechanism for halting therapy in the case of an adverse event. We systematically optimized these switches and demonstrated selective activation of CAR-T cells against multiple cancer antigens. This platform provides a CAR-T cell therapy that is both safe and versatile and can be universally applied to nearly any cancer associated antigen.

Inflammatory Bowel Disease

OR.49. Genetically-guided Bioassays Reveal Distinct Immune Profiles in Subjects with Inflammatory Bowel Disease (IBD) Compared to Controls

***Sabine Ivison**[1,2], Sylvie Lesage[3,4], Gabrielle Boucher[3,4], Rita Kohen[5], Rosa Garcia[1,2], Livia Odagui[3,4], Mathieu*

Goupil[3,4], The iGenoMed Consortium[3,4], Alain Bitton[5], John Rioux[3,4] and Megan Levings[1,2]. [1]Child and Family Research Institute, Vancouver, BC, Canada; [2]★University of British Columbia, Vancouver, BC, Canada; [3]University of Montreal, Montreal, QC, Canada; [4]Montreal Heart Research Institute, Montreal, QC, Canada; ★[5]McGill University, Montreal, QC, Canada

Inflammatory bowel diseases (IBD) are common and genetically well-characterized diseases with 163 validated genetic risk factors. Many IBD risk factors target different points of the same biological pathway, suggesting that despite the variation in genetic risk, there are a limited number of biological pathways that underlie disease. Identification of genetically-driven and patient-specific alterations in these biological pathways will enable a better understanding of disease mechanisms and heterogeneity, as well as development and selection of more targeted therapies. The IBD Genomic Medicine Consortium has identified ~300 genes which are putatively affected by these IBD risk factors and is mapping each gene into common biological pathways. In keeping with the central role of host-microbe interactions in the etiology of IBD, many of these pathways are components of the immune response. We developed a series of genetically-guided bioassays that each integrate the functional effects of multiple risk factors. Measuring the relative activity of these pathways revealed significant differences in responses in subjects with IBD versus healthy controls. For example, PBMCs from IBD patients had a dysregulated response to LPS as well as a reduced ability of prostaglandin E2 to modulate this pathway. Production of several innate cytokines after stimulation of B cells (IL-10, TNFa) or the IL-23 receptor (IL-1b, IL-22) was also significantly higher in IBD patients compared to healthy controls. These data illustrate the power of using genetic risk factors to guide the development of assays that interrogate the activity of biological pathways, which could ultimately be used to measure patient-specific profiles.

OR.59. A Defect in a Small-intestinal Lymphoid Structure Affects Colonic B Cell Development

***Neeraj Surana**[1,2] and Dennis Kasper[2]. [1]★Boston Children's Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA*

Peyer's patches (PPs) are small-intestinal secondary lymphoid organs that represent an important inductive site for intestinal immunity. In this study, we prevented formation of PPs to examine its influence on intestinal immunity. Pregnant dams were injected at E14 with an IL-7Ra antibody, resulting in inhibition of PP formation in the pups. Flow cytometric analysis revealed no change in the numbers of immune cells in the spleen, lymph nodes, or small-intestinal lamina propria (SI LP). Surprisingly, the colon of PP-deficient mice was lymphopenic, with a specific decrease in $CD19^+$ B cells, including colonic plasmablasts. We extensively looked for other defects (e.g., changes in other lymphoid structures, changes in cecal or peritoneal B cells, B cell trafficking, microbiota-related effects) that might explain this colonic phenotype, but everything else was normal. In control animals, the number of B cells present in PPs was strongly correlated to the number of B cells in the colon but not the SI LP. Moreover, if pregnant dams were injected with the IL-7Ra antibody at E18, the resulting pups had normal-appearing PPs and the normal number of colonic B cells. Interestingly, PP-deficient mice have more severe disease in infectious and chemically induced models of colitis, demonstrating that these colonic B cells are functionally important. Taken together, our data establishes that a portion of colonic B cells is regulated by PPs, indicating a regulatory link between the small- and large-intestinal immune systems. Ultimately, our findings suggest a new approach to thinking about immunoregulation of the colonic immune system.

W.34. CCR9 Expression Correlates with Promoter Demethylation in Colon-derived $CD4^+$ T Cells During IBD

***Ludvig Linton**, Helena Jonsson Rolandsdotter, Yigael Finkel and Ola Winqvist. ★Karolinska Institutet, Stockholm, Sweden*

Background: In mice, it is established that $CD4^+$ T cells migrate to the small intestinal mucosa during chronic inflammation through CCR9-CCL25 interactions. However, the role and regulation of CCR9 in human colonic inflammation remain unclear. We have studied CCR9 expression in $CD4^+$ T cells isolated from the colonic mucosa in

patients with inflammatory bowel disease (IBD), and also investigated whether demethylation of the *CCR9* promoter accompanies expression of the CCR9 protein.

Methods: We have included 10 juvenile patients with active IBD. Flow cytometry was used to analyze the expression of CCR9, CD4, CD45RA and CCR7 in colon and peripheral blood. $CD4^+$ T cells were subsequently sorted and subjected to DNA isolation and bisulphite conversion followed by pyrosequencing.

Results: We present three main findings: I) Approximately 50% of colon $CD4^+$ T cells express CCR9. Expression levels are slightly higher in memory cells ($CD4^+$CD45RA-$CD45RO^+$) than in naïve cells (CD4+CD45RA+CD45RO-). In comparison, CCR9 is barely detectable on circulating $CD4^+$ cells. II) CCR9 levels are significantly lower in tissue specimens from inflamed colon compared to non-inflamed sections from the same patient ($p<0.01$). III) We have identified an evolutionary conserved, CpG-rich region approximately 1.5kB upstream of the *CCR9* transcription start site. This region is consistently demethylated in CCR9-expressing $CD4^+$ memory cells, whereas the CCR9-negative memory cells remain methylated.

Discussion: CCR9 expression is higher in non-inflamed sections of the colon, possibly indicating a regulatory role of CCR9-expressing $CD4^+$ T cells. Furthermore, we show that CCR9 is regulated by promoter methylation in colon-derived $CD4^+$ T cells.

3841. Features of the Immune Status of Children with Bronchopulmonary Pathology

N. Fayzullaeva, G. Khakimova, T. Aripova and Diloram Musakhodjaeva. Academy of Sciences of Uzbekistan, Tashkent, Uzbekistan

The increased incidence of inflammatory diseases and the presence of chronic respiratory tract inflammations accompanied by disturbances of immunological reactivity. The purpose of research: to study features of immunological reactivity in children with chronic bronchitis. The study included 58 children with chronic bronchitis in age from 7 to 14 years. 20 healthy children of the same age in the control group. The diagnosis of chronic bronchitis (CB) was put in accordance with the WHO criteria. We studied lymphocyte levels (CD3, CD4, CD8, CD16, CD20, CD25, CD95) and the concentration of IgA, IgG iIgM. It was found that the level of $CD3^-$, $CD4^-$ and $CD8^-$ cells in sick children decreased ($P <0,01$), and the number of B-lymphocytes increased ($P <0,01$). The concentration of IgG and IgA decreased ($P <0.05$), aIgM-enhanced. Revealed that in bronchopulmonary pathology observed increased expression of activation markers as early activation - $CD25^+$ cells, and late activation $CD95^+$. It is shown that the more difficult the case, the more appeared $CD95^+$ cells. Consequently, the development of secondary immunodeficiency with bronchopulmonary pathology, may be related to a violation of activation of effector cells resulting from violations of the signal. Thus, a significant pathogenetic role of immune disorders in the formation of a chronic inflammatory process in bronchopulmonary diseases in children.

Innate Immunity

OR.01. Glibenclamide Induces Impaired Responses of Neutrophils Against *Burkholderia pseudomallei* by Reduction of Intracellular Glutathione Level

Chidchamai Kewcharoenwong[1], Darawan Rinchai[1], Arnone Nithichanon[1], Manabu Ato[2] and Ganjana Lertmemongkolchai[1]. [1]Khon Kaen University, Khon Kaen, Thailand; [2]National Institute of Infectious Diseases, Tokyo, Japan

The major risk factor for melioidosis, an infectious disease caused by *B. pseudomallei*, is type 2 diabetes mellitus. More than half of melioidosis patients with diabetic condition in Thailand are prescribed glibenclamide to control blood glucose levels. Recent evidence from our group illustrates that glibenclamide reduces pro-inflammatory cytokine production by polymorphonuclear neutrophils (PMNs) of diabetes in response to this bacterial infection. However, mechanisms by which glibenclamide affects cytokine production are unknown. Here we found that *B. pseudomallei* infected PMNs from glibenclamide-treated diabetic subjects showed lower intracellular glutathione (GSH) levels when compared with healthy subjects. Interestingly, glibenclamide decreased GSH level of *B. pseudomallei* infected PMNs. Moreover, PMNs from diabetic patients who had been treated by glibenclamide exhibited reduced cytokine production, migration capacity and bactericidal activity against *B. pseudomallei* infection, whereas GSH could restore these functions. Taken together, our data show a link between the effect of glibenclamide on GSH and PMN functions in response to *B. pseudomallei* that may contribute to the host susceptibility to bacterial infection in diabetic patients.

OR.02. Increased Survival and Exocytosis of Toxic Primary Granules by Neutrophils in an *in vitro* Model of Cystic Fibrosis Airway Disease

Osric Forrest[1,2], Sarah Ingersoll[1,2], Marcela Preininger[2], Julie Laval[1,2], Milton Brown[1,2] and Rabindra Tirouvanziam[1,2]. [1]Children's Healthcare of Atlanta, Atlanta, GA; [2]★Emory University School of Medicine, Atlanta, GA

Cystic Fibrosis (CF) lung disease is characterized by the massive recruitment of neutrophils (PMNs) into the bronchiolar lumen. Extracellular neutrophil elastase (NE) that is released from PMN primary granules, is a strong predictor of lung function and survival in CF patients. However, the process by which CF airway PMNs exocytose HNE is not well understood and not reproduced in animal models of CF airway disease. Methods: We used a surface collagen-coated 3D porous polystyrene scaffold (Alvetex) to mimic the airway lamina propria and grew human bronchiolar H441 cells at air-liquid interface on top. In the transepithelial migration assay, blood PMNs were placed on the basal side and allowed to migrate towards to CF airway fluid placed apically, after which PMNs were analyzed by flow cytometry for rate of migration, survival, and degranulation. Results: Using this model we show that (i) CF airway fluid induces rapid transepithelial migration and survival of neutrophils, (ii) live PMNs actively release of primary granules upon exposure to CF airway fluid, and (iii) migration towards CF airway fluid induces PMN reprogramming allowing for the activation of the inflammasome and pinocytosis. Conclusions: These results suggest a primary role for the CF microenvironment in inducing increased survival and phenotypic reprogramming of PMNs and establish our model as a robust platform for deconstructing CF airway PMN dysfunction and developing new therapies for CF inflammation.

OR.03. Estrogen-mediated TLR8 Expression via STAT1 Facilitates Endogenous miRokine Ligand Activation by Exosomes Containing miR-21: A Novel Innate Inflammatory Pathway in Systemic Lupus Erythematosus

Nicholas Young, Lai-Chu Wu, Stephanie Amici, Mireia Guerau, Mary Severin, Amy Lovett-Racke, Giancarlo Valiente, Craig Burd, Jeffrey Hampton and Wael Jarjour. The Ohio State University Wexner Medical Center, Columbus, OH

While the adaptive immune response has been investigated extensively in systemic lupus erythematosus (SLE), recent work suggests that innate immunity may play a significant pathological role. Recently, we demonstrated that estrogen lowers the threshold of immune cell activation and leads to enhanced TLR8 expression with agonist stimulation; a response that was more robust in primary cells from females. TLR8 is an innate immune system receptor that binds to single-stranded RNA sequences present in viruses. Here, we examined this pathway to better understand innate immune responses via TLR8 in SLE. Estrogen treatment of primary cells significantly elevated the expression of many genes, including STAT1 and TLR8. ChIP-seq and EMSA analysis in cells stimulated with estrogen identified a putative estrogen response element to promote STAT1 expression. Subsequent EMSA analysis confirmed STAT1-mediated transcriptional activation of TLR8 with estrogen stimulation. In lieu of viral RNA to

activate TLR8, we explored the potential contribution of miR-21, which has been shown to act as an endogenous ligand in carcinogenesis via exosome signaling. Here, we detected miR-21 in exosomes isolated from SLE serum and induced TLR8 expression *in vitro* by stimulating cells with synthetically produced pseudoexosomes containing fluorescently-labeled miR-21. Thus, just as a cytokine or chemokine, exosome-encapsulated miR-21 can act as an inflammatory signaling molecule, or miRokine, between cells by virtue of being an endogenous ligand of TLR8. Collectively, our data elucidates a novel innate inflammatory pathway in SLE where estrogen-mediated expression of STAT1 promotes TLR8 expression, which can also be triggered by miR-21.

OR.18. Human Type 1 Innate Lymphoid Cells Accumulate in the Inflamed Synovium in Spondyloarthritis

Nataliya Yeremenko[1,2], Troy Noordenbos[1,2], Iris Blijdorp[1,2], Hulda Hreggvidsdottir[1,2], Kristine Germar[1,2], Jochem H. Bernink[2], Hergen Spits[2] and Dominique Baeten[1,2]. [1]Amsterdam Rheumatology and Immunology Center, Amsterdam, Netherlands; [2]★Academic Medical Center/University of Amsterdam, Amsterdam, Netherlands

Spondyloarthritis (SpA) is a chronic inflammatory arthritis characterised by inflammation of axial and peripheral joints and by pathologic new bone formation. Several lines of evidences indicate that IL-23/IL-17 immune axis plays a pivotal role in the pathophysiology of SpA. It remains, however, unknown which IL-23-responsive cells are the major source of IL-17 in SpA. Innate lymphoid cells (ILCs) are an emerging family of innate immune cells that produce various cytokines including IL-17 and IL-22, and play critical roles in regulation of inflammation and tissue remodeling. Here we characterise ILCs in the peripheral blood (PB), synovial fluid (SF) and synovial tissue (ST) of patients with SpA.

ILCs were present in all three compartments of patients with SpA. Analysis of ST revealed a significantly increased frequency of total ILCs in the joint compared with PB (0.37% of the lymphocyte population in ST versus 0.06% in PB, p=0.016). Immunophenotyping of ILC subsets showed a significant increase in the frequency of ILC1 ($CRTH2^-$ $NKp44^-$$ckit^-$) in ST (37.8%) versus SF (7.27%, p=0.008) and PB (3.45%; p=0.004). The second most prominent ILCs in the joint were NCR-negative ILC3 ($CRTH2^-NKp44^-ckit^+$), composing 33.45% of the total ILCs. NCR-positive ILC3 and ILC2 populations were present in synovium at lower frequencies.

We observed an absolute and relative enrichment of both ILC1 and NCR-negative ILC3 in the ST of patient with spondyloarthritis. As studies in other tissues revealed that these IL-23-responsive ILC subsets can be an important source of IL-17 and/or IL-22, we will further investigate the cytokine production by these cells.

OR.24. Soluble CD52 is a Potential Therapeutic for Inflammatory Diseases

Maryam Rashidi, Yuxia Zhang, Esther Bandala-Sanchez, John M. Wentworth, James Vince and Leonard Harrison. Walter & Eliza Hall Institute of Medical Research, Melbourne, Australia

Background: We recently showed that glycosyl-phosphatidylinositol-anchored cell surface glycopeptide CD52 suppresses T cell function. Following activation, T cells release CD52, which inhibits T cell receptor signaling in bystander T cells via interaction with the sialic acid-binding immunoglobulin-type lectin 10 (Siglec 10) receptor. Whether CD52 signaling can also modulate innate immune cell function is not known.

Methods: Monocytic THP-1 cells, primary human monocytes, mouse bone marrow-derived macrophages (BMDM) and dendritic cells (BMDC) were cultured in the presence of purified recombinant CD52-Fc or control Fc proteins with or without immune stimulants, and the inflammatory response assessed using NF-kB activation assays and cytokine ELISAs. The effect of soluble CD52 *in vivo* was assessed by treating mice with lipopolysaccharide (LPS) i.p. and CD52-Fc or control Fc i.v.

Results: CD52-Fc bound to Siglec-10 in human monocytes and Siglec-E in mouse macrophages. CD52-Fc potently inhibited NF-kB activation and cytokine secretion by innate immune cells following stimulation with ligands for Toll-like receptors and the TNF receptor. When injected into mice, CD52-Fc significantly blunted the hypothermic and cytokine response to intravenous LPS.

Conclusion: Soluble CD52 inhibits innate immune responses via disruption of NF-kB activation. Its ability to blunt the response to LPS *in vivo* raises the possibility that CD52-Fc could be used to treat inflammatory disorders.

OR.27. Self-glycerophospholipids Regulate Immune Response and Inflammation via Induction of 'Anti-inflammatory' Myeloid-derived Suppressor Cells

Ram Singh, Ramesh Halder, Cynthia Tran and Priti Prasad. ★*University of California, Los Angeles, CA*

Glycosphingolipid (GSL) and glycerophospholipid (GPL) antigens bind CD1d and activate T cells. Here, we report that the normal immune repertoire contains αβ T cells that recognize abundant self-GPLs in a CD1d-restricted manner. CD1d tetramers loaded with GPLs, including phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylethanoloamine (PE), phosphatidylinositol (PI), and phosphatidylserine (PS), and BMP (bis(monoacylglycero)phosphate), identify 0.4–4% T cells in the lymphoid organs of wild-type and iNKT–deficient Jα18$^{-/-}$ mice but not in CD1d$^{-/-}$ mice. GPL-reactive T cells don't recognize GSL-loaded tetramers and don't respond to αGalCer, suggesting that GPL-reactive T cells are distinct from iNKT cells. GPL-reactive T cells expand, express CD69, and produce cytokines upon *in vivo* priming. However, self-GPL antigens potently inhibited the proliferation and cytokine production by invariant natural killer T cells (iNKT) via induction of monocytic myeloid-derived suppressor cells (M-MDSC) that inhibited iNKT cells in an IL-10-dependent manner. Treatment with a GPL as well as adoptive transfer of GPL-modified M-MDSC ameliorated liver inflammation, whereas it reversed the iNKT cell-mediated protection against tumor. These observations support a new role for self-GPLs to help maintain homeostasis between the diverse populations of lipid-reactive T cells, with important implications for immune pathogenesis and intervention in conditions associated with altered lipid metabolism and inflammation.

OR.30. The Role of XIAP in BCL10-mediated Dectin-1 Signaling

Wan-Chen Hsieh[1,2], I-Hsuan Chiang[2,3], Shi-Chuen Miaw[3] and Ming-Zong Lai[1,2,3]. [1]National Defense Medical College, Taipei, Taiwan; [2]Academia Sinica, Taipei, Taiwan; [3]National Taiwan University, Taipei, Taiwan

X-linked inhibitor of apoptosis protein (XIAP) is known for its critical role in cell survival. Recent studies found that XIAP deficiency leads to X-linked lymphoproliferative syndrome type 2 (XLP-2). However, the molecular mechanisms on how XIAP deficiency generates XLP-2 are not clear. Here we showed that XIAP is selectively involved in NF-κB activation induced by dectin-1, but not by TNF-α or TLRs. XIAP interacted with BCL10 and enhanced BCL10-mediated NF-κB activation that is stimulated by dectin-1, TCR, LPA and EGFR. Furthermore, XIAP promoted K63-polyubiquitination of BCL10. As a consequence of defective dectin-1 response, *Xiap*$^{-/-}$ mice were highly susceptible to *C. albicans* infection. At early stage of infection, *Xiap*$^{-/-}$ mice generated weak innate immune responses, followed by the unsolved inflammation including inflammatory cytokines elevation and neutrophils accumulation. We also demonstrated that dectin-1 ligand curdlan induced XLP-2-like syndromes in *Xiap*$^{-/-}$ mice. XIAP deficiency also impaired dectin-1-induced Rac1 activation and phagocytosis. We further found that resolvin D1 restored Rac1 activity and phagocytic ability of XIAP-deficient-macrophages. In addition, resolvin D1 and resolved the persistent inflammation and rescued *Xiap*$^{-/-}$ mice from lethal *C. albicans* infection. Therefore, sustained inflammation as a consequence of defective BCL10-dependent innate immunity to control specific infection in XIAP-deficient individuals may contribute to the development of XLP-2. Our results also suggest that resolvin D1 may be a potential therapeutic reagent for XLP-2 and other innate deficiency diseases.

OR.58. Dendritic Cells and Bronchial Epithelial Cell Interaction in the Elderly

*Sudhanshu Agrawal, Sreerupa Ganguly, Pega Hajian and **Anshu Agrawal**. University of California Irvine, Irvine, CA*

Increased susceptibility to respiratory infections is a hallmark of advancing age and is considered a major cause of morbidity and mortality associated with aging. The underlying mechanisms are not well understood. The crosstalk between the dendritic cells (DCs) and epithelial cells is essential in maintaining tolerance as well as in generating immunity in the respiratory mucosa. DCs from aged subjects display an enhanced basal level of activation which can affect the function of epithelial cells. Our investigations suggested that this is indeed the scenario as exposure of primary bronchial epithelial cells (PBECs) to supernatants from unstimulated DCs of aged subjects resulted in activation of PBECs. The expression of chemokines, CCL20, CCL26, CXCL10 as well as the permeability of the epithelial cell barrier was significantly increased in the PBECs cultured with aged DC supernatants compared to young DC supernatants. Further investigations suggest that DCs from the elderly are also impaired in their capacity to respond to signals from the epithelial cells. These signals are essential to maintain tolerance in the airway to prevent immune response to harmless antigens. Culture of DCs from the elderly with PBECs did not lead to induction of T regulatory cells. Thus age-associated alterations in DC-epithelial interactions contribute to chronic airway inflammation in the elderly, increasing their susceptibility to respiratory diseases.

W.35. Neuronal Innate Immunity Negative Regulates Dendrite Outgrowth

***Hsin Yu Liu**[1], Yun-Fen Hong[2], Chiung-Ya Chen[1], Tzyy-Nan Huang[1] and Yi-Ping Hsueh[1]. [1]Academia Sinica, Taipei, Taiwan; [2]Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan*

Toll-like receptor (TLR) family has been known as a critical danger sensor in innate immune system through recognition of both pathogen- and damage-associated molecular patterns to against invading pathogens. In addition to immune cells, some of TLRs are expressed in neurons and involved in the regulation of neurogenesis, neurite growth and neurodegeneration. However, the downstream signal pathways and effectors for TLRs in neurons are still controversial. From our TLR agonists screening, we identified that TLR7 agonist impaired dendritic growth of cultured mouse cortical neurons. To investigate the function of *Tlr*7 in neuronal development, we measured the difference of neuronal morphology between wild-type (WT) and *Tlr*7 knockout (KO) neurons in primary cortical cultures, and we found that *Tlr*7 KO neurons have longer dendrites and primary axons. Similarly, *Tlr*7 knockdown neurons also showed more complex dendritic arborization and misoriented of apical dendrites. In addition, the *ribonuclease A* (RNase A) treatment further promoted dendritic growth by removing the single-stranded RNA, the ligand of TLR7, in cultures. Furthermore, TLR7 activation by specific endogenous ligands and synthetic agonists restricted dendritic growth. Moreover, we found that TLR7 activation induced the expression of IL-6 and TNF-α cytokines as well as c-FOS transcription factor. Using different deficient neurons, including *Myd88*, *Trif*, *IL-6*, *Tnf-α* and *IL-1R1* KO neurons, demonstrated that the MYD88 and IL-6 are essential for TLR7 signaling to restrict dendritic growth. Finally, lower exploratory activity of juvenile *Tlr*7 KO mice were observed in an open field, indicating that the changes of neuronal morphology also influence mouse behaviors.

W.36. Neutrophil Extracellular Traps and Activation Markers in Patients with Liver Cirrhosis

*Juan M Agraz-Cibrian[1] Jorge Segura-Ortega[2], Vidal Delgado-Rizo[1], Karla Bustos-López[1] and **Mary Fafutis-Morris**. [1]Universidad de Guadalajara, Guadalajara, Mexico; [2]Antiguo Hospital Civil de Guadalajara, Guadalajara, Mexico*

Background: Patients with liver cirrhosis (LC), have a high susceptibility to bacterial infections. Neutrophils from these patients exhibit defects in production of oxygen radicals, which are essential to the elimination of bacteria through the activation of various microbicides mechanisms as is the release of neutrophil extracellular traps (NETs).

Aim: Evaluate the ability to release NETs and activation markers in patients with LC.

Methods: Purification of neutrophils with Ficoll Histopaque was conducted from a sample of peripheral blood of patients with compensated and decompensated LC. Neutrophils were activated with PMA for evaluate NETs by fluorescence microscopy and fluorometry, expression of activation markers (CD69, CD80, perforin and CAP-18) was assessed by flow cytometry.

Results: A significant decrease in the release ability of NETs was observed in decompensated cirrhotic patients. When comparing activation markers of neutrophil, results not significant were found, however, a correlation between the decrease in the expression of CD69 and CD80, with decompensated patients was observed. Conversely an increase in intracellular expression of CAP-18 and perforin was observed.

Conclusion: Neutrophils play an important role in the defense against infections, this study showed that patients with LC have deficiencies in the release of NETs, which increases the risk to the establishment of complications.

W.37. NAD Kinase Acts as a Negative Regulator of TLRs-mediated Immune Responses

Jing-Yiing Wu, Yu-Jie Wu and Cheng-Chin Kuo. National Health Research Institutes, Miaoli, Taiwan

The activation of TLR signaling by pathogenic components results in the induction of specific gene expression those are committed to ensure efficient removal and destruction of the invading pathogens, control tissue homeostasis and activate adaptive immunity. However, excessive TLR activation causes to an inflammation persists states which can disrupt immune homeostasis and then may harm the host and result in inflammatory diseases. To avoid harmful and inappropriate inflammatory responses, the strength and duration of TLR signaling needs to be tightly regulated. Here we found that ligand activation of TLR2, TLR4 and TLR9 resulted in a time-dependent induction of the mouse macrophages cellular level of NAD kinase which plays a crucial role in the regulation of NAD(H)/NADP(H) conversion and oxidative defense systems. Kinase inhibitor studies indicated that both p38 /PI3K/Akt and JNK dependent pathway were involved in the upregulation of NAD kinase mediated by TLR2, TLR4 or TLR9 in macrophagrs. Inhibition of NADK by shRNA enhanced TLR-mediated production of cytokines and ROS in macrophages, whereas ectopic expression of NADK in macrophages suppressed TLR-induced responses. Additionally, cytokine productions induced by LPS were enhanced in NAD kinas knockout mice as compared with those in wild type mice. Overall, these results suggest that NAD kinase behaves as a negative regulatory molecule in TLR2, TLR4 and TLR9-mediated immune responses in macrophages.

W.38. Altered Cellular Metabolism in Monocytes of Infants Born Prematurely

Bernard Kan[1,2], Ashish A. Sharma[1,2], Kelsey Lee[1,2], Colin Ross[1,3] and Pascal Lavoie[1,2,4]. [1]★University of British Columbia, Vancouver, BC, Canada; [2]Child and Family Research Institute, Vancouver, BC, Canada; [3]Centre for Molecular Medicine and Therapeutics, Vancouver, BC, Canada; [4]British Columbia Children's Hospital, Vancouver, BC, Canada

Compared to adults, newborn infants suffer from a high rate of morbidity and mortality from infectious diseases. We have previously demonstrated that pathogen recognition and cytokine mediated responses are deficient in human neonates, particularly in those born prematurely. Previous research points toward broad mechanisms of attenuation in innate immune responses at the cellular level. Cellular metabolism plays a critical role in the regulation of innate immune functions. Specifically, skewing of metabolic pathways related to oxidative phosphorylation or glycolysis can result in innate immune cells that are hypo-responsive to Pathogen Associated Molecular Patterns. Methods: Monocytes were extracted from the cord blood of infants born extremely preterm (gestational age ≤ 28 weeks, n=8), cord blood from infants born at term (n=12) and from adult peripheral blood (n=12). Whole genome expression profiling following LPS stimulation was performed using Illumina BeadChip arrays. Results: A total of 1298 genes were differentially expressed in preterm monocytes upon LPS stimulation; with 264 genes being unique to the preterm group (>2 fold differential expression, adjusted p<0.01). Strikingly, only 9% of these genes were related to

immune functions. Pathway analysis of these genes reveals a profound down-regulation of genes necessary for oxidative phosphorylation, and protein translation upon LPS stimulation. In contrast, resting preterm monocytes overexpressed genes related to glycolysis and glucose transport, compared to their term and adult counterparts. We conclude the cell metabolism is globally altered in monocyte from human neonates during gestation, which might play a role in their attenuated immune protections against infections.

W.39. A Systems-level Analysis of the Human Immune System Throughout Acute Psychological Stress

Michael Breen[1], Nadejd Beliakkova-Bethell[2], Wayne Ensign[3], Christopher Woelk[1] and Brinda Rana[2]. [1]Univerisity of Southampton, Southampton, United Kingdom; [2]★University of California, San Diego, San Diego, CA; [3]Space and Naval Warfare Systems Center, San Diego, CA

Acute psychological stress can influence immune system function and subsequent disease risk. Studies linking stress to disease pathology often focus on individual genes and cell-types, while no reports evaluate the longitudinal effects of acute psychological stress on the immune system in humans. Here, we used a multifaceted systems-level analysis based on whole genome transcriptional profiles of peripheral blood leukocytes (PBL) and absolute cell counts together with immunoassay measurements of the endocrine system sampled throughout the sequence of events leading up to and following a real-world acute psychological stress, induced by first-time tandem skydive. Transcriptomic analysis revealed the rapid up-regulation of genes post-stressor involved in IL-12 signaling and Natural Killer (NK) cell cytotoxicity mediated by transcription-factors *RUNX3*, *FOS*, *JUN*, *CDKN1A*, *GATA3*, *CREBBP*, and down-regulation of genes involved in MyD88-dependent toll-like receptor signaling mediated by *ME2* and *MAPK3*, all returning to baseline levels one hour later. While transient mobilization of leukocyte cell subsets was also confirmed by flow cytometry throughout the stress response, accounting for these fluctuations revealed stress-induced gene expression changes associated with the adrenal cortex and production of cortisol. Further integrated analysis of hormone and gene co-expression network data revealed suppression and activation of functional PBL gene-modules influenced by specific hormonal cues at critical points throughout the stress response. These results provide a comprehensive characterization of acute psychological stress-induced immune system alterations which contain broad implications for understanding the role of stress in immunity and health, offering a spring-board for future stress-related research.

W.40. High Molecular Weight Hyaluronan Inhibits Dendritic Cell Maturation and Antigen Presentation in a NFkB and CD44-dependent Manner

Shannon Ruppert[1], John Gebe[2], Payton Marshall[1], Ben Falk[2], Johanna Sweere[1], Gernot Kaber[1] and Paul Bollyky[1]. [1]★Stanford University School of Medicine, Stanford, CA; [2]★Benaroya Research Institute, Seattle, WA

The interactions of immature dendritic cells (DCs) with pro-inflammatory “danger signals”, such as lipopolysaccharide (LPS), are critical for maturation and for eliciting antigen-dependent T cell responses. We find that high-molecular weight hyaluronan (HMW-HA), an anti-inflammatory polymer abundant in healing tissues, inhibits DC maturation and, thereby, efficient antigen presentation. Immature DC were pre-incubated for 24 hours with HMW-HA that was crosslinked to prevent its rapid catabolism, a preparation we called “XHA”, and this treatment prevented changes in markers associated with DC maturation, including CD80, CD86, MHC class II, and CD14. This immature, tolerogenic phenotype in DCs persisted despite the addition of pro-inflammatory mediators, including lipopolysaccharide (LPS), TNFα, or Pam3CSK4 and limited the efficiency of antigen presentation to ovalbumin (OVA)-specific T cells *in vitro*. These effects were due to CD44-mediated inhibition of NFkB activation and nuclear translocation. Consistent with this, XHA co-administered with OVA intranasally to OVA-sensitized mice prevented airway hypersensitivity *in vivo*. These effects were not seen with fragmented HA or other polymers tested. We conclude that XHA alters pro-inflammatory function of DCs by providing tissue-contextual cues via CD44-mediated inhibition of NFkB signaling.

W.41. ER Stress and TLR Activation Interact Through p38, STAT3, and XBP1s to Modulate Inflammation in Cystic Fibrosis

Anthony Tang[1] and Stuart Turvey[2]. [1]★University of British Columbia, Vancouver, BC, Canada; [2]Child & Family Research Insitute, Vancouver, BC, Canada

Background: There is increasing appreciation for the inflammatory role of ER stress in a spectrum of diseases. Previous work showed increased ER stress responses in airway epithelial cells, blood cells, and lung tissue from patients with cystic fibrosis (CF)(1). Although its role in disease progression is unclear, there are many potential sources of ER stress in CF airways including misfolded CFTR, pathogens, and oxidizing conditions, which can increase inflammation and subsequent damage.

Aim: To determine the mechanisms by which ER stress regulates inflammation in CF.

Methods: CF airway cells (IB3-1, CuFi-1), ΔF508-transfected A549 cells, and monocytic cells (THP-1) were used to determine ER stress/inflammatory pathway interactions with a panel of ER stressors and inflammatory ligands. Signaling was examined by cell fractionation, immunoblot, and reporter assays, and inflammatory output by qPCR and ELISA.

Results: ER stressors alone increased cytokine mRNA but not NF-κB activation or cytokine secretion. However, ER stress in conjunction with subsequent TLR stimulation significantly augmented IL-6, IL-8, and IL-1β levels ($P<0.05$). This was reflected by broad changes in inflammatory signaling including increases in p38 and IRF3 activation but STAT3 inhibition. Activation of TLRs 4 and 5 modulated levels of the ER stress transcription factor XBP1s ($P<0.001$) but not ATF4, both of which when knocked down decreased cytokine levels ($P<0.05$).

Conclusion: ER stress sensitizes inflammatory pathways to TLR stimulation and drives XBP1s-mediated cytokine production. ER stress inhibition has potential therapeutic value in decreasing excessive inflammatory responses.

[1]Blohmke et al. 2012. JI: 1, 189 (11): 5467-75

W.42. CD40L, a Bridge Between Innate and Adaptive Immune Responses Linking Inflammation and Atherosclerosis

Jack A. Ragheb[1], Nicholas Busar[1], E. Michael Gertz[2], Alejandro Schaffer[2], Anuja Patnaik[1], Hyoung (Fred) Oh[1], Sagarika Chakrabarty[3,4], Allan Kirk[5,6] and David Roberts[7]. [1]Food and Drug Administration, Silver Spring, MD; [2]National Center for Biotechnology Information, Bethesda, MD; [3]National Eye Institute, National Insititues of Health, Bethesda, MD; [4]Scripps Institute, La Jolla, CA; [5]The National Institute of Diabetes and Digestive and Kidney Diseases, National Instuties of Hleath, Bethesda, MD; [6]★Duke University, Durham, NC; [7]National Cancer Institute, National Institues of Health, Bethesda, MD

CD40 ligand (CD40L) is a well-known regulator of the adaptive immune response that induces and modulates the activation and differentiation of monocytes, dendritic cells, and lymphocytes. Less appreciated is its critical role in promoting the inflammatory response in CD40 expressing cells such as vascular endothelium. Expression of human CD40L on $CD4^+$ T cells is biphasic and dependent upon monocyte cell contact early and IL-2R signaling late. Here we report upon a previously unidentified β_1 integrin pathway that costimulates early and late phase CD40L expression on $CD4^+$ T cells independently of monocytes and IL-2 through interaction with the vascular endothelial cell adhesion molecule 1 (VCAM-1) or members of the extracellular matrix (ECM) family, such as fibronectin (FN). This innate form of costimulation is modulated by the type and quantity of ECM protein present, with thrombospondin-1 inhibiting FN mediated costimulation. Our findings demonstrate the existence of distinct pathways that differentially regulate the expression of CD40L. The novel link established between VCAM-1, ECM proteins, and CD40L expression on $CD4^+$ T cells suggests CD40L may serve as a bridge between innate and adaptive responses, an insight which should further our understanding of the establishment and progression of inflammatory

thrombovascular diseases such as atherosclerosis.

W.43. Determination of Gene Expression in Blood Granulocytes from Tuberculosis Patients

Priscilla Silva, Fabiana Zambuzi, Valdes Bolella, Rogerio Rosada and ***Fabiani Frantz****. Universidade de São Paulo, Ribeirão Preto, Brazil*

According to recent data from the World Health Organization, it is estimated that more than 30% of the world population is infected with the bacillus *Mycobacterium tuberculosis* (Mtb). The hallmark of pulmonary tuberculosis is the local immune response that results in the granuloma formation. Together with resident macrophages, neutrophils appear to be important in the control of infection and granuloma formation, releasing potent inflammatory mediators and being involved on the initiation and maintenance of type 1 profile of immune response against the bacilli. Further, some authors have been described the presence of eosinophil on the site of infection, which can release various cytotoxic mediators after its activation. These cells not only secrete cytokines such as IL-4, IL-5 and IL-10, but also accordingly with the environment, can secrete proinflammatory cytokines such as IFN-γ and TNF-α tumor necrosis factor, typically detected in patients with TB. Accordingly, in the present study we evaluated the gene expression profile of eosinophils and neutrophils obtained from peripheral blood of active or latent TB patients. In general, neutrophils shown increased expression of CD206, Arg-1 and IL-4 in latent PPD+ patients and those genes were decreased in active TB patients. Additionally, eosinophils had a diminished CD206, NOS-2 ARG-1 and IL-4 in both groups, but further decreased even more the expression of CD206 and IL-4 in patients with TB. This scenario supports the role described for neutrophils in the disease but suggests that there are changes in markers for eosinophils that could be correlated with disease progression.

W.44. Effect of Adrenal Steroid Hormone Dehydroepiandrosterone (DHEA) in Neutrophil Microbicidal Activity

Veronica Brauer, Milena Espindola, Luana Soares, Leonardo Lima, Fabiana Zambuzi, Caroline Fontanari, Cristina Cardoso and ***Fabiani Frantz****. Universidade de São Paulo, Ribeirão Preto, Brazil*

Neutrophils or polymorphonuclear leukocytes (PMNs) are immune cells that constitute the first line of defense of the organism against bacteria and fungi infection. PMNs interact with pathogens molecules or molecules produced as a result of damage or cell death, triggering various microbicidal mechanisms that aim to restore the homeostasis. Among those mechanisms are phagocytosis, degranulation, reactive oxygen species production and NET - Neutrophil Extracellular Trap. NETs are structures formed by DNA strands associated with histones, proteins and antimicrobial peptides, capable of holding and exposing microorganisms to a toxic environment. It is well known that hormones have the ability to modulate the immune response and vice versa, comprising the neuroendocrine and immune system relationship. Based on this information, our objective was to evaluate the influence of adrenal steroid hormone dehydroepiandrosterone (DHEA) in the microbicidal activity of neutrophils infected *in vitro* with *Salmonella enterica* serovar Typhimurium. To understand how the hormone regulates the function of neutrophils in the biological system, we evaluated the microbicidal activity of PMNs via phagocytosis and NET release. Here we showed that neutrophils from human peripheral blood, stimulated or not with GM-CSF are capable to phagocytize *S. enterica* serovar Typhimurium. Further, when cells were pre-incubated with DHEA the phagocytosis was enhanced. Moreover, the NET formation was also stimulated when neutrophils are incubated with bacteria, but the presence of DHEA induced an opposite effect, drastically reducing the formation of NETs. Our results suggest that DHEA is a mediator that can modulates the neutrophil mechanisms of action.

OR.05. Evolution of Innate and Adaptive Cytokine Responses in Ischemia Reperfusion Injury in Orthotopic Liver Transplantation

Ping Rao, Ali Zarrinpar, Charles R. Lassman, Vatche G. Agopian, Fady M. Kaldas, Douglas G. Farmer, Ronald W. Busuttil, Yuan Zhai, Jerzy W. Kupiec-Weglinski and Elaine F. Reed. ★University of California, Los Angeles, Los Angeles, CA

Background: Ischemia reperfusion injury (IRI) in orthotopic liver transplantation (OLT) can lead to primary graft non-function, re-transplantation or recipient death. Data from experimental models indicate that OLT-IRI is mediated by complex interactions between the innate and adaptive arms of the immune system. This study aims to determine the evolution of cytokine response over time following OLT-IRI.

Methods: Blood samples from 27 adult OLT recipients were collected pre-operative, intraoperative (blood liver flush, LF) and post-transplant on day 1, weekly for 1 month, and bi-monthly for 3 months. Graft biopsies were collected pre-transplant and 2 hours after reperfusion. LF and plasma samples were analyzed using Luminex 38-plex cytokine/chemokine arrays (Millipore). H&E stained slides of liver biopsies were graded for IRI (0=none, 1=minimal, 2=mild, 3=moderate, 4=severe) based on the following parameters: necrosis, inflammatory infiltrates, large droplet macrovesicular steatosis, congestion, and ballooning. IRI scores and cytokine levels were analysis to detect significant associations.

Results: 44% of transplant recipients (12/27) were IRI+ (score ≥2). Immediately post-transplant, IRI+ patients showed significant ($P<0.05$) increase inflammatory innate cytokines in the LF including IL-12p70, MCP-3 and IFN-α2. Early post transplantation (day1), both innate and adaptive cytokines/chemokines, IFN-γ, GM-CSF, MIP-1a and Fractalkine, were significantly higher in IRI+ patients compared to IRI-. Late after IRI (day 7 post transplantation), T cell-associated cytokines/chemokines dominate the immune response in IRI+ recipients showing significant increases in IL-17A, IL-8, IL-2, IL-4, IL-7 and G-CSF.

Conclusion: Activation of innate immunity during human OLT-IRI triggers adaptive immunity and correlates with the degree of IRI injury.

OR.52. Mixed Chimerism and Two Way Alloimmune Responses in Human Intestinal Allografts

***Julien Zuber**, Brittany Shonts, Sai Ping Lau, Shana M. Coley, Suxiao Yang, Susan Dewolf, Joshua I. Weiner, Donna Farber, Yufeng Shen, Mercedes Martinez, Tomoaki Kato and Megan Sykes. ★Columbia University Medical Center, New York, NY*

The impact of alloreactivity on the turnover rate and repertoire of gut-resident lymphoid cells populating human intestinal allografts is unknown. We analyzed the origin and alloreactivity of graft-resident T cells in human intestinal allografts using flow cytometry and high-throughput TCR sequencing in 11 consecutive intestinal transplant recipients.

The replacement rate of intestinal T cells was significantly ($p<0.01$) faster over the first 200 days in patients who experienced an early moderate to severe acute rejection compared to those with mild or no rejection. We next investigated the presence of alloreactive T cell clones in intestinal allografts. Host-vs-graft (HvG)- and graft-vs-host (GvH)-reactive clones were identified via pre-transplant CFSE-MLR (recipient anti-donor and donor anti-recipient, respectively) sorted into $CFSE^{low}$ populations followed by high-throughput sequencing of TCRb CDR3 regions. HvG-reactive clones were significantly enriched in cellular rejection biopsies relative to pre-transplant blood and post-transplant rejection-free biopsies ($p<1E-20$). The enrichment in HvG-reactive clones was much lower in peripheral blood T cells isolated at the time of rejection than in the graft ($p<1E-20$). We further investigated one patient in whom

there was low donor cell replacement in the graft over the first year post-transplant despite two mild rejection episodes. We hypothesized that an intra-graft GvH-reactive response might prevent the replacement of donor cells by recipient cells. Consistently, GvH-reactive clones were significantly enriched in post-transplant biopsies ($p<1E-20$), and accounted for approximately 50% of the $CD8^+$ graft-resident clones identifiable as donor-derived.

This study provides new tools to assess the processes that drive human intestinal graft repopulation by host cells.

W.45. IL-6 Inhibits T Cell Death and Promotes the Development of Alloimmune-mediated Arteriosclerotic Disease

Anna von Rossum, Winnie Enns and Jonathan Choy. Simon Fraser University, Burnaby, BC, Canada

Blockade of IL-6 bioactivity is clinically approved for the treatment of a number of rheumatological diseases and is under investigation for treatment of the immune-mediated arteriosclerotic condition giant cell arteritis and for the prevention of alloimmune responses in transplantation. However, the mechanisms by which IL-6 contributes to immunopathological responses are incompletely understood. In our studies using a mouse model of allograft artery rejection that resembles transplant arteriosclerosis, an alloimmune-mediated arteriosclerotic condition that is a leading cause of heart transplant failure, the absence of IL-6 production from artery cells reduced pathological arterial thickening. There was a marked and significant reduction in the accumulation of both CD4 and CD8 T cells in artery grafts lacking IL-6 and this was accompanied by an increase in the frequency of effector T cells undergoing cell death. No difference in the frequency of splenic T regs was observed. Stimulation of T cells with allogeneic antigen presenting cells (APCs) that lacked IL-6 resulted in the expansion of effector T cells that were more susceptible to Fas-induced cell death as compared to those stimulated with wild-type APCs. Taken together, our data indicate that IL-6 protects alloantigen-activated T cells from cell death and that this may be a mechanism by which IL-6 promotes the development of alloimmune-mediated arteriosclerotic thickening.

W.46. Antibody Mediated Rejection of Renal Transplant- Clinico-pathological Correlation

Suhas Bavikar, Oswal Ajay, Swarnalata Gowrishankar. Utkarsh Hospital, Aurangabad, Maharashtra, India

Antibody mediated (C4d positive and or DSA by luminex positive) rejection vs T cell mediated rejection in 81 graft biopsies done over 3 year in 138 kidney transplant recipients - comparison for treatment outcomes , histopathology slides, DSA testing by luminex Reports. Effective treatment given was methylprednisolone 500 mg daily for 3 days C4d staining positive cases with positive DSA in 3 first 30 post-operative day period cases recd. rabbit thymoglobulin 3 mg/kg over 3 days and 5 mg/kg over 5 days in one case. All these patients recovered. Late onset after 3 months of transplant to 3 year - 5/14 DSA^+, C4d positive recipients were treated by 4 sessions of daily Plasmapheresis removing 2 litre each time and low dose ivig (100 mg/kg/day after TPE) with 1 gm of rituximab after last plasmapheresis. Bortezomib 1.2 mg/m2 on day 1,4,7,11 and repeat courses with dsa titres follow up were used in 5 chronic humoral denovo antibody mediated rejections with beneficial effects in 2 cases - DSA dropped to MFI < 1000 Class 1 and class 2 IgG with improvements in proteinuria and stabilisation of creatinine. Conclusion: Antibody mediated rejections with dsa positivity behaved differently to treatment in immdte post-operative period (4/14recovered), late onset acute rejections (5/14 did not respond - 1 died, 4 progressed to end stage) and chronic, denovo antibody mediated rejection (5/14 had creeping creatinine progressing very slowly).

W.47. Mesenchyma-meidated Immune Control in Liver Transplant Tolerance

Lina Lu, Miwa Morita, John Fung and Shiguang Qian. ★Cleveland Clinic, Cleveland, OH

Organ transplantation requires lifelong immunosuppression with severe side effects. Induction of tolerance is deal, but remains elusive. Indeed, liver transplant tolerance occurs in both animals and humans (mechanisms unknown).

We were inspired by liver transplant tolerance that absolutely requires interferon (IFN)-γ, an inflammatory cytokine, and revealed that the rejection of IFN-γR1$^{-/-}$ liver graft was not mediated by the graft CD45^{+} hematopoietic cells as they were rapidly replaced by recipient IFN-γR1$^{+/+}$ cells. It is rather mediated by the CD45^{-} mesenchymal cells. Graft demonstrated ability to counterattack host immunity trough the mesenchyma-mediated immune control (MMIC) mechanisms, which were triggered by IFN-g produced by graft infiltrating T effectors (Tef) cells to activate IFN-γ signaling pathways in graft mesenchymal cells, resulting in upregulated expression of end product B7-H1 to facilitate Tef cell apoptosis. Comparable elevations of Treg cells and MDSC were seen in both rejection and tolerance groups, suggesting a critical role of Tef cell elimination in tolerance induction. We identified potent MMIC activity of hepatic stellate cells and liver sinusoid endothelial cells in significantly prolong the co-transplanted islet allografts. MMIC represents the negative feedback loop cascade reactions between graft mesenchymal cells and effector T cells leading to establishment of liver tolerance. MMCI is unlikely exclusive to the liver, as spontaneous acceptance of kidney allograft has been reported, although less common, probably reflecting variance in MMIC activity. MMCI is an important homeostatic mechanism that supports peripheral tolerance. Enhancement of MMIC is a novel strategy for induction of tolerance and treatment of autoimmune diseases.

Reproductive Immunology

3684. Representation of Iimmunological Parameters in Peripheral Blood of Patients with Fibrocystic Mastopathy and Uterine Myoma

Diloram Musakhodjaeva[1] and Aida Ergesheva[2]. [1]Institute of Immunology, Tashkent, Uzbekistan; [2]Tashkent Institute of Postgraduate Education, Tashkent, Uzbekistan

To obtain reliable results was conducted immunological study of blood from 84 women. From them, 34 women awarded fibrocystic breast disease and 30 women disease combined with uterine myoma. The results indicated that patients with fibroid breast disease a moderate degree of epithelial proliferation of the tumor, there was a significant increase in all subpopulations of T lymphocytes, CD16 $^{+ -}$ and CD20 $^{+-}$ lymphocytes compared with the control group. With the combination of fibrocystic breast disease and uterine fibroids showed a significant decrease in CD4^{+-} lymphocytes. Analysis of the research revealed that the severity of the clinical manifestations of the disease in patients with fibrocystic breast disease has a significant impact on the level of production of IL- 2 and the number of CD25^{+-} lymphocytes. Decreased production of TNFα in women with multiple forms of fibrocystic breast disease.

3696. Mechanism of the Local Immune Response in Women with Cervical Intraepithelial Neoplasia on the Background of Human Papillomavirus Infection

***Diloram Musakhodjaeva**[1] Amina Kalandarova[2] and Gulsara Eshimbetova[3]. [1]Institute of Immunology, Tashkent, Uzbekistan; [2]Tashkent Pediatric Medical Institute, Nukus, Uzbekistan; [3]Tashkent Institute of Postgraduate Education Medical Academy, Tashkent, Uzbekistan*

The importance of Th1-response in the elimination of HPV infection (PVI) or lack of response associated with the development of HPV-associated neoplasia. The aim of the study was to examine the levels of cytokines in the cervical mucus of women, depending on the severity of cervical intraepithelial neoplasia (CIN). Were examined 36 women of reproductive age with varying degrees of CIN. Immunological studies were performed to examine the levels of cytokines in the cervical mucus by ELISA. Analysis of the results showed that increased TNF-a women c CIN I detected in 63% of cases, IL-1β - in 38%, and IL-8 - only 5%, while INF-γ - defined high frequency (92%) and its concentration does not exceed the value of women without HPV (P> 0.05). Women c CIN II-III degree studied cytokine levels were significantly higher than in patients without HPV and CIN I. In the cervical mucus of women c CIN II-III often determined by IL-8 (22%), and its concentration was 3 times greater (P <0.001) than in those without HPV and CIN I (P <0.05). IL-1β was detected in 56% of women with CIN II-III and its concentration is 2-3 times

higher than in the two groups (P <0.05). In addition, the level of IL-8 directly correlated with the content and INFγ, INFα (r = 0.56 and 0.62, P <0.05), as well as TNF-α (r = 0.66, P <0.05), indicating that the local prolonged inflammation. In the group of women without HPV revealed more favorable condition immune reactivity.

Therapeutics/Pharmacology

OR.41. Modulating IL-2/IL-2R Interactions to Therapeutically Shift the Balance between Regulatory T Cells and Effector Lymphocytes

Li Li[1], Dina Alcorn[1], Cyr De Imus[1], Mei Han[1], Chris Spahr[2], Abraham Guerrero[1], Mehrdad Ameri[2], Margaret Karow[2], Jacqueline Kirchner[1], Mark Kroenke[2], Kevin Gorski[2], David Martin[1], Katsu Ishida[2], Guna Kannan[2], Joshua Pearson[1] and Marc Gavin[1]. [1]Amgen, Seattle, WA; [2]Amgen, Thousand Oaks, CA

IL-2-based therapeutics that enhance $CD4^+$ regulatory T cells (Treg) represent a promising new approach for the treatment of inflammatory disease. IL-2-mediated Treg enrichment inhibits disease progression in multiple mouse models of autoimmunity, and in initial clinical studies, low dose IL-2 (Proleukin) enriches Treg and alleviates symptoms in patients suffering from various inflammatory conditions. One concern with this approach, however, is that efficacy will be compromised in certain diseases or patients due to IL-2 activity on autoreactive T cells, NK cells, or innate lymphoid cells, or that IL-2-mediated lymphocyte activation will exacerbate pathology. To explore ways to increase Treg-selectivity, we have generated human IL-2 muteins with reduced potency and increased dependence on high IL-2Rα/CD25 expression. In humanized mice and cynomolgus monkeys, Fc-fused IL-2 muteins (Fc.muteins) were highly effective at stimulating Treg growth, but were poor agonists of conventional T cells and NK cells relative to Fc-fused wild-type IL-2 (Fc.WT) or Proleukin. Furthermore, unlike with Fc.WT or Proleukin, proinflammatory mediators and elevated body temperature were not induced with Fc.muteins. Unexpectedly, certain Fc.muteins were more effective than Fc.WT or Proleukin at increasing Treg frequency and upregulating FOXP3. This property was found to correlate with an ability to stably associate with cell surface CD25 and stimulate low levels of IL-2R signaling for extended periods of time. Thus, optimal Treg-selectivity resulted from a combination of reduced activation of effector cells and better agonism of Treg. Our results demonstrate that a high degree of Treg-selectivity can be achieved through subtle changes in IL-2/IL-2R interactions.

F.67. Tofacitinib Modulates Both Adaptive and Innate Immune Response and Ameliorates Lupus-like Phenotype and Vascular Dysfunction in MRL/lpr Mice

Massimo Gadina[1], Yasuko Furumoto[1], Wenpu Zhao[1], Carolyne Smith[1], Wanxia Tsai[1], Anna Trier[1], Victoria Hoffmann[2] and Mariana Kaplan[1]. [1]National Institute of Arthrits Musculoskeletal and Skin Diseases, National Instututes of Health, Bethesda, MD; [2]Division of Veterinary Resources, National Institues of Health, Bethesda, MD

Tofacitinib is an inhibitor of a Janus kinases (JAKs) that blocks signaling downstream of multiple cytokines. Ongoing and completed clinical trials have shown that is efficacious in inflammatory and autoimmune diseases such as rheumatoid arthritis, psoriasis and inflammatory bowel disease.

Systemic lupus erythematosus (SLE) is a complex, multi-organ autoimmune disease characterized by the production of autoantibodies and abnormalities of both adaptive and innate immune cells. Inflammatory cytokines synthesized by these cells are critical for the pathogenesis of the disease. Several studies have shown that blockade of inflammatory cytokines is a feasible approach for the treatment of SLE.

To evaluate the impact of tofacitinib on the pathology of SLE, we utilized the MRL/lpr lupus-prone mouse model. Upon inception of the disease (around 10 weeks of age), we orally administered tofacitinib for 8 weeks. Tofacitinib-treated mice displayed lower serum levels of anti-nuclear antibody and decreased albumin/creatinine ratio in the

urine. Histological analysis of kidney and skin tissue indicated decreased renal and skin inflammation. Splenocytes taken from tofacitinib-treated mice, showed reduced expressions of type I/II IFN signature genes (IP-10, MX1, and STAT1). Reduced levels of several serum pro-inflammatory cytokines (IL-17, IFN-γ, and TNF-α) as well as significant improvements in innate immune response as well vascular damage were also observed.

These data suggest that tofacitinib ameliorates the SLE phenotype and vascular dysfunction in lupus-prone mice by modulating both the adaptive and innate arms of the immune system. This supports the concept that tofacitinib and other Jak inhibitors may be beneficial in SLE.

F.68. The Anti-pulmonary Fibrosis Activity of *Osmanthus Fragrans* and its Major Compound

Yi-Ling Ye. National Formosa University, Huwei Yunlin, Taiwan

Pulmonary fibrosis is a chronic inflammation, progressive and fatal interstitial lung diseases. Idiopathic pulmonary fibrosis is the most common, but the pathogenesis remains unclear. It is revealed that transforming growth factor-β (TGF-β) is a master switch cytokine during pulmonary fibrosis. The cause of pulmonary fibrosis is characterized by the excessive deposition of extracellular matrix proteins within the pulmonary interstitium. The flower roots, flowers, leaves, fruits and seeds of *Osmanthus fragrans* flower can be medical used, *Ben Cao Gang Mu*, traditional Chinese medical literature, describes the usefulness of these flowers for phlegm and stasis reduction . In some *in vivo* and *in vitro* studies have also been shown that have a widely pharmacological activity, but the effect of improving pulmonary fibrosis is still unclear. In this study, we used mouse lung fibroblast cell line to investigate the anti-fibrotic effect of *Osmanthus fragrans* and its major compound.

The results showed that the proliferation and collagen type I expression of fibroblast cells induced by TGF-β were significantly inhibited by verbascoside. Furthermore, TGF-β related Smad 2/3signaling pathways also are evaluated in order to understand this anti-fibrotic mechanism. In the future, we hope this study can provide the possibilities of verbascoside for prevention or treatment of pulmonary fibrosis.

F.69. Molecular Impact of Graphene Oxide with Different Shape Dimensions on Immune Cells

Marco Orecchioni[1], Dhifaf Jasim[2], Mario Pescatori[1], Davide Bedognetti[3], Alberto Bianco[4], Kostas Kostarelos[2] and Lucia Delogu[1]. [1]University of Sassari, Sassari, Italy; [2]University of Manchester, Manchester, United Kingdom; [3]Sidra Medical & Research Centre, Doha, Qatar; [4]Centre National de la Recherche Scientifique, Strasbourg, France

Graphene oxide (GO) is gaining the interest of the scientific community for its revolutionary future applications [Geim AK et al. Nature Materials 2007]. In this context, the possible immune cell impact of GO is fundamental area of study for a translational application in medicine [Orecchioni M. et al. JTM 2014, Pescatori M et al. Biomaterials 2013]. In our work we focused on the effects, on human lymphomonocytes (PBMCs), of two types of GOs, deeply characterized, which differed in lateral size dimension (GO-Small and GO-Large). To clarify the immune impact of GOs we provided a wide range of assays looking at cells viability, cell activation, cytokines release and genome expression. We let in lights also the impact of GOs on immune correlated 84 genes. A whole genome analysis was conducted on T cells and monocytes to deeply evaluate the GO-cell molecular interactions. GOs didn't impact the cell viability. We identified 37 upregulated genes in the GO-Small samples compared to 8 genes for GO-Large, evidencing a clear impact of the GO dimension on cell activation. We confirmed the size-related impact at the protein level by multiplex ELISA. Results were supported also by microarray analysis. Data evidenced a clear GO-Small-induced downregulation of oxidative phosphorylation followed by a glycolitic switch-on in both cell types giving future perspectives for anticancer nano-graphene system. Our work represents a wide activity characterization of different sized GOs on immune cells giving crucial information for the chemical and physical design of graphene for biomedical applications.

F.70. Nano-emulsified Curcumin (NEC), a Patented Anti-inflammatory Drug Developed at Ohio State, Reduces Renal Pathology in an Animal Model of Lupus Nephritis When Added to Drinking Water

Nicholas Young, Lai-Chu Wu, Mark Gardner, Jeffrey Hampton, Michael Bruss and Wael Jarjour. The Ohio State University Wexner Medical Center, Columbus, OH

Nutraceuticals are food components that have therapeutic value in disease treatment or prevention. Curcumin is the bioactive component of turmeric, which is a nutraceutical that has been used for centuries in Eastern medicine as an anti-inflammatory agent. Although many molecular targets have been identified *in vitro*, the inability to successfully translate curcumin to the clinic results from inadequate bioavailability. Specifically, curcumin is poorly absorbed in the intestinal tract and is metabolized rapidly. Recently, pharmacology researchers at Ohio State developed a novel formulation of nano-emulsified curcumin (NEC), which enhanced bioavailability over 10-fold in mice. Subsequently, our laboratory demonstrated that NEC suppresses NFκB-mediated inflammatory responses by inhibiting macrophage infiltration in several animal models of acute inflammation. Here, we supplied NEC in drinking water to determine therapeutic potential in the treatment of chronic inflammation. NZM2410 mice spontaneously develop severe glomerulonephritis at 22-40 weeks of age and were used as a model of lupus. Mice were supplied approximately 40 mg/kg/day NEC beginning at 18 weeks of age; overall health was assessed by weekly weight measurements and kidney function was gauged by weekly blood urea nitrogen (BUN) testing. After 40 weeks, while only 62% of the mice in the control group had maintained BUN levels under 50 mg/dL and not lost more than 20% body weight, none of the mice receiving NEC met the increased BUN or the weight loss criteria. These results indicate that NEC indeed has therapeutic potential in treating the chronic inflammation associated with lupus nephritis as well as other autoimmune diseases.

F.71. Anti-CD184-FK506 Antibody-drug Conjugate to Treat Autoimmunity and Graft Rejection

Robin Humphreys, Shiva Bhowmik, Andrew Beck, Nick Knudsen, Anthony Manibusan, Jianing Wang, Ying Sun, Tim Buss, Veronica Gutierrez, Bill Brady, Kiah Smythe, Susan Richards, Dennis Gately, Trung Phuong, Christine Phung, Alyssa Powell, Marvin Paterson, Kari Cox, Lillian Skidmore, Jessica Kirtley, Alan Wahl and Damien Bresson. Ambrx Inc., San Diego, CA

Tacrolimus (FK506) is a very potent immunosuppressant used to prevent organ rejection post-transplantation and reduces inflammation in various autoimmune diseases. However, severe off-target effects and generalized immune-suppression are seen after prolonged treatment, which limit its effectiveness. We hypothesized that directing FK506 to activated T cells would improve its therapeutic index. With this goal, we aimed to develop a novel antibody-drug conjugate (ADC) with immunomodulatory capability. We selected CD184 as a candidate T cell targeting antigen. A chimeric anti-human CD184 antibody was engineered to carry two FK506 drugs per antibody using Ambrx's proprietary site-specific conjugation technology. We showed that the naked CD184 antibody was able to traffic into the lysosomes of activated human T cells for optimal intracellular drug delivery. *In vitro* activity studies were conducted (1) to select an optimal linker which possessed serum stability and efficient release of the payload inside the cells; and (2) to evaluate how different linker-payload conjugation sites on the antibody would impact activity. The potency of CD184-FK506 ADCs was assessed by measuring inhibition of NFAT activation in Jurkat-NFAT-Luc reporter cells. We demonstrated that both the linker chemistry and the conjugation site play an important role in the ability of ADCs to deliver active FK506 into the target cells. CD184-FK506 ADC significantly reduced pro-inflammatory cytokines released by anti-CD3/anti-CD28 activated primary human T cells. In addition, *in vivo* data using a xenogenic graft-versus-host disease (GVHD) mouse model will be presented. Our data support the utility of site-specific conjugated ADCs for the treatment of immune-mediated diseases.

F.72. MEDI-551 Treatment Effectively Depletes B Cells and Reduces Serum Titers of Autoantibodies in *Sle*1-Human CD19 Transgenic Mice

Yue Wang, Sandra Gallagher, Roland Kolbeck and Ronald Herbst. MedImmune, Gaithersburg, MD

B cells and their auto-antibody products play an important role in multiple autoimmune diseases and the use of monoclonal antibodies (mAb) targeting B cells for treatment has produced promising results. MEDI-551 is an anti-CD19 mAb targeting B cells for depletion and is currently being evaluated in Phase I trials in systemic sclerosis and multiple sclerosis. Here we studied MEDI-551 in the *Sle*1-human CD19 transgenic (*Sle*1-hCD19Tg) mice, which develop classical autoimmune symptoms, including hyper-activation of B and T cells and presence of auto-reactive antibodies in sera. *Sle*1-hCD19Tg mice were treated with either a single dose of MEDI-551 or repeated doses for up to twelve weeks. The B cell numbers were detected by FACS assay and the number of antibody secreting cells (ASC) by ELIspot. Serum autoantibody levels were determined by ELISA and cytokine levels by RBM multiplex assay. MEDI-551 treatment resulted in significant (>90%) and sustained depletion of naïve B cells in spleens. In addition, spleen germinal center B cells and ASC were reduced by ≥70%; whereas, ASC in bone marrow are largely unchanged. After prolonged treatment of MEDI-551 in *Sle*1-hCD19 Tg mice, autoantibodies specific for dsDNA, histone, and ANA were reduced by 40-80% and levels of inflammatory cytokines, such as IL-6 and PAI-I were significantly reduced, too. Our findings highlight the ability of MEDI-551 of depleting B cells and ASC in the autoimmune *Sle*1-hCD19Tg mice. Thus, MEDI-551's novel ability to remove a broad range of B cells and eliminate most disease-driving autoantibodies in an autoimmune mouse model warrants continued research.

F.73. Manocept, a Derivative of FDA-approved 99mTc-Tilmanocept, Exhibits Diagnostic Potential in Rheumatoid Arthritis: A Novel Application of an Existing Drug

Nicholas Young[1], Thomas Rosol[2], Larry Schlesinger[1], Frederick Cope[3], Ramiro Toribio[2] and Wael Jarjour[1]. [1]The Ohio State University Medical Center, Columbus, OH; [2]The Ohio State University, Columbus, OH; [3]Navidea Biopharmaceuticals, Inc., Dublin, OH

99mTc-Tilmanocept is a receptor-targeted gamma-emitting metastable radiopharmaceutical currently used in humans to facilitate preoperative lymphoscintigraphy, and more accurate intraoperative lymphatic mapping with discrete sentinel node detection (SLN) of solid tumors. This SLN target specificity is the result, in part, of 99mTc-Tilmanocept binding to CD206 on tumor-associated macrophages. CD206 is a mannose-binding receptor that is expressed following alternative activation of macrophages. Using a fluorescently-labeled derivative of 99mTc-Tilmanocept, Manocept-Cy3, we examined the potential use of this pharmaceutical in rheumatoid arthritis (RA). Immunohistochemistry of synovial tissue from RA patients demonstrated significant presence of Manocept-Cy3 positive cells relative to osteoarthritis and healthy tissue controls, which displayed little to no fluorescent detection. These results also demonstrated the co-localization of CD206 and Manocept-Cy3, indicating that alternatively activated macrophages expressing CD206 are novel biomarkers of the inflammatory process in RA. Macrophage-specific labeling was confirmed by flow cytometry in RA synovial fluid, as positive detection was observed in conjunction with CD14, CD16, CD11b, and CD163. To evaluate the feasibility of Manocept as a diagnostic imaging agent, RA was induced in Dba1 mice and epifluorescent imaging was performed *ex vivo* and *in vivo* following intravenous injection with Manocept-Cy3. Data acquired by two-photon imaging indicated significantly greater signals in knees and elbows of arthritic mice where these signals were totally concordant with the level of histiocytosis. Our results suggest that Manocept is a viable candidate to pursue clinically as an imaging biomarker for disease activity in RA to facilitate early diagnosis and to help guide therapeutic strategies in RA patients.

F.74. Evaluation of CCR6 as a Target for Selective Calcineurin Inhibition in Th17 Driven Autoimmunity

Emily L. Williams[1,2], Lauren P. Schewitz-Bowers[1,2], Philippa J. P. Lait[1,2], Madeleine L. Stimpson[1,2], Andrew D. Dick[1,2,3], Lai Wei[4], Robert B. Nussenblatt[5,6,7] and Richard W. J. Lee[1,2,3]. [1]University of Bristol, Bristol, United Kingdom; [2]National Institute for Health Research (NIHR) Biomedical Research Centre at Moorfields Eye Hospital and University

College London Institute of Ophthalmology, London, United Kingdom; [3]University Hospitals Bristol NHS Foundation Trust, Bristol, United Kingdom; [4]Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China; [5]National Centre for Complementary and Alternative Medicine, National Institutes of Health, Bethesda, MD; [6]National Eye Institute, National Institutes of Health, Bethesda, MD; [7]★Centre for Human Immunology, Autoimmunity and Inflammation, National Institutes of Health, Bethesda, MD

Th17 cells play a key role in the pathogenesis of autoimmunity and allergy. However, monoclonal antibodies which inhibit IL-17 have variable clinical efficacy. This provides a rationale for the development of therapeutics which selectively inhibit Th17 cells rather than their canonical soluble mediators. Human Th17 cells are exclusively derived from $CCR6^+$ expressing $CD4^+$ memory cells. CCR6 internalises its cognate ligand, CCL20, upon engagement and therefore targeting of Th17 cells via CCR6 is a candidate route for selective drug delivery. Previously we have demonstrated that calcineurin inhibitors selectively inhibit Th17 cells. However, when administered systemically, they have a plethora of side-effects. Targeted delivery of calcineurin inhibitors via CCR6 using an antibody-drug conjugate has the potential to maximise their therapeutic effect while minimising unintended harm in non-immune tissues. Here we evaluate CCR6 as an immunotherapeutic target by assessing anti-CCR6 mAb internalisation *in vitro* and examining CCR6 distribution and receptor density across immune cell subsets in peripheral blood. CCR6 in both human and murine $CD4^+$ cells becomes internalised following mAb engagement. Assessment by multi-coloured flow cytometry confirms that CCR6 expression within human immune cell populations, in terms of both receptor distribution and density predominates in memory $CD4^+$ T cells and B cells, however B cells are not modulated by calcineurin inhibition. These data therefore support the use of CCR6 as an immunotherapeutic target for the selective delivery of calcineurin inhibitors to the effector memory T cell pool from which Th17 cells are derived in the treatment of autoimmune and allergic diseases.

F.75. Antibody-mediated Targeting of Polymeric Nanoparticles to Inflamed Human Endothelium

***Gregory Tietjen**, Alexander Engler, Nancy Kirkiles-Smith, Jiajia Cui, W. Mark Saltzman and Jordan S. Pober. ★Yale University School of Medicine, New Haven, CT*

Polymeric nanoparticles have the capacity to act as therapeutic reservoirs for the sustained release of a wide array of drugs (e.g. anti-inflammatory agents). Additionally, the surface of these nanoparticles can be modified to allow conjugation of targeting ligands, such as antibodies, to provide the potential to manipulate the biodistribution of systemically delivered nanoparticles, as well as improve cellular uptake of nanoparticles via receptor-mediated endocytosis. Cytokine-inducible adhesion molecules on the surface of vascular endothelium play a critical role in recruitment of leukocytes to inflamed tissues. We hypothesize that polymeric nanoparticles have tremendous therapeutic potential for the treatment of inflammatory conditions via targeting of adhesion molecules on endothelium in order to delivery anti-inflammatory agents capable of suppressing leukocyte recruitment. Here we describe targeting of both commercially available and custom made polymeric nanoparticles to human endothelium *in vitro* using either TNF-induced or constitutively expressed endothelial markers. Utilizing a microfluidic cell-culture platform, we further show the importance of performing nanoparticle-targeting experiments under physiological shear stress, as static incubation of nanoparticles with cells leads to artificially high background levels, which can confound optimization of targeting specificity. Finally, we show that human microvessels in TNF-treated human skin grafts on mouse hosts will express an inducible adhesion molecule, E-selectin, that can be targeted by systemically administered antibodies. Preliminary observations in this model support the feasibility of targeting antibody-conjugated nanoparticles to inflamed human endothelium *in vivo.*

3956. Immunological Status of Children in Drug Interactions Viferon on the Background of Timoptin Usage

***Z. Kamalov**, T. Aripova and Z. Rakhmankulova. Academy of Sciences of the Republic of Uzbekistan, Tashkent, Uzbekistan*

Were studied parameters of cellular, humoral immunity and cytokine profile in neonates with HSV and CMV before and after treatment in 2 weeks. Confirmation of infection carried by two methods: ELISA and PCR or by detecting viral RNA in peripheral blood mononuclear cells. There was a significant increase in the total pool of indicators of T lymphocytes. In the study of the absolute values also revealed a significant increase in the number of $CD3^+$ cells (P<0,001) in relation to indicators at baseline. The study of the level of immunoregulatory subpopulations of T lymphocytes showed that the number of $CD4^+$ -and $CD8^+$ -lymphocytes significantly changed, and it was at the level of control (P>0,05). The study of non-specific protective factors shows that the content of $CD16^+$ cells, reduced pre-treatment and traditional treatment, after treatment Timoptin to normal (P<0,001). Results of the study humoral immunity showed that elevated levels of B-lymphocytes after turning on the background viferon Timoptin significantly decreased compared to its level before treatment. The concentrations of immunoglobulins A, M, G also significantly decreased and reached the end of the study an average of 13,7±1,21 mg /%, 11,3±1,11 mg /% and 895,6±43,83 mg /%, respectively (P<0,05). Elevated concentrations of IL-1β, and IL-8 treatment, under the influence of Timoptin decreased, and decreased levels of IL-4 and γ-interferon rose to control values. Thus, after a complex therapy with viferon against Timoptin normalized all the indicators of immune system, ie recovered from the cellular and humoral immune responses, which contributed to significant clinical effect.

Transplantation

OR.38. Enhanced Mixed Hematopoietic Chimerism and Immune Tolerance in Monkeys After BMT Across MHC Barriers and Infusion of Polyclonal Recipient Tregs

Paula Alonso[1], Jonah Zitsman[1], Hugo Sondermeijer[1], David Woodland[1], Yojiro Kato[1], Joshua Weiner[1], Adam Griesemer[1], Leo Bühler[2], Alicia McMurchy[3], Megan Levings[3], Megan Sykes[4] and Raimon Duran-Struuck[1].
[1]★Columbia Center for Translational Immunology, New York, NY; [2]Harvard University, Boston, MA; [3]★University of British Columbia, Vancouver, BC, Canada; [4]★Columbia University Medical Center, New York, NY

Transient mixed hematopoietic chimerism leads to renal allograft tolerance across MHC barriers in about 70% of cynomolgus monkeys (CM) when the kidney is co-transplanted with donor bone marrow (BM), but fails with more immunogenic organs. In mice, co-infusion of regulatory T cells (Tregs) with BM using a non-myeloablative protocol permits durable mixed chimerism, BM engraftment, and skin graft tolerance. We aim to extend this approach into the pre-clinical CM model.

Cryopreserved Tregs expanded *in vitro* are infused over a week to recipients under mild conditioning consisting of thymic and low-dose total body irradiation, ATGAM, anti-CD40L and cyclosporine or rapamycin (monotherapy) for 30 days. Control animals (no Tregs (n=4)) lost chimerism within 40 days. Anti-donor proliferative T cell responses were observed and donor BM was rejected. Treg recipients (n = 2) developed remarkably high (>90% in myeloid lineages) and long-lasting chimerism (110-335 days after BMT). T cell chimerism was observed only in Treg recipients and Treg animals were donor-hyporesponsive at day +42. In one animal, a kidney graft from the BM donor transplanted 126 days post BMT was accepted without immunosuppression until euthanasia (day+420 after BMT). A second Treg recipient received skin grafts on day +119 post-BMT. Rejection of the donor graft was delayed compared to a third party graft and compared to a control animal. In summary, host Tregs significantly prolong donor chimerism without GVHD and promote the survival of donor organs transplanted four months later without immunosuppression.

W.61. Phenotype, Function and Expansion of Regulatory T Cells in the Cynomolgus Macaque (*Macaca fascicularis*)

Paula Alonso-Guallart[1], Jonah Zitsman[1], Hugo Sondermeijer[1], Leo Bühler[2], Christopher Ovanez[1], Alicia McMurchy[3], Megan Levings[3], Megan Sykes[1] and Raimon Duran-Struuck[1]. [1]★Columbia University, New York, NY; [2]Harvard University, Boston, MA; [3]★University of British Columbia, Vancouver, BC, Canada;

★ = member of a FOCIS Center of Excellence

Safe and reliable *in vitro* Treg expansion protocols are key for clinical translation of regulatory T cell (Treg) therapy.

We have characterized the Tregs of the Cynomolgus Macaque (CM), a pre-clinical animal model. An average of 3.67% of circulating $CD4^+$ T cells co-express CD25 and FoxP3 (n=15). Mean 3.53% express CD25 and are negative for CD127. Using CD45RA expression, a mean of 1.63% of $CD3^+CD4^+$ cells are naïve or resting Tregs, and 2.4% are activated effector Tregs. The suppressive activity of $CD4^+$CD25hiCD127-$FoxP3^+$ CM Tregs is comparable to that of human Tregs, supporting the clinical relevance of the monkey model.

In CM, four *in vitro* Treg expansion protocols were developed, ranging over 21-56 days. 25-100 thousand CD4+CD8-CD25hi or $CD4^+CD8^-$CD25hiCD127$^-$ Tregs were isolated from 15-30mL of blood and cultured with IL-2, αCD3, rapamycin, and either irradiated donor PBMCs, irradiated artificial APCs (murine fibroblasts expressing human CD80, CD58 and CD32), or both. Cultures that had artificial APCs yielded 10-100 fold more Tregs than cultures without artificial APCs. All protocols successfully prevented proliferation of bead (CD2CD3CD28)-stimulated self-PBMCs with >50% suppression at a 1:2-1:4 Tregs:PBMCs ratio (up to 1:32 ratio). Re-sorting Treg cultures successfully eliminated CD8 contamination, but led to significant losses of Tregs and required longer culture periods for expansion. Tregs in culture >40 days maintained function and phenotype. In addition, re-cultured cryopreserved Tregs maintained suppression and phenotype, and the freeze/thaw process seemed to eliminate contaminating CD8 T cells.

In summary, we document four Treg protocols ready to be tested in the pre-clinical setting.

W.62. Human NK Cell Tolerance Induced by Mixed Xenogenic Chimerism

Paresh Vishwasrao[1], HaoWei Li[1], Markus Holzl[1], Stephanie Chen[2], Guiling Zhao[1], Goda Choi[3] and Megan Sykes[1]. [1]★Columbia University, New York, NY; [2]College of Physicians and Surgeons, New York, NY; [3]★Academisch Medisch Centrum (AMC), Amsterdam, Netherlands

Xenotransplantation is a potential solution for the shortage of solid organ. The potent rejection of xenogeneic organs remains the main barrier. Mixed xenogeneic hematopoietic chimerism has been shown in mouse models to induce xenogeneic tolerance of T, B and NK cells. NK cells play an important in the rejection of xenogeneic tissues. We have shown previously in a rat-to-mouse transplantation model that induction of mixed chimerism led to tolerance of recipient mouse NK cells to donor rat cells, which was associated with global hyporesponsiveness. In this study, we investigated whether pig-human mixed chimerism could tolerize human NK cells in a humanized mouse model. Our results showed that induction of human NK cells in pig-human mixed chimeras did not lead to rejection of pig cells. NK cells developing in the presence of pig chimerism did not show an immature phenotype or enhanced activation. We have delineated the NK cell subpopulation $CD56^{bright}$ and $CD56^{dim}$ immunophenotypically from various tissues showing reduced cytotixicity. NK cells from pig-human mixed chimeric mice were specific tolerant to pig cells as NK cells from these mice mount decreased cytotoxic responses to pig cells while maintaining normal cytotoxicity against K562 cells.

W.63. Effect of Infusion of mAbs to TNFRSF25 on Skin Graft Survival in Mice Receiving Autologous Marrow Transplantation

Reginald Gorczynski and Ismat Khatri. University Health Network, Toronto, Ontario, Canada

CD200 overexpression in transgenic recipients, or by skin allografts themselves, increases graft survival and increased $Foxp3^+$Treg graft infiltration in mice receiving rapamycin without producing long-term survival. Immune ablation by busulphan/cyclophosphamide, followed by autologous CD45-congenic marrow reconstitution (BMTx), increased survival and allowed cessation of immunosuppression in some cases. Tregs of host and donor origin (by CD45 phenotyping) were implicated in augmented survival. We produced rat mAbs to a peptide of mouse

TNFRSF25, a molecule reported to be expressed on Tregs, and examined the effect of infusion of this reagent in mice receiving BMTx post skin grafting.

C57BL/6 mice received BALB/c skin grafts with rapamycin (1mg/Kg/36hr) for 7 days, followed by busulphan/cyclophospamide for 6 days, with subsequent CD45.1 BL/6 bone marrow. Beginning 7d post BMTx (21d post skin transplantation) all mice received rapamycin for a further 14d (to 35d post transplant). A subgroup of these mice also received mAb (50µg/iv/mouse) to TNFRSF25 at 72hr intervals for 4 doses. All immunosuppression was withdrawn at 35d post transplant. Graft survival was monitored throughout, and MLCs measured with splenocytes from individual mice at 80d.

After BMTx, graft survival in WT mice was ~35% at 80d with attenuated MLC responses relative to controls (no BMTx-100% rejection at 16d). Infusion of anti-TNFRSF25 increased survival to ~80% at day 80 and increased graft-infiltrating Tregs without further immunosuppression. Adoptive transfer of splenocytes from these latter mice to naïve animals induced increased graft survival and suppression of MLCs.

Conclusion: Anti-TNFRSF25 mAbs increase skin allograft survival in mice with autologous BMTx.

W.64. Intradermal Injection of IDO Expressing Fibroblasts Prolongs Skin Graft Survival

Mohammadreza Pakyari. ★University of British Columbia, Vancouver, BC, Canada

Background: Despite the effectiveness of skin autotransplantation, the high degree of immunogenicity of skin precludes the use of allogeneic skin grafts. Systemic immunosuppression is generally felt to be inappropriate for isolated skin grafts. This study examines the potential to create an allogeneic skin transplant that delays rejection by inducing localized immonosupression. Specifically, IDO (indoleamine 2 3-dioxygenase) expressing fibroblasts are introduced into the dermis and subcutaneous area of donor subjects to provide a tryptophan depleted environment and therefore local immune supression toward the graft.

Method: 4-days post injection of the cells; grafts with regular and IDO fibroblast were transplanted to allogeneic recipients and monitored until graft rejection. To investigate any possible cumulative effect of multiple injections on the survival rate of grafts, cells are injected at different time point (days 0,3,6) to the same area and a 6mm graft was harvested from that region and transplanted to the allogeneic subjects.

Results: Skin transplantation studies demonstrates that IDO expressing grafts remain viable for significantly longer than control allogeneic grafts ($p=0.01$). Following 3-times injection of the IDO cells to the allogeneic full-thickness graft, average survival graft rate in IDO group increased up to 35-days in comparison to 13-days for control group ($p<0.001$).

Conclusion: These data suggest that local immunosuppression can be provided by the delivery of IDO-expressing fibroblasts in allogeneic skin transplantation. The potential of this research goes far beyond the promising role for skin transplantation. This “cell-based” approach to localized immunosupression can also provides potential opportunities to autoimmune skin disorders such as Alopecia Areata.

W.65. Superiority of *in vitro*-generated MDSC over Mreg and TolDC in a Mouse Model of Skin Transplantation

Lucile Drujont[1], Laura Carretero-Iglesia[1], Laurence Bouchet-Delbos[1], Gaelle Beriou[1], Emmanuel Merieau[1], Marcelo Hill[2], Yves Delneste[3], Aurelie Moreau[1], Maria Cristina Cuturi[1] and Cedric Louvet[1]. [1]★Inserm, Nantes, France; [2]Institut Pasteur de Montevideo, Montevideo, Uruguay; [3] Le Centre Régional de Recherche en Cancérologie Nantes/Angers, Angers, France

★ = member of a FOCIS Center of Excellence

Controlling the immune response is a major therapeutic challenge in the field of transplantation. Adoptive transfer of regulatory cells such as Treg has already proved its efficacy in various pathological contexts. Myeloid-derived suppressor cells (MDSC), originally identified in cancer, represent one of the new attractive strategies for cell therapy development. We recently showed that weekly injection of *in vitro*-generated MDSC without adjunct immunosuppressive treatment, in a mouse model of skin transplantation, resulted in a significant prolongation of allograft survival up to 40 days (versus 21 days for untreated mice). Here we compared MDSC with two other *in vitro*-generated regulatory myeloid cells: regulatory macrophages (Mreg) and tolerogenic dendritic cells (TolDC). In a polyclonal T cell proliferation assay, TolDC and Mreg showed stronger inhibition than MDSC for both $CD4^+$ and $CD8^+$ T cells. The lack of proliferation observed in TolDC co-cultures was associated with decreased T cell activation whereas T cells displayed an activated phenotype with Mreg or MDSC. However, this activation correlated with increased T cell death only for MDSC. Contrastingly with *in vitro* predictions, a single injection of MDSC induced a better prolongation of skin allograft survival (45 days) than Mreg or TolDC (27 and 31 days). Thus, despite the diversity of phenotypes and immunoregulatory mechanisms, these three cell types offer promising therapeutic applications and need to be further investigated. Our *in vivo* observations also reinforce the particular potential of MDSC for cell therapy development.

W.66. Dramatic Primary Expansion of Allo-specific Indirect CD4 T Cells *in vivo*

Nicholas Bishop[1] and Ronald Gill[2]. [1]Unversity of Colorado Anschutz Medical Campus, Aurora, CO; [2]★University of Colorado Denver, Aurora, CO

The relative contributions of 'direct' (donor MHC-restricted) versus 'indirect' (host MHC-restricted) pathways of allorecognition *in vivo* are uncertain. *In vitro* assays of primary alloreactivity may not model *in vivo* scenarios where donor APCs are eliminated while host APCs abound. Also, indirect T cells *in vivo* are thought to be 10^2-10^3 times fewer than direct T cells, making their role in primary alloresponses unclear. We tracked the primary response of direct versus indirect T cell receptor transgenic C57Bl/6 (B6; H-2^b) CD4 T cells to allogeneic BALB/c (H-2^d) cells *in vivo*. To model relative indirect and direct alloreactive frequencies, 100 fold fewer CD45.1 congenic indirect TCR75 (1e4) versus direct 4C (1e6) TCR transgenic CD4 T cells were transferred to CD45.2 B6 recipients. Mice were challenged with 1e6 BALB/c splenocytes. Spleens and draining popliteal lymph nodes were harvested from separate mice on days 3-5. TCR Vβ staining distinguished direct 4C ($V\beta13^+$) from indirect TCR75 ($V\beta8.3^+$) cells and Ki67 marked dividing cells. The frequency of $CD45.1^+$ cells among total $CD4+Ki67^+$ cells increased between days 3 and 5 (1.8 % to 27%). Importantly, the percentage of direct 4C cells within $CD4+Ki67^+CD45.1^+$ cells decreased from 94% on day 3 to 50% on day 5. Meanwhile, the percentage of indirect TCR75 cells increased from 0 on day 3 to 50% on day 5. Despite a numeric disadvantage, the indirect response rapidly increases and approximates the absolute magnitude of the direct response. These results suggest the indirect pathway may play a critical role in primary alloresponses *in vivo*.

W.67. Mechanistic Insights into Poor Outcomes of Cell Transplantation: Exploring the Possible Solutions

Shiguang Qian, Hong-Shiue Chou, Daniel Joyce, John Fung and Lina Lu. ★Cleveland Clinic, Cleveland, OH

Organ transplantation has applied for decades, but outcomes of cell transplantation remain poor, implicating the immune regulatory activity of non-parenchymal cells. This is case in animal models. Liver allografts are spontaneously accepted, while hepatocyte transplants are acutely rejected. We have shown that cotransplantation with hepatic stellate cells (HpSC) protected islet allografts in recipients; here HpSC must be syngeneic to recipient (patient) which limits its applications. HpSC are potent inducers of MDSC. Addition of HpSC (any strain) into BM-derived DC culture generated MDSC. Cotransplantation with 2 x 10^6 MDSC achieved >60% long-term survival of islet allografts with less graft CD8 T cells, increased Tregs. B7-$H1^{-/-}$ MDSC lost ability to protect islet allografts, indicating a crucial role of B7-H1. We attempted to generate MDSC from iPS cells (iPSC) from somatic cell). iPSC were cultured with OP9 feeder layers to become hematopoietic progenitors ($CD309^+$/$CD34^+$/c-kit^+/$Sca\text{-}1^+$), followed by culture with

GM-CSF to commit to myeloid lineage ($CD11b^+$), and then cultured with GM-CSF+IL-4 with or without HpSC. The iPSC Without HpSC, iPSC became DC. With HpSC, cells (iPS-MDSC) demonstrated similar phenotype and function to BM-MDSC, suppressed T cell proliferative response in an MLR with reduced IFN-γ. To test *in vivo*, OVA-pulsed iPS-MDSC, BM-MDSC or DC were injected into the footpad of mice who had been adoptive transferred of OTII T cells. T cells recovered from popliteal LN of iPS- or BM-MDSC group had markedly reduced proliferative response, compared to DC group. Generation of MDSC from iPSC could be a more feasible approach in clinical practice.

W.68. Combined Bone Marrow Plus Liver Transplantation in Cynomolgus Monkeys

*Joshua Weiner[1] Yojiro Kato, **Raimon Duran-Struuck**, Mercedes Martinez, Samuel Baker, Paula Alonso, Jonah Zitsman[1], Anette Wu, Philipp Houck, Teeda Pinyavat, Jay Lefkowitch, Sulemon Chaudhry, Tomoaki Kato, Megan Sykes and Adam Griesemer. ★Columbia University, New York, NY*

Introduction: Combined kidney/bone marrow transplantation (CKBMT) induces tolerance in primates and humans. Since liver is thought to be more tolerogenic than kidney, we studied combined liver/bone marrow transplantation (CLBMT) for tolerance induction in cynomolgus monkeys.

Methods: Liver and bone marrow were transplanted between four MHC-mismatched monkey pairs. Induction consisted of total body/thymic irradiation, ATGAM (Pfizer), rituximab (Genentech) (recipients 2-4), and 28 days of either IM cyclosporine (Novartis) or continuous IV tacrolimus (Astellas) followed by 4-week taper (tacrolimus only). Animal 4 additionally received LoCD2 (Mass Biologics) and anti-CD40 mAb (Mass Biologics).

Results: Recipient 1 survived 42 days with donor-specific hyporesponsiveness *in vitro* and multilineage mixed chimerism (MC) up to day 36 but died during repair of a biliary stricture. Recipients 2 and 3 were sacrificed on days 69 and 57. They maintained MC for 40 and 23 days and had normal to increased anti-donor cellular responses and severe histological cellular rejection. Flow cytometry showed high percentages of CD8 effector memory cells in the blood, graft, and draining node but not the periphery. Animal 4, who received additional costimulatory blockade and depletion of $CD2^+$ memory cells, survived 61 days (final 3 weeks with negligible immunosuppression levels) and died of diarrhea/electrolyte abnormalities on day 61 with normal LFTs, no histological rejection or GVHD, significant MC, decreased anti-donor cellular responses, and extremely low levels of CD8 effector memory cells.

Conclusion: CLBMT induces MC, but memory cell expansion is associated with rejection. Enhanced T cell/memory cell depletion prevents rejection, prolongs chimerism, and may permit tolerance.

W.69. Dual Function of *Ex Vivo*-expanded Human Double-negative Regulatory T Cells: Suppressor and Cytotoxic

Paulina Achita[1], Dzana Dervovic[1,2], Dalam Ly[1,2], Jong Bok Lee[1] and Li Zhang[1,2]. [1]★University of Toronto, Toronto, ON, Canada; [2]University Health Network, Toronto, ON, Canada

$TCR\alpha\beta^+$, NK lineage marker negative, CD4 and CD8 double-negative regulatory T cells (DN Tregs) have been shown to prevent allograft rejection, inhibit type I diabetes and attenuate graft-versus-host disease (GvHD) in rodents. Since DN Tregs comprise only ~1% of peripheral blood T lymphocytes, clinical applications of DN Tregs in humans are limited by their scarce number and lack of effective expansion method. Here we developed a novel protocol, which allows for ~4000-fold expansion of human (hu)DN Tregs in 3 weeks with >97% purity. Expanded huDN Tregs upregulate expression of IL-2 receptor alpha, maintain expression of lymphoid homing receptor CD62L, produce IL-10 and IFN-γ, and suppress proliferation of autologous $CD4^+$ and $CD8^+$ T cells *in vitro* in a cell-contact dependent manner. In addition, we show that huDN Tregs expanded in the presence of IL-7 augment, while huDN Tregs expanded in the presence of IL-15 abrogate the magnitude of *in vitro* suppression, respectively. Thus, we demonstrated for the first time that IL-7 and IL-15 expanded huDN Tregs have distinct roles in immunosuppression.

In vivo, infusion of huDN Tregs does not cause xenogeneic GvHD in NSG mice in contrast to mice injected with peripheral blood mononuclear cells. Conversely, expanded huDN Tregs are cytotoxic towards lung cancer and leukemic cell lines *in vitro*, highlighting their additional therapeutic potential. Taken together, these results indicate a dual function of *ex vivo*-expanded huDN Tregs *in vitro* and suggest their therapeutic potential in suppression of allograft rejection and treatment of malignancies.

F.76. Allograft Immunity in the Diabetic Host

Nicholas Bishop[1] and Ronald Gill[2]. [1]University of Colorado Anschutz Medical Campus, Aurora, CO; [2]★University of Colorado Denver, Aurora, CO

Diabetes is considered to be associated with impaired immunity, particularly to certain pathogens. However, the impact of diabetes on transplant immunology is uncertain. Moreover, diabetes represents a significant problem in the field of transplantation. Therefore, we sought to determine the influence of diabetes on cellular alloreactivity *in vivo*. We used spontaneously diabetic C57BL/6-Ins2^{Akita}/J mice (Akita) to model severe, chronic hyperglycemia. Akita mice harbor an Ins2 gene polymorphism resulting in severe, non-autoimmune diabetes. After challenging Akita mice and unaffected littermates with allogeneic BALB/c splenocytes, we harvested the draining popliteal lymph node and measured the T cell numbers, proliferation (Ki67$^+$) and effector function (IFN-gamma$^+$). Endogenous CD4 and CD8 T cell responses (Ki67$^+$ IFN-gamma$^+$) were comparable between Akita and control animals. We also examined the responses of marker populations of antigen-specific (BALB/c-reactive) T cell receptor transgenic T cells. Adoptively transferred, BALB/c-reactive T cell receptor transgenic CD4 (4C or TCR75) and CD8 (2C) T cells responded equally well in either Akita or non-diabetic littermates. Thus, results indicate that diabetes does not result in any dramatic global or antigen-specific impairment in T cell alloreactivity. In parallel studies, islet allograft rejection was actually accelerated in chronically diabetic Akita hosts relative to SZ-induced diabetic littermates ($p<.05$). However, long-term BALB/c islet allograft survival following costimulation blockade (anti-CD154 therapy was reduced in Akita mice relative to SZ-induced diabetic mice ($p=.01$). Taken together, these results suggest that while alloreactivity is normal in the chronically diabetic host, regulation of this response may be impaired.

Other

OR.26. Ly9 (CD229) Functions as a Regulator Controlling Innate B Cell Homeostasis and Antibody Responses

Marta Cuenca[1], Xavier Romero[1], Jordi Sintes[2] and Pablo Engel[2]. [1]Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain; [2]★University of Barcelona, Barcelona, Spain

Marginal zone (MZ) and B1 B cells are distinct B lymphocyte subsets that differ from conventional follicular B cells both developmentally and functionally. MZ B cells and B1 B cells have the capacity to respond to foreign antigens more rapidly than conventional B cells, and are the earliest lymphocytes to encounter invading pathogens acquired through the blood stream. Ly9 is a unique SLAM family receptor with 4 Immunoglobulin-like domains that interacts with itself. *In vitro* and *in vivo* studies suggest that it functions as an inhibitory receptor negatively regulating iNKT and CD8 innate-like cells development and activation. Moreover, aged Ly9-deficient mice spontaneously developed features of systemic autoimmunity. Our main goal was to elucidate the function of Ly9 in B cell development and homeostasis, and to explore the use of monoclonal antibodies against Ly9 as a novel therapeutic approach. Ly9-deficient mice showed that Ly9 absence leads to a marked expansion of T1 (transitional), MZ B and B1a B cells without affecting the development of conventional B2 lymphocytes. Ly9-deficient mice presented an increased antibody production following immunization, and elevated serum levels of IgG3 natural antibodies. These data demonstrate that Ly9 functions as a negative regulator controlling innate B cell homeostasis. Moreover, treatment with an agonistic anti-Ly9 mAb was able to inhibit innate B cell development in the spleen and antibody responses.

Thus, our data indicate that anti-Ly9 treatment could be useful for treating lympho-proliferative disorders caused by an aberrant expansion of B cell populations, and for ameliorating autoimmune diseases with B cell hyperactivation.

W.48. The Actin Remodeling Protein Cofilin is Crucial for Early T Cell Development

Isabel John[1], Daniel Deibel[1], Sandra Prokosch[1], Günter Küblbeck[2], Bernd Arnold[2] and Yvonne Samstag[1].
[1]★Institute of Immunology, Heidelberg, Germany; [2]German Cancer Research Center, Heidelberg, Germany

Costimulation of antigen-specific T cells by antigen-presenting cells leads to dephosphorylation and thereby activation of the actin depolymerizing and severing protein cofilin. The resulting cofilin mediated dynamic rearrangement of the actin cytoskeleton is essential for T cell activation, function and migration. We generated knock-in mice in which, T cell specifically, functional inactive cofilin was expressed together with eGFP instead of endogenous wild type cofilin. Lck-Cre mediated knock-in occurred very early in T cell development as eGFP expression was observed from DN1 stage on. Homozygous knock-in mice lacked peripheral T cells and showed a severe thymus atrophy. The small number of cells present in the thymus were double negative (DN) for CD4 and CD8. In detail, most of the DN cells accumulated in the DN3 stage. By generation of mixed bone marrow chimeras it was proven that the reason for impaired T cell development in these mice was T cell intrinsic and was not influenced by extrinsic factors. Heterozygous knock-in mice were completely normal in their T cell development when compared with B6 control mice. These mice can serve as reporter mice for cofilin promoter activity under different physiological and pathological conditions. Overall, we could show that functional cofilin is indispensable for proper T cell development, most likely due to a crucial role of cofilin during migration of early T cells or during signal transduction of pre-TCR signaling in the thymus.

W.49. Computational Infrastructure for Single Cell-based Biomarker Identification from Flow/Mass Cytometry Data

Yu Qian[1], Hyunsoo Kim[2], Shweta Purawat[3], Jianwu Wang[3], Ilkay Altintas[3], Holden Maecker[4] and Richard Scheuermann[1,3]. [1]J. Craig Venter Institute, La Jolla, CA; [2]J. Craig Venter Institute, Rockville, MD; [3]★University of California, San Diego, San Diego, CA; [4]★Stanford University School of Medicine, Stanford, CA

Recent advances in cytometry instrumentation are enabling the generation of increasing data volume and dimensionality at the single cell level for the identification of cell-based biomarkers. These advances are fundamentally changing the current paradigms of diagnosis and personalized treatment of immune system disorders, blood diseases, and cancer. However, traditional cytometry data analysis based on manual gating is not effectively scaling to address this new level of data complexity. Even though the true value of multiparameter cytometry is that all dimensions could be considered simultaneously for deciding cell populations boundaries, without computational technology to explore the high-dimensional space, hematopathologists still logically focus on identifying known populations of cells based on a subset of parameters, ignoring whether other minor populations exist and/or are diagnostic or prognostic.

We have developed a novel prototype cyberinfrastructure (http://flowgate.jcvi.org) for computational cytometry data analysis, which for the first time integrates graphical user interfaces, workflow engines, and parallel computing resources together for exhaustive and reproducible cell-based biomarker identification. This computational infrastructure is making multiple data analysis workflows easily accessible to immunology researchers, without requiring comprehensive informatics training. The utility of the infrastructure is being demonstrated through the analysis of the cytometry datasets from two published clinical studies: one quantifying immune responses to tolerance-inducing immunotherapy for seasonal allergies by flow cytometry, and the other revealing human NK cell repertoire diversity by mass cytometry. The results demonstrate computational analysis reveals unexpected diversity of potentially interesting cell types and identifies cell-based biomarkers that are otherwise difficult to find using manual gating.

W.50. Human Delta-like 1-expressing Human Mesenchymal Stem Cells Promote Human T Cell Development in Humanized NOD/SCID/IL-2rgnull (NSG) Mice

Jae Berm Park[1], Miyoung Kim[2], Bongkum Choi[3], Jinkyung Son[2], Kyo Won Lee[2], Hyojun Park[2] and Sung Joo Kim[2]. [1]Samsung Medical Center, Seoul, South Korea; [2]Sungkyunkwan University School of Medicine, Seoul, Korea, Seoul, South Korea; [3]Harvard Medical School, Boston, MA;

Herein, we investigate the functional role of human delta-like 1 (hDlk1)-expressing mesenchymal stem cells (MSC) in humanized mice. The co-infection of MSCs derived from human fetal liver with hCD34$^+$ cord blood (CB) cells markedly suppresses the development of human T cells. In contrast, the co-infection of hDlk1-expressing MSCs with hCD34$^+$ CB cells dramatically promotes the development of human T cells that are markedly diverse in terms of TCR Vb usages, functionally active, and restricted in human MHC molecules. Interestingly, the co-injected hDlk1-expressing MSCs are strongly detected in the liver tissue of humanized mice, and co-localized with hCD34$^+$ and hCD3$^+$ T cells. Additionally, hDlk1-expressing MSCs are positively involved in the T cell development through the notch signaling. Upon the challenge of Epstein-Barr virus (EBV) into the humanized mice, furthermore, EBV-specific hCD8+ T cells were effectively generated, activated and differentiated to effector memory T cells. Taken together, our data suggest for the first time that the hDlk1-exprresing MSCs can effectively promote the development of human T cells in humanized mice, and this humanized model might have potential advantages for the development of immunotherapy by using CD8$^+$ T cells targeting viral diseases or cancer.

W.51. Hyaluronan-mediated T Cell/T Cell Clustering Drives STAT5 Signaling and Proliferation Early After Activation

Vivekananda Sunkari[1], Shannon Ruppert[1], Nadine Nagy[1], Melissa Pickett[2], Ben Falk[2], S. Alice Long[2], Manish Butte[1] and Paul Bollyky[1]. [1]★Stanford University School of Medicine, Palo Alto, CA; [2]★Benaroya Research Institute, Seattle, WA

It is well-established that T cells form proliferative clusters upon activation but the functional significance of these aggregates is unclear. We find that cell-cell contact early after activation is important for human T cell proliferation and that these interactions are mediated by hyaluronan (HA), an extracellular matrix polymer, and the HA receptor, CD44. Inhibition of HA synthesis or blockade of HA interactions with CD44 prevented both the clustering and proliferation of human T cells. Proliferation could be restored in the absence of HA by antibody-mediated crosslinking of CD44 or by high concentrations of IL-2. These findings are explain augmentation of IL-2 signaling by HA and CD44. Both plate-bound HA and CD44 crosslinking strongly amplify STAT5 phosphorylation, and CD44 physically associates with the IL-2R complex. CD44$^{-/-}$ mice and CD44$^{-/-}$ human T cell lines transfected with CD44 indicate that CD44 is a negative regulator of IL-2R signaling in naïve cells. In effector cells, however, this inhibition is relieved by HA-mediated CD44 crosslinking. Together, these data indicate that HA functions as an intercellular glue that promotes T cell clustering and T cell proliferation in newly activated cells.

W.52. Lyophilized Human PBMCs (Lyo-PBMC), A Superior Control for Flow Cytometry Application

Anagha Divekar, Michael Lee, Myra Gordon, David Soper, Xifeng Yang, Craig Monell and Gene Lay. BioLegend, Inc, San Diego, CA

Reference laboratories, clinical research organizations and multi-center clinical trials need stabilized cells as controls to validate lot-to-lot consistency and for daily machine set up. We have developed a lyophilized peripheral blood mononuclear cell (Lyo-PBMC) preparation that can be successfully used for detecting a wide range of surface markers (on resting and activated PBMC) including CD3, CD4, CD8, CD16, CD19, CD20, CD21, CD22, CD25, CD27, CD56, CD69, CD154, CD360 and IL-21R. Additionally, Lyo-PBMCs can be used for detecting intracellular

cytokines such as IL-2, IFNɣ and TNFα and transcription factors such as Foxp3. Upon reconstitution, Lyo-PBMCs maintain forward/side scatter profiles (of lymphocytes and monocytes) similar to freshly isolated PBMC and expression of the above mentioned markers is stable. Ongoing studies are investigating the long term stability (> 6 months) of Lyo-PBMC preparations.

W.53. Expansion of Regulatory T Cells Reduces Hepatic Lymphocyte Responses and Blocks Hepatobiliary Injury in Murine Sclerosing Cholangitis

Amy Taylor[1], Alexandra Menchise[2], Celine Silva-Lages[1], Julia Simmons[1], Kumar Shanmukhappa[1] and Alexander Miethke[1]. [1]★Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [2]The Children's Hospital of Philadelphia, Philadelphia, PA

Background: IL2RA mutation and reduced number of regulatory T cells (Tregs) were previously shown to confer susceptibility to primary sclerosing cholangitis (PSC). We have shown before that initiation of cholangitis in mdr2$^{-/-}$ mice, a model of small duct PSC, coincides with expansion of effector lymphocytes. Here we test the hypothesis that expansion of Tregs attenuates hepatobiliary injury in this model.

Methods: Male double transgenic mdr2$^{-/-}$ and FoxP3-GFP mice received intraperitoneal injections with (0.01 ug/g) of recombinant IL-2 combined with (0.05 ug/g) anti-IL2 (clone Jes6.1) complex (IL2C) or PBS twice weekly between day of life 7 and 30.

Findings: Treatment with IL2C increased the frequency of splenic Tregs (mean % of Foxp3^{+}CD25^{+}/CD4^{+}CD3^{+} of 9.28% vs 7.77% in IL2C vs PBS; p=0.05; n=3-8/group) and concomitantly decreased hepatic CD8+ (10.0% vs 18.0% of CD8+CD3+/lymphocytes; p=0.02) and NK lymphocytes (7.0% vs 9.4% of NK+CD3-/lymphocytes; p=0.03;) at day 30. Suppression of hepatic lymphocyte responses was associated with reduction of serum alkaline phosphatase levels (172.14 vs 185.38 IU/L; p=0.01; n=4-5/group), a biomarker of biliary injury. Review of H&E^{-} and trichrome-stained liver sections showed reduced sclerosing cholangitis scores for IL2C vs PBS treatment on a 1 to 4+ scale (grade of inflammation: 1.8 vs 2.6; fibrosis 1.5 vs 2.6; p<0.05 for both, n=8-13/group). Immunohistochemical staining of bile duct epithelium using CK19 demonstrated decreased ductular proliferation, signifying decreased biliary injury and cholestasis.

Conclusions: IL2C induced expansion of regulatory T cells suppressed hepatic lymphocyte responses and blocked progression of cholangiopathy and fibrosis in a murine model of PSC.

W.54. Immune Response in Gene Therapy: Proof of Principle of Oral Tolerance for Better Efficacy

Romain Hardet[1,2], Olivier Boyer[1,2,3] and Sahil Adriouch[1,2]. [1]Normandy University, Rouen, France; [2]Inserm, U905, Rouen, France; [3]Rouen University Hospital, Rouen, France

Background and objective: Gene therapy strategies that aim to treat disease by providing, through a viral vector, a functional copy of an otherwise defective gene are hampered by immune responses elicited against vector and/or transgenic proteins. Most proposed approaches to combat these side effects intend to inhibit anti-vector immune responses. Here, we considered a new strategy for inducing specific tolerance toward the transgene product, using oral tolerization.

Methods: We used a murine model of adeno-associated virus (AAV)-mediated muscle gene transfer of a secreted form of Ovalbumin (Ova) as transgene. Oral tolerance was induced by feeding mice with Ova for 7 days. Ova-specific immune responses and transgene persistence were monitored overtime.

Results: Prophylactic oral Ova administration before AAV-mediated gene transfer completely prevented Ova-specific antibody formation and cytotoxic $CD8^+$ T cell responses. This state of immunological unresponsiveness allowed persistence of transgenic DNA in muscle and RNA expression in transduced muscles, together with maintenance of Ova production in serum. Mechanistically, oral tolerance induced abortive proliferation of transgene specific $CD8^+$ T cells, followed by their deletion, whereas regulatory T cells were not found to have a major role. Oral tolerance could not be induced in Ova immune mice.

Conclusion: This work provides the first proof-of-principle of oral tolerization as a candidate approach to circumvent immune responses directed toward transgenic proteins in the framework of AAV-mediated gene transfer. Gene therapy may represent a prototypic clinical situation in which prophylactic oral tolerization may be effective in immunologically naive individuals, *i.e*. before gene transfer in genetically-deficient patients.

W.55. Estrogen Enhanced Immune Response in Females with Cystic Fibrosis and a Role of Regulatory T Cells

Rahul Kushwah[1], Stéphane Gagnon[2] and Neil Sweezey[2]. [1]National Research Council of Canada, Human Health Therapeutics, Ottawa, ON, Canada; [2]Hospital for Sick Children, Toronto, ON, Canada

Cystic Fibrosis (CF) is a clinically complex, multi-system, autosomal recessive disease. Females with CF are more likely to have acute pulmonary exacerbations than their male counterparts and CF females are at a disadvantage in survival and morbidity. The mechanisms involved in this "CF gender gap" remain unknown. In response to menstrual elevations in estrogen (E2), we identified in CF, but not controls, a significant increase in sputum levels of the Th17 cytokine IL-17 along with the neutrophil chemo-attractant IL-8, indicating an E2 enhanced inflammatory response in female CF patients. Additionally, IL-1beta and IFN-gamma, as well as the WBC count, showed a non-significant trend to be increased when E2 levels were higher.

E2 is known to drive expansion of Regulatory T cells (Tregs) which suppress overactivation of the immune response. Increased Treg levels are normally observed during the follicular phase of the menstrual cycle, when E2 levels are maximal. As such, T cells from non-CF controls showed a significant increase in their Treg differentiation potential following *in vitro* stimulation with E2. Surprisingly, T cells from CF patients failed to undergo E2-induced Treg differentiation/expansion. Our previous findings have identified a selective predisposition of T cells from CF patients to differentiate along a Th17 lineage and Tregs are known to suppress a hyperactive Th17 response. Therefore in CF females, a lack of E2 dependent Treg expansion likely contributes to a Th17/Treg imbalance, which in turn may contribute to the CF gender gap.

W.56. The Immunology Database and Analysis Portal (ImmPort): A Data Science Resource in Immunology

Ashley Xia, Quan Chen and Dawei Lin. National Institute of Allergy and Infectious Disesases, National Institues of Health, Bethesda, MD

Rapid technological advances and emerging data science approaches are changing the landscape of immunology studies. Research is now generating datasets in such large volume and complexity that specialized infrastructure is required to support data sharing as well as integrative and reproducibility analyses. Recognizing this growing need, DAIT, NIAID has provided funding for over a decade to a multidisciplinary team of bioinformaticians, computer scientists, and experts in immunology and other scientific disciplines to create a resource called ImmPort under the Bioinformatics Integration Support Contract (BISC) awarded to Northrop Grumman.

ImmPort is a unique resource that supports data sharing in all areas of immunological research and clinical studies including normal immune function, immune-mediated diseases, and biodefense. To date, it has released more than 100 datasets (including de-identified patient level clinical data) from NIAID-funded clinical trials, associated

mechanistic studies, and other basic research programs. ImmPort also provides immunology data standards, ontology, knowledge-base (Comprehensive List of Immune-Related Genes, immuneXpresso: comprehensive mapping of cytokines and immune cells), and data analysis tools. The data analysis tools, such as Flow Cytometry Analysis (FLOCK), and Sequence Feature Variant Type Analysis (MHC SFVT Analysis) provide user-friendly access for researchers to easily and quickly analyze ImmPort data or their own data.

ImmPort can be accessed at http://immport.niaid.nih.gov.

W.57. A High Throughput Screen Approach for the Identification of Novel Regulators of Human T Follicular Helper (Tfh) Cell Differentiation

Michela Locci[1], Jennifer Wu[1], Carol Dahlberg[2] and Andrew Miller[2]. [1]La Jolla Institute for Allergy and Immunology, La Jolla, CA; [2]Genomics Institute of the Novartis Research Foundation, San Diego, CA

T follicular helper (Tfh) cells are a subset of CD4 T cells specialized in aiding antibody responses. Besides being necessary for the generation of affinity-matured memory B cells and long-lived plasma cells, Tfh cells are limiting for the quality and magnitude of B cell responses. Therefore, there is therapeutic potential in manipulating Tfh cells. Thus far, several studies have uncovered a multiplicity of pathways regulating mouse Tfh cell differentiation. Human and mouse Tfh cells are phenotypically similar, and share some conserved requirements for their generation. However, recent evidence highlights the existence of substantial evolutionary differences in signaling pathways driving Tfh differentiation. In light of these differences, studies in mice aimed to identify unknown Tfh cell regulators might have limited relevance to human Tfh cell biology.

To overcome this shortcoming, we set up an unbiased high throughput human Tfh cell differentiation screen using recombinant proteins. Overall, over 3000 unique human proteins were tested for their ability to induce expression of Tfh signature markers, as CXCR5 and PD-1, in naive CD4 T cells upon activation. Importantly, our screen approach allowed us to identify both positive and negative regulators of human Tfh gene expression program. Additional follow up studies on the molecule emerging from the screen as the most potent Tfh cell inducer revealed that this molecule controls the acquisition of Tfh cell phenotype and effector function in humans and macaques, but not in mice. A high throughput screen approach is a powerful tool for uncovering novel regulators of human Tfh cell biology.

W.58. Automated Discovery of Rare Cell Subsets: Identification of Leukemic Cells as Minimal Residual Disease (MRD)

Tiffany J. Chen, Chris Ciccolella and Nikesh Kotecha. Cytobank, Inc, Mountain View, CA

Using publicly available 35-parameter CyTOF-based clinical data from Amir 2013 (Nature Biotechnology), we computationally combine a sample of leukemic bone marrow at 0.2% to a sample of otherwise healthy marrow to mimic an MRD condition. We then seek to capture the aberrant cells from the healthy background without prior knowledge of significant biomarkers or type of cancer, and then characterize the significant biomarkers of the cancer cells. Three computational workflows of varying degrees of automation are used in order to reach this goal: 1) dimensionality reduction using viSNE, 2) viSNE followed by clustering via SPADE, and 3) automatic identification of differential biomarkers that separate cancer cells from healthy, using a logit LASSO-based classifier.

Using viSNE alone we reproducibly identify the rare subset of leukemic blasts with complete separation from the healthy background. Extending this analysis to clustering by SPADE resulted in output of a targeted cluster of cells of interest that was confirmed to match the known aberrant cells, shortcutting their path to discovery. Using the LASSO-based classifier with no prior knowledge of biomarker profile, we were able to automatically identify CD10 and CD34 as the strongest markers that distinguish ALL blasts from healthy cells. 100% of the expected aberrant cells were recovered and no cells were found to be misidentified when their location was unblinded after analysis..

W.59. Development of a Malaria Transmission Blocking Nanoparticle Vaccine

Petr Ilyinskii[1], Conlin O'Neil[1], Takashi Kishimoto[1], Patrick Duffy[2], Kazutoyo Miura[2] and Carole Long[2].[1]Selecta BioSciences, Watertown, MA; [2]National Institute of Allergy and Infectious Diseases, National Instutues of Health, Rockville, MD

We have developed a potent transmission-blocking malaria vaccine via the encapsulation of recombinant Pfs25 antigen into Selecta's biodegradable, synthetic vaccine particle platform (SVP™) and combining it with nanoparticle formulations containing TLR7/8 or TLR9 agonists. Pfs25 is a sexual stage antigen of Plasmodium falciparum expressed on the surface of its zygote and ookinete forms. It has been long known that antibodies to Pfs25 can block the development of P. falciparum oocysts in mosquito midgut, and thus Pfs25 has been extensively studied as a candidate antigen for transmission-blocking vaccination approach. Several formulations of SVP.Pfs25 nanoparticles were produced and co-administered into BALB/c mice with adjuvant nanoparticles containing either ODN CpG oligonucleotides (SVP.CpG), or a novel polymer-resiquimod conjugate prodrug (SVP.R848). SVP immunizations resulted in robust antibody titers over a wide range of nanoparticle-encapsulated Pfs25 doses. Titer comparison showed that SVP formulation containing just 2 ng of Pfs25 resulted in titers that were comparable to that obtained with 8 µg Pfs25 in alum, indicating a 4000-fold dose sparing effect. When tested in the standard membrane feeding assay, serum from SVP-immunized mice showed 97-99% inhibition in oocyst intensity compared to 10-70% with a standard alum formulation. These results indicate that SVP.Pfs25 formulations provide strong potency and efficacy *in vivo* thus holding an excellent promise of generation of durable antibody titers.

W.60. Global Analysis of the Inter-cellular Communication Network Reveals Novel Network Properties and Disease Similarities

Shai Shen-Orr[1], Ksenya Kveler[1], Amit Ziv-Kenet[1], Netta Aizenbud-Reshef[1], Gali Shalev-Malul[1], Nuaman Asbeh[1], Yuval Kalugny[1], Yuri Gorelik[1], John Campbell[2], Patrick Dunn[2], Jeff Wiser[2], Elina Starosvetsky[1] and Tatiana Dubovik[1]. [1]Technion-Israeli Institute of Technology, Haifa, Israel; [2]Northrop Grumman IT Health Solutions, Rockville, MD

Protective immunity is not the end outcome of any single cell, but rather draws on functionality elicited by many cell types communicating between one another. To date, using reductionist techniques, immunologists have elucidated many of the basic principles of individual cell type behaviour at a given condition. Complex inter-cellular circuitry and whole system effects, however, are difficult to capture or understand. We used Natural Language Processing techniques to assemble for the first time a global map immune inter-cellular interaction from the primary literature, covering all known disease conditions. Global analysis of this network and its comparison to gene expression data identifies central 'hub-like' and specialized cytokines whose perturbation (*e.g.* by biologics) may produce more major and specific effects respectively. Moreover, by comparative analysis of the cell-interaction network and its comparison to existing disease we assemble a new high resolution disease similarity network, that is immune centric. The assembled system (immuneXpresso), paves the way for novel paradigms of reasoning over heterogeneous cell populations.

3819. The Importance of Immune Mechanisms during Bacterial Translocation in Surgical Infection

K. Suvonov and N. Nuraliev. Research Institute of Sanitation, Hygiene and Occupational Diseases MH, Tashkent, Uzbekistan

The aim was to study the immunological markers of pathogens odontogenic infections that developed as a consequence of bacterial translocation from the gastrointestinal tract and the development trend of redistribution of immune cell component of the immune system *in vitro*. Experiments were carried out on 152 white mongrel mice (2-3 months old, weighing 18-20 grams) with surgical infections. The control group consisted of 20 similar animals. Study

found fundamental differences in the structure of the immune response in surgical infections caused by bacterial translocation from the gastrointestinal tract. The basic mechanisms of the immune response responsible for the development of surgical infections, the phenotype of the receptor apparatus of immune cells and their change in the dynamics of the pathological process. The data on the immune status of the individual units of immunological reactivity depending on the microorganism, which allowed to formulate recommendations for the usage of the tactics of immunomodulatory drugs on the stages of the empirical and causal treatment of surgical infections.

3831. The Level of Microelements in the Spleen of Rats Under the Influence of the Exhaust Gases

K. Grigoryants and T. Aripova. Academy of Sciences of Uzbekistan, Tashkent, Uzbekistan

Microelements are necessary for normal biochemical reactions in all organs of man. Violation of the ratio of microelements under the influence of the exhaust gases leads to the development of a variety of pathological conditions in all organs. In the spleen, under the influence of the exhaust gas varies trace level that leads to disruption of hematopoietic function. The aim of the study was to investigate the quantitative content of certain microelements in the spleen of rats under the influence of the exhaust gases. We used 185 white rats of both sexes, weighing 160-180 g., 4-6 months of age. Animal poisoning with exhaust gases carried in the chamber volume of 50 liters for 4 hours. Acute exposure was held for 2 days. Subacute exposure was held within a month. Chronic exposure was held within four months. All groups of animals were kept under standard vivarium conditions with free access to food and water. Determination of the microelemental composition of the organs of the immune system was performed by neutron activation analysis. It was found that acute exposure of exhaust gases in the spleen decreased levels of antimony ($P < 0.05$). In subacute exposure reduced the content of cobalt, selenium and antimony ($P < 0.05$). Thus, the most pronounced changes in the minerals content in the spleen where observed after subchronic exposure to exhaust gases.

Abstract Index by Subject Area

Allergy/Asthma
OR.11, OR.33, T.1, T.2, T.3, T.4, T.5, T.6, T.7, T.8

Autoimmune Neurologic Disease
OR.13, OR.15, OR.16, OR.23, OR.40, T.9, T.10, T.11, T.12, T.13, T.14, T.15, T.16, T.17, T.18, T.19, T.20, T.21, T.22, T.23

Autoimmune Rheumatologic Disease
OR.04, OR.08, OR.12, OR.20, OR.21, OR.22, OR.32, OR.48, OR.51, W.1, T.24, T.25, T.26, T.27, T.28, T.29, T.30, T.31, T.32, T.33, T.34, T.35, T.36, T.37, T.38, T.39, T.40, F.1, F.2, F.3, F.4, F.5, F.6, F.7, F.8, 3967

Bone Marrow or Stem Cell Transplantation
OR.06, T.41, T.42, T.43, T.44, T.45

Cytokines/Chemokines
OR.17, OR.44, T.46, T.47, T.48, T.49, T.50, T.51, T.52, T.53, T.54, T.55

Diabetes and Other Autoimmune Endocrine Diseases
OR.14, T.56, T.57, T.58, T.59, T.60, T.61, T.62, T.63, T.64, T.65, T.66, T.67, T.68, T.69, T.70, T.71, T.72

General Autoimmunity
OR.31, OR.37, OR.43, F.9, F.10, F.11, F.12, F.13, F.14, F.15, F.16, F.17, F.18, F.19, F.20, F.21, F.22, F.23, F.24, F.25, F.26, 3958

Genetics
F.27, F.28, F.29, F.30, F.31

Immune Monitoring
OR.10, OR.35, OR.45, OR.57, F.32, F.33, F.34, F.35, F.36, F.37, F.38, F.39, F.40, F.41, F.42, F.43, F.44, F.45, F.46

Immunity and Infection
OR.07, OR.25, OR.28, OR.46, OR.47, OR.50, OR.54, OR.55, OR.60, F.47, F.48, F.49, F.50, F.51, F.52, F.53, F.54, F.55, F.56, F.57, F.58, F.59, F.60, F.61, F.62, F.63, F.64, F.65, F.66, 3823, 3828

Immunodefiency- Primary or Acquired
OR.09, OR.29, OR.39, OR.53, OR.56, W.2, W.3, W.4, W.5, W.6, W.7, W.8, W.9, W.10

Immunodermatology
OR.19, OR.34, OR.42, W.11, W.12, W.13, W.14

Immunology of the Eye
W.15, W.16, W.17

Immunoncology
OR.36, W.18, W.19, W.20, W.21, W.22, W.23, W.24, W.25, W.26, W.27, W.28, W.29, W.30, W.31, W.32, W.33

Inflammatory Bowel Disease
OR.49, OR.59, W.34, 3841

Innate Immunity
OR.01, OR.02, OR.03, OR.18, OR.24, OR.27, OR.30, OR.58, W.35, W.36, W.37, W.38, W.39, W.40, W.41, W.42, W.43, W.44

Organ Transplantation
OR.05, OR.52, W.45, W.46, W.47

Reproductive Immunology
3684, 3696

Therapeutics/Pharmacology
OR.41, F.67, F.68, F.69, F.70, F.71, F.72, F.73, F.74, F.75, 3956

Transplantation
OR.38, W.61, W.62, W.63, W.64, W.65, W.66, W.67, W.68, W.69, F.76

Other
OR.26, W.48, W.49, W.50, W.51, W.52, W.53, W.54, W.55, W.56, W.57, W.58, W.59, W.60, 3819, 3831

Abstract Index by FOCIS Pillar

Host Defense
OR.01, OR.10, OR.25, OR.28, OR.30, OR.50, OR.55, W.36, W.41, W.43, W.56, W.59, W.66, T.47, T.54, F.36, F.47, F.48, F.49, F.50, F.54, F.55, F.56, F.58, F.60, F.61, F.62, F.63, F.64, F.65, F.76

Immunodiagnosis
OR.21, OR.45, OR.52, OR.57, W.2, W.9, W.14, W.17, W.52, W.58, W.62, T.13, T.15, T.24, T.28, T.29, T.36, T.38, T.39, T.48, T.52, T.56, T.62, F.33, F.34, F.35, F.37, F.40, F.41, F.44, F.46, F.59, F.73, 3819, 3823, 3828, 3958, 3967

Immunogenetics/Genomics
OR.03, OR.12, OR.16, OR.22, OR.49, OR.51, OR.54, W.8, W.10, W.12, W.28, W.29, W.31, W.39, W.60, T.2, T.9, T.40, T.44, T.49, T.65, T.66, T.72, F.2, F.3, F.4, F.13, F.27, F.28, F.29, F.30, F.31, F.32, F.43, F.57

Immunoregulation
OR.02, OR.05, OR.07, OR.08, OR.09, OR.14, OR.15, OR.17, OR.18, OR.19, OR.20, OR.23, OR.24, OR.26, OR.27, OR.29, OR.31, OR.32, OR.33, OR.34, OR.35, OR.37, OR.40, OR.41, OR.42, OR.44, OR.46, OR.47, OR.48, OR.53, OR.56, OR.58, OR.59, OR.60, W.1, W.3, W.4, W.5, W.6, W.7, W.11, W.13, W.15, W.16, W.20, W.22, W.24, W.25, W.27, W.30, W.32, W.34, W.35, W.37, W.38, W.40, W.42, W.44, W.45, W.47, W.48, W.50, W.51, W.53, W.55, W.57, W.64, W.68, T.1, T.3, T.4, T.5, T.7, T.8, T.10, T.14, T.16, T.17, T.18, T.19, T.20, T.23, T.25, T.26, T.27, T.30, T.31, T.32, T.34, T.35, T.45, T.50, T.53, T.55, T.57, T.59, T.60, T.61, T.63, T.67, T.68, F.1, F.6, F.8, F.9, F.10, F.11, F.12, F.14, F.15, F.16, F.18, F.19, F.20, F.22, F.25, F.26, F.39, F.42, F.45, F.51, F.52, F.53, F.67, F.75, 3684, 3696, 3831, 3841, 3956

Immunotherapy
OR.04, OR.06, OR.11, OR.13, OR.36, OR.38, OR.39, OR.43, W.18, W.19, W.21, W.23, W.26, W.33, W.46, W.49, W.54, W.61, W.63, W.65, W.67, W.69, T.6, T.11, T.12, T.21, T.22, T.33, T.37, T.41, T.42, T.43, T.46, T.51, T.58, T.64, T.69, T.70, T.71, F.5, F.7, F.17, F.21, F.23, F.24, F.38, F.66, F.68, F.69, F.70, F.71, F.72, F.74

Abstract Index by Author

A
Abdollahi, Amir OR.36
Abella, Raul T.72
Abtin, Fereidoun T.28
Abudho, Bernard OR.50
Achita, Paulina W.69
Adeiga, Adesina T.47
Adekambi, Toidi F.44
Ådén, Ulrika T.17
Adijaya, Ciputra W.21
Adriouch, Sahil W.54
Agarwal, Sudha F.14
Agopian, Vatche G. OR.05
Agrawal, Anshu OR.58
Agrawal, Sudhanshu OR.58
Agraz-Cibrian, Juan M W.36
Aguiar, Cassyanne T.26, T.33
Ahlén Bergman, Emma W.20, W.21
Åhlin, Anders T.43
Ahmed, Rafi OR.10
Aizenbud-Reshef, Netta W.60
Akram, Ali F.48
Akramkhodjaeva, D. 3958
Albani, Salvatore OR.48, OR.51, F.6
Albaud, Benoit F.39
Albert, Matthew F.39
Alcorn, Dina OR.41
Aleppo, Grazia T.56
Alexandre, Bonnin T.20
Alonso, Paula OR.38, W.68
Alonso-Garcia, Laura W.9
Alonso-Guallart, Paula W.61
Alpan, Oral W.10
Alt, Frederick W.7
Altintas, Ilkay W.49
Aluquin, Shirley W.1
Aluquin, Vincent W.1
Alvarado-Navarro, Anabell W.12
Álvarez, Élida W.27
Amaravadi, Lakshmi F.42
Amengual, Olga F.5
Ameri, Mehrdad OR.41
Amici, Stephanie OR.03
Amin, Shreyasee F.2, F.3
Anandhan, Annandurai F.11
Andaluz, Consuelo T.5
Anderson, Mark F.22
Andolfi, Grazia OR.06
Anegon, Ignacio OR.43
Angulo, Ana F.55
Angus, Brian OR.25
Anjum, Samreem W.31
Anstadt, Emily OR.23, T.9
Anthony, Donald OR.28
Antonni, Sonir F.15
Aqel, Saba F.14
Araki, Manabu OR.13, T.11
Araujo, Flavio F.64
Araujo, Thaisa F.62
Arikan, Soykan F.32
Aripova, T. 3831, 3841, 3956
Ariza, Maria F.52
Arnold, Bernd W.48
Asbeh, Nuaman W.60
Aslam, Shahin F.60
Atkinson, Mark T.68
Ato, Manabu OR.01
Atsumi, Tatsuya F.5
Aussy, Audrey T.36
Awasthi, Amit T.8
Awoderu, Oluwatoyin T.47
Azizov, Yu. 3823

B
Babcook, John T.50
Bacchetta, Rosa OR.39
Badaro Garcia, Stephanie F.31
Baenkler, Hans T.6
Baeten, Dominique OR.18, T.25, T.31
Bagos, Art F.21
Baker, Samuel W.68
Bakiev, B. 3967
Bakker, Arjen T.31
Banchereau, Romain T.23
Bandala-Sanchez, Esther OR.24
Bansal, Reema W.15
Barochia, Amisha V T.3
Barra, Giusi W.5
Barraza, Monique F.54
Basavalingappa, Rakesh F.25
Bashratyan, Roman F.19
Battaglia, Manuela OR.14
Bavikar, Suhas W.46
Beam, Craig T.69
Beck, Andrew F.71
Bedognetti, Davide W.31, F.69

Beeton, Christine F.24
Behrens, Timothy W.8
Belanger, Simon OR.10
Beliakkova-Bethell, Nadejd W.39
Belkina, Anna T.32
BenMohamed, Lbachir F.65, F.66
Bennett, Jeffrey T.13
Bennett, Jeffrey L T.20
Bensmail, Halima W.31
Bentebibel, Salah-Eddine OR.07
Berger, Thomas T.13
Bergua, Cecile T.24
Beriou, Gaelle W.65
Bermejo López-Muñiz, Maria Paz T.38
Bernal, Paula OR.25
Bernink, Jochem H. OR.18
Berry, Kelly T.50
Bertoletti, Antonio OR.46
Bhangale, Tushar W.8
Bhowmik, Shiva F.71
Bhumiratana, Sarindr T.41
Bianco, Alberto F.69
Biancotto, Angelique T.3
Bigler, Jeannette OR.42
Bishop, Nicholas W.66, F.76
Bitton, Alain OR.49
Bjerkan, Louise OR.34
Blackburn, Tessa F.57
Blahnik, Gabriele OR.47
Blanco, Luz OR.20
Blanco, Patrick F.20
Blancou, Philippe OR.43
Blazar, Bruce OR.08
Blijdorp, Iris OR.18
Blish, Catherine F.50
Blogowski, Wojciech W.22
Blohmke, Christoph J OR.25
Blomberg, Bonnie B. OR.55
Blum, Lisa OR.12
Boes, Marianne L. T.42
Bogdani, Marika T.64
Bohgaki, Toshiyuki F.5
Bolella, Valdes W.43, F.62
Bolen, Christopher F.43
Bollyky, Paul OR.40, W.40, W.51, T.19, T.64
Bondanza, Attilio OR.06
Bondar, Galyna F.34
Bongen, Erika OR.21
Borghans, Jose A.M. OR.53
Boucher, Gabrielle OR.49
Bouchet-Delbos, Laurence W.65
Boyer, Olivier W.54, T.24, T.36
Bradley, Linda F.54
Brady, Bill F.71
Brahmandam, Archana T.59
Brandao, Izaira F.64
Brauer, Veronica W.44
Braun, Kathy OR.40
Breckwoldt, Michael OR.36
Breen, Michael W.39
Bresson, Damien F.71
Bridges, S. Louis OR.04, F.28
Brimberg, Lior T.20
Broen, Jasper C.A. OR.53
Broere, Femke T.42
Bronson, Paola W.8
Brouard, Sophie OR.60, F.33
Brown, Milton OR.02
Brusko, Todd T.68, T.70
Bruss, Michael F.14, F.52, F.70
Buckner, Jane H. OR.47, T.16, T.59, T.63, F.45
Budkowska, Marta W.22
Bulun, Serdar E T.27
Burd, Craig OR.03
Burkhardt, Amanda T.46, T.49
Burrows, Peter F.57
Busar, Nicholas W.42
Buss, Tim F.71
Bustos-López, Karla W.36
Busuttil, Ronald W. OR.05
Butcher, Eugene T.14
Butte, Manish W.51
Bühler, Leo OR.38, W.61

C

Caddy, Sarah F.63
Cadeiras, Martin F.34
Cagle, Stephanie F.44
Caielli, Simone T.23
Calvo Martins Okuyama, Nádia F.31
Camisa, Barbara OR.06
Campbell, Daniel J. T.64
Campbell, John W.60
Cantaert, Tineke T.25
Cao, Yonghao OR.15
Capuder, Kelly W.7
Cardoso, Cristina W.44
Carmona-Rivera, Carmelo OR.20
Carnevale, Christina D. T.46
Carretero-Iglesia, Laura W.65

★ = member of a FOCIS Center of Excellence

Carrette, Florent F.54
Casanova, Jean-Laurent T.53
Caspi, Rachel OR.17, W.16
Casteras, Anna T.72
Causton, Benjamin OR.29
Cebinelli, Guilherme Cesar Martelossi F.31
Ceccarelli, Michele W.31
Cerosaletti, Karen T.62, T.63
Cervantes-Trujano, Edgar T.5
Chakrabarty, Sagarika W.42
Chan, Brian T.50
Chan, Chi-Chao OR.17
Chan, Cliburn F.40
Chan, Meagan F.21
Chandler, Phillip R. T.67
Chaudhry, Sulemon W.68
Chaussabel, Damien T.62
Chávez, Raúl T.7
Chen, Chiung-Ya W.35
Chen, Ding T.21
Chen, Janice T.62
Chen, Jinmiao OR.46
Chen, Karin W.2
Chen, Quan W.56
Chen, Ruey-Hwa F.9
Chen, Stephanie W.62
Chen, Tiffany J. W.58
Chen, Wei OR.08
Chen, Ying Yu F.12
Chen, Yuan-Tsong T.2
Cheng, Yang OR.46
Chess, Leonard F.1
Chester, Cariad F.35
Chiang, I-Hsuan OR.30
Chiavelli, Helene T.24
Chiblak, Sara OR.36
Chin, Monica OR.12
Chio, Vera F.59
Choi, Bongkum W.50
Choi, Goda W.62
Choi, Woo Jun W.17
Chou, Hong-Shiue W.67
Chou, Shiu-Huey T.45
Chou, Ting-Fang F.9
Chowdhary, Vaidehi F.2, F.3
Choy, Jonathan W.45
Chrabot, Beverly OR.04
Chua, Camillus OR.48
Chuang, Ya-Hui F.16
Chuang, Ya-Ting F.9
Chum, Marla F.59
Chung, James OR.42
Ciccolella, Chris W.58
Ciceri, Fabio OR.06
Clark, Robert OR.23, T.9
Clark, Rutilio T.50
Cleaver, Ondine OR.56
Clemencio, Aline F.15
Coffman, Robert L. F.20
Coley, Shana M. OR.52
Colley, Dan OR.50
Collins, Justin T.32
Colobran, Roger W.9, T.72
Colonna, Marco OR.29, W.7
Conroy, Brittany W.28
Contin-Bordes, Cecile F.20
Cook, Laura F.49
Coon, V, John S T.27
Cope, Frederick F.73
Costa Brandão Berti, Fernanda F.31
Cox, Kari F.71
Craft, David T.52
Crampton, Jordan F.61
Cristillo, Anthony T.55
Crotty, Shane OR.10, F.56, F.61
Cserny, Judit T.70
Csizmadia, Eva W.7
Csomos, Krisztian OR.29, W.6, W.7
Cuenca, Marta OR.26
Cuento, Rosemarie A T.3
Cui, Jiajia F.75
Cupo, Albert F.61
Cutler, Antony F.63
Cutucache, Christine W.28
Cuturi, Maria Cristina W.65

D
Dagur, Pradeep K T.3
Dahlberg, Carin OR.29
Dahlberg, Carol W.57
Dai, Yang F.19
Daley, Stephen W.3
Dan, Jennifer F.56
Dapena, Jose Luis W.9
Darton, Thomas OR.25
Davini, Dan F.10
Davis, Laurie F.7
Davis, Mark F.43
Day, Anthony T.64
Day, Cheryl F.44

De Beca, Francisco W.6
De Imus, Cyr OR.41
de la Morena, M.Teresa OR.56
De Palma, Raffaele W.5
De Ravin, Suk OR.20
De Sessions, Paola OR.46
de Taeye, Steven F.61
de Vries, Niek T.31
Dechanet-Merville, Julie F.43
Decker, Corry F.43
Deenick, Elissa T.53
DeFranco, Anthony F.22
Degauque, Nicolas OR.60, F.33
Dehner, Carina T.26, T.33
Deibel, Daniel W.11, W.48
Delaney, John T.50
Delemarre, Eveline OR.53
Delemarre, Eveline M. T.42
Delgado-Rizo, Vidal W.36
Delneste, Yves W.65
Delogu, Lucia F.69
Deng, Mario F.34
Deng, Songyan OR.31
Dent, Alexander OR.09
Dervovic, Dzana W.69
Dewolf, Susan OR.52
Di Fonte, Roberta OR.14
Diamond, Betty T.20
Diaz, Alain OR.55
Diaz de Heredia, Cristina W.9
Dick, Andrew D F.38, F.74
DiToro, Daniel T.22
Divekar, Anagha W.52, T.28
Djebali, Raouf F.39
Dolegowska, Barbara W.22
Dong, Haidong OR.35
Dooms, Hans T.32
Dorschner, Jessica OR.04, F.2, F.3
Dougan, Gordon OR.25
Drake, Alison F.50
Drennan, Michael F.18
Dronca, Roxana OR.35
Drouot, Laurent T.24, T.36
Drujont, Lucile W.65
Dubey, Seema W.26
Dubovik, Tatiana W.60
Dudaronek, Justyna T.52
Duffy, Darragh F.39
Duffy, Patrick W.59
Duncton, Matthew F.21
Dunn, Patrick W.60
Duran-Struuck, Raimon OR.38, W.61, W.68
Durning, Sean T.61
Dutz, Jan W.13, T.71
Duzkoylu, Yigit F.32

E
Elashoff, David T.28
Elewaut, Dirk F.18
Elkon, Keith OR.20
Elliott, Thomas T.71
Engel, Pablo OR.26, F.55
Engelhardt, Monika W.27
Engler, Alexander F.75
Enns, Winnie W.45
Ensign, Wayne W.39
Enzor, Jennifer F.40
Ergen, Arzu F.32
Ergesheva, Aida 3684
Eriksson, Anna T.30
Erin, Nuray W.19
Erkan, Doruk T.26, T.33
Ernste, Floranne F.2, F.3
Eshimbetova, Gulsara 3696
Espindola, Milena W.44, F.62
Esquivel, Yeraldin F.8
Esser, Mark F.59, F.60
Evans, Hayley G OR.44
Evavold, Brian T.15

F
Faccioli, Lucia F.64
Fafutis-Morris, Mary W.36
Falck-Ytter, Yngve OR.28
Falk, Ben OR.40, W.40, W.51, T.64
Falsey, Ann F.59
Fan, Wei F.2
Faneye, Adedayo T.47
Farber, Donna OR.52, OR.54
Farmer, Douglas G. OR.05
Fasano, Alessio W.6
Fasano, Kayla F.22
Fathman, C. Garrison T.65, T.66
Faustin, Benjamin F.43
Fayzullaeva, N. 3841
Faz, David F.8
Felsenstein, Susanna T.40
Ferbas, John T.4
Ferrë, Domenec F.55
Ferreira, Ricardo W.8, F.63

Ferreres, Joan Carles W.9
Ferrini, Maria OR.33
Fessler, Michael F.47
Fesus, Laszlo W.6
Finger, Eduardo F.13
Finkel, Yigael W.34
Finzel, Stephanie T.6
Fischer, Karen F.41
Fleskens, Veerle OR.44
Fleury, Michelle T.32
Flynn, Ryan OR.08
Fontanari, Caroline W.44, F.62
Forrest, Osric OR.02
Fousteri, Georgia OR.14
Frances, Roy F.21
Franco Cruz, Rodrigo F.11
Frank, Ian F.45
Frank, Stuart T.22
Frantz, Fabiani W.43, W.44, F.62, F.64
Frasca, Daniela OR.55
Frederiksen, Klaus S OR.44
Fredholm, Bertil T.17
Freiberger, Tomas W.8
Fresnay, Stephanie OR.25
Froňková, Eva W.4
Fuhlbrigge, Rebecca T.65, T.66
Fuhrman, Christopher T.68
Fujiwara, Mai OR.23, T.9
Fukazawa, Toshiyuki T.10
Fukuyama, Julia F.50
Fung, John W.47, W.67
Funk, Andrew T.59
Furman, David F.43
Furst, Daniel T.28
Furumoto, Yasuko F.67
Furuzawa-Carballeda, Janette F.8

G
Gabor, Kristin F.47
Gadina, Massimo F.67
Gadola, Stephan T.60
Gagnon, Stéphane W.55
Galicia CarreÃ³n, Jorge T.7
Gallagher, Sandra F.72
Ganguly, Sreerupa OR.58
Gao, Jiliang T.44
Garcia, Rosa OR.49
García González, Ilian Janet F.30
García Iglesias, Trinidad F.4
Gardner, Mark F.70
Gasser, Martin W.24, W.25
Gately, Dennis F.71
Gattorno, Marco F.6
Gavin, Marc OR.41, T.50
Gebe, John W.40, T.64
Gentien, David F.39
Germar, Kristine OR.18, T.31
Germer, Christoph W.24, W.25
Gersuk, Vivian T.62
Gertz, E. Michael W.42
Ghahary, Aziz W.13
Gharib, Sina F.58
Ghodke, Yogita F.3
Gill, Ronald W.66, F.76
Gimenez-Barcons, Mireia T.72
Giral, Magali OR.60, F.33
Goergen, Krista F.27
Goker-Alpan, Ozlem W.10
Golding, Hana OR.07
Gooden, Michel T.64
Goodfellow, Ian F.63
Goodman, Andrew OR.31, OR.32, T.26, T.33
Goodnow, Chris W.3
Goods, Brittany A. OR.15
Gorczynski, Reginald W.19, W.63
Gordon, Elizabeth M T.3
Gordon, Myra W.52
Gorelik, Yuri W.60
Gorski, Kevin OR.41
Goto-Inoue, Naoko OR.34
Gould, Douglas F.22
Goupil, Mathieu OR.49
Govindarajan, Srinath F.18
Graciano Machuca, Omar W.12, F.4
Graham, Kareem T.14
Graham, Robert W.8
Grau-Lopez, Laia F.37
Grauer, Stephanie T.50
Gravano, David F.10
Green, Lydia T.50
Greenbaum, Carla T.59, T.62, T.63, F.45
Gregersen, Peter K. OR.04
Gregori, Silvia OR.06
Griesemer, Adam OR.38, W.68
Grigoryants, K. 3831
Grill, Diane F.27
Grimmig, Tanja W.24, W.25
Grinshpun, Boris OR.54
Guerau, Mireia OR.03
Guerrero, Abraham OR.41

Gupta, Amod W.15
Gupta, Saloni T.34
Gutierrez, Miguel-Angel T.35
Gutierrez, Veronica F.71
Guzman, Alyssa T.21

H
Habib, Tania T.59
Haddad, Francois F.43
Hafler, David A. OR.15, OR.16
Hagelberg, Stefan T.43
Hajian, Pega OR.58
Halder, Ramesh OR.27, T.35
Haller, Michael T.70
Hammarström, Lennart W.8
Hampton, Jeffrey OR.03, F.14, F.52, F.70
Han, Mei OR.41, T.50
Hansen, Elizabeth F.58
Hansson, Johan W.20, W.21
Haralambieva, Iana F.27
Hardet, Romain W.54, T.36
Harrison, Leonard OR.24
Hartana, Ciputra W.18
Hausser, Ingrid OR.34
Havenar-Daughton, Colin OR.10, F.56
Hay, Arielle T.34
Hayakawa, Yoshihiro T.67
Haynes, Winston OR.21
Hedrick, Michael F.40
Helikar, Tomas W.28
Hendrickx, Wouter W.31
Herbst, Ronald F.72
Herek, Tyler W.28
Hernandez-Molina, Gabriela F.8
Herold, Kevan OR.31, T.61, T.69
Hervás, Jose Vicente F.37
Hevezi, Peter T.49
Hevezi, Peter A. T.46
Hill, Harry W.2
Hill, Marcelo W.65
Hiltensperger, Michael OR.32
Hirakawa, Yasuko OR.13
Hoffmann, Victoria F.67
Hohjoh, Hirohiko T.12
Holland, Sacha F.21
Holland, Steve T.53
Holmes, Susan F.50
Holmström, Benny W.20, W.21
Holt, Richard T.60
Holzl, Markus W.62
Homberg, Toni T.5
Hong, Enrique T.7
Hong, Wei Hsin F.12, F.17
Hong, Yun-Fen W.35
Hoover, Ashley OR.56
Horita, Tetsuya F.5
Horwitz, Marc T.57
Houck, Philipp W.68
Housley, William OR.16
Hoyer, Katrina F.10
Hreggvidsdottir, Hulda OR.18
Hsieh, Wan-Chen OR.30
Hsu, Hailing T.50
Hsueh, Yi-Ping W.35
Hu, Joyce F.61
Hu, Yijuan F.44
Huaman, Cecilia T.55
Huang, Chiang-Ching T.27
Huang, Lei T.67
Huang, Shau-Ku T.1
Huang, Tzyy-Nan W.35
Huerta, Patricio T.20
Humphreys, Robin F.71
Hundhausen, Christian T.63
Hung, Tun-Ywin F.17
Huq, Redwan F.24
Hwang, Yihshiou F.17

I
Ibegbu, Chris F.44
iGenoMed Consortium, The OR.49
Ilowite, Norman T.34
Ilyinskii, Petr W.59
Ingersoll, Sarah OR.02
Inman, Robert F.48
Inoue, Taeko F.24
Ireland, Sara T.21
Isanova, D. 3823
Ishida, Katsu OR.41
Isitmangil, Gulbu F.32
Ives, Megan T.53
Ivison, Sabine OR.49
Iwata, Daiju F.38
Iwawaki, Takao F.18

J
Jacobs, Paejonette T.44
Jacquemin, Clement F.20
Jaffar, Zeina OR.33
Jahnsen, Frode OR.34

Jalili, Reza W.13
James, Eddie OR.47
Jansen, Koos J.G. OR.53
Janssen, Willemijn J.M. OR.53
Jarjour, Wael OR.03, F.14, F.52, F.70, F.73
Jarrell, Justin F.50
Jasim, Dhifaf F.69
Jeanne, Marion F.22
Jensen, Mark OR.04, F.2, F.3
Jeon, Sohyun W.16
Ji, Shaoquan T.54
Jia, Ting F.11
Jiménez-Martínez, María T.5, T.7
Jin, Jing T.56
Jin, Zhongbo F.2
Jittayasothorn, Yingyos W.16
Joaquín, Fernández-Doblas T.72
Jofra, Tatiana OR.14
Johansson, Markus W.20, W.21
John, Isabel W.11, W.48
John-Stewart, Grace F.50
Johnson, Pamela T.64
Jojic, Vladimir F.43
Jones, Claire OR.25
Jones, Kendra F.14
Jonsson Rolandsdotter, Helena W.34
Joo, Sung-Yeon W.29
Joyce, Daniel W.67
Ju, Chia-Hsin OR.12
Judge, Chelsey OR.28
Jurisic, Vladimir T.48

K
Kaber, Gernot W.40, T.64
Kachaochana, Akadia T.27
Kadowaki, Atsushi T.18
Kalandarova, Amina 3696
Kaldas, Fady M. OR.05
Kalina, Tomáš W.4
Kalokhe, Ameeta F.44
Kalugny, Yuval W.60
Kamalov, Z. 3956, 3958
Kan, Bernard W.38
Kanai, Mariko T.41
Kang, Hee-Kap T.56
Kannan, Guna OR.41
Kannen, Vinicius W.25
Kaplan, Mariana OR.20, F.67
Kaplan, Mark OR.09
Karan, Dev W.26
Karanja, Diana OR.50
Karow, Margaret OR.41
Kasper, Dennis OR.59
Kasturi, Sudhir OR.10
Kato, Tomoaki OR.52, W.68
Kato, Yojiro OR.38, W.68
Kayserová, Jana W.4
Kennedy, Richard F.27
Kerkman, Priscilla T.31
Kern, Marlena OR.04
Kersh, Anna T.15
Keselowsky, Benjamin T.70
Ketas, Thomas F.61
Kewcharoenwong, Chidchamai OR.01
Khakimova, G. 3841
Khan, Arif F.65, F.66
Khan, Fatima S. F.24
Khan, Shaheen OR.56
Khanna, Dinesh T.28
Khanna, Kamal T.9
Khass, Mohamed F.57
Khatri, Ismat W.63
Khatri, Purvesh OR.21
Khnykin, Denis OR.34
Khosravi-Maharlooei, Mohsen W.13
Khurana, Surender OR.07
Kikuchi, Seiji T.10
Kilani, Ruhangiz W.13
Kim, Hyunsoo W.49
Kim, Miyoung W.29, W.50
Kim, Peter T.30
Kim, Sung Joo W.29, W.50
Kim, Woojin T.26, T.33
Kim, Yong Chan OR.37
Kimchi, Adi F.9
Kimura, Kimitoshi T.11
King, Jennifer T.30
Kinsella, Mike OR.40
Kinuthia, John F.50
Kiran, Bayram F.32
Kiran, Huseyin F.32
Kirchner, Jacqueline OR.41
Kirk, Allan W.42
Kirkiles-Smith, Nancy F.75
Kirtley, Jessica F.71
Kis-Toth, Katalin W.7
Kishimoto, Hidehiro T.51
Kishimoto, Takashi W.59
Kita, Mariko T.16
Kittur, Nupur OR.50

Kivelevitch, Dario W.14
Klareskog, Lars T.31
Klein, Clarissa T.34
Klekotka, Paul OR.42
Kleschenko, Yuliya W.10
Klocperk, Adam W.4
Knize, Anna T.50
Knudsen, Nick F.71
Kodama, Keiichi T.66
Kohen, Rita OR.49
Kolbeck, Roland F.72
Kongpachith, Sarah OR.12
Kono, Michihito F.5
Korin, Yael F.34
Korponay-Szabo, Ilma R. W.6
Kostadinova, Lenche OR.28
Kostarelos, Kostas F.69
Kotecha, Nikesh W.58
Kottschade, Lisa OR.35
Krantz, David W.20, W.21
Kriegel, Martin OR.31, OR.32, T.26, T.33
Kroenke, Mark OR.41
Kuçukhuseyin, Ozlem F.32
Kuipers, Hedwich OR.40, T.19, T.64
Kumanovics, Attila W.2
Kumar, Ravi W.15
Kumar, Varsha F.26
Kuo, Cheng-Chin W.37
Kuo, Ming-Ling F.51
Kupiec-Weglinski, Jerzy W. OR.05
Kushwah, Rahul W.55
Kveler, Ksenya W.60
Kwakkenbos, Mark T.31
Kwok, William W. OR.15
Kwon, Murray F.34
Kwon, Yeongbeen W.29
Küblbeck, Günter W.48

L
Lafyatis, Robert T.32, T.39
Lahey, Lauren OR.12
Lai, Jacqueline W.13
Lai, Liyun OR.48
Lai, Ming-Zong OR.30, F.9
Lait, Philippa J P F.38, F.74
Lambert, Stacie F.60
Landais, Elise OR.10
Landin, Ana Marie OR.55
Laplaud, David-Axel OR.60
Lara, Ivan T.5
Lasaro Mangieri, Luis Fernando F.31
Lassman, Charles R. OR.05
Lau, Sai Ping OR.52
Laval, Julie OR.02
Lavoie, Pascal W.38
Law, Hsei Di W.3
Lawson, Jonel F.41
Lay, Gene W.52
Lazar-Molnar, Eszter W.2
Lear, Sean T.4
Ledbetter, Jeffrey OR.20
Lee, Jong Bok W.69
Lee, Justin T.34
Lee, Kelsey W.38
Lee, Kyo Won W.50
Lee, Michael W.52
Lee, Richard W J F.38, F.74
Lee, Thomas F.24
Lefkowitch, Jay W.68
Lemos, Henrique T.67
Leonardson, Tansy F.59
Leong, Jing Yao OR.48
Leong, Mei-Ling OR.46
Lertmemongkolchai, Ganjana OR.01
Lesage, Sylvie OR.49
Levels, Maartje T.25
Levine, Myron OR.25
Levine, Stewart J T.3
Levings, Megan OR.38, OR.49, W.61, T.71, F.49
Lewis, Jamal T.70
Li, HaoWei W.62
Li, Juntao OR.48
Li, Li OR.41
Li, Quan-Zhen OR.45
Li, Yueh-Yuan T.1
Liang, Emily F.50
Lim, Ai Ching T.50
Lim, Bentley OR.31
Lim, Seng Gee OR.46
Lima, Leonardo W.44, F.62
Lin, Chia-Hsien T.2
Lin, Dawei W.56
Lin, Dongxia F.36
Lin, Meei-Hua OR.34
Lin, Ming-Chieh F.51
Lin, Youwei OR.13, T.11
Lincez, Pamela T.57
Linton, Ludvig W.34
Lipinski, Shawn W.10
Litzman, Jiri W.8

★ = member of a FOCIS Center of Excellence

Liu, Chaolin F.17
Liu, Dennis T.55
Liu, Hong OR.09
Liu, Hsin Yu W.35
Liu, Pan T.56
Liu, Qian T.44
Liu, Xin OR.35
Liu, Yang F.20
Lloyd Hibberd, Martin OR.46
Lo, Yu-Chieh T.45
Locafaro, Grazia OR.06
Locci, Michela OR.14, W.57
Loizou, Denise W.10
Lombardi Pereira, Ana Paula F.31
Long, Carole W.59
Long, S. Alice W.51, F.45
Lood, Christian OR.20
Lopes, Patricia T.54
López, Margarita W.27
López Pulido, Edgar W.30
Lopez-Lerma, Ingrid W.9
Lougaris, Vassilios W.8
Loureiro-Amigo, Jose T.38
Louvet, Cedric W.65
Love, J. Christopher OR.11, OR.15
Lovell, Daniel OR.51, F.6
Lovett-Racke, Amy OR.03
Low, Andrea Hsiu Ling OR.48
Lu, Chun-Hao F.51
Lu, Daniel OR.12
Lu, Lina W.47, W.67
Lucas, Anna T.72
Ludvigsson, Johnny T.69
Luger, Dror OR.17
Lundberg, Karin T.31
Lundgren, Christian W.21
Luo, Xunrong T.56
Ly, Dalam W.69

M
Ma, Cindy W.3
Ma, Stephen T.41
Macaubas, Claudia T.34
MacCallum, Colleen T.69
Madej-Michniewicz, Anna W.22
Mader, Simone T.20
Maecker, Holden W.49, F.35, F.36
Magni-Manzoni, Silvia F.6
Maifeld, Sarah F.59, F.60
Makol, Ashima F.2, F.3
Malech, Harry OR.20
Malik, Sakshi T.8
Maliszewska, Agnieszka OR.48
Malysz, Jozef W.1
Manfredo Vieira, Silvio OR.31, OR.32, T.26, T.33
Manibusan, Anthony F.71
Manilay, Jennifer F.10
Mansilla, Maria Jose F.37
Mansour, Salah T.60
Mansouri, Bobbak W.14
Maranian, Paul T.28
Maravillas-Montero, Jose Luis T.46
Marchiarullo, Daniel T.52
Maric, Veljko T.48
Marin-Sánchez, Ana T.38, T.72
Marincola, Francesco W.31
Marits, Per W.20, W.21
Markovic, Svetomir OR.35
Marshall, Payton W.40
Martens, Craig T.44
Martin, David OR.41, OR.42
Martin, Javier W.8
Martinez, Mercedes OR.52, W.68
Martinez, Olivia F.36
Martinez-Caceres, Eva Maria F.37
Martinez-Gallo, Monica W.9
Martínez-Vicente, Pablo F.55
Martini, Alberto F.6
Martins, Thomas B. W.2
Marusic, Suzana OR.08
Marwaha, Ashish T.71
Massa, Margherita F.6
Massilamany, Chandirasegaran F.11, F.25
Masson, Ana F.64
Masuda, Esteban F.21
Mattapallil, Mary OR.17
Matthew, Divij W.7
Matyas, Stephanie T.4
McAuliffe, William F.42
McCoy, J Philip T.3
McDermott, David T.44
McMurchy, Alicia OR.38, W.61
McWilliams, Rob OR.35
Medoff, Benjamin OR.29
Meerding, Jenny T.42
Mehta, Devangi F.42
Mei, Henrik F.35
Mejstříková, Ester W.4
Mellins, Elizabeth T.34
Mellor, Andrew L. T.67

★ = member of a FOCIS Center of Excellence

Menchise, Alexandra W.53
Mendez-Flores, Silvia F.8
Menter, Alan W.14
Merieau, Emmanuel W.65
Miaw, Shi-Chuen OR.30
Miethke, Alexander W.53
Mikacenic, Carmen F.58
Miller, Andrew W.57
Miller, Corey F.22
Miner, Jeffrey H. OR.34
Minnich, Anne F.40
Miura, Kazutoyo W.59
Miyake, Sachiko OR.13, T.18
Miyazaki, Yusei T.10
Moder, Kevin F.2, F.3
Moench, Romana W.24, W.25
Mohamed, Eslam T.67
Mok, Hoyin F.59
Mokry, Michal OR.22
Monell, Craig W.52
Monson, Nancy T.21
Monson, Natalie T.56
Montero, Enrique F.23
Montoya Buelna, Margarita F.4
Moon, James OR.11
Moore, John F.61
Moreau, Aurelie W.65
Moreau, Jean-Francois F.20, F.43
Morgan, Gabrielle A. T.27
Morita, Craig W.23
Morita, Miwa W.47
Mota Sena, Michelle F.31
Moyer, Kimberly T.14
Munn, David T.67
Muñoz Valle, Jose Francisco W.30, F.4, F.30
Murakami, Akikazu T.51
Murata, Miho OR.13, T.11, T.12
Murphy, Philip T.44
Musakhodjaeva, Diloram 3684, 3696, 3841

N
Nagy, Nadine W.51, T.64
Nakamura, Masakazu OR.13, T.11, T.12
Nakazawa, Kenneth T.41
Nakazawa, Soken T.37
Narayanan, Priya F.20
Nascimento, Margarida F.62
Ndombi, Eric OR.50
Nemati, Reza OR.23
Nepom, Gerald T. OR.15, OR.40
Newell, Evan OR.46
Ng, Yolanda T.59
Ni, Chester T.62
Nicholas Wisniewski, Nicholas F.34
Nichols, Frank OR.23
Niewold, Timothy OR.04, F.2, F.3
Niino, Masaaki T.10
Nirula, Ajay OR.42
Nithichanon, Arnone OR.01
Noordenbos, Troy OR.18
Norris, Paula W.32
Notarangelo, Luigi D. OR.29, W.6, W.7
Nuraliev, N. 3819
Nussenblatt, Robert B F.74
Nyuydzefe, Melanie OR.08

O
O'Neil, Conlin W.59
Oberg, Ann F.27
Obermoser, Gerlinde W.14
Obiols, Gabriel T.72
Ochs, Katharina OR.36
Odagui, Livia OR.49
Oh, Hyoung (Fred) W.42
Okamoto, Tomoko OR.13, T.11
Oki, Shinji T.12
Oku, Kenji F.5
Olek, Sven T.42
Oliveira, Guilherme F.64
Oliveira, Joao F.63
Oliveira, Karen Brajão de F.31
Olsen, Nancy W.1
Olsson, Richard T.43
Ong, Raymond OR.48
Orecchioni, Marco F.69
Ortmann, Ward W.8
Osborn, Thomas F.2, F.3
Ostrower, Adam T.27
Ott, Martina OR.36
Ovanez, Christopher W.61
Ovsyannikova, Inna F.27
Owyang, Alexander F.21

P
Pachman, Lauren T.27
Pacholczyk, Rafal T.67
Padilla-Gutiérrez, Jorge Ramón F.30
Pais, Fabiano F.64
Paiva Trugilo, Kleber F.31
Pakyari, Mohammadreza W.13, W.64

Palafox Sánchez, Claudia Azucena F.4
Paliard, Xavier F.59, F.60
Palmer, Jerry T.69
Palucka, A. Karolina OR.07
Pan-Hammarström, Qiang W.8
Parhy, Bandita F.59
Park, Hyojun W.29, W.50
Park, Jae Berm W.29, W.50
Park, Samuel F.59
Pascual, M. Virginia T.23, T.34
Pasquale, Giuseppe W.5
Passerini, Laura OR.39
Paterson, Marvin F.71
Patil, Sarita OR.11
Patnaik, Anuja W.42
Pautler, Robia F.24
Payan, Donald F.21
Pearson, Joshua OR.41, T.50
Pecqueur, Claire OR.60
Pepple, Kathryn W.17
Pereira-SuÃ¡rez, Ana W.30
Pérez-Carmona, Natalia F.55
Pérez-Tapia, Mayra T.5
Pérez-Tapia, Sonia Mayra T.7
Perry, Daniel T.70
Pescatori, Mario F.69
Petrelli, Alessandra OR.22
Petryna, Olga F.1
Phelan, Stephanie T.55
Philips, Rachael T.30
Phung, Christine F.71
Phuong, Trung F.71
Pickett, Melissa W.51
Piening, Brian Donald OR.39
Pileggi, Gecilmara F.15
Pineda, Oscar T.5
Pinyavat, Teeda W.68
Plassmeyer, Mattew W.10
Platten, Michael OR.36
Plebani, Alessandro W.8
Plonk, Kelly F.40
Pober, Jordan S. F.75
Podnos, Anna W.19
Pogu, Julien OR.43
Poidinger, Michael OR.46
Poignard, Pascal OR.10
Poland, Gregory F.27
Pollard, Andrew J OR.25
Pollastro, Sabrina T.31
Poormasjedi-Meibod, Malihesadat W.13
Porter, Forbes F.47
Possémé, Céline F.39
Powell, Alyssa F.71
Powell, Nicole F.14
Prakken, Berent J. OR.22, OR.53, T.42
Prasad, Priti OR.27
Preininger, Marcela OR.02
Presnell, Scott F.45
Preston-Hulburt, Paula T.61
Preston-Hurlburt, Paula OR.31
Proekt, Irina F.22
Prokosch, Sandra W.48
Protocol C Team, Iavi OR.10
Puhalo Sladoje, Dragana T.48
Pujol Borrell, Ricardo W.9, T.38, T.72
Pulendran, Bali OR.10
Purawat, Shweta W.49
Purmalek, Monica OR.20

Q
Qian, Shiguang W.47, W.67
Qian, Yu W.49
Quintana-Murci, Lluis F.39
Quirant-Sanchez, Bibiana F.37

R
Raddassi, Khadir OR.15
Radella, Frank F..58
Ragheb, Jack A. W.42
Rahman, M. Jubayer F.19
Raithel, Martin T.6
Raj, Prithvi OR.45
Rajasekaran, Rajkumar F.11, F.25
Rajasekhar, Megha OR.44
Rakhmankulova, Z. 3956
Ramakrishna, Lakshmi OR.48
Raman, Nandhini W.14
Ramirez de Arellano, Adrian W.30
Ramírez Dueñas, María Guadalupe W.12, F.4
Ramo-Tello, Cristina F.37
Rana, Brinda W.39
Randall, Katrina W.3
Rao, Ping OR.05, F.46
Rasheed, Mustafa T.55
Rashidi, Maryam OR.24
Rathanaswami, Palaniswami T.50
Ratnam, Manohar W.1
Raveney, Ben T.12
Rawlings, David J. T.59
Ray, Soma F.42

★ = member of a FOCIS Center of Excellence

Ray, Susan F.44
Raychaudhuri, Siba OR.19
Raychaudhuri, Smriti OR.19
Recher, Mike W.7
Reddy, Jay F.11, F.25
Reed, Elaine F.34
Reed, Elaine F. OR.05, F.46
Reed, Evan T.31
Reed, James OR.31
Rees, William T.4
Regn, Danielle F.19
Reiff, Andreas T.40
Reimold, Andreas F.7
Reindl, Markus T.13
Reiss, Samantha OR.10
Remy, Séverine OR.43
Rengarajan, Jyothi F.44
Reyes, Cynthia T.49
Reynolds, Richard F.28
Rice, Lisa T.39
Richards, Susan F.71
Rieck, Mary T.19
Rigden, Rachael C OR.17
Rims, Cliff OR.47
Rinchai, Darawan OR.01
Riner, Diana OR.50
Ringden, Olle T.43
Rios, Wendy F.64
Rioux, John OR.49
Ristic, Sinisa T.48
Rizzi, Thais T.31
Ro, Bodrey F.59
Roan, Florence T.16
Roberts, Ceri A. OR.44
Roberts, David W.42
Roberts, Kevan OR.33
Roberts, Robert F.53
Robinson, William OR.12
Rodgers, David W.33
Rodrigues, Rodrigo F.64
Romão Pereira, Érica F.31
Romero, Maria OR.55
Romero, Xavier OR.26
Roncarolo, Maria-Grazia OR.06, OR.39
Rong, Fan OR.57
Rosada, Rogerio W.43, F.64
Rosati, James T.56
Rosenblatt, Robert W.20
Rosol, Thomas F.73
Ross, Colin W.38
Rossetti, Maura OR.51, F.6, F.46
Rossi Mel, Eva OR.39
Roth, Alena T.63
Rouilly, Vincent F.39
Rucci, Francesca W.7
Ruff, William OR.32, T.26, T.33
Rui, Jinxiu T.58
Ruppert, Shannon W.40, W.51
Russell, Chris OR.42
Russo, Fabio OR.06
Ruzmetov, F. 3828
Ryan, Caitrona W.14

S

Sachdeva, Naresh W.15
Sadhu, Srikanth T.8
Saga, Ryoko T.18
Saggar, Rajan T.28
Salata, Daria W.22
Salazar, Luis Alberto T.7
Salim, Anna F.64
Salimi-Elizeh, Sanam W.13
Saltzman, W. Mark F.75
Samstag, Yvonne W.11, W.48
Samuel, E. Loic F.24
San Pablo, Florentino F.21
Sánchez Hernández, Pedro Ernesto W.12, F.4
Sandborg, Christy T.34
Sanders, Philip F.10
Sanders, Rogier F.61
Sandoval Pinto, Maria F.30
Sartirana, Claudia OR.39
Sato, Wakiro OR.13, T.11, T.12
Schaenman, Joanna F.34
Schaffer, Alejandro W.42
Schaffert, Steven F.36
Schanda, Kathrin T.13
Scherer, Hans T.31
Scheuermann, Richard W.49
Schewitz-Bowers, Lauren P F.74
Schiller, Bodil T.43
Schleimer, William W.32
Schlesinger, Larry F.73
Schmitt, Nathalie OR.07, F.20
Schroeder, Harry F.57
Schumann, Kathrin F.29
Scott, David OR.37
Seay, Howard T.68, T.70
Sediva, Anna W.4
Sedy, John W.32

★ = member of a FOCIS Center of Excellence

Seegren, Philip T.55
Segura-Ortega, Jorge W.36
Seldin, Michael W.8
Severin, Mary OR.03
Shalev-Malul, Gali W.60
Shambaugh, Cindy F.60
Shanmukhappa, Kumar W.53
Sharma, Ashish A W.38
Sharma, Mamta W.14
Sharma, Surya Prakash W.15
Sheckler, Chris T.4
Shen, Chia-Rui F.12, F.17
Shen, Yufeng OR.52, OR.54
Shen-Orr, Shai W.60
Sheridan, John F.14
Sherif, Amir W.20, W.21
Shew, Timothy W.28
Shima, David F.38
Shimamura, Sanae F.5
Shimizu, Tomohiro F.5
Shimizu, Yuka F.5
Shonts, Brittany OR.52
Shreffler, Wayne OR.11
Sidwell, Tiffany F.34
Silva, Celio F.15, F.64
Silva, Priscilla W.43, F.64
Silva-Lages, Celine W.53
Silver, Phyllis B OR.17
Simeone, Ines W.31
Simmons, Julia W.53
Simon, Thomas OR.43
Simon-Vecsei, Zsofia W.6
Simons, Bryan OR.33
Singh, Rajinder F.21
Singh, Ram OR.27, T.28, T.30
Singh, Ram Raj T.35
Sinquett, Frank T.52
Sintes, Jordi OR.26
Sismová, Kristýna W.4
Siu, Stacey F.21
Siwicki, Marie T.44
Skidmore, Lillian F.71
Sliepen, Kwinten F.61
Smit, Martine T.46
Smith, Carolyne OR.20, F.67
Smith, Dirk T.4
Smythe, Kiah F.71
Snyder, Laurie F.40
Snyder, Michael OR.39
Snyder-Cappione, Jennifer T.32
Soares, Luana W.44, F.62
Sokolove, Jeremy OR.12
Solans-Laqué, Roser T.38
Son, Jinkyung W.50
Sondermeijer, Hugo OR.38, W.61
Soper, David W.52
Spadaro, Giuseppe W.5
Spahr, Chris OR.41
Spits, Hergen OR.18, T.31
Spreafico, Roberto OR.51, F.6
Spurgin, Heather W.28
Srivastava, Ruchi F.65, F.66
Staats, Janet F.40
Stablini, Angela OR.14
Stapleton, Renee F.58
Starosvetsky, Elina W.60
Starzynska, Teresa W.22
Steffen, David F.25
Steinbrecher, Johanna OR.11
Steiner, Theodore F.49
Steinman, Lawrence T.19
Stevens, Erin T.4
Stifano, Giuseppina T.39
Stimpson, Madeleine L F.74
Strauss-Albee, Dara F.50
Sturgeon, Craig W.6
Suen, Jau-Ling T.1
Sun, Binggang T.54
Sun, Ying F.71
Sunga, Gemalene F.34
Sunkari, Vivekananda W.51
Surana, Neeraj OR.59
Suvonov, K. 3819
Suwannasaen, Duangchan F.45
Sweere, Johanna W.40
Sweezey, Neil W.55
Sykes, Megan OR.38, OR.52, W.61, W.62, W.68
Szanto, Attila W.7
Szekely, Laszlo W.18
Sztein, Marcelo OR.25

T

Taams, Leonie S OR.44
Tai, Ernest F.21
Tajhya, Rajeev F.24
Takahashi, Eri T.10
Takahashi, Ryosuke T.11
Takahata, Masahiko F.5
Tang, Anthony W.41
Tangye, Stuart W.3, T.53

★ = member of a FOCIS Center of Excellence

Tanner, Mark R. F.24
Tatakihara, Nathália F.31
Taylor, Amy W.53
Teklenburg-Roord, Sarah T.A T.42
Teniente-Serra, Aina F.37
Tesselaar, Kiki OR.53
Teuscher, Paulla OR.09
Thome, Joseph OR.54
Thompson, Mike OR.35
Thompson, Susan T.34
Thon, Vojtech W.8
Thorpe, Jerill F.45
Thum, Rebecca W.24
Thumboo, Julian OR.48
Tiao, Janice T.29
Tietjen, Gregory F.75
Tilly, Gaelle OR.60, F.33
Ting, Emily T.45
Tinoco, Roberto F.54
Tirouvanziam, Rabindra OR.02
Toapanta, Franklin OR.25
Tocheva, Anna T.60
Todd, John F.63
Toes, Rene T.31
Tonacchera, Massimo T.72
Tonra, James OR.08
Toribio, Ramiro F.73
Torres, Richard OR.31
Tour, James M. F.24
Tran, Cynthia OR.27
Trappe, Raif F.36
Trier, Anna F.67
Truedsson, Lennart T.43
Tsai, Wanxia F.67
Tseng, Michelle W.10
Tsokos, George W.7
Tuller, Friederike T.13
Tung, James F.41
Turan, Saime F.32
Turvey, Stuart W.41

U
Uchida, Yoshikazu OR.34
Uchtenhagen, Hannes OR.47
Udi, Josefina W.27
Ueno, Hideki OR.07, F.20
Ujhazi, Boglarka OR.29, W.6, W.7
Umetsu, Dale OR.29
Uno, Kazuko T.37
Ur-Rasheed, Ata OR.10
Ureta, Daniela W.27
Urrutia, Alejandra F.39
Ushach, Irina T.49
Utz, Paul OR.21
Uzel, Gulbu T.53

V
Vadalia, Aarti T.55
Valdés Alvarado, Emmanuel F.30
Valdez Haro, Angélica F.30
Valera, Isela T.28, T.35
Valiente, Giancarlo OR.03
Valle, Yeminia F.30
van den Broek, Theo OR.53, T.42
van der Voort, Ellen T.31
van Gils, Marit F.61
van Herwijnen, Martijn J.C. T.42
van Loosdregt, Jorg F.6
Van Oers, Nicolai OR.56
van Tok, Melissa T.25
van Uden, Nathalie T.31
van Wijk, Femke OR.22, OR.53, T.42
Vashisht, Priyanka OR.04
Vazquez, Monica T.49
Vazquez Mateo, Cristina T.32
Velarde de la Cruz, Erandi W.12, F.4
Venkatanarayanan, Hari Balaji OR.48
Vera, Erika T.5
Verheugen, Eveline F.18
Vernon, Robert T.64
Villanueva-Quintero, Guadalupe W.12
Villinger, Francois OR.10
Vinas, Laura W.9
Vince, James OR.24
Virk, Sonia W.15
Vishwasrao, Paresh W.62
Vlodavsky, Israel OR.40
Voll, Reinhard W.27
Volpe, Bruce T T.20
Volpi, Stefano W.7
von Rossum, Anna W.45
Vsetecka, Danielle F.2, F.3
Vudattu, Nalini OR.31
Vunjak-Novakovic, Gordana T.41

W
Waaga Gasser, Ana Maria W.24, W.25
Wabnitz, Guido W.11
Waddington, Claire OR.25
Wahl, Alan F.71

Wakeland, Edward OR.45
Waksal, Samuel OR.08
Waldron-Lynch, Frank F.63
Wallace, Carol OR.51, F.6
Walsh, Edward F.59
Walter, Jolan OR.29
Walter, Jolan E. W.6, W.7
Walter, Otto W.7
Wampler Muskardin, Theresa OR.04
Wang, C. Kathy F.59
Wang, Ena W.31
Wang, Hong W.23
Wang, Jianing F.71
Wang, Jianwu W.49
Wang, Kevin Yehsheng F.53
Wang, Ruikang W.17
Wang, Xuan W.14
Wang, Yue F.72
Wang, Yun F F.44
Ware, Brian W.32
Ware, Carl W.32
Wassif, Christopher F.47
Watanabe, Maria Angelica Ehara F.31
Weaver, Casey T. T.22
Wehrens, Ellen J.M. T.42
Wei, Lai F.74
Weiner, Joshua OR.38, W.68
Weiner, Joshua I. OR.52
Weinhold, Kent F.40
Weiss, Jonathan OR.08
Wentworth, John.M OR.24
Werner, Bonnie T.14
Werth, Victoria T.29
Westerberg, Lisa OR.29
Whalen, Elizabeth T.59
Wherrett, Diane T.69
Wick, Wolfgang OR.36
Wicker, Linda F.63
Wight, Thomas OR.40, T.64
Willaims, Marshall F.52
Williams, Emily L F.38, F.74
Wilson, Robert T.53
Wilson, Vicki T.50
Winerdal, Malin W.20, W.21, T.17
Winerdal, Max W.20, T.17
Winqvist, Ola W.18, W.20, W.21, W.34, T.17
Wiser, Jeff W.60
Wobma, Holly T.41
Woelk, Christopher W.39
Wolfson, Martin T.50
Wong, May F.49
Woodland, David OR.38
Wrammert, Jens OR.10
Wren, Lisa T.56
Wu, Anette W.68
Wu, Hao OR.09
Wu, Jennifer OR.10, W.57
Wu, Jing-Yiing W.37
Wu, Lai-Chu OR.03, F.14, F.70
Wu, Tai-Ting T.1
Wu, Yu-Jie W.37
Wucherpfennig, Kai OR.37
Wurfel, Mark F.58
Wäsch, Ralph W.27

X
Xia, Ashley W.56
Xu, Dong T.27
Xu, Linlin OR.09

Y
Yamamura, Takashi OR.13, T.11, T.12, T.18
Yan, Mei OR.45
Yang, Cian-Ru T.45
Yang, Suxiao OR.52
Yang, Wei Cheng F.12
Yang, Xifeng W.52
Yao, Jingjing T.54
Yao, Xianglan T.3
Yao, Xudong OR.23
Yap, Michelle OR.60, F.33
Yasuda, Shinsuke F.5
Yaylim, Ilhan F.32
Ye, Yi-Ling F.68
Yeh, Wen-I T.68
Yenilmez, Ezgi Nurdan F.32
Yeremenko, Nataliya OR.18, T.25
Yi, Sothy F.21
Yim, Erin T.44
Yip, Linda T.65, T.66
Yoshizaki, Kazuyuki T.37
Young, Chi F.21
Young, Nicholas OR.03, F.14, F.52, F.70, F.73
Young, Travis W.33
Yu, Andrew OR.32, T.26, T.33
Yu, Gang T.50
Yu, Li F.59
Yu, Sanhong OR.29

Z

★ = member of a FOCIS Center of Excellence

Zambuzi, Fabiana W.43, W.44, F.62
Zanin-Zhorov, Alexandra OR.08
Zárate-Bladés, Carlos R OR.17
Zarrinpar, Ali OR.05
Zeybek, Umit F.32
Zhai, Yuan OR.05
Zhang, Aihong OR.37
Zhang, Li W.69
Zhang, Ming T.50
Zhang, Sisi OR.12
Zhang, Yujuan T.34
Zhang, Yuxia OR.24
Zhao, Guiling W.62
Zhao, Hong T.27
Zhao, Wenpu F.67
Zhu, Honglin OR.45
Ziegler, Steven T.16
Zimmermann, Heiner F.36
Zirakzadeh, Ali W.20, W.21
Zitsman, Jonah OR.38, W.61, W.68
Ziv-Kenet, Amit W.60
Zlotnik, Albert T.46, T.49
Zoudilova, Maria T.14
Zuber, Julien OR.52
Zuo, Fengrong F.59
Zuo, Xiaoxia OR.45