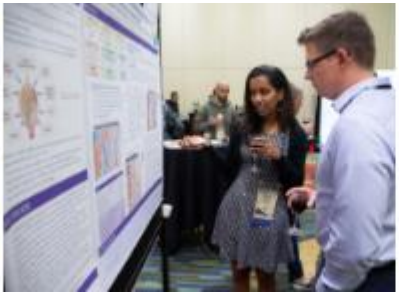
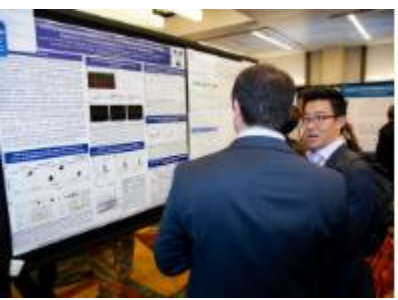
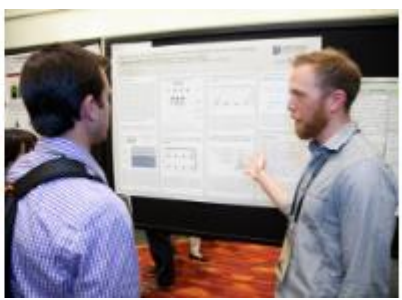



2019 FOCIS Abstract Supplement






Federation of Clincial Immunology Societies
June 18 – 21, 2019
Boston, Massachusetts

FOCIS 2019
Abstract Supplement

Table of Contents

Abstracts by Subject Area

Allergy/Asthma

T. 13. Towards a data-driven approach to dissect interactions between diet and autoimmunity

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Autoimmunity is on the rise around the globe. An estimated 70% of autoimmune disease cases are due to environmental factors. Diet has been proposed as a risk factor for autoimmunity and shown to modulate the severity of several autoimmune disorders. Yet, the interactions between diet and autoimmunity in humans remains largely unstudied. Here, we systematically interrogated commonly consumed animals and plants for peptide epitopes previously implicated in seventy seven human autoimmune diseases. A total of fourteen species could be divided into three broad categories regarding their content in human autoimmune epitopes. Strikingly, however, pig contains a disproportionately high number of autoimmune epitopes not found in any of the other species analyzed. Importantly, these epitopes were found to be expressed across all tissues collectively. Ongoing analyses focus on mapping autoimmune epitopes present in food onto specific HLA alleles and inspecting epidemiological data on diet and autoimmune disease incidence. This work sheds light on potential new links between diet and autoimmunity in humans and lays the foundation for future mechanistic studies on the impact of diet on the pathogenesis and progression of autoimmune disorders.

H. 1. Natural Tr1-like Cells Do Not Confer Long-Term Tolerogenic Memory

Paul Bollyky[1], Carlos Medina[1], Koshika Yadava[1], Heather Ishak[1], Irina Gurevich[1], Hedwich Kuipers[1], Elya Shamskhou[1], Ievgen Koliesnik[1], James Moon[2], Casey Weaver[3] and Kari Nadeau[4]

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IL-10-producing Tr1 cells promote tolerance but their contributions to tolerogenic memory are unclear. Using 10BiT mice that carry a Foxp3-eGFP reporter and stably express CD90.1 following IL-10 production, we characterized the spatiotemporal dynamics of Tr1 cells in a house dust mite model of allergic airway inflammation. CD90.1+Foxp3-IL-10+ Tr1- like cells arise from memory cells and rejoin the tissue-resident memory T-cell pool after cessation of IL-10 production. Persistent antigenic stimulation is necessary to sustain IL-10 production and Irf1 and Batf expression distinguishes CD90.1+Foxp3-IL-10+ Tr1-like cells from CD90.1+Foxp3-IL-10- “former” Tr1. Depletion of Tr1-like cells during primary challenge exacerbates allergic airway inflammation. However, neither transfer nor depletion of CD90.1+Foxp3-IL10- “former” Tr1 cells influences the inflammatory response during subsequent allergen re-challenge weeks later. Together these data suggest that naturally- arising Tr1 cells do not contribute to tolerogenic memory. This instability may limit efforts to re-establish tolerance by expanding Tr1 in vivo.

H. 2. Phospholipid Phosphatase 6 Selectively Regulates Phagocyte Function and Immune Responses

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Phospholipid phosphatase 6 (PLPP6) regulates polyisoprenoid diphosphate signaling in cell activation. In response to pro-inflammatory stimuli, PLPP6 converts presqualene diphosphate (PSDP) into its monophosphate form (PSMP). Because polyisoprenoids serves fundamental roles in cell immunology, we generated mice deficient in Plpp6 (Plpp6 -/-) to investigate its role in isoprenoid remodeling and cellular responses *in vivo*. Naïve Plpp6-/- had lower total and cellular cholesterol levels. In house dust mite (HDM)-induced lung allergic inflammation, Plpp6-/- mice had reduced conversion of PSDP into PSMP. Plpp6-/- mice had lower numbers of lung eosinophils, neutrophils and dendritic cells (DCs) relative to WT. In addition, Plpp6-/- mice also had lower expression of type 2 cytokines and serum IgE levels. Uptake of labeled HDM by DCs *in vivo* was decreased in Plpp6-/- mice, and *in vitro* Plpp6-/- DCs uptake of labeled dextran by macropinocytosis was decreased with lower phosphoinositide 3-kinase (PI3K) expression and phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) formation compared to WT. Together, these results indicate that PLPP6 deficiency leads to lower cellular cholesterol levels, reduced polyisoprenoid diphosphate remodeling, decreased DC macropinocytosis and reduced allergen-driven tissue inflammation, and suggest a pivotal role for PLPP6 in mediating allergic responses to environmental stimuli.

H. 3. Randomized Controlled Phase 2a Study Results Using Anti IL-33 in Food Allergy

R. Sharon Chinthrajah[1], Andrew Long[1], Shu-Chen Lyu[1], Marco Londei[2], Kari Nadeau[1] and Cherie Liu[3]

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Objectives of this placebo-controlled study were to assess the safety and tolerability of single dose ANB020 administration in peanut-allergic adult patients and to measure the response during a standardized oral food challenge (OFC) after ANB020 or placebo administration compared to baseline OFCs. On Day 1, fifteen highly allergic adult patients were administered 300 milligrams of ANB020 by IV infusion, and five patients were administered placebo injections. To measure patient response, patients were administered ex vivo whole blood peanut antigen challenges on Day 2 and Day 5 and OFCs on Day 15 and Day 45. The clinical outcomes and cellular responses of patients were analyzed. For patients given ANB020, 67% on Day 15 and 57% on Day 45 passed the OFC; no placebo patients passed on either day. Patients given ANB020 had fewer adverse events (abdominal pain, nasal congestion) compared to placebo patients (abdominal pain, vomiting, nausea, asthma, allergic reaction). The median percentages of IL-4 and IL-9 cells in CD69 T cells were lower for patients given ANB020 compared to patients given placebo at Baseline, Day 2, and Day 5, and higher at Day 15. Peanut-specific and histamine skin test wheal sizes among all patients stayed consistent, except at Day 15 for patients given ANB020, where both wheal sizes decreased to less than 5 mm. Volumes of peanut-specific IgE and total IgE were smaller for patients given ANB020 at Baseline, Day 15, and Day 45. Study results show ANB020 to be potentially helpful in increasing patient tolerability and safety during OFCs.

H. 4. Robustness of the Evidence Supporting the Association Between Air Pollution and Immune-based Diseases

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The evidence on the impact of air pollution on human health shows inconsistency partly due to bias in the individual studies and in the overall field. We endorsed an umbrella review approach to evaluate the strength of the evidence on the effect of air pollution on immune-based diseases.

We searched PubMed (up to 6/2018) for meta-analyses of observational studies investigating the association between air pollution and any health outcome using a search algorithm incorporating both individual pollutants and general terms. Meta-analyses pertaining to any immune-based disease outcome were identified. The validity of the evidence was evaluated using the following criteria: statistical significance (random-effects estimate), statistical significance (largest study), cumulative number of cases, between-study heterogeneity, 95% prediction intervals, small-study effects bias, and excess significance bias.

We identified 46 associations (8 publications) pertaining to 6 pollutants. Asthma dominated the evidence-base with only 5 non-asthma associations present (allergen sensitization, atopy, eczema). NO_2 was most frequently studied (12 comparisons) followed by PM_{10} and $PM_{2.5}$ with 9 comparisons each. Only 2 associations showed strong level of evidence scrutinizing the link between NO_2 and PM_{10} and hospital admissions or ED visits for asthma in the elderly. One additional association on O_3 and adult hospital admissions or ED visits for asthma was highly suggestive. The rest of the comparisons presented suggestive (N=7), weak (N=17) or not significant (N=19) associations.

Although the detrimental effect of air pollution on immune-based diseases is frequently discussed, a very small number of associations on hard clinical outcomes are supported by strong evidence.

H. 5. Toward fully leveraging the capabilities of basophil activation test in clinical research through workflow simplification and standardization

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Background: The basophil activation test (BAT) is a flow-based functional assay that rely on *ex vivo* basophil activation through exposure to allergenic substances.

Objective: This work aims at assessing our capabilities to simplify, standardize and miniaturize the experimental procedure of BAT.

Method: BATs were performed on whole blood and all tests were carried out within 24h of blood collection. To enable workflow simplification and standardization, we leveraged a dry and room temperature stable reagent technology. Not only staining reagents but also allergenic substances and anti-IgE for positive controls were dried. CD203c and C63 expression on basophils were monitored to

characterize basophil activation upon exposure to different allergenic extracts. Water soluble protein extracts of milk and peanut were prepared in house and used throughout the study. A robotic platform was also used to fully automate sample preparation when plate-based assays were considered.

Results: After having optimized the staining reagent panels, flow cytometry performances as well as intermediate precision were characterized. Utility and relevance of the developed strategy were demonstrated through the analysis of blood samples from non-allergic and allergic donors. We further demonstrated that the developed procedure can be realized on plates with a fully automated sample preparation, extending further the standardization capabilities of the method while enabling its miniaturization.

Conclusion: A no-wash, whole blood based procedure for BAT, relying on the use of dry, room temperature stable and ready to use reagents was developed. This could be a major step toward fully leveraging the capability of BAT in clinical research.

H. 6. Understanding the pathogenesis of pulmonary fibrosis using the Fra2-Tg model

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Pulmonary fibrosis is a hallmark pathology seen across many interstitial lung diseases (ILDs) including idiopathic pulmonary fibrosis (IPF), scleroderma, sarcoidosis, and severe asthma. The drivers behind initiation and progression of pulmonary fibrosis are unclear and chemically induced preclinical models, such as bleomycin-induced lung fibrosis, have poor predictive value for drugs going towards the clinic. In contrast, Fos-related antigen 2 overexpressing transgenic mice (Fra2-Tg), develop a spontaneous, age-dependent progressive lung fibrosis and allowed us to evaluate the signatures of inflammation and fibrosis at three stages corresponding to pre-onset, inflammatory-dominant, and fibrosis-dominant biology.

Transcriptomic analysis of lungs revealed early increases in cytokine-cytokine receptor interactions and antigen processing and presentation pathways followed by enhanced Th2 and M2-macrophage driven type 2 responses. This type2 Inflammation progressed to extensive fibrotic signatures by 14-16 weeks of age. Further immune profiling showed highly enhanced $GATA3^+$ Th2 cells and mannose-receptor (Mrc1) positive M2-macrophages. Treatment of Fra2-Tg mice with a bispecific antibody targeting IL-4 and IL-13 during the intermediate phase of the disease abrogated the Th2 and M2-responses; however the ECM deposition was only marginally impacted. Analysis of lungs and PBMCs from IPF patients revealed a similar increase in Th2 and M2-related genes and the abundance of $CD206^+$ M2-Macrophages correlated with disease progression.

These data suggest that Fra-Tg mice recapitulate important features of pulmonary fibrosis and may be an valuable tool for both understanding the pathobiology of IPF and for testing therapeutic-agents targeting pulmonary fibrosis.

H. 9. Effective Treatment of Experimental Asthma by Lung-restricted Inhibition of Janus Kinase 1

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Preclinical and clinical evidence indicates that a subset of asthma is driven by type 2 cytokines such as Interleukin-4 (IL-4), IL-5, IL-9, and IL-13. Additional evidence predicts pathogenic roles for IL-6 and type I type II interferons (IFN). Since each of these cytokines depends on Janus kinase 1 (JAK1) for signal transduction, and because many of the asthma-related effects of these cytokines manifest in the lung, we hypothesized that lung-restricted Jak1 inhibition may confer therapeutic benefit. To test this idea, we synthesized iJak-381, an inhalable small molecule specifically designed for local JAK1 inhibition in the lung. In pharmacodynamic models, iJak-381 suppressed Signal Transducer and Activator of Transcription 6 (STAT6) activation by IL-13. Furthermore, iJak-381 suppressed ovalbumin (OVA)-induced lung inflammation in both murine and guinea pig asthma models, and improved allergen induced airway hyper-responsiveness in mice. In a model driven by human allergens, iJak-381 had a more potent suppressive effect on neutrophilic inflammation than a systemic corticosteroid. The inhibitor iJak-381 strongly reduced lung pathology, without affecting systemic Jak1 activity. Our data show that local inhibition of Jak1 in the lung can suppress lung inflammation without conferring systemic Jak inhibition in mice, suggesting that this strategy might be effective for treating asthma.

W. 1. Anti-inflammatory Role of 6-Gingerol In Mouse Model of House Dust Mite (HDM)-Induced Asthma.

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Asthma is a chronic inflammatory airway disease that affect over 234 million people worldwide. 6-gingerol, a bioactive component of *Zingiber officinale* have been reported to possess several pharmacological activities. However, there is a dearth of information on the effect of 6-Gingerol on asthma. This study investigated the effect of 6-Gingerol on mouse model of house dust mite (HDM)-induced asthma. Fifty Balb/c male mice (17±1g) were assigned into 5 groups of 10 animals each. Group 1 and 2 received normal saline and 6-Gingerol (50mg/kg) orally for 8 weeks. Groups 3 was intranasally administered house dust mite (HDM) (25µg in 10µl of saline) for 5days/week for up to 7 consecutive weeks. Group 4 and 5 were administered 6-Gingerol (50mg/kg) and dexamethasone (1mg/kg)1 hour prior to intranasally administered HDM (25µg in 10µl of saline) for 5days/week for up to 8 consecutive weeks. Serum IgE and neutrophils, eosinophils, lymphocyte, macrophages, IL-6, TNF-α, IL-7, Eotaxin and chemokine (C-X-C motif) ligand 1 was determined by in BALF by ELISA. Data were analyzed using ANOVA at $\alpha_{0.05}$.. 6-Gingerol significantly decreased HDM-induced increase in IgE, neutrophils, eosinophils, lymphocyte, macrophages, IL-6, TNF-α, IL-7, Eotaxin and CXCL-1 when compared with HDM-induced mice. Additionally, 6-gingerol markedly reduced total collagen content, peribronchial inflammation infiltrates, subepithelial collagen deposition and goblet cell hyperplasia in lung tissues of HDM-induced mice. In conclusion, 6-Gingerol elicited a protective effect on HDM-induced asthma in mice via anti-inflammatory properties and attenuation of airway remodeling.

W. 2. A Novel Role for Long noncoding RNAs in Airway Responses to Type 2 Cytokines.

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Duringtype 2 inflammation, such as in allergic asthma, IL13 signals through STAT (Signal Transducer and Activator of Transcription) proteins to drive pathophysiological changes in the airway epithelium, including increased mucus production, goblet cell metaplasia, and loss of ciliated cells. How these changes are coordinated is poorly understood. Long non-coding RNAs (lncRNAs) do not encode proteins; rather, some produce functional RNA transcripts that are powerful regulators of cellular identity and function. We have developed an approach to test the function of lncRNAs in an air liquid-interface organoid culture system of primary human bronchial epithelial cells (BECs) that recapitulates much of the *in vivo* physiology of the airway epithelium and its response to type 2 inflammation. Using CRISPR/Cas9, ATAC-Seq and single-cell RNA-Seq, we have identified a lncRNA that coordinates the response of the airway epithelium to IL13 and hypothesize it provides a mechanistic link between IL13 signaling and lung pathology seen in asthma. The work highlights a new role for lncRNAs as central regulators of airway epithelial cell biology, and as potential therapeutic targets for asthma.

W. 3. Alternative dosing strategy of omalizumab during multi-allergen oral-immunotherapy in multi-food allergic patients

Sayantani B. Sindher[1], Andrew Long[1], Natasha Purington[1], Divya Kumar[1], Dana Tupa[1], Katherine L. O'Laughlin[1], Margaret A Woch[1], Tiffany Tan[1], R. Sharon Chinthrajah[1], Stacey Skura[2], Maria Garcia-Lloret[3] and Kari Nadeau[1]

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Rationale: Adjunct therapy with omalizumab with multiallergen food oral immunotherapy (mOIT) has been shown to be safe and effective, however optimal omalizumab dosing strategies have not been explored in the setting of mOIT.

Methods: In a pilot study, participants received 3 monthly doses of 150 mg of omalizumab prior to initiating mOIT and were randomized 1:1 to receive total protein maintenance mOIT doses of either 300 mg (Group A) or 1200 mg (Group B). Each participant's 150 mg omalizumab dose was divided by their weight and total IgE in post-hoc analysis. Those receiving less than 0.016 mg/kg/(IU/mL) were categorized as low-dose (LD) and the remainder were categorized as standard-dose (SD). Time to mOIT maintenance (TTM) was compared for groups A and B and within each group for SD versus LD.

Results: Sixty participants aged 4-20 years were randomized to 30 in each treatment arm, with 9 and 21 in each arm receiving the SD and LD omalizumab, respectively. Within Group A, TTM was not different between SD and LD ($p=0.66$). However, TTM was significantly different between SD and LD ($p<0.001$) within Group B, where those in SD reached maintenance much faster than those in the LD arm and a

higher proportion of participants reached the maintenance dose at week 12 (p=0.01; 42% in LD vs 100% in SD).

Conclusions: Our preliminary data suggest that when pursuing higher dose mOIT, SD omalizumab dosing significantly reduces TTM. Further randomized controlled trials are needed to better define optimal dosing strategies for omalizumab in food allergy.

W. 4. CD9+ Regulatory B cells Induce T Cell Apoptosis via IL-10 and are Reduced in Severe Asthmatic Patients

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Background: CD9 was recently identified as a marker of murine IL-10-competent regulatory B cells. Functional impairments or defects in $CD9^+$ IL-10-secreting regulatory B cells are associated with enhanced asthma-like inflammation and airway hyperresponsiveness. In mouse models, all asthma-related features can be abrogated by $CD9^+$ B cell adoptive transfer. We aimed herein to decipher the profiles, features and molecular mechanisms of the regulatory properties of $CD9^+$ B cells in human and mouse.

Methods: The profile of $CD9^+$ B cells was analyzed using blood from severe asthmatic patients and normal and asthmatic mice by flow cytometry. The regulatory effects of mouse $CD9^+$ B cells on effector T cell death, cell cycle arrest, apoptosis and mitochondrial depolarization were determined using yellow dye, propidium iodide, Annexin V and JC-1 staining. MAPK phosphorylation was analyzed by westernblotting.

Results: Severe asthmatic patients and asthmatic mice both harbored less $CD19^+CD9^+$ B cells, although these cells displayed no defect in their capacity to induce T cell apoptosis. Molecular mechanisms of regulation of $CD9^+$ B cells characterized in mouse showed that they induced effector T cell cycle arrest leading to apoptosis in an IL-10-dependent manner. This process occurred through MAPK phosphorylation and activation of both the intrinsic and extrinsic pathways.

Conclusions: This study characterizes the molecular mechanisms underlying the regulation of $CD9^+$ B cells to induce effector T cell apoptosis in mice and humans via IL-10 secretion. Defects in $CD9^+$ B cells in blood from patients with severe asthma reveal new insights into the lack of regulation of inflammation in these patients.

W. 5. Change in immune markers after rapid desensitization to a minimum oral immunotherapy maintenance dose in multi-food allergic patients

R. Sharon Chinthrajah[1], Andrew Long[1], Natasha Purington[1], Divya Kumar[1], Dana Tupa[1], Katherine L. O'Laughlin[1], Margaret A Woch[1], Tiffany Tan[1], Maria Garcia-Lloret[2], Stacey Skura[3], Kari Nadeau[1] and Sayantani B. Sindher[1]

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Rationale: Multiple food allergen oral immunotherapy (mOIT) with adjunct omalizumab is associated with increased tolerability and faster desensitization, however, the minimum maintenance dose of each food protein in mOIT required to achieve successful clinical outcomes is not yet known.

Methods: In a pilot, randomized controlled multicenter trial, 60 participants, aged 4-20 years, received 3 monthly doses of omalizumab prior to initiating mOIT. Participants were randomized 1:1 to receive individualized mOIT containing 2-5 allergens and escalate to a maintenance dose of either 300 mg (Group A) or 1200 mg (Group B) of total food protein. Immune markers, including sIgG4 and sIgE were tested at baseline and week 18 to evaluate whether a minimum maintenance dose could induce a $\geq$25% increase in the IgG4/IgE ratio after rapid desensitization.

Results: Peanut was the most common allergen included in the mOIT (n=36). The median percent change in the peanut sIgG4/sIgE ratio from baseline to week 18 was 91% (range: -76%, 877%) and no difference was detected between groups A and B (p=0.23). An increase by 25% or more in the peanut sIgG4/sIgE ratio was achieved by 65% (11/17) in Group A and 74% (14/19) in Group B (p=0.72).

Conclusions: An increase in peanut sIgG4/sIgE ratio was achieved within 18 weeks of mOIT with adjunct omalizumab with no significant difference between treatment group A (300 mg) and B (1200 mg) suggesting that biomarker changes are induced early and at a lower maintenance dose than previously known. Larger phase 2 trials are needed to confirm these findings.

W. 6. Characterization of a Novel Clinical Assay for Peanut-Specific Immunoglobulin A in the Stool

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Food allergy is a growing problem, with an estimated 8% of children affected. Among food allergies, peanut allergy is particularly associated with severe allergic reactions. Despite the prevalence and risk of peanut allergy, there are limited therapeutic options, in part due to incomplete immunologic understanding of food allergy. Immunoglobulin A (IgA) is the most prevalent antibody in the gut, and it regulates commensal flora balance, neutralizes toxins, and opsonizes pathogenic microbes. It has been presumed that IgA may also be able to neutralize food antigens and prevent development of allergy, but that has yet to be shown. To address the question of whether peanut-specific IgA can prevent the development of

allergy, we have developed and validated a clinical assay that can detect peanut-specific IgA in human stool. This ELISA-based assay is precise, with results replicable from the same sample over multiple tests. The assay is also specific to peanut IgA. We have also found that results are closely clustered when using multiple samples from the same person over time, indicating that production is consistent over time. Using this assay, we have found that there is a range of peanut-specific IgA levels in healthy, non-allergic people. The peanut-specific IgA levels do not correlate with total IgA levels in the stool. We aim to use this assay to examine the stools of peanut-allergic individuals to determine whether stool peanut-specific IgA is correlated with protection against peanut allergy.

W. 7. Follicular Regulatory T Cells Promote the Germinal Center Reaction and IgE Response in a Peanut Food Allergy Model

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T follicular regulatory (TFR) cells are a specialized subset of Foxp3+ T cells found within the germinal center (GC) reaction and its role remains controversial. Here, we have used a peanut allergy model in mice to examine the role of TFR cells in controlling the production of antigen-specific IgE and IgE-controlled anaphylaxis. Using a mouse model with a specific loss of TFR cells (Foxp3-cre Bcl6-flox), we found TFR cells play an essential role in promoting and maintaining GC B cells, IgE production and anaphylaxis, as well as the GC reaction. Compared to control mice, TFR-deficient mice lacked circulating peanut-specific IgE four weeks after challenges and anaphylaxis was significantly weakened. Similarly, deletion of regulatory T cells using $FOXP3^{DTR}$ mice abolished peanut-specific IgE and IgG1 responses. We identified a positive role for TFR cells in the IgE response using Foxp3-cre Pten-flox mice, which have augmented TFR cell responses, higher peanut-specific IgE and increased GC B cells. Mechanistically, TFR cells require Blimp1 controlled IL-10 to promote GC B cell survival and IgE production. Further studies using mice deficient of IL-10Ra (Mb1-cre Il10ra-flox) showed IL-10 signaling was pivotal for GC B and Ab responses. Blocking IL-10 signals *in vivo* mimicked the loss of IgE levels in TFR-deficient mice and rescued mice from anaphylaxis. Our data unexpectedly show that TFR cells have a critical helper function in the production of GC-dependent IgE, in part by producing IL-10. These studies have provided greater understanding of how allergic immune responses are controlled by the GC reaction.

W. 8. Computational Identification and Comparison of GR+ Circulating Leukocyte Subsets in Healthy and Asthmatic Adolescents in Response to Exercise

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Background: Glucocorticoid receptor (GR) is a key receptor involved in inflammatory responses. Previous studies showed that aerobic exercise training could reduce GR expression on circulating leukocytes, reflecting a reduced responsiveness to stress, and thereby improve health in asthmatics. However, the cellular mechanism of this response is not completely understood.

Methods: We reanalyzed the multi-panel (Basophils, Eosinophils, Combo) flow cytometry (FCM) data in a recently published study using cutting-edge computational approaches. The study measured GR expression in leukocytes in response to a 30-minute acute exercise challenge before and after an 8-week training intervention of healthy and asthmatic adolescents. Both predefined and novel leukocyte subsets were identified using data clustering methods. Characteristics of the identified cell populations and the experiment variables and clinical parameters were associated using a mixed-effect regression model.

Results: Unbiased computational analysis identified 25 GR+ leukocyte subtypes across healthy and asthmatic groups, in comparison to the 10 subtypes reported in the original manuscript. A significant difference in CD3-CD14-CD16int NK cells was identified between baseline and peak of the acute exercise. CD14lowCD16- T lymphocytes significantly decreased after the 8-week exercise training for both healthy and asthmatic groups. At baseline, CBC-normalized frequency of CD3-CD14lowSSC-Aint granulocytes were lower in the asthmatic group, in comparison with the healthy group. Before the training, the frequency change of CD3lowCD14+CD16intGRhigh basophils in response to the acute exercise was significantly different between asthmatic and healthy adolescents; but the difference disappeared after the 8-week training. Sexual dimorphism for GR expression levels of many granulocyte subtypes is also identified.

W. 9. Effect of anti-IgE treatment in Peripheral T Cell Subpopulations in Chronic Urticaria

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Introduction

Chronic urticaria (CU) is an spontaneous urticaria that lasts for at least 6 weeks without an allergic trigger. In 40% of the patients an autoimmune pathogenesis, IgE -mediated, is involved. Severity and prognosis are highly variable among patients, and therapeutic management needs to be personalized. Omalizumab, a monoclonal antibody that binds free human IgE has shown therapeutic effect in some patients.

Objective

To analyze the effect of Omalizumab in peripheral blood T -cell subpopulations of CU patients.

Materials and Methods

Naïve, central memory (CM), effector memory (EM), effector (Th1, Th2, Th17)T-cell subpopulations and activation markers (CD38, HLA-DR) were analyzed in peripheral blood of 49 CU patients (Omalizumab treatment (n=22); non immunomodulatory drugs (NID; n=27)) and 50 healthy donors (HD).

Results

Compared to HD, CU patients treated with NID showed lower percentages of $CD4^+DR^+CD38^+$ [0,8(0,7-1)vs1,23(1,01-1,54),p<0,0001], and $CD4^+DR^+CD38^-$ [1,1(0,8-1,4)vs3,07(2,5-4,7),p<0,0001]. Percentages of $CD8^+DR^-CD38^+$ [21±3vs8±0,5%, p<0,0001] and $CD4^+$ naïve [55±3vs41±2, p<0,0001] subpopulations were increased.

CU patients treated with Omalizumab, showed lower percentage of $CD4^+$HLA-DR^+CD38^+[0,75(0,6-1,1)vs1,23(1,01-1,54)%,p<0,0001], $CD4^+DR^+CD38^-$[1,7(0,7-3,15)vs3,07(2,5-4,7)%,p=0,0006] and higher percentage of $CD8^+DR^-CD38^+$ [14±2 vs 8±0,5%,p=0,0001] and Th1 CM [12±0,8 vs 9±0,4%, p<0,0001] than HD.

Patients under Omalizumab showed a decrease in $CD4^+$ naïve [42±3 vs 55±3%;p=0.001] and $CD4^+DR^-CD38^+$ [36±3vs52±3, p=0.0002] compared to NID patients.

Th1CM [12±1vs9±1, p=0.013], EM [5,7(4,6-10,2)vs4,2 (3,6-6,8))%, p=0.033] and Th2 CM [8,5(6,7-12,3)vs6,4(4,3-8))%, p=0.019] cells were increased in Omalizumab- treated patients

Conclusions

Omalizumab induces changes in peripheral blood T-cell subpopulations, promoting a decrease of activated T subsets, and an increase of effector Th1 and Th2 cells. The impact of these changes on treatment response deserves further investigation

W. 10. Combined blockade of IL13 or IL5 with IL33 leads to a greater inhibition of Type-2 inflammation over inhibition of either pathway alone

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Uncontrolled Type-2 inflammation, mediated by IgE , eosinophils and the cytokines IL13 and IL5, is associated with allergic/atopic diseases. Clinical benefit from blocking these factors has been observed in asthma, particularly in patients displaying elevated expression of pathway specific Type-2 biomarkers. Multiple factors, including IL33, regulate expression of these and other pro-inflammatory mediators following lung injury. While perturbation of IL13 or IL5 ameliorates disease in pre-clinical and clinical studies, how each cytokine contributes to individual facets of lung pathology is unclear. We used allergic and infectious models to assess the contribution of IL5 and IL13 on pathways implicated in asthma. Mucus-related pathologies were driven by IL13, while eosinophilia was more sensitive to IL5 activity. To

determine whether inflammation could be further reduced by targeting pathways upstream of IL13 or IL5, we compared IL33 inhibition alone or with IL13 or IL5. IL33 obstruction dampened expansion/activation of Type-2 effector cells and granulocyte progenitors, attenuating disease. Simultaneous blockade of IL33 with IL5 or IL13 completely abrogated these pathologies. This highlights the distinct functions of IL5 and IL13, and suggests there is a level of crosstalk between pathways which ultimately manifests in a reduction in overall inflammation upon single cytokine blockade. Moreover, additional therapeutic benefit over single axis therapies may be achievable by combinatorial blockade of IL33 with IL5 or IL13. As many systems can regulate IL5 and IL13, this approach would circumvent this redundancy in addition to obstructing other IL33 regulated inflammatory pathways contributing to disease.

W. 11. Increased Allergen Specific CD8+ T Cells in Peanut Allergic People

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$CD8^+$ T cells are involved in atopic conditions such as atopic dermatitis, where they have been shown to secrete T_H2 cytokines, and asthma. Despite this, the involvement of $CD8^+$ T cells in IgE mediated food allergy is less studied, particularly in humans. We incubated PBMCs from 16 peanut allergic pediatric subjects with or without peanut protein and compared them to another 16 nonallergic children. Based on changes in activation marker expression, we observed a significant ($p<0.0001$) increase in peanut specific $CD8^+$ T cells in association with peanut allergy. We then incubated PBMCs from 15 HLA-A*02:01$^+$ peanut allergic subjects with a pool of peanut peptides (as opposed to peanut protein in the previous experiment) containing HLA-A*02:01 binding motifs. In 8 of these subjects, the activation of peanut specific $CD8^+$ T cells was observed. Peanut specific $CD8^+$ T cell clones derived from one of these subjects were shown to recognize a single peanut peptide in a sequence dependent manner. In addition, expression of the T_H2 chemokine receptor CCR4 was detected in two ways: 1) *ex vivo* on peanut specific $CD8^+$ T cells from peanut allergic individuals; and 2) on the peanut peptide specific $CD8^+$ T cell clones described above. These results show that peanut specific $CD8^+$ T cells are increased in peanut allergic humans, recognize peanut peptide in a sequence specific manner, and express a homing marker associated with allergic immune responses.

W. 12. Airway Dendritic Cells Distinguish Allergic Asthmatics from Allergic Controls

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It remains unknown why only some allergic individuals develop asthma. Previous work has shown that allergic asthmatics (AA) recruit more pathogenic Th2 effector cells into the airway after allergen challenge compared to allergic controls (AC). We hypothesized that airway dendritic cells (DC) regulate this effector

response and drive the asthma phenotype. We used segmental allergen challenge (SAC) to compare the airway response to allergen in AA (n=16) and AC (n=14). We characterized airway DC with flow cytometry and measured airway cytokines and IgE. Airway DC were sorted for RNA sequencing (n=4 per group). While AA and AC had similar baseline numbers of airway CD141$^+$DC, AA had a higher percentage expressing the high-affinity IgE receptor (FcεRI). After SAC, CD141$^+$DC only increased in AC. AA had higher CCL19 and CCL20 levels, and endobronchial brushings suggested mucosal accumulation of CD141$^+$DC after SAC in AA compared to AC. AA also had higher airway levels of cytokines that promote tertiary lymphoid organ (TLO) formation and higher Th2 cytokines and IgE levels after SAC compared to AC. Airway DC in AA had 110 differentially-expressed genes in the allergen- compared to the control-challenged segment, with down-regulation of genes that promote tolerance. Our data suggest that CD141$^+$DC in AA are primed to respond to allergen via expression of FcεRI and down-regulate tolerogenic genes in response to allergen challenge. CD141$^+$DC may respond to DC-active chemokines by accumulating in the mucosa to serve as the nidus of TLO formation, enhancing the tissue effector response to allergen characteristic of allergic asthma.

W. 14. IRF4 Expression by Lung Dendritic Cells Drives a Th2 Program throughout the Effector Allergic Response, but is not Essential during the Memory Response

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Mechanisms by which DCs initiate Th2 responses and the role for DCs throughout allergic memory responses are not completely understood. We previously demonstrated that expression of the transcription factor IRF4 in mature DCs is needed for type 2 lung inflammation in response to house dust mite extract (HDM) in mice. We hypothesized that IRF4 regulates specific functions in mature DCs that are required for Th2 induction and lung resident memory T cell (T_{RM}) responses. Here, we demonstrate that mice having IRF4-deficient DCs display impaired recruitment of activated effector T cells to the lung soon after sensitization. While IRF4-deficient DCs exhibit minimal defects in the lungs, we find that their migration to the draining lymph nodes is limited. Moreover, DC-intrinsic defects in Th2 priming exist. IRF4-deficient DCs express less of the Th2-associated costimulatory molecule OX40L and less of the Th2-promoting cytokines IL-33 and IL-10. The instillation of IL-33 and IL-10 into the lungs during sensitization restores T cell production of some Th2 cytokines but does not entirely restore inflammation, suggesting other effector molecules downstream of IRF4 play an important role in initiating the Th2 response. Further, we demonstrate that mice lacking IRF4-expressing DCs during sensitization display impaired memory responses to HDM, but T cells educated by IRF4-competent DCs mediate potent memory responses independently of IRF4-expressing DCs. Together, these findings suggest that IRF4 controls a program in mature DCs that governs Th2 priming during sensitization and Th2 effector responses during challenge, but that mitigated T_{RM}-dependent responses stem from defects in earlier education.

W. 15. Targeting the IL-7Rα as a potential therapeutic approach for allergic asthma

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Asthma remains an important cause of morbidity and mortality. We herein provide for the first time a preclinical proof-of-concept for a novel therapeutic approach for allergic asthma using an anti-IL-7Rα mAb, which blocks both IL-7R and TSLPR. We used a murine asthma model in which mice received 4 weekly percutaneous sensitizations followed by 2 weekly intranasal (IN) challenges with total house dust mite (HDM) extracts as allergen. This model corresponds to a mixed asthma phenotype in which the bronchoalveolar inflammation comprises both neutrophils and eosinophils. Asthmatic mice were then treated with an anti-IL-7Rα mAb or an isotype control every other day during the 2 weeks of IN challenges. Anti-IL-7Rα mAb blocks STAT5 phosphorylation in mouse CD4+ T cells induced by either IL-7 or TSLP. Compared to control animals, anti-IL-7Rα-treated mice showed significantly lower airway resistance in response to methacholine as measured by flexiVent, associated with an improvement in lung histology. Anti-IL-7Rα treatment significantly decreased the mRNA expression of Th2 cytokines (IL-4, IL-5, and IL-13) and chemokines (CCL5/RANTES) in lung tissue, decreased the secretion of Th2 cytokines (IL-4, IL-5, and IL-13) and chemokines (CXCL1 and CCL11/eotaxin) in bronchoalveolar lavage fluid as measured by luminex, and decreased serum HDM-specific IgE as measured by ELISA. Leukocyte phenotyping by flow cytometry revealed a reduction of eosinophils, total lymphocytes, T cells, and especially ILC2 in lung and in BALF in anti-IL-7Rα-treated mice. Targeting the IL-7Rα by a mAb, through its broad mechanisms of action, presents as a potential therapeutic approach for asthma.

W. 16. Comprehensive Mapping of Immune-Epithelial Interactions in Severe Asthma Using Machine Learning Analysis of Multi-'omics Data

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Asthma is common, affecting more than 300 million people worldwide. Though well controlled in most, a subset experience symptoms refractory to treatment, accounting for nearly half the US healthcare expenditure on asthma. Time-of-flight mass cytometry (CyTOF) offers an opportunity to characterize the inflammatory milieu of bronchoalveolar lavage (BAL) in unprecedented detail. Pairing this with gene expression data from bronchial epithelial cells (BECs) and BAL yields the most comprehensive understanding of SA to date.

Flow cytometry from 7 healthy control, 15 mild-to-moderate and 19 SA patients reveals 31 cells types. Network construction of covariant cells demonstrates neighborhoods that define 4 patient clusters. One SA-enriched cluster shows high levels of IFN-ɣ production driven by T-cells. Other

clusters demonstrate varying abundance of innate immune lineages that differ in Type 2 (T2) cytokine elaboration. BEC gene expression analysis identifies 3 clusters, two of which enriched for SA cases. One SA-cluster demonstrates high levels of T2 inflammation and strong relationship with mast cell/basophils in BAL. Another shows low levels of T2 inflammation despite housing a third of SA patients. GSEA identifies interferon and IL-6 pathway activation, suggesting Type 1 (T1) immune response. These data were corroborated with transcriptional profiling of BAL cells, underscoring networks of interacting cytokines between cellular compartments.

Comprehensive immunophenotyping of BAL identifies stereotyped derangements associated with cytokine signatures and reciprocal epithelial response. Correlative mapping of receptor-cytokine pairings across BEC and BAL offers insight into pathways driving inflammation and may guide future targeted interventions.

Autoimmune Neurologic Diseases

H. 10. 'Older' T cell and B cell compartments in patients with Parkinson's Disease

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Background: Accumulating evidence now suggests that the immune system plays important roles in the pathogenesis of Parkinson's disease (PD). In preliminary work, abnormal T cell responses have been described in the circulation of patients with PD. The aim of the current study was to comprehensively immunophenotype different immune cell subsets in the patients with PD and controls.

Method: Peripheral blood were collected from patients with PD (n=34) as well as age and sex matched controls (n=17). Multi-color flow cytometry using validated panels of up to 16 parameters capturing a broad range of immune-related molecules was performed blindly on either whole blood leukocytes or peripheral blood mononuclear cells (PBMC) to deeply characterized distinct immune-cell subsets and their ex-vivo responses.

Result: The percentage of $CD4^+$T cells was decreased (p=0.008), while $CD8^+$T-cell frequencies increased (p=0.0015) in the PD patients, resulting in a decrease of CD4/CD8 ratios (p=0.002). Of note, PD patients seemed to harbor more $CD28^-CD27^-CD57^+KLRG1^+$immunosenescent T cells in their peripheral blood (p=0.05). In keeping with T cell compartment changes, PD patients also harbored increased frequencies of age-associated $CD11c^+$ B-cells (p=0.009), while frequencies of newly generated transitional B cells with immune-regulatory capacity were decreased in the PD group (p=0.0072).

Conclusion: Taken together, our data indicate that the aging of particular T cell and B cell compartments seems to be accelerated in PD, which may offer an explanation for the pro-inflammatory immune-

response profile implicated in these patients. Further functional and mechanistic cellular-immune study is warranted in PD.

H. 11. Anti-inflammatory B cell shift induced by Bruton's Tyrosine Kinase (BTK) inhibition

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B cells are strongly implicated in new multiple sclerosis (MS) relapses, through antibody-independent functions including antigen presentation and pro-inflammatory cytokine-mediated activation of T cells and myeloid cells. B cells also persist in meninges of patients where they are now thought contribute to non-relapsing (progressive disease) mechanisms. Therapies able to target B cells within the CNS may effectively limit disease progression. Bruton's Tyrosine Kinase (BTK) plays a crucial role in early B cell development. Specific BTK inhibitors have now been used for treatment of B cell malignancies and some can access the CNS. Here, we studied the impact of BTK inhibition (BTKi) on human B cell functions.

Using purified peripheral $CD19^+$B cells from healthy volunteers or MS patients as well as human tonsillar B cells, we show that the presence of BTKi strongly decreased B cell activation (limiting up-regulation of CD69 and CD40) without apparently affecting B cell survival. BTKi significantly limited the induction of co-stimulatory molecule (CD80, CD86) expression on activated B cells, which in turn resulted in a decreased capacity of the B cells to support both polyclonal as well as antigen-specific T cell activation. Finally, BTKi treatment also significantly reduced pro-inflammatory B-cell cytokine (GM-CSF, TNF-alpha and IL-6) secretion with only marginal influence on B cell IL-10 production, resulting in a decreased capacity of the B cells to promote myeloid cell pro-inflammatory responses. BTKi may thus effectively limit antibody-independent pro-inflammatory B cell responses that are now implicated in both peripherally-mediated relapsing disease, and in CNS-compartmentalized progressive disease.

H. 12. B cell:Myeloid cell interaction and contribution to the CNS compartmentalized inflammation of Multiple Sclerosis

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B cells are abnormally fostered within central nervous system (CNS) meninges of patients with multiple sclerosis (MS). These B-cell rich immune-cell collections in the meninges are adjacent to substantial cortical injury that is now strongly associated with disease progression. This cortical demyelination involves a gradient of microglia ad macrophage activation. We previously reported that MS patient cells produce abnormally higher levels of pro-inflammatory (IL-6$^+$, TNF$^+$, LTa$^+$ and GM-CSF$^+$) cytokines, capable of inducing pro-inflammatory T cell responses, while IL-10 expressing B cells were diminished in MS patients. Here, we hypothesized that bi-directional interactions between MS-implicated B cell subsets and underlying CNS myeloid cells can propagate CNS-compartmentalized inflammation associated with disease progression.

First, we showed that human M1 microglia-derived supernatants increase B cell activation (CD86 and CD95 expression), while M2c microglia-derived supernatants induced B cell death. Next, MS-implicated pro-inflammatory B cell supernatants increased human microglia/macrophage pro-inflammatory cytokine (TNF, IL-12 and IL-6) responses, in part due to B cell-derived GM-CSF, while down-regulating the myeloid cell IL-10 production, and increasing their expression of the T-cell co-stimulatory molecule CD80. In contrast, IL-10 expressing B cell supernatants enhanced microglia/macrophage expression of TREM-2, known to be functionally associated with phagocytosis. Finally, supernatants of the pro-inflammatory and anti-inflammatory B cells reciprocally regulated myelin phagocytosis by human microglia/macrophage.

In conclusion, cross-talk between B cells/myeloid cells may support ongoing cascades of inflammation and injury during disease progression, offering the possibility to use novel therapeutic strategies for progressive MS – an unmet clinical need.

H. 13. B cells regulate CD8+ MAIT cell effector functions: implications to multiple sclerosis

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B-cell depletion therapy (BCDT) substantially reduces new disease activity in multiple sclerosis (MS), without impacting the abnormal cerebrospinal fluid immunoglobulin levels. Recent work has demonstrated that B cells engage in bi-directional interactions with CD4$^+$ T cells, and contribute to their abnormal immune responses in MS as substantiated by findings that T-cell effector functions are attenuated in individuals receiving BCDT. Curiously, BCDT is also found to decrease CD8$^+$ T cell responses, and B cells and CD8$^+$ T cells (including mucosal associated invariant T (MAIT) cells), are found together within the MS central nervous system (CNS). Little is known about interactions between B cells and CD8$^+$ T cells, including MAIT cells. To this end, we generated an *in vitro* cell culture system, in which human polyclonally activated CD8$^+$ T cells are co-cultured with autologous B cells. We found that while primed B cells suppressed the proliferation of naïve CD8$^+$ T cells, they enhanced the proliferation of CD8$^+$ MAIT cells. Primed B cells also selectively boosted MAIT-cell degranulation and cytotoxicity. These effects were not contact-dependent and involved B-cell derived soluble factors. In turn, activated CD8$^+$ T cells enhanced B-cell cytokine production. Together this data indicates cross-talk may exist between B cells

and CD8+ T cells, including the capacity of B cells to reciprocally impact distinct CD8+ T cell subsets. The particular capacity of B cells to induce activation and effector responses of MAIT cells may be relevant to MS pathophysiology and to the therapeutic mode of action of BCDT in MS.

H. 14. Comparison of Immunoprofiling and Gene Expression Analysis in Benign and Secondary Progressive Multiple Sclerosis

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Objectives: We tested the hypothesis that SPMS has a different immunological and gene expression profile than benign MS.

Background: In contrast to SPMS, BMS is diagnosed primarily retrospective and weighted towards motor progression, specifically an Expanded Disability Status Scale (EDSS) £3 after at least 15 years of disease onset. Utilizing phase III EXPAND trial cohort of SPMS baseline blood samples, our study aims to identify immunological and gene expression changes between SPMS and BMS.

Methods: We conducted experiments with flow cytometry, mircroarray, luminex, and CyTOF comparing 40 SPMS patients to 20 age, sex, and disease duration matched BMS. We also used RRMS and HC. State-of-the-art CyTOF technology allowed the identification additional immune subsets changes.

Results: We found that 1) RRMS and SPMS has a significantly higher % of CD8+ T cells and higher % of B cells as compared to BMS. SPMS have significantly lower % of Th2 compared to HC. 2) Microarray analysis indicate that SPMS has a different gene expression profile than BMS; many of which are involved in immune response including both T and B cell mediated immunity. 3) B cell and myeloid cell dysregulation is a feature of RRMS and SPMS; 4) Elevation of sCD40L was found only in plasma of SPMS, but not in BMS nor RRMS.

Conclusion: SPMS has a different immunological and gene expression profile than BMS. Future longitudinal studies will allow us to determine immunological and gene expression changes which ultimately lead to discovery of new therapeutic targets for disease progression.

H. 15. CysLTR2 Signaling on T cells Is Required For Experimental Autoimmune Encephalomyelitis

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Cysteinyl leukotrienes receptors CysLTR1 and CysLTR2 are members of a trans-membrane G-coupled receptor family known for its role in airway inflammation. They can mediate leukocyte chemotaxis, vascular leakage and endothelial cell migration upon binding of the cysteinyl leukotrienes (cysLTs) LTC4, LTD4 and LTE4. The particular role of CysLTR2 in T cell mediated immune responses, and its participation in autoimmune diseases has not been studied. We investigated the expression of CysLTR2 on murine CD4+ T helper cell subsets and found the highest expression on pathogenic Th17 cells.

These cells are important contributors to the pathogenesis of a variety of autoimmune diseases, including Multiple Sclerosis (MS). To elucidate the potential role of CysLTR2 in the pathogenesis of MS we used the murine model of experimental autoimmune encephalomyelitis (EAE). Immunizing CysLTR2-/- mice with MOG_{35-55} we demonstrated that deficiency of CysLTR2 led to ameliorated disease with lower degree of paralysis, histopathologically decreased number of inflammatory parenchymal and meningeal lesions and lower frequencies of CNS-infiltrating IFNg and IL-17A producing CD4+ T cells.

This clinical phenotype was reproducible in LTC4s-/- mice, which are lacking all CysLTs. To further establish if loss of CysLTR2 was T cell intrinsic we utilized MOG35-55 transgenic mice and could indeed recapitulate our findings from the global KO mice. Together these results show that CysLTR2 signaling on CD4 T cells is required for autoimmune CNS inflammation.

Therapeutic blocking antibodies selectively binding to CysLTR2 might therefore be a promising future therapy in inflammatory diseases of the CNS and other autoimmune diseases.

H. 16. Dimethyl Fumarate Therapy Reduces the Frequency of Memory T cells and the Potential of CNS Trans-migration in Patients with Multiple Sclerosis

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Background: Dimethyl fumarate (DMF) is a disease modifying therapy used for patients with relapsing-remitting multiple sclerosis (RRMS). T cells are major contributors to the pathogenesis of RRMS, where they regulate the inflammatory immune response and participate in infiltration and lesion development in the CNS. The impact of DMF on T cell subpopulations, their CNS trans-migration potential and effector functions remains unsolved.

Objectives: We evaluated the effects of DMF on T cell subsets, their CNS trans-migration potential and their effector functions.

Methods: Blood and cerebrospinal fluid (CSF) cells from untreated and DMF-treated patients with RRMS and healthy donors were analyzed by flow cytometry.

Results: We found that DMF reduced the prevalence of circulating memory T cells. This reduction was observed in proinflammatory CD4+ and CD8+ T cell subsets whereas regulatory T cells were unaffected. Furthermore, DMF reduced the frequency of peripheral CD4+ T cells expressing CNS-homing markers. Additionally, we found a reduced recruitment of CD4+ T cells but not CD8+ T cells to the CSF. We also found that monomethyl fumarate (MMF), the primary metabolite of DMF, dampened T cell proliferation *in vitro* and reduced the frequency of TNFα, IL-17 and IFN-γ producing T cells.

Conclusion: These data show that DMF influences the balance between proinflammatory and regulatory T cells in the periphery by reducing the proinflammatory potential. Furthermore, DMF reduces the CNS-migratory potential of CD4+ T cells whereas CD8+ T cells migration is less affected by the treatment. Altogether, our study suggests an anti-inflammatory effect of DMF on the CD4+ T cell compartment

H. 17. Eomes+ Th cells: A crucial biomarker for Secondary Progressive Multiple Sclerosis (SPMS) disease status

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Secondary progressive multiple sclerosis (SPMS) is a chronic neuroimmflammatory disease that develops in many patients following relapsing/remitting multiple sclerosis (RRMS). Whilst RRMS is recognized as an autoimmune disease of the central nervous system, the mechanisms that drive SPMS are yet to be established, but growing evidences indicate immune components also play an important role in SPMS pathogenesis.

We previously reported that the cytotoxic-like CD4+ T cells expressing the transcription factor Eomes (Eomes+ Th cells) are increased in the blood and in cerebrospinal fluid of patients with SPMS, but not RRMS (Raveney *et al*. Nat. Commun. 2015).

Using a novel mouse model for SPMS, in addition to patient samples, we have carried out further analysis of Eomes+ Th cells to reveal their functional mechanism in chronic autoimmune disease. Importantly, using flow cytometry on post-mortem tissue samples, we have observed for the first time a very high proportion of Eomes+ Th cells amongst brain-infiltrating Th cells only in SPMS, strongly linking these unusual cells with pathogenic processes in this disease.

Furthermore, longitudinal studies confirm our preliminary findings showing an association between Eomes+ Th cell level in blood and current disease activity. High levels of Eomes+ Th cells predicted worsening of disease symptoms as measured by subsequent EDSS changes (positive predictive value, PPV=0.793; negative predictive value, NPV=0.778; FDR=0.179).As SPMS diagnosis currently lacks effective biomarkers, our studies into Eomes+ Th cells and their functional phenotype may provide much-needed prognostic monitoring SPMS activity as well as new therapeutic targets for SPMS treatment.

H. 18. Follicular helper T cells may promote T-B interaction in lymphoid organs through IL-21 production in myasthenia gravis

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[Intoroduction]

Anti Acetylcholine receptor antibody is produced with the help of C-X-Chemokine Receptor 5 (CXCR5)-positive follicular helper T cells (Tfh) in myasthenia gravis (MG). CXCR5, which is also expressed on B cells, is important for homing to lymphoid organs. Here, we analyzed the alteration of Tfh phenotype in MG and production of Interleukin (IL)-21 by peripheral Tfh in MG and healthy controls (HC), and the effect of IL-21 on B and Tfh cells.

[Methods]

We analyzed phenotypes of peripheral blood T cells from MG before and after treatments and IL-21 production of Tfh. Effect by IL-21 on B and Tfh cells was analyzed. These analyses were mainly performed by flow cytometry.

[Results]

Total frequency of CXCR5+ cells in Th cells were elevated in MG, additionally Inducible T-cell co-stimulator (ICOS) were upregulated on Tfh cells compared with HC. Immunotherapy reduced the frequency of CXCR5+ and ICOShighCXCR5+ in Th cells in parallel with their clinical improvement. The production of IL-21 by Tfh upon a stimulation from MG patients was about nine times as high as that from HC. Interestingly, IL-21 improved B cell survival and maintained CXCR5 expression on B and Tfh cells.

[Conclusion]

Activated CXCR5+ Th cells were significantly higher in MG and immunotherapy corrects this alternation. IL-21 production by Tfh cells, its maintenance of CXCR5 on B and Tfh cells may promote Tfh-B interaction in lymphoid organs in MG.

H. 19. High-Dimensional Profiling of Single Memory CD8+T cells Reveals a Specific Pattern in Multiple Sclerosis Patients

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Numerous researches highlight the involvement of CD8+T cells in Multiple Sclerosis (MS) but the culprit CD8+T cells driving autoimmune inflammation have not been identified. We hypothesized that the cells able to provoke damages in the Central Nervous System (CNS), may have a specific phenotypic and functional pattern.

To identify this cell subtype, we analyze the single CD8+T cells molecular signatures by isolating single memory non MAIT CD8+T cells from the blood and the CSF of MS and Clinically Isolated Syndrome patients and from the blood of Healthy Controls and patients with other inflammatory neurological diseases. We then perform on each single-cells, qPCR of 96 genes involved in the CD8+T cell.

We thus are able to define subsets of CD8+T cells specific of MS by a set of genes including CCR7, CD62L, CD58, CD94, transcription factors (TBET and TCF7) and cytotoxic molecules (Granzyme A/B, perforin and granulysin) with unbiased analyses. Using T-SNE analysis, we identify a CD8+ effector memory T cell subtype with highly cytotoxic properties present only in MS patients. This MS cell subtype identification is confirmed by flow cytometry experiments on another cohort of patients. Interestingly, we also find that, in MS patients, the CSF CD8+T cells and those from the blood have a highly similar profile suggesting migration of the cells from the blood to the CNS.

Our data are the first to describe a specific cell subtype present only in MS patients made up of single cells strongly oriented toward an activated, effector and highly cytotoxic profile.

H. 20. Influence of a SESN3 risk allele on CD4+ T cells and Multiple Sclerosis susceptibility

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To date, 233 genetic variants have been associated with multiple sclerosis (MS) susceptibility. We prioritized 8 MS susceptibility genes that are highly expressed in human Th17 cells polarized *in vitro* and focused on one, sestrin 3 (SESN3) gene. We found that the rs4409785 risk allele (C) associated with MS risk also leads to a significant decrease in SESN3 RNA expression in naïve CD4+ T cells (p=1.58E-16). We characterized chromatin state of four T-cell subsets, naïve, memory, T helper 17 and regulatory CD4+ T cells using the Roadmap Epigenomics data **and found that** the rs4409785 SNP overlaps with quiescent chromatin state in all four T cell subsets. Furtheremore, using DNA methylation data from CD4+ T cells of MS patients, we observed a genome-wide significant cis-mQTL effect between rs4409785 risk allele (C) and hypermethylation of cg26564895 (p=8.65E-7), which is located close to the SESN3 transcription start site

Analysis of SESN3 gene expression in naïve and memory CD4+ T cells in healthy, untreated and glatiramer acetate (GA)-treated MS patients (n=196) shows that SESN3 expression is decreased in memory T cells from untreated MS patients compared to healthy controls, however SESN3 expression is induced in MS patients that received GA-treatment. These data suggest that SESN3 may be involved in downregulating the autoimmune response in MS. Functional studies using conditional knockout mice in addition to gene deletion strategies in human T cells are ongoing to further characterize the function of SESN3 in the regulation T cell pathogenicity.

H. 21. Multiple sclerosis-associated IL2RA gene variants, rs2104286 and rs11256593, affect expression of CD25 on CD4+ , but not CD8+, T cell subsets in genotype-selected healthy controls

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The interleukin-2 (IL-2) receptor α (CD25) is important for T cell functions. Single nucleotide polymorphisms (SNPs) rs2104286 and rs11256593 in or near the *IL2RA* gene, that encodes CD25, increase the risk of multiple sclerosis (MS), an immune-mediated disease causing neurological dysfunction and disability. However, the mechanism is poorly understood.

We investigated how MS-associated *IL2RA* SNPs affected CD25 expression on T cells *ex vivo* by multiparameter flow cytometry in genotype-selected healthy controls (HC).

The HCs were homozygous carriers for either the risk or protective alleles of *IL2RA* SNPs rs2104286 and rs11256593. Risk allele carriers were paired with protective allele carriers regarding sex and age and pairs were sampled and analyzed jointly.

We observed that risk allele carriers had a higher frequency of $CD25^+$ recent thymic emigrant $CD4^+$ T cells ($p = 0.006$) and a lower frequency of $CD25^+$ $T_{FH}1$ cells ($p = 0.001$) compared to protective allele carriers. Also, we found that risk allele carriers had lower surface expression of CD25 on post-thymic

expanded $CD4^+$ T cells ($CD31^-CD45RA^+$) (p = 0.01), $CD39^+$ T_{Reg} cells (p = 0.02) and on several $CD4^+$ non-follicular memory subsets. In $CD8^+$ T cells, we found no association between CD25 expression and MS-associated *IL2RA* SNPs.

In summary, we found novel effects of MS-associated *IL2RA* SNPs on expression of CD25 on $CD4^+$ T cells. Expression of CD25 is central for T cell responsiveness to IL-2. Thus, risk allele carriers may have differential IL-2 responsiveness that contribute to the immunological alterations in $CD4^+$ T cells observed in MS patients.

H. 22. Pentraxin 3 is useful to differentiate infections from flares in SLE patients with Systemic Inflammatory Response.

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Introduction: Differentiating systemic lupus erythematosus (SLE) activity from infections in febrile patients is difficult because of similar initial clinical presentation. The aim of this study is to evaluate the usefulness of pentraxin 3 (a protein released by a response to primary inflammatory signals, such as TLRs) for differentiating infections from activity in SLE patients admitted with a systemic inflammatory response (SIRS). **Methods:** Patients with SLE and SIRS admitted to the emergency room were included in this study. Measurements of serum Pentraxin 3 were performed by an enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (Abcam-ab21570). Infection was considered present when positive cultures and/or polymerase chain reaction were obtained. Mann-Whitney U test and ROC curves were used to evaluate the pentraxin 3 performance. **Results:** Twenty-four patients were admitted, 19 women (79%), mean age 33,5 ± 13.7 years. Infectious disease was confirmed in 10 cases. Markers for SLE activity including anti-DNA titers by ELISA (p=0.04) and complement were used for differentiating SLE flares from infection. On the contrary, increased pentraxin 3 levels were observed in infected SLE patients (35357 pg/mL, IQR: 12839-145625) compared to flares (median: 8321 pg/mL, IQR: 3678-36696 pg/mL), p=0,03. **Conclusions**: High Pentraxin levels are useful to differentiate infections from activity in SLE patients.

H. 24. Interleukin-34 Provides Neuroprotection in a Mouse Model of Experimental Autoimmune Uveitis

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Interleukin-34 is a novel cytokine produced primarily by neuronal cells that promotes differentiation, survival and proliferation of macrophages, monocytes, and microglia under inflammatory conditions. Increased levels of IL-34 have been reported in patients with various autoimmune diseases and correlate positively with levels of pro-inflammatory cytokines. However, IL-34 has also been shown to induce Foxp3+ T regulatory cells through M2 polarization of monocytes. Since retina contains both neuronal and (macrophage-like) microglial cells, we examined the role of IL-34 in autoimmune uveitis using the mouse model of Experimental Autoimmune Uveitis (EAU). Detectable levels of IL-34 were present in sera of uveitis patients and some healthy controls. In mice, retinal photoreceptor cells and retinal glial Müller cells constitutively expressed IL-34 whereas its receptors, Csf1r and Ptpr-b, were expressed by retinal microglia and by photoreceptors, respectively. Expression of IL-34 in the mouse retina gradually decreased with progression of EAU. Importantly, local overexpression of IL-34 within the eye by adenoviral gene transfer completely protected the neural retina from EAU damage. We conclude that locally produced IL-34 has a role in inhibiting inflammation and enhancing neuroprotection, through still unknown mechanisms. We propose that, during autoimmune uveitis inflammation and/or breakdown of blood-retinal barrier inhibits production and or causes loss of endogenous IL-34. Overexpression of IL-34 by gene transfer restores ocular levels of IL-34, resulting in protection. Our findings suggest that IL-34 could offer a treatment strategy in a wide spectrum of sight-threatening neurodegenerative and inflammatory ocular conditions.

W. 17. Phenotypic and Functional Alterations of Monocytes in Adult-onset Leukoencephalopathy with Axonal Spheroids and Pigmented Glia

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OBJECTIVE: Adult-onset Leukoencephalopathy with axonal Spheroids and Pigmented glia (ALSP) is an adult-onset leukoencephalopathy caused by mutations in colony stimulating factor 1 receptor, which expresses mainly on monocyte lineage cells. Although microglial dysfunction is considered critical in the pathogenesis, little has been reported on peripheral blood monocytes. The objective of this study is to reveal phenotypic and functional changes of monocytes in patients with ALSP. METHODS: Four genetically determined ALSP patients and healthy subjects were recruited. Peripheral blood monocytes were analyzed by flow cytometry. Surface molecules and intracellular cytokine production after lipopolysaccharide stimulation were examined. The phagocytic analysis was performed by using latex

beads coated with fluorescent-labeled immunoglobulin G. RESULTS and DISCUSSION: CD80, CD86, CD62L, CX3CR1, and CCR2 were highly expressed by monocytes from patients with ALSP. Interleukin 10 production by monocytes was relatively reduced in patients while tumor necrosis factor alpha secretion tended to be increased. Upregulated expression of antigen presentation- and migration-related molecules, and the inflammatory shift in cytokine production suggest inadequate activation of ALSP monocytes. Phagocytic impairment was observed in monocytes and probably in microglia, which we assume to be crucial in the ALSP pathology leading to the characteristic pathological features. CONCLUSION: Our results prove phenotypic changes of peripheral blood monocytes in ALSP and probably indicate microglial dysfunction in the brain.

W. 18. Response to Plasmapheresis is Predicted by the Th1 Cell Frequency in the Blood in Multiple Sclerosis

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Multiple sclerosis (MS) is an autoimmune disease of the central nervous system, in which T helper 1 (Th1) cells have a pivotal pathogenic role targeting oligodendrocytes. Although plasmapheresis is effective in some patients with MS, it is still difficult to predict treatment response. Here, we analyzed lymphocytes in the peripheral blood of 21 patients who were treated with immunoadsorption plasmapheresis (IAPP). Twelve patients (57.1%) responded to IAPP with objective improvement in EDSS (Expanded Disability Status Scale). There was no significant difference in clinical parameters such as sex, age, disease duration, disease severity between responders and non-responders. The frequency of Th1 cells (IFN-γ^+ memory $CD4^+$ T cells) in the peripheral blood just before treatment was significantly higher in responders than in non-responders (14.9±8.1% vs 3.9±2.0% (average±SD), $p<0.01$). There was no difference in the frequencies of Th2, Th17, regulatory T, and several B cell subsets. The Th1 cell frequency was not significantly changed by the treatment. In contrast, several key transcripts including *IFNG*, *STAT1*, and *STAT4* in Th1 cells decreased after plasmapheresis (p=0.016, 0.002, 0.013, respectively). This study suggests that the Th1 cell frequency could be used to predict responders to IAPP (Area under the ROC curve (AUC): 0.95). Moreover, the effect of IAPP might be mediated by phenotypic change of Th1 cells in addition to removal of autoreactive antibodies.

W. 19. Synergistic Effect of Vitamin D3-Tolerogenic Dendritic Cells Combined with Interferon-beta in Multiple Sclerosis

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Background:

Our group has developed an autologous antigen-specific therapy based on vitamin-D3 tolerogenic-dendritic cells (VitD3-tolDC) for MS patients (ClinicalTrials.gov: NCT02903537). A combined treatment of tolDC with an immunomodulatory drug, such as IFNβ, might have a synergistic effect.

Objective:

To evaluate the effect of combined VitD3-tolDC and IFNβ treatment in the EAE model and, *in vitro*, in peripheral blood lymphocytes from MS patients.

Methods:

C57BL/6-EAE induced mice were treated daily from day 10 to 17 post-immunization (pi) with IFNβ (5,000 IU) and/or on day 13, 17 and 21pi with VitD3-tolDC-MOG (1·10^6 cells) (n=7 mice/group) and monitored for 34 days.

Human monocyte derived VitD3-tolDC were generated from healthy donors and MS patients (n=6/group) and co-cultured with allogenic PBMC with/without IFNβ. T-cell phenotype and proliferation were analysed after 4 days.

Results:

Treatment of EAE mice with combined therapy ameliorated the disease course compared to each monotherapy (mean score day 34pi: Sham (PBS)= 3.71±1.60; IFNβ= 3.29±0.49; VitD3-tolDC-MOG= 2.79±0.81; VitD3-tolDC-MOG+IFNβ= 2.29±1.29). In addition, VitD3-tolDC-MOG and VitD3-tolDC-MOG+IFNβ treatments reduced MOG-reactivity ($p<0.01$ and $p<0.05$, respectively). No differences in the percentage of Treg were found.

Allogenic proliferation of human T cells was reduced in VitD3-tolDC+IFNβ compared to VitD3-tolDC co-cultures (HD: $p<0.05$; MS: $p=0.09$). VitD3-tolDC treatments reduced the percentage of activated CD4+ and CD8+ T cells compared to control mature DC ($p<0.05$, both group). Interestingly, VitD3-tolDC+IFNβ treatment decreased the percentage of Th17 and increased Th2 lymphocytes compared to VitD3-tolDC ($p<0.05$ in all comparisons).

Conclusions:

Combined therapy of antigen-specific VitD3-tolDC and IFNβ may be a promising strategy for MS patients.

W. 20. Systems immunology using Mass Cytometry identified functional rewiring of the architecture of the Immunome in refractory epilepsy

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Drug refractory epilepsy (RE) is a chronic neurological disease with varied etiology that represents a group of patients whose seizures do not respond to anti-epileptic drugs. In children, refractory epilepsy is often associated with significant disability and even mortality. The immune system may have a role in seizure and epilepsy development, but the specific mechanisms of inflammation that lead to epileptogenesis and contribute to RE are unknown. Autoimmune encephalopathy (AIE), a group of acquired, often antibody-associated central nervous system inflammatory disorders, can certainly present with frequent seizures in affected children, but there is little if any data regarding such mechanisms specifically in pediatric RE . Here, we used high dimensional mass cytometry to comprehensively study the immune system of pediatric patients with RE and compared their immune profile and function with patients with age-matched autoimmune encephalitis (AIE) and healthy controls. Patients with RE and AIE displayed similar immune profiles overall, with changes in CD4+ and CD8+ T-cell subsets and an unbalance toward pro-inflammatory IL-17 production. In addition, patients with RE uniquely showed an altered balance in natural killer cell subsets. A systems level intercellular network analysis identified rewiring of the immune system leading to loss of inhibitory/regulatory intercellular connections and emergence of pro-inflammatory pathogenic functions in neuro-inflammatory immune-cell networks in patients with AIE and RE. These data underscore the contribution of systemic inflammation to the pathogenesis of seizures and epileptogenesis and have direct translational implications in advancing diagnostics and therapeutics design

W. 21. The modulation IL-17/IL-10 balance in experimental autoimmune encephalomyelitis through thyroid hormone receptor beta signaling

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Thyroid hormone-thyroid hormone receptor beta (TRβ) signaing is widely accepted as an orchestrator of lipid metabolism in our body, however its role in immune system is rarely known. Here we report a novel insight of TRβ signaling in immune system. *Thrb* gene is highly expressed in CNS-infiltrating CD4 T cells during the development of experimental encephalomyelitis (EAE). Silencing of TRβ ameliorated the severity of EAE with the reduction of the transcriptional levels of IL-17 in encephalitogenic CD4 T cells. On the other hand, the administration of sobetirome, the selective TRβ agonist, exacerbated the disease course of EAE with the reduction of IL-10 in encephalitogenic CD4 T cells and promoted *in vitro* Th17 differentiation of naïve CD4 T cells in the presence of IL-1β. Intracellular staining of TRβ revealed the

expression of TRβ in Th17 cells and the silencing of TRβ reduced the IL-17 production in vitro, indicating a T cell-intrinsic role of TRβ. In addition, sobetirome increased glycolytic and lipogenic genes of hypoxia-inducible factor-1α (HIF-1α) and acetyl-CoA carboxylase-1 (ACC-1) during Th17 differentiation. And a chemical inhibition of *de novo* lipogenesis completely restrained Th17 skewing via sobetirome, suggesting the possible involvement of *de novo* lipogenesis through TRβ signaling. Finally, TGF-β1-induced IL-10 productions in naïve CD4 T cells were severely reduced in the presence of sobetirome. Taken together, we propose a novel mechanism for modulation of IL-17/IL10 balance via TRβ signaling as an example of active cross-talk between immune and endocrine systems.

W. 22. TIGIT signaling restores suppressor function of Th1-Tregs

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Th1 regulatory T cells (Th1-Tregs) are characterized by the acquisition of pro-inflammatory cytokine secretion and reduced suppressor activity. Th1-Tregs are found at increased frequency in autoimmune diseases including type 1 diabetes and multiple sclerosis (MS). We have previously reported that *in vitro* stimulation with IL-12 recapitulates the functional and molecular features of MS-associated Th1-Tregs, revealing a central role for hyper-activation of the Akt pathway in their induction. TIGIT is a newly identified co-inhibitory receptor which marks Tregs that specifically control Th1 and Th17 responses. Here we report that signaling through TIGIT counteracts the action of IL-12 in inducing the Th1 program. Specifically, TIGIT signaling represses production of IFNg, T-bet expression and restores suppressor function in Tregs treated with IL-12. FoxO1 functional inhibition abolishes the protective effect of TIGIT, indicating that TIGIT signaling promotes FoxO1 nuclear localization. Consistent with this observation, signaling through TIGIT leads to a rapid suppression of Akt function and FoxO1 phosphorylation. Finally, TIGIT stimulation reduces the production of IFNg and corrects the suppressor defect of Tregs from patients with MS. Our results indicate an important role for TIGIT in controlling the functional stability of Tregs through repression of Akt, suggesting that the TIGIT pathway could be targeted for immune-modulatory therapies in human autoimmune disorders.

W. 23. TMEM119-tdTomato reporter mice: a new tool to study microglia in MS and other neurodegenerative diseases

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Microglia, the resident immune cells in the central nervous system (CNS), play a critical role in the pathogenesis of neurodegenerative diseases including multiple sclerosis (MS). Although microglia are thought to contribute to the inflammatory component of neurodegeneration and axonal loss in MS, the relationship between MS progression and inflammation in the brain remains ambiguous because microglia and monocyte-derived macrophages are difficult to distinguish from one another in an inflamed brain. Transmembrane protein 119 (TMEM119) is a microglia-specific marker, and because it

has no reported expression in macrophages or other immune cells, can be useful for discriminating resident microglia from any infiltrating macrophages in inflamed CNS tissues. Here, we characterized a new reporter mouse model, which contains a tdTomato reporter transgene linked to TMEM119 (knockin). By tracking the fluorescence of tdTomato, we can track and isolate the TMEM119-expressed microglia. Our immunostaining in brain sections showed that all tdTomato+ cells express Iba1 in both neurocortex and hippocampus, while a small amount of Iba1+ cells did not express tdTomato, particularly in the hippocampus. Using two-photon microscope in experimental autoimmune encephalomyelitis (EAE) mice, we found that microglia differentiate into an activated phenotype revealed by a rounded shape and are recruited to inflammatory sites in the spinal cord, and form synapses with adoptively transferred regulatory CD4+Foxp3/GFP+ T cells (Tregs). Overall, our model is a valuable tool to study the function of microglia compared to infiltrating macrophages and their crosstalk with pro-inflammatory and regulatory T cells.

W. 24. Topical immunomodulation for the treatment of experimental autoimmune encephalomyelitis (EAE)

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Multiple sclerosis (MS) is an autoimmune disease characterized by T cell-mediated destruction of myelinated axon sheaths in the central nervous system. Topical application of the vitamin D analogue, calcipotriol, can abrogate skin hypersensitivity responses in a T cell-dependent manner. We aim to test whether topical calcipotriol or tretinoin (vitamin A), a similarly immunomodulatory compound, can modulate the disease course of experimental autoimmune encephalitis (EAE), a mouse model of MS. We hypothesize that topical treatment with calcipotriol or tretinoin reduces EAE disease severity by reducing the generation of encephalitogenic T cells and increasing the number of regulatory T cells. Mice were pre-treated with tretinoin, calcipotriol, or vehicle for 2 days once daily before induction of EAE. Mice were monitored for up to 28 days, scored and weighed daily. Lymph nodes and spleens were harvested for flow cytometric analysis of T cell subsets and cytokine expression, and spinal cord sections were collected for histological study. Topical pre-treatment with tretinoin, but not calcipotriol, reduced the severity of EAE as reflected by lower clinical disease scores and sustained maintenance of body weight. Immune cell phenotype analysis showed reduced expression of interferon-gamma in CD4 and CD8 T cell compartments from lymphoid organs of animals pre-treated with tretinoin. The nature of topical immunomodulation as a non-invasive therapeutic intervention makes it an appealing treatment option. Further studies are warranted to verify the efficacy and mechanisms of topical retinoids on modulation of systemic immunity.

W. 25. Trajectories of effector T cell (Teff) and regulatory T cells (Treg) imbalance in pediatric multiple sclerosis

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We previously demonstrated in cross-sectional analysis that, on average, children with MS exhibited increased frequencies and pro-inflammatory responses of particular Teff subsets, and diminished Treg function such that the Teff/Treg balance was dysregulated relative to children with other (monophasic) acquired demyelinating syndromes (monoAS) and healthy controls. Whether this Teff/Treg imbalance is a stable feature, or fluctuates over time in individual children is unknown. **We serially measured** frequencies of the implicated $CD4^+CCR2^+CCR5^+$ and $CD8^+CD161^{high}TCR\text{-}V\alpha7.2^+$ MAIT Teff subsets, and the ratios of Teff to Treg ($CD4^+CD25^{hi}CD127^{low}FOXP3^+$) cells, in prospectively followed children with MS and controls. Children with MS (n=14) harbored increased frequencies of the two Teff subsets (p=0.021, and p=0.008), yet deficient Treg suppressive capacity (p=0.041), including diminished capacity to suppress the implicated Teff (p=0.01), compared to children with monoADS (n=7). In turn, the implicated Teff of MS patients were relatively resistant to suppression by normal Tregs (p=0.008). An abnormal Teff/Treg ratio at the individual level best distinguished MS children from controls (p<0.001). In a subset of serially studied children (n=10), Teff/Treg ratios of the individual children were relatively stable over a 3-5 period, in both MS and controls. Overall, we find that higher ratios persist in MS patients over time whether during or between relapses. Although further analyses are ongoing, we posit that the elevated Teff/Treg ratios in children with MS may reflect intrinsic abnormalities in their immune response propensities as opposed to transient abnormalities associated with emergence of disease relapse.

W. 26. Treatment with ILT3.Fc of C57BL/6 mice delays the onset and attenuates the evolution of MOG35-55-induced-EAE

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Immunoglobulin Like Transcript 3 (ILT3) also called LILRB4, LIR-5, CD85k, a member of the leukocyte immunoglobulin like receptors, is a marker of human tolerogenic dendritic cells which induce anergy in T helper cells and elicit the differentiation of CD8 T suppressor cells. The recombinant ILT3.Fc protein engineered from the extracellular Ig like domains of ILT3 binds to its ligand CD166/ALCAM suppressing human T cell effector function both *in vitro* and *in vivo*. We found that ILT3.Fc also binds to the mouse $CD166^+$ immune cells. Therefore, we explored the immunotherapeutic potency of ILT3.Fc in the classical model of MOG_{35-55} peptide-induced-EAE in C57BL/6 mice. Treatment was initiated at the time of immunization or 5 days thereafter. A significant delay of disease onset, progression to partial or complete paralysis and increased survival was achieved in mice treated with ILT3.Fc. T cells from control mice proliferated strongly and produced large amounts of IFN-γ and IL-17A in response to MOG_{35-55} peptide while ILT3.Fc treated mice showed significantly lower responses. Flow cytometry and PCR studies indicated that IL-4, TNF, IL-6, IL-10 and IL-2 were also inhibited in cells from spinal cord, lymph nodes or spleen of ILT3.Fc treated mice. Neuropathological studies showed a reduction in inflammatory infiltrates and demyelinated areas in brains and spinal cords of treated mice. ILT3.Fc induced $CD8^+$ mouse T suppressor cells and anergy in $CD4^+$ T helper cells when added to in vitro activated cultures, suggesting its potential immunotherapeutic value.

W. 27. Foxp3+ regulatory T cells use heparanase to access IL-2 bound to extracellular matrix at sites of autoimmune inflammation

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Foxp3+ regulatory T cells (Treg) depend on exogenous IL-2 for function and homeostasis. However, circulating levels of IL-2 are generally low, making it unclear how Treg access this resource in vivo. We report that IL-2 is sequestered by heparan sulfate (HS) moieties within inflammatory lesions in the experimental autoimmune encephalomyelitis model of multiple sclerosis. HS-bound IL-2 supports Treg homeostasis more potently than soluble IL-2, but accessing this sequestered IL-2 requires heparanase (HPSE). Both murine and human Treg express HPSE more abundantly than conventional CD4+ T cells. Accordingly, Hpse-/- Treg are compromised in their ability to utilize HS-bound IL-2 and display impaired homeostasis and suppressive function in vitro and in vivo. Moreover, CAR-Treg engineered to express HPSE show enhanced phenotypic stability in vitro and in vivo compared to conventional CAR-Treg. These data indicate that HPSE and HS-bound IL-2 contribute to Foxp3+ Treg homeostasis and function and provide new avenues for improving Treg-based cell therapy of autoimmunity.

Autoimmune Rheumatologic Diseases

H. 25. Compartmentalization, persistence and conserved motifs of dominant (regulatory) T cell clones in autoimmune inflammation

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In autoimmune diseases, inflammation is often limited to specific target tissues, but within tissues, multiple sites can be affected. An important outstanding question is whether affected sites are infiltrated with the same (pathogenic) T cell clones and whether these clones persist over time. In Juvenile Idiopathic Arthitis it is possible to analyze large number of cells derived from the site of inflammation, i.e. inflamed joints. Here, we performed CyToF analysis and T cell receptor (TCR) sequencing to study immune cell composition and (hyper)expansion of inflamed joint-derived T cells. Samples were taken from different joints affected at the same time, and joints that were affected multiple times during the relapsing remitting course of the disease. CyTOF analyses revealed that the composition and functional characteristics of the immune infiltrates are strikingly similar between joints within one patient.

Furthermore we observed a strong overlap between dominant T cell clones, especially Treg, in inflamed joints affected at the same time. Some of the most dominant clones could also be detected in circulation. Dominant Treg and Teff cell clones were found to persist over time and to expand during relapses, even after full remission of the disease. Finally, despite having little overlap between patients for the exact TCR sequence, we found several shared immune fingerprints, based on sequence motifs.. These data suggest that in autoimmune disease there is (dominant) auto-antigen driven expansion of both Teff and Treg clones that are highly persistent and (re)circulating. Therefore these dominant clones can be interesting therapeutic targets.

H. 26. PPP2R2B Facilitates the Persistence of Chronic Inflammation through an Epigenetic Mechanism

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PPP2R2B encodes B55β, a regulatory subunit of the phosphatase PP2A that controls apoptosis of activated T cells. Impaired expression of B55β in patients with systemic lupus erythematosus (SLE) is associated with T cell resistance to cytokine withdrawal-induced death (CWID).

We analyzed the transcriptional regulation of *PPP2R2B* in T cells from patients with systemic autoimmune diseases (AID: SLE, rheumatoid arthritis [RA], Sjögren's syndrome, *n*=86) and healthy donors (HD, n=25), to identify hereditary and acquired factors that could decrease its expression. Failed transcription of B55β was not confined to SLE and was documented in ~50% of patients. This was not associated with expansion or contraction of a CAG repeat that has been linked to altered B55β expression in patients with hereditary neurodegenerative diseases. Methylation-sensitive PCR indicated that a CpG island in the promoter of *PPP2R2B* was hypermethylated in patients. Pyrosequencing identified the cytosines whose methylation affected *PPP2R2B* transcription.

To determine whether *PPP2R2B* hypermethylation could be acquired during chronic inflammation, we analyzed the effects of cytokines on *PPP2R2B* methylation and transcription, and on T cell CWID. Healthy T cells exposed to TNF-α became resistant to CWID. This was linked to increased *PPP2R2B* methylation and decreased B55β expression. *PPP2R2B* methylation correlated with erythrocyte sedimentation rate (r=0.76, *P*=0.01). Moreover, disease activity (DAS28) was significantly higher in patients with RA who displayed *PPP2R2B* hypermethylation (*P*<0.0001).

These results identify a gene whose expression is affected by inflammation through an epigenetic mechanism. By decreasing the expression of B55β, inflammation may perpetuate itself.

H. 27. R848 (Resiquimod), a TLR 7/8 Agonist, accelerates disease and causes a fatal myeloproliferative disorder in NZM 2410 lupus mice

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Spontaneous murine models of lupus-like disease are used to study the pathogenesis of SLE, but require extended observation. Some accelerants, (i.e. Type I IFN) are used to trigger earlier disease onset. We used a TLR 7/8 agonist, previously reported to induce lupus-like disease in WT mice within weeks, as an accelerant in lupus prone mice. C57BL/6J mice (n=29) and pre-disease NZM2410 (n=31) were treated with topical R848 for 8 wks. Compared to vehicle-treated mice, R848-treated B6 and NZM mice had profoundly enlarged spleens, and survival was significantly reduced ($p<0.009$ and $p<0.001$). Treated B6 mice trended towards a higher ANA ($p=0.059$), but not anti-dsDNA, while treated NZM mice had higher levels of ANA ($p=0.07$) and dsDNA ($p=0.004$). Albuminuria and renal pathology in treated NZM mice indicated acceleration of nephritis, but not sufficient to cause death. Treated mice had significantly reduced splenic B cells (4% vs. 40%) and T cells (8% vs. 31%) compared with vehicle. CD11b+ cells were significantly expanded (66% vs. 45%) in BM from treated mice. Spleen IHC and histopathology revealed a massive expansion of F4/80+ cells, extramedullary hematopoiesis and changes consistent with histiocytic sarcoma. In summary, topical TLR7/8 agonist treatment induced mild autoimmunity in B6 mice and accelerated autoimmunity in NZM2410 mice. Both had a severe immunophenotype and early death most consistent with malignant histiocytosis. Care should be taken in using TLR7/8 as a disease accelerant in NZM2410 mice as data suggest that death is accelerated by myeloproliferative disease rather than nephritis.

H. 28. Salivary IgA as biomarker of disease activity in Systemic Lupus Erythematosus.

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Immunoglobulin A (IgA) is the main antibody isotype present in the body fluids such as tears, intestinal mucus, colostrum, and saliva. There are two subtypes of IgA in humans: IgA1 mainly present in blood and IgA2 preferentially expressed in mucosal sites. In clinical practice, immunoglobulins are typically measured in venous or capillary blood; however, alternative samples including saliva are now being considered given its non-invasive and easy collection nature.
Since IgA deficiency could be frequently detected in patients with autoimmune diseases, we decided to evaluate the levels of both IgA subtypes in serum and saliva of systemic lupus erythematosus (SLE)

patients. Specific IgA1 and IgA2 levels were measured by a light chain capture-based ELISA in a cohort of 32 patients with SLE that were compared with antibody levels of healthy volunteers.
Surprisingly, our results indicated that in the saliva of SLE patients, both IgA subtypes were significantly elevated; however, serum IgA1 levels were decreased when compared with control subjects.
Interestingly, we also found that salivary IgA levels, most specifically IgA1, positively correlate with the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) values as well with the amount of serum anti-nucleosomes and anti-dsDNA IgG autoantibodies. Strikingly, we also were able to detect the presence of salivary anti-nucleosome IgA antibodies in SLE patients.
According to our results, IgA characterization in saliva could be used as a pre-diagnostic or follow-up clinical tool in SLE with easier, faster and safer handling than blood samples.

Supported by CONACyT 240314 and UNAM-DGAPA-PAPIIT IA202318.

H. 29. Serine Arginine-rich Splicing Factor 1 (SRSF1) controls T Lymphocyte Homeostasis through the Control of Anti-apoptotic Bcl-xL in Patients with SLE

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Lymphopenia is one of the most common clinical features in patients with systemic lupus erythematosus (SLE), and associates with severe disease and comorbidities, yet the underlying mechanisms remain unclear. By discovery approaches we previously identified the serine arginine-rich splicing factor 1 (SRSF1) binding to the 3`UTR of CD3 zeta in human T cells, and showed the role of SRSF1 in T cell function. We showed that SRSF1 levels are decreased in T cells from patients with SLE, and associate with severe disease. Because SRSF1 is an essential pro-survival factor and controls the expression of Bcl-2-related anti-apoptotic genes, we hypothesized that SRSF1 controls T cell homeostasis and its deficiency may contribute to lymphopenia. To this end, we generated T cell-conditional *Srsf1*-deficient mice. We observed a peripheral T cell lymphopenia in these mice, with increased apoptosis of T cells ex vivo, and after Fas crosslinking. Transcriptomics profiling of effector CD4 T cells from Srsf1-cko mice revealed aberrant expression of apoptosis-related genes. We confirmed the decreased expression of the anti-apoptotic long (L) isoform of Bcl-x (Bcl-xL) in T cells from *Srsf1*-deficient mice. Of clinical relevance is our finding that lower expression of SRSF1 correlated with low levels of Bcl-xL in T cells and with lymphopenia in SLE patients. Importantly, overexpression of Srsf1 in T cells from patients with SLE, improved cell survival. These results suggest that SRSF1 controls T cell homeostasis via Bcl-xL in patients with SLE, and reduced SRSF1 expression is an underlying molecular defect which contributes to lymphopenia in SLE.

H. 30. Sulforaphane Inhibits Inflammatory Responses of Primary Human T-cells by increasing ROS and depleting Glutathione

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The activity and function of T-cells are influenced by the intra- and extracellular redox milieu. Oxidative stress induces hypo-responsiveness of untransformed T-cells. Vice versa increased glutathione (GSH) levels or decreased levels of reactive oxygen species (ROS) prime T-cell metabolism for inflammation, e.g. in rheumatoid arthritis (RA). Therefore, balancing the T-cell redox milieu may represent a promising new option for therapeutic immune modulation. Here we show that sulforaphane (SFN), a compound derived from plants of the Brassicaceae family, e.g. broccoli, induces a pro-oxidative state in untransformed human T-cells of healthy donors or RA patients. This manifested as an increase of intracellular ROS and a marked decrease of GSH. Consistently, increased global cysteine sulfenylation was detected. Importantly, a major target for SFN-mediated protein oxidation was STAT3, a transcription factor involved in the regulation of T_H17-related genes. Accordingly, SFN significantly inhibited the activation of untransformed human T-cells derived from healthy donors or RA patients, and specifically downregulated the expression of the T_H17-related cytokines IL-17A, IL-17F, and IL-22, which play a major role within the pathophysiology of many chronic inflammatory/autoimmune diseases. The inhibitory effects of SFN could be abolished by the GSH replenishing antioxidant N-acetyl-cysteine (NAC).

Together, our study provides mechanistic insights into the mode of action of the natural substance sulforaphane. It specifically exerts T_H17 prone immunosuppressive effects on untransformed human T-cells by decreasing GSH and accumulation of ROS. Thus, SFN may offer novel clinical options for the treatment of T_H17 related chronic inflammatory/autoimmune diseases such as rheumatoid arthritis.

H. 31. Suppression of MyD88 in B cells after Disease Onset Improves Outcomes in Murine Lupus

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Systemic lupus erythematosus (SLE) is a complex autoimmune disease defined by immune dysregulation. MyD88 is a central immune adaptor protein that regulates disease pathogenesis in SLE. Previously, we showed that global and B cell-specific MyD88-deficient mice exhibited ameliorated disease, including reduced organ damage and suppressed autoantibody formation. The role of MyD88 in B cells during disease initiation compared to disease perpetuation is still unclear.

Lupus prone (MRL.*Fas*^{lpr}) mice develop autoantibodies, proteinuria, dermatitis, and glomerulonephritis, with disease onset occurring between 9-11 weeks of age. In order to study the therapeutic potential of MyD88 suppression, we used hCD20-Tam Cre mice crossed to Myd88^{floxed} mice in which B cell-specific depletion of MyD88 is induced by tamoxifen. Tamoxifen was orally administered biweekly starting after disease onset (12 weeks of age). Deletion of MyD88 was confirmed functionally and via qPCR.

Most notably, mice with induced B cell-specific deletion of MyD88 (Tam,B-MyD88$^{fl/fl}$) exhibited a significant survival advantage over control mice ($p< 0.05$) and reduced kidney histologic disease, including both glomerulonephritis ($p< 0.01$) and interstitial inflammation ($p< 0.01$). Additionally, Tam,B-MyD88$^{fl/fl}$ mice had reduced autoantibody formation as assessed by ANA immunofluorescence and ELISA.

These experiments suggest that there is a continued role for MyD88 signaling in B cells throughout the course of disease in MRL.*Fas*^{lpr} lupus prone mice, rather than simply disease initiation. Numerous genetic deletions have resulted in suppressed disease onset in lupus models, but herein, we observed disease amelioration after disease onset. This portends that targeting MyD88 or its upstream activators may be a viable therapeutic option in SLE.

H. 32. Detection of Extracellular Vesicles from Patients with Systemic Lupus Erythematous Serves as a Novel Therapeutic

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Introduction
Systemic lupus erythematosus is an autoimmune disease characterized by chronic inflammation. We have previously shown that TLR7 and TLR8 are significantly upregulated in PBMCs of Lupus patients. Recent studies have discovered that miR-21, mir-29a, and miR-29b packaged and secreted in extracellular vesicles (EVs) can also bind to these receptors.
Methods
Lupus patients meeting revised ACR guidelines and healthy controls provided informed consent to participate in this IRB-approved study. Plasma-derived EVs were isolated by differential ultracentrifugation and validated by Nanosight and ELISA. A novel human-mouse chimeric model of Lupus was created by adoptively transferring Lupus PBMCs into immunodeficient mouse recipients. Prior to transfer, PBMCs were incubated with synthetic liposomal EVs containing miR antagonists to miR-21, mir-29a, and miR-29b, or a control. After 21 days, PBMCs were collected for immunophenotyping and ELISA.
Results
There was an upregulation of EVs found in the plasma of Lupus patients compared to healthy controls. RNAseq data resulted in a collection of statistically significant small RNA reads, such as miR-142-3p and let-7b-5p. Human CD4+, CD8+, B-cells, monocytes, and NK cells were successfully recovered from whole blood of chimeric mice at similar levels, but levels of human IL-2, IL-6, IL-10, and TNF-α were reduced with miR inhibition. Moreover, miR inhibition significantly reduced histopathology in the small intestine, liver, and kidney, demonstrated by H&E and human CD3 immunohistochemistry.
Conclusion
Our data shows elevated levels of EVs in Lupus patients and reveals unique EV-derived small RNA signatures that may be targeted therapeutically or used as diagnostic biomarkers for Lupus.

H. 33. Serine Arginine-rich Splicing Factor 1 (SRSF1) restrains effector T cell responses via the PTEN/mTORC1 pathway in systemic lupus erythematosus

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T cells from patients with systemic lupus erythematosus (SLE) exhibit a hyperactive phenotype with aberrant cytokine production. By discovery approaches we previously identified the serine arginine-rich splicing factor 1 (SRSF1) to bind to the CD3 zeta mRNA in human T cells. We showed that SRSF1 levels are decreased in T cells upon stimulation. Importantly SRSF1 expression levels are decreased in T cells from patients with SLE, and associate with worse disease. To define the role of SRSF1 in T cell physiology and in autoimmune disease, we generated *Srsf1*-T cell-conditional knockout (*Srsf1*-cko) mice. These mice develop systemic autoimmune disease with elevated serum autoantibodies and lupus nephritis. CD4 T cells from Srsf1-cko mice exhibit an activated/effector phenotype with increased frequencies of aberrant (IL-17, IFN-γ, IL-4) cytokine producing cells upon e*x vivo*stimulation and an elevated T cell activation gene signature. Mechanistically, we found increased activity of the mechanistic target of rapamycin complex 1 (mTORC1) pathway and decreased expression of its inhibitor PTEN. Rapamycin suppressed proinflammatory cytokine production from Srsf1-deficient T cells. Of direct clinical relevance is our finding that in T cells from SLE patients,the expression levels of PTEN were decreased along with SRSF1, and the overexpression of Srsf1 rescued PTEN, and suppressed mTORC1 activity and proinflammatory cytokine production. Our results reveal that SRSF1 is a novel negative regulator of T cell activation and its deficiency in T cells from SLE patients contributes to the pathogenesis of disease.

H. 35. Estrogen induction of Interstital Lung Disease is mediated through PD-1+Th17 cell-dependent and -independent signaling pathways

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Background: Prior reports noted the profibrotic properties of the PD-1 pathway signaling in patients with autoimmune interstitial lung disease, such as pulmonary sarcoidosis and rheumatic lung disease. Both are notable for female predominance of disease, invoking the notion of estrogen- mediated pulmonary fibrosis.

Methods: We assessed for baseline distinctions in sarcoidosis patients with pulmonary progression by gender. Concurrently with these analyses, we used the bleomycin-induced pulmonary fibrosis in C57BL/6 mice and assessed for distinctions in pulmonary fibrosis and pSTAT3 signaling, according to gender.

Results: We noted significantly higher percentages of systemic PD-1+CD4+ T cells in the sarcoidosis females, compared to males. This gender distinction was absent in healthy control subjects. Pulmonary-derived PD-1+CD4+ T cells were also distinct by gender in C57BL/6 mice following bleomycin administration. Bleomycin administration to PD-1 null mice revealed significantly less pulmonary fibrosis among female mice, compared to males. Administration of anti-PD-L1 antibody following bleomycin administration also revealed significantly less weight loss and pulmonary fibrosis among the females, compared to males. Investigation for relevant mechanisms demonstrated distinctions in phospho-STAT3

expression in females compared to males. In addition, isolated naïve CD4+ T cells from wild-type and estrogen receptor-α deficient (ESR1-/-) mice revealed significant reductions in IL-17A production and IL-23 receptor expression following anti-CD3/anti-CD28 stimulation. Disparate IL-23R expression by gender was noted in PD-1 null mice following bleomycin administration.

Conclusion: Estrogen induction of profibrotic IL-17A production involves mechanisms dependent and independent of PD-1 pathway signaling.

T. 1. The Incidence of Cancer in Giant Cell Arteritis: A Single Center Study

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Objective: Giant cell arteritis (GCA) is a large vessel vasculitis with persistent interferon-gamma and interleukin-12 elevation seen in temporal artery biopsies (TAB) despite glucocorticoids. These cytokines can exert anti-tumor attributes that can help protect against cancer. We evaluated the incidence of cancer in TAB-proven GCA versus non-GCA controls.
Methods: Using the Penn State electronic/pathology database from 2006-2017, we reviewed records of 100 biopsy-proven-GCA and 100 biopsy-negative non-GCA subjects (ensuring the absence of clinical GCA /other autoimmune rheumatic diseases in this control) and compared the incidence and distribution of cancer between these groups<.br />Results: GCA subjects were older than non-GCA (76.6 ± 7.2 years vs. 69 ± 10.5 years ;p< 0.001). Majority of subjects were women with no difference between the groups. Cancer was found in 8/40 (20%) GCA compared to 18/40 (45%) non-GCA (p=0.03). The median time interval from TAB to incident cancer was not significant between the 2 groups (42.5 vs 22 months; p=0.80). The GCA group had basal cell (5/8) , squamous cell (2/8) cancer and 1 fatality from lymphoblastic leukemia while the non-GCA had basal cell (1/18), squamous cell (4/18) and melanoma (2/18), breast (2/18), hematologic (2/18),1 each of prostate, lung ,colon cancer with 3 deaths from metastatic (2/18) and parotid cancer (1/18)
Conclusion: Compared to controls, GCA subjects have a lower incidence of malignancy and may have a potential anti-tumor immune response program that deserves further study

T. 2. A Novel NLRC4 Mutation Leads to Elevated IL-18 without Increased Caspase-1 Activation in a Lupus Patient with Macrophage Activation Syndrome

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Inflammasomes are complex cytoplasmic structures that become activated in the presence of bacterial ligands or cellular damage. Inflammasome activation leads to massive amplification of pro-inflammatory signals, and so is tightly regulated. Formation of the NLRC4 inflammasome leads to incorporation and activation of procaspase-1 and results in the release of IL-1 and IL-18. Human gain-of-function (GOF) mutations in *NLRC4* impair its autoinhibition and lead to inappropriate inflammasome activation and increased levels of IL-18. A spectrum of human autoinflammatory disease is associated

with *NLRC4* mutations ranging from mild cutaneous involvement to fatal neonatal entercolitis with macrophage activation syndrome (MAS). Autoimmunity has not been reported in NLCR4-inflammasomopathy. We present a unique patient with systemic lupus erythematosus and a novel NLRC4 variant (p.I287T) who displayed highly elevated levels of serum IL-18. Using a bimolecular fluorescence complementation (BiFC) assay, we show that while previously published NLRC4 mutations (p.V341A, p.T337S and p.S171F) lead to enhanced activation of caspase-1 compared to wild-type (WT) NLRC4, p.I287T activation of caspase-1 was significantly less than WT. Neither p.I287T nor known NLRC4 GOF mutations lead to increased activation of other inflammatory caspases (-4 and -5). Co-immunoprecipitation studies using WT NLRC4 or known NLRC4 GOF variants revealed protein complex formation with caspase-1, but p.I287T did not immunoprecipitate with caspase-1. We describe a phenotypic expansion of the clinical features associated with *NLRC4* mutations to include autoimmunity. We further show p.I287T led to elevated IL-18 without enhanced activation of caspase-1. Further investigations to determine how p.I287T leads to elevated IL-18 are ongoing.

T. 3. ANCA Associated Vasculitis Following Related Bone Marrow Transplantation

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A 16 year old male who underwent a donor-related (brother) hematopoietic stem cell transplant for aplastic anemia due to paroxysmal nocturnal hemoglobinuria > 1 year prior was transferred to our children's hospital for new-onset fever, chills, cough, dyspnea on exertion and hemoptysis. Prior treatment for community acquired pneumonia was not effective. Initial laboratory analysis revealed a normal complete blood count and differential, elevated C-reactive protein (CRP) and elevated procalcitonin. Urinalysis was normal. Imaging revealed bilateral pulmonary infiltrates. Broad-spectrum antibiotics and anti-fungal agents were not effective. Studies to identify viral, bacterial, disseminated fungal, and mycobacterial infections were negative. Patient continued to deteriorate clinically. High dose steroid therapy was initiated. The patient rapidly improved on the second day of steroid administration. Due to positive response to steroids, rheumatologic studies were performed. Anti-myeloperoxidase antibody was positive at high titer while anti-proteinase 3, anti-glomerular basement membrane, anti-nuclear antibody and rheumatoid factor were negative. Sinus imaging demonstrated ethmoid, sphenoid and maxillary sinus mucosal thickening and fluid. Upon further history, mother noted a cousin who died of Wegener's disease. Recent chimerism analysis revealed >98% donor engraftment. Animal models of organ specific and systemic autoimmunity are transferable by bone marrow transplantation. We have identified two reports of ANCA associated vasculitis, one following an autologous and the other following allogenic bone marrow transplantation. This and the positive family history leaves open the possibility that susceptibility to ANCA associated vasculitis rests in the hematopoietic stem cell.

T. 4. Autoinflammatory syndrome, secondary to de novo variant in CDC42 (p.C188Y), improved with IL-1b inhibition.

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Monogenic autoinflammatory syndromes (MAIS) are characterized by primary over-activation of the innate immune system. Induction of the inflammasome complex by innate immune sensors and increased production of IL-1b are implicated in its pathogenesis. Macrophage activation syndrome (MAS) is defined by acute hyper-inflammation and unopposed cytokine release. The early therapeutic use of IL-1b inhibition has profoundly improved the prognosis MAS. Significant overlap in clinical presentation and laboratory markers between patients with MAIS and MAS led us to explore the role of free IL-18 and therapeutic use of IL-1b inhibition in a patient with MAIS due to CDC42 mutation. We report the case of an 20 months-old female who presented with hydrops fetalis in-utero, and later developed failure-to-thrive, splenomegaly, anemia, thrombocytopenia, rashes, frequent febrile episodes along with massive increase in CRP, ESR and ferritin. Whole Exome Sequencing (WES) identified a heterogenous likely pathogenic de novo variant in cell division control protein 42 homolog (CDC42) c.563G>A (p.C188Y).

Because of significant clinical overlap to MAS, we measured IL-6, IL-18, free IL-18 and IL-18 binding protein, all of which were highly increased. Her IL1-b level was normal, but an increase in IL-1b is hardly ever detectable in the serum despite playing a critical role in this type of inflammation. With this rationale we started the IL-1 receptor antagonist anakinra, with immediate, dramatic clinical improvement.

Significant increase in free IL-18 and extremely encouraging clinical response to therapy with anakinra in a patient with novel CDC42 mutation suggests a mechanistic link between MAS and defects in CDC42.

T. 5. Computational Analysis of Citrulline-Specific CD4+ T Cell Frequency and Phenotype Reveals Differences that are Driven by Antigen Specificity and Disease Characteristics Among Rheumatoid Arthritis Patients

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The presence of anti-citrullinated protein antibodies in rheumatoid arthritis (RA) indicates that an immune response directed toward citrullinated antigens participates in disease development. Using a combinatorial HLA class II tetramer staining approach and applying a new computational algorithm for phenotyping rare cell populations, we characterized cit-specific CD4+ T cells in a cross-sectional cohort of 80 RA subjects and 30 matched healthy control (HC) subjects. We assayed the frequency and phenotype of CD4+ T cells specific for five citrullinated autoantigens expressed in the joint: alpha-enolase, aggrecan, cartilage intermediate layer protein (CILP), fibrinogen and vimentin. While the overall frequency of cit-specific CD4+ T cells was increased in RA subjects compared to HC subjects, antigen-specific differences were observed. Further, antigen-specificity influenced the predominant immunophenotype of cit-specific CD4+ T cells. Cit-aggrecan-specific cells were primarily Th2-like; cit-alpha-enolase-specific cells were principally Th1; and CILP-specific cells were mostly naïve. Lastly, certain cit-specific cell immunophenotypes were significantly associated with disease characteristics. Th17-like cit-aggrecan-specific cells were more frequent in early disease whereas Th1-like cells were abundant in longstanding RA. CILP-specific cells shifted out of the naïve compartment with increased disease duration. Vimentin/fibrinogen-specific stem cell memory cells were more frequent than predicted from the CD4+ landscape and were linked to disease activity. These data reveal the heterogeneity of autoantigen-specific CD4+ T cells across and within individuals and suggest that specific antigens may drive distinct immune responses. Future work may help classify RA subjects based on the specificity and dominant phenotype of their T cell response, leading to more targeted therapies.

T. 6. Cytokine dependence of enthesis-resident lymphocytes in murine spondyloarthritis

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Background: Enthesis-resident CD4-CD8- DN T cells have been implicated in the pathogenesis of spondyloarthritis. Overexpression of IL-23 in adult mice is thought to activate these cells to produce IL-17A and other pathogenic cytokines leading to spondyloarthritis-like disease. Many innate lymphocytes require IL-1 for IL-23-induced production of IL-17A *in vitro*. We therefore investigated the role of IL-1 receptor signals on CD4-CD8- DN T cell homeostasis and induction of murine spondyloarthritis.

Methods: Lymphocytes were isolated from the spleen and Achilles enthesis of WT, Il23r-/-, Il1r1-/- and Il23r-gfp reporter mice (C57BL/6 background) and analyzed *in vitro*. Spondyloarthritis was induced by hydrodynamic injection of IL-23 minicircles in B10.RIII mice. IL-1 signals were blocked *in vivo* using a cocktail of three monoclonal antibodies against IL-1α, IL-1β and IL-1R1.

Results: Enthesial CD4-CD8- DN T cells comprise γδ T cells and DN αβ T cells. Both subsets secreted IL-17A *in vitro* upon stimulation with IL-23 + IL-1β but not IL-23 alone. Cell frequencies were not substantially different in Il23r-/- or Il1r1-/- mice compared with WT mice. Disease induction in Il1r1-/- mice could not be tested as C57BL/6 WT mice did not develop arthritis upon IL-23 minicircle injection. Antibody blockade of IL-1 in susceptible B10.RIII mice had no impact on disease onset but diminished arthritis

severity.

Conclusions: C57BL/6 mice do not develop IL-23 minicircle-induced spondyloarthritis, which diniminshes the utility of this model for pathogenesis studies. Mediators other than IL-1 may provide a second signal for IL-23-induced secretion of IL-17A *in vivo*.

T. 8. Detection of Patients with Systemic Lupus Erythematosus in Electronic Health Records Using Clinical Classification Criteria Based Algorithms

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Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease with diverse manifestations. Electronic health records (EHR) are a rich source of information that can be used to understand the presentation of SLE. We assessed three SLE clinical classification criteria as a foundation for phenotype-based detection of SLE patients in EHR data. Performance was evaluated over 600 medical records from the Northwestern Medicine EHR system, 472 with definite SLE and 128 without, based on chart review. We developed three algorithms, based on the American College of Rheumatology (ACR), Systemic Lupus International Collaborating Clinics (SLICC) and proposed European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) classification criteria using only structured data elements (diagnosis codes and lab results) to determine whether patients met the classification criteria for SLE. The SLE identification rate ranged from 49-73% across the algorithms; all had >=97% specificity and >=99% positive predictive value (PPV). Sensitivity ranged from 49-73% and negative predictive value (NPV) from 43-58%. The SLICC-based algorithm performed best, detecting 73% of patients with SLE, with 99% PPV, 73% sensitivity, 97% specificity and 58% NPV. All 3 algorithms detect a significant proportion of patients with SLE, with high PPV and specificity. Low NPV of the algorithms likely reflects undetected cases of SLE resulting from low detection of clinical and laboratory criteria that are not consistently documented in structured data. The algorithms may improve through use of natural language processing of physician notes for criteria that were difficult to detect using diagnosis codes and labs.

T. 9. Differential expansion of Innate-lymphoid populations in Spondyloarthritis and Crohn's Disease

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Clinical overlap between Spondyloarthritis (SpA) and Crohn's disease (CD) indicates they may share common pathogenic mechanisms originating in the intestinal mucosa. There is minimal data comparing these disease populations to each other and to patients having an overlap of the two conditions (CD-SpA). To uncover underlying immunologic commonalities between the disease states in an unbiased manner, we performed single cell RNA sequencing on viable CD45+ cells isolated from colon biopsies taken from 2 patients each with SpA, CD, and CD-SpA as well as 2 controls. In the SpA and IBD-SpA

patients, we observed an expansion of a TCR-negative population that clustered with T-cells by TSNE. We hypothesized that these cells were ILCs. To validate this initial finding, we expanded our subject cohort to 62 individuals and assayed colon biopsy tissue for ILC expansion by flow cytometry. We observed increased ILC3s in the SpA and CD groups, and increased ILC1s in the CD-SpA group, in contrast to previously reported ILC1 expansion in Crohn's. Additionally, while others have shown that the expanded ILC3 population in AS is primarily Natural-Cytotoxicity-Receptor positive (NCR+), we see the most significant increase in the NCR- population. Finally, the unique expansion of ILC1s in IBD-SpA implies the presence of a distinct disease process in affected individuals. In addition to confirming a role of ILC3 in the pathogenesis of SpA, these data suggest a role of ILC3s in CD and ILC1s in CD-SpA. Additional investigation will be needed to further elucidate the contribution of these populations to disease.

T. 10. Early Rheumatoid Arthritis (eRA) is a Perturbation of the Architecture of the Immunome: a System Biology Approach

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We have created a high dimensionality atlas of the healthy human immunome (EPIC: Extended Poly-dimensional Immunome Characterization) by interrogating PBMC of 200+ healthy subjects with 63 unique mechanistic and phenotypic markers byCytof. EPIC provides a detailed depiction of the architecture of the human healthy Immunome. We tested here the hypothesis that eRA as a whole pathological process can be studied as a perturbation of the healthy immunome, thus allowing the identification of pathways and cell subsets, and their overall organization, in a way that is mechanistically and clinically poignant.

We tested eRA patients at diagnosis, with active disease and before establishing therapy, using the EPIC panels and analysis tools and compared the resulting Immunome architecture against the healthy EPIC atlas. Dimensionality reduction and clustering were used to group the cells into subsets. Correlation-based Immune cell networks were created to depict and dissect the immunome of both groups<./p>

System level analysis of immune cell network showed a decrease in regulatory cellular networks in eRA. Also, the eRA immune cell network showed higher modularity and centralization when compared to healthy network, suggesting the emergence of specific functions dominated by few subsets, mainly of inflammatory, experienced adaptive nature, all related to each other. This approach enables the identification of relevant individual immune subsets and underscores the functional relations of all subsets organised within an architecture which profoundly differs from normal. This knowledge can be exploited to understand pathogenesis and also understand and predict effects of disease activity or therapy.

T. 11. Effects of the Malondialdehyde-Acetaldehyde Adduct (MAA) on Biological Responses to Citrullinated Proteins

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Background/Purpose. Malondialdehyde-acetaldehyde adducts (MAA) are products of oxidative stress that modify self-proteins and stimulate potent cellular and humoral immune responses. However, mechanism(s) by which citrullinated (Cit) proteins/peptides initiate immune responses are not clear. The purpose of these studies were to evaluate whether co-modification of proteins with MAA and Cit drives anti-Cit autoimmune responses observed in RA.

Methods. DBA/1 mice i.p. weekly x 5 wks with human serum albumin (HSA), or HSA modified with Cit and/or MAA. Serum was evaluated for antibodies to HSA, HSA-MAA, HSA-Cit and HSA-MAA-Cit by ELISA at week 6. Type II collagen (Col) was modified with MAA and/or Cit and mice were immunized as above. $CD4^+$ T cells were evaluated by proliferation against all antigens used for immunization at week 6. Finally, activated peritoneal macrophages were stimulated with Col unmodified or modified with MAA and/or Cit, and assessed for TNF-α secretion.

Results. ACPA concentrations were significantly higher in mice immunized with HSA-MAA-Cit than mice immunized with HSA-Cit. Col-MAA-Cit immunization resulted in increased T cell responses compared to either modification alone. Activated macrophages were shown to increase TNF-α secretion following exposure to Col-MAA-Cit and Col-Cit compared to Col or unimmunized controls.

Conclusion. Co-modification with MAA and Cit serves to increase the immunogenicity of citrullinated proteins to initiate marked antigen specific responses, and the secretion of the pro-inflammatory cytokine TNF-α. Taken together these data suggest that MAA protein adduct formation and resulting immune responses to both MAA and Cit antigen play an important pathogenic role in RA.

T. 12. Estrogen Controls the Expression of Serine/Arginine-rich Splicing Factor 1 (SRSF1) at the Transcriptional and Post-transcriptional Levels in Human T lymphocytes

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Systemic lupus erythematosus (SLE) is a chronic debilitating autoimmune disease that primarily afflicts women in the childbearing years. Female hormones especially estrogen are implicated in disease pathogenesis, yet the precise molecular events regulated by estrogen in immune cells are not clear. We previously showed that the expression levels of serine/arginine-rich splicing factor (SRSF)1 are decreased in T cells from SLE patients and associate with severe disease activity. We recently generated T cell conditional *Srsf1*-deficient mice, which exhibit T cell hyperactivity, aberrant cytokine production and develop lupus-like disease. These studies imply that SRSF1 is an important molecule in T cell function and its deficiency contributes to autoimmune disease. Yet, little is known of the regulation of SRSF1 in T cells. We hypothesized that estrogen may regulate SRSF1 expression in human T cells. We found that exposure to estrogen led to a dose dependent increase in *Srsf1* mRNA levels but a decrease in protein levels in human T cells. This discrepancy between mRNA and protein levels suggests that estrogen

controls SRSF1 via multiple mechanisms. Accordingly estrogen increased the activity of an *Srsf1*-promoter-luciferase construct indicating that estrogen activates transcription of *Srsf1*. In parallel, the addition of the proteasome inhibitor MG132 to estrogen-treated cells rescued SRSF1 protein levels, indicating that estrogen leads to proteasome-mediated degradation of SRSF1. Our results suggest that estrogen can modulate the expression of SRSF1 via transcriptional and post-transcriptional mechanisms in human T lymphocytes, thus revealing a potential molecular link between hormones, immune cells and autoimmune disease.

T. 14. Extensive Immunophenotypic analysis of co-inhibitory an co-stimulatory molecules in Juvenile Idiopathic Arthritis (JIA) peripheral lymphocytes

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Objective: To analyze the expression of co-inhibitory and costimulatory molecules in different cellular subpopulations in JIA patients stratified by age and treatment.

Methods: Exploratory cross-sectional study. 53 patients fulfilling Oligoarticular JIA (ILAR) criteria and 22 controls were included. All of them were children or young adults (2 to 35 years of age). JIA patients were either not treated (n=14) or treated with MTX (n=14), anti-TNF (n=8), or a combination of both (n=16). Four flow cytometry panels were designed for the analysis of co-stimulatory/co-inhibitory markers, some typical of "exhausted" cells (PD1, TIM 3, TIGIT, CD226, CD137, HVEM, LIGHT, BTLA), assessed in memory, effector and naïve CD4+ and CD8+ T lymphocytes, and also in B lymphocytes and NK cells. FDR correction was applied for p values.

Results: No correlation was found among the exhaustion/activation markers and age neither in JIA nor in controls. Interestingly, age-related differential trends were observed in the CD8+ compartment in controls, while in JIA patients were detected in the B cell compartment. We didn't observe, after correcting for treatment, any significant difference in lymphocyte subpopulations defined by the above co-inhibitory and co-stimulatory markers in JIA patients in relation to indexes of disease activity. However, there was a tendency towards a higher expression of CD137 in several CD8+ subpopulations of active patients as compared to inactive.

Conclusion: No clear differences were found in PBL subpopulations of oligoarticular-JIA patients regarding co-stimulatory/co-inhibitory molecules, perhaps, because of the good therapeutic control achieved in this patient population.

T. 15. Failed STAT4 Downregulation Underlies the Pathogenicity of SLE-associated Variants

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Genome-wide association studies have identified more than 40 loci that may be pathologically relevant for systemic lupus erythematosus (SLE). Variants in *STAT4*, are robustly associated with lupus nephritis (LN). Although these variants have been shown to increase STAT4 expression in peripheral blood mononuclear cells and monocytes, the molecular mechanisms that underlie this effect and its functional consequences are still unclear.

Naïve CD4 T cells obtained from healthy human donors homozygous for the protective (Prot) or risk (Risk) *STAT4* alleles, were cultured in Th1 polarizing conditions. Cells carrying the Prot alleles, downregulated *STAT4* transcription in response to sustained exposure to IL-12. In contrast, cells with the Risk alleles did not modulate *STAT4* and consequently accumulated increased amounts of the transcription factor. This was accompanied by increased levels of STAT4 protein and pSTAT4. Further, Risk cells produced significantly more IFN-γ. To dissect the regulatory effects of the SLE-associated variants, we edited Jurkat cells using CRISPR/Cas9 technology. Disruption of the *STAT4* intronic region that contains the SLE-associated SNPs unleashed the production of IFN-γ. The effect of STAT4 overexpression was evaluated in vivo by comparing the pathogenic capacity of STAT4-overexpressing and control OT-I cells. Upon transfer to RIP-mOVA mice, STAT4-OT-I cells exhibited increased migration into the pancreas and established insular inflammatory infiltrates.

This work has identified a key regulatory region within the third intron of *STAT4* and has provided evidence of how SLE-associated STAT4 SNPs contribute to increase the pathogenic capacity of T cells.

T. 16. Glutaminase 1 is a target for Th17-related autoimmune diseases

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Objective: Glutaminase 1 (Gls1) is the first enzyme in glutaminolysis. We previously showed that the selective Gls1 inhibitor Bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl)ethyl sulfide (BPTES) suppresses Th17 cell development and suppresses experimental autoimmune encephalomyelitis (EAE). However, the involved mechanisms and whether inhibition of glutaminolysis can be useful in the treatment of systemic lupus erythematosus (SLE) remain unknown.

Methods: MRL/*lpr* mice were treated by BPTES or vehicle control and disease activity was examined. Then naïve $CD4^+$ T cells from patients with SLE were cultured under Th17 conditions with BPTES or the vehicle. Furthermore, using newly generated Gls1 conditional knockout mice in IL-17 producing cells, in vitro Th17 differentiation and EAE disease were investigated. The expression of hypoxia-inducible factor

1α (HIF1α) and the von Hippel–Lindau tumor suppressor protein (VHL) which degrades HiF1α were examined by qPCR and Western blot.

Results: MRL/*lpr* mice with BPTES improved autoimmune pathology in a Th17-dependent fashion. T cells from patients with SLE treated with BPTES displayed decreased Th17 differentiation. Using the conditional knockout mice we demonstrated that both the in vitro Th17 differentiation and the development of EAE depend on Gls1. Gls1 inhibition reduced the expression of HIF1α protein, while the expression levels of VHL were found to be increased suggesting excessive degradation of HIF1α.

Conclusion: We have provided evidence that inhibition of glutaminolysis can be used to treat patients with SLE and Th17-related autoimmune diseases. Mechanistically we showed that glutaminolysis inhibition suppresses Hif1α expression in Th17 cells by increasing VHL.

T. 17. Granzyme K+ CD8 T Cells are Highly Enriched and Have Pro-inflammatory Effects in Synovium and Synovial Fluid in Rheumatoid Arthritis

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CD8 T cells are enriched in synovium and synovial fluid of patients with rheumatoid arthritis (RA), yet little is known about their role in autoimmune arthritis. As suggested by recent single-cell RNA-sequencing data from the Accelerating Medicines Partnership, we have found that the majority of CD8 T cells in synovial tissue and fluid express granzyme K (GzmK), a marked enrichment compared to blood. GzmK is a protease which, unlike granzyme B (GzmB), does not activate apoptotic caspases. In contrast, very few synovial CD8 T cells express GzmB alone, the pattern seen in cytotoxic T lymphocytes (CTLs). Relative to GzmK-negative CD8 T cells, GzmK$^+$ CD8 T cells in blood express higher frequencies of chemokine receptors CCR2 and CCR5, which direct cells towards sites of inflammation, suggesting that GzmK$^+$ CD8 T cells are preferentially recruited to inflamed joints in RA. We have found that CD8 T cells play several roles in RA synovium. First, synovial CD8 T cells express IFNγ at a higher frequency and TNF at a similar frequency as CD4 T cells after stimulation. Second, GzmK itself has pro-inflammatory effects on synovial fibroblasts, inducing them to produce IL-6, CCL2, and reactive oxygen species, all of which are upregulated in inflamed synovium. Together, these findings form the basis of a new model of CD8 T cell migration and function in RA and potentially other immune responses. We have also developed methods for isolation of RNA from fixed and intracellularly stained cells to further study GzmK$^+$ CD8 T cells by low-input RNA-seq.

T. 18. Human genetic variation within the MHC influences the hypervariable region of T cell receptor

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Major histocompatibility complex (MHC) is the strongest genetic risk factor for many immune diseases. Disease modulating amino acid (AA) polymorphisms are often localized in the antigen binding pocket of MHC, and affect epitope bindings. To understand their impact on immune systems, we need to understand how such MHC-epitope complexes influence the repertoire of T cell receptors (TCRs). We assessed TCR frequencies using RNA-seq data of $CD4^+$ T cells from BLUEPRINT consortium (n=169). After extracting reads from TCR regions, we identified the AAs of complementary determining region 3 (CDR3), and quantified them in each position of CDR3 (275 AAs in total). We imputed AA polymorphisms for *HLA-A*, *B*, *C*, *DPA1*, *DPB1*, *DQA1*, *DQB1* and *DRB1* using SNP2HLA and the reference panel from Type 1 Diabetes Genetics Consortium (n=5,225). We analyzed the associations between MHC AA polymorphisms and the AA usage in each CDR3 position. Consistent with the MHC class II restriction of $CD4^+$ T cells, most associations were observed within MHC class II regions, prominently in *DRB1* loci: six out of eight significant associations were led by AAs of *DRB1* (Bonferroni corrected P value < 0.05). One such example includes the association between the 4-th AA of CDR3 of beta chain and AA position 11 of *DRB1* (P value = 7.7×10^{-13}), which is also a reported risk AA position of rheumatoid arthritis (RA) and type I diabetes. Our study showed novel associations of MHC-epitope-CDR3 interactions, and may help to further decipher the pathogenesis of RA and other autoimmune diseases.

T. 19. In Situ Lymphocyte and Dendritic Cell Characterization in Idiopathic Inflammatory Myopathies

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Background/Purpose: Idiopathic Inflammatory Myopathies (IIM) are the most common acquired muscle disorders. In this pilot study, we utilized confocal microscopy using fluorescent antibodies for characterization of dendritic cell (DC) and lymphocyte populations in Dermatomyositis (DM) and Inclusion Body Myositis (IBM) patient biopsies.

Methods: A total of 4 DM and 4 IBM samples were stained for myeloid dendritic cells (mDCs)(BDCA1 and CD11c) and plasmacytoid DCs (pDCs)(BDCA 2 and CD123); or plasma cells (CD138), B-cells (CD20), and T cells (CD4 and CD8), along with cell nuclei (DAPI). Slides were imaged with the SP8 confocal microscope. Regions of Interest were randomly acquired by means of tiling. The resulting data was manually analyzed for number of DCs and lymphocytes by a blinded observer (IBV). Mann-Whitney U test was used for all comparisons.

Results: IBM biopsies had larger proportions of plasma cells (mean of 22 versus 8 per biopsy, p=0.003), $CD4^+$ lymphocytes (73 versus 38, p=0.03), and $CD8^+$ lymphocytes (36 versus vs 10, p=0.002) in comparison with DM. Double-positive staining for mDCs was also greater in IBM (8 versus 1, p<0.001). Moreover, IBM biopsies also had higher numbers of $BDCA2^+$ cells (p=0.008). Double positive pDC and CD20 staining was similar between DM and IBM.

Conclusion: We observed a rich inflammatory milieu in IBM biopsies, with greater amounts of $CD8^+$ and $CD4^+$ T cells as well as mDCs and $BDCA2^+$ cells. Other cell markers considered characteristic of DM, such as the pDC marker CD123, did not differ among the groups<./p>

T. 20. Integration of single cells from inflamed tissue in RA and SLE reveals shared immune and stromal cell populations

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Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are considered related autoimmune diseases that share similar genetic variants and lymphocyte infiltration. Recently, single-cell technologies have provided an opportunity to understand cellular heterogeneity by querying damaged tissue. Across diseases, similarities in autoimmune and inflammatory cell states at the single-cell level in human tissues are currently understudied. To this end, we applied an integrative pipeline to harmonize 5,265 RA synovial cells and 6,857 lupus kidney cells from parallel single cell RNA-seq data generated by the Accelerated Medicines Partnership (AMP) RA/SLE consortium[1,2,3,4]. We observed multiple shared major cell types including T cells, B cells, myeloid cells, and fibroblasts. We analyzed each identified cell type across diseases and observed multiple shared subpopulations, including inflammatory and tissue resident macrophages, Tph and Treg CD4+ cells, GzmB+ and GzmK+ T cells, naïve and activated B cells. Across shared Tph cells, we observed an upregulation in type I interferon signaling (FDR p=1e-5) among SLE Tph cells, while RA Tph cells showed upregulation in cyclic adenosine monophosphate (FDR p=1e-4). Integrative analyses between damaged tissue from RA and SLE by single-cell transcriptomics focusing on shared cellular populations has identified shared and disease-specific gene expression modules, and should help predict potential therapeutics for RA and SLE in the future.

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W. 28. Invalidation of the ICOS costimulation pathway in NOD mice : shift of autoimmunity from diabetes to myositis and oxydative stress in target tissue.

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Autoimmune-prone NOD mice represent an invaluable model of type 1 diabetes (T1D). Inducible T cell co-stimulator (ICOS) is involved in induction of helper T cell responses, T-dependent antibody responses and germinal center reaction. Our objective was to investigate the consequences of ICOS invalidation on the autoimmune manifestations in NOD mice and study myositis pathogenesis.

Neither *Icos*$^{-/-}$ nor *Icosl*$^{-/-}$ NOD mice developed T1D. In contrast, myositis spontaneously occurred by 28 wks in both lines with decreased grip strength, impaired cadence and death around 40 wks. Pathological muscle analysis revealed necrotic myofibers and important inflammatory infiltrates (CD4^{+} T cells, macrophages). Muscle lesions yielded T2 hypersignals in MRI that correlated with histopathology and regressed under steroid therapy. CD4^{+} T cells were Th1 biased. Myositis developed in CD8- but not CD4-deficient mice. Disease was adoptively transferred to NOD.*scid* recipients by *Icosl*$^{-/-}$ CD4^{+} T cells. Activating IL-2/anti-IL-2 complexes exacerbated myopathy. Oxydative stress was present in muscle, as attested by transcriptomic and proteomic analyses, histo-enzymology, reactive oxygen species production and evaluation of mitochondrial function. Abnormalities in mitochondria were found by electron microscopy. Anti-oxydant therapy ameliorated disease. Similar oxidative stress was found in biopsies from patients with dermatomyositis.

The ICOS pathway is indispensable for T1D development in NOD mice. ICOS/ICOSL deficiency shifts autoimmunity from pancreas to muscle. Myositides are severe diseases leading to bedridden state and possibly death. This work establishes *Icos*$^{-/-}$ and *Icosl*$^{-/-}$ NOD mice as a unique paradigm of myositis. Inflammation causes oxidative stress in muscle, prompting to evaluate anti-oxydant therapy in myositides.

W. 29. Mass cytometry reveals elevated phosphorylated STAT3 (pSTAT3) in circulating CD4+ T cells during active Psoriatic Arthritis

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Psoriatic Arthitis (PsA) is a chronic inflammatory arthritis affecting up to 30% of patients with psoriasis. Immune factors, especially T cells, are involved in PsA. We used mass cytometry to profile proteins on circulating immune cells in active and inactive patients with Psoriatic Arthritis.

Blood from adults PsA patients with active (n=15) or inactive disease (n=13), and from patients with active Rheumatoid Arthritis (RA, n=14) was fixed with Proteomic Stabilizer (Smart Tube) and frozen at -80ºC. After thawing and red cell lysis, surface and intracellular antigens were stained using metal-labeled antibodies (Fluidigm). Cells were acquired on a Helios CyTOF instrument and data analyzed using FlowJo and VisNE (Cytobank). Statistical analysis was performed using GraphPad Prism.

The frequencies of activated CD8 T cells (CD38+HLA-DR+), and classical (CD14+CD16-) monocytes were elevated in active versus inactive PsA patients. Higher frequency of T regulatory cells, defined as CD4+CD127^{low}CD25^{high}FoxP3+, was found in active PsA versus active RA.

Levels of phosphorylated STAT3 (pSTAT3) was elevated in total CD4+ T cells in active PsA, and in the Th1, Th2, Th17 and Treg CD4+ T cell subsets. pSTAT3 was also elevated in CD14+CD16- monocytes from active versus inactive PsA patients.

Elevated pSTAT3 is associated with active PsA in comparison to inactive PsA in CD4+ T cells. Recent mouse studies showed that overexpression of STAT3 in CD4+ T cells was sufficient to elicit all the major characteristics of PsA, reinforcing a potential role for STAT3 signaling in the promotion of an inflammatory environment in PsA.

W. 30. Microenvironment driven re-shaping of pathogenic HLA-DR+ T subsets in active Juvenile Idiopathic Arthritic patients

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We have previously identified two pathogenic CD4 subsets in both Teff (CPLs) and Treg (iaTreg) compartments that are HLA-DR$^+$, antigen experienced, pro-inflammatory, correlating with disease activity and sharing TCR sequence oligoclonality with JIA synovial T cells. Despite being two functionally distinct T cell subsets, their phenotype and association with clinical fate suggests that these subsets may originate from a common precursor. To elucidate the common pathogenic gene drivers, we decided to perform next-generation RNA sequencing on sorted CPLs/iaTregs and their conventional Teff/Treg counterparts in both circulation and synovium. Comparative DEG analysis indicate transcriptomic convergence between circulatory CPLs/iaTreg and divergence from conventional effector/regulatory pools. Circulatory CPLs/iaTregs exhibit (a) common pathway dysregulation in T cell signalling, (b) restriction in TCR oligoclonality and (c) common transcription factor drivers (SPL1 and E2F1), suggesting a common antigenic selection pressure. Comparing healthy/JIA circulatory and paired synovium reveals a gradual transcriptomic convergence between Teff/CPLs, Treg/iaTreg and CPLs/iaTreg across the spatial/disease states. This is paralleled by an antigenic convergence in shared TCR clonotypes in CPLs/iaTreg across the same conditions. Synovium CPLs/iaTreg reveal 7 common dysregulated

pathways; MHC II antigen presentation, T cell costimulation, IFNg pathway, apoptosis, viral response, bacteria response and chemotaxis. Overall the data indicate immune-phenotypic convergence between CPLs/iaTregs, that is strengthen across disease/spatial states. These findings underscore a potential mechanistic role of the inflammatory microenvironment in shaping two functionally dichotomic populations, relevant to disease pathogenies and progression.

W. 31. Molecular and cellular biomarkers that discriminate juvenile idiopathic arthritis from septic arthritis

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Juvenile idiopathic arthritis (JIA) and septic arthritis (SA) are the most frequent cause of arthritis among children under the age of 16 years. Although these diseases have different physiopathological basis, they share clinical similarities.

To date, there are no markers sufficiently reliable to discriminate between these two forms of arthritis at the onset of the disease. Our goal is to identify diagnostic biomarkers capable of discriminating between JIA and SA and to study their functions. We focused on microRNAs (miRNAs) and myeloid cell subsets, both playing a major role in inflammatory processes.

We analyzed serum and synovial fluid (SF) samples from patients with a next-generation sequencing detection technology to measure the expression level of 2083 miRNAs and validated our results using RT-qPCR (oJIA: n=9; SA: n=9). In parallel, we performed a phenotypic characterization of peripheral blood (PB) and SF myeloid subpopulations using a flow cytometer (oJIA: n=9; SA: n=8).

Serum miRNAs analysis did not show significant differences between oJIA and SA. However, we observed a distinct miRNA profile in SF with 16 upregulated miRNAs and 5 down-regulated that discriminated oJIA and SA (p

In this study, we propose for the first time a synovial fluid-based miRNA signature as well as 5 myeloid cell subsets that discriminate between oJIA and SA. They represent potential diagnosis markers and help to understand the physiopathological mechanisms involved in these diseases.

W. 32. Orphan Receptors in an Orphan Disease: Identification of the NR4A Family as Key Players of Dendritic Cell Dysregulation in Systemic Sclerosis

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Systemic sclerosis (SSc) is a complex, heterogeneous autoimmune disease characterized by vascular abnormalities, immune involvement, and extensive fibrosis of the skin and internal organs. Myeloid dendritic cells (mDCs) are shown to be dysregulated in SSc and are implicated in the pathogenesis. To further explore the role of mDCs in SSc, we performed a transcriptomic profiling of circulating mDCs from SSc patients and healthy donors, and built a gene co-expression network to identify genes potentially involved in disease pathogenesis.

Within the co-expression network we identified gene clusters (modules) that significantly correlated with SSc and/or associated clinical traits. By applying enrichment analysis we observed that one module mainly consisted out of immune-regulatory genes down-regulated in the most fibrotic SSc patients. Using network parameters and literature-driven regulatory network analysis, the orphan nuclear receptor 4A subfamily (NR4A1, NR4A2, NR4A3) were identified as the key regulators of this module. The role and functionality of NR4As has not been studied hitherto in mDCs and in SSc, but they have recently emerged as important regulators of inflammation and fibrosis in other cell types. The down-regulation of NR4A1/2/3 was validated in an independent SSc cohort.

To further elicit the regulatory potential of NR4As in mDCs and their implication in SSc, we are currently performing siRNA knowdown assays, functional assays and ChIP-sequencing. Thus, by applying both bioinformatic and experimental approaches, we are exploring the functional role of the NR4A orphan receptor subfamily and establishing them as key players in SSc pathogenesis.

W. 33. Patient Characteristics and Current Management of Systemic Lupus Erythematosus Patients in a Large, Representative US-based Real World Cohort

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Background: Systemic lupus erythematosus (SLE) is a heterogenous, multifactorial disease with a debilitating and highly variable clinical course. Real world data are critical to better understanding these patients and their unmet clinical needs, particularly as inclusion and exclusion criteria for clinical studies are by design highly restrictive and typically not representative of the overall SLE population. Methods: The OM1 SLE Registry (OM1, Boston), an ongoing, continually enrolling, representative sample of patients with SLE in the U.S. who are followed prospectively, was used to assess clinical, laboratory, , symptomatic and disease activity information. Results: The average age of the 35,484 SLE Registry patients was 49.9 years (SD 15.1), 92% of patients were female, 18% had evidence of lupus nephritis, and 2% had lupus endocarditis or pericarditis. While 8.5% of patients were treated with the disease-

modifying therapy (DMT) Benlysta, 75% were treated with anti-malarials and many with other 'off label' therapies. One-third of patients had at least a one Health Assessment Questionnaire functional ability index score (HAQ) and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scores were reported for over 1400 patients. Among patients with SLEDAI scores available, 20% showed high or very high disease activity. Conclusions: Use of a representative, real-world cohort of SLE patients followed by rheumatologists provides unique information on treatment patterns and outcomes. Treatment options are currently limited to a single DMT and more typically a combination of off-label immunosuppressants and steroids, demonstrating an unmet clinical need for patients with this debilitating condition.

W. 34. Performance of automated indirect immunofluorescence assay for antinuclear autoantibodies in cancer patients

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Background: The presence of anti-nuclear antibody (ANA) has reported its correlation with a worse prognosis. ANA test using indirect immunofluorescence assay (IIFA) needs to detect because there is no indication of a common protein specificity. Cancer prevalence has increased worldwide. Labor burden for ANA IIFA also has become higher. We evaluated suitability of automated ANA analyzer for ANA detection in cancer patients.

Materials and Methods: NOVA View software-based classification and conventional visual interpretation were compared with NOVA View in 51 solid cancer patients, 30 systemic autoimmune disorder patients, and 90 health check-ups<./p>

Results: Total agreement was 97.1% (Kappa value 0.91) between NOVA View software-based classification and NOVA View visual confirmation. In cancer patients, Kappa values showed very good (0.82) agreements. Agreements were 96.7% (Kappa value 0.92) in systemic autoimmune disorder patients and 98.9% in health check-up group.

Conclusion: NOVA View system provided not only high-accuracy ANA positive/negative identification but also reliable digital image. Reducing test time and clerical error and labor force was suitable for routine use in cancer patients.

W. 35. PP2A B55ß limits the lifespan of self-reactive and pathogen-specific CD8 T cells through the pro-apoptotic molecule Hrk

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Activated T cell apoptosis represents an essential mechanism of peripheral tolerance. Defects in this process cause autoimmunity and cancer. Here, we demonstrate the role of PP2A B55β as a key regulator of T cell lifespan, essential for immune homeostasis.

T cell-specific deficiency of B55β caused an accumulation of activated/memory CD8 T cells in aging mice. Following an infection with *Listeria monocytogenes*, B55β-deficient CD8 T cells exhibited significantly higher survival than their WT counterparts. The accumulating cells expressed an activated/memory phenotype and produced high levels of IFN-γ. The failure to remove activated T cells was due to defective apoptosis. Proteomic analyses revealed that, during cytokine withdrawal, WT T cells inactivate AKT. This leads to the activation of FoxO factors which promote the transcription of Hrk. In B55β-deficient T cells, AKT remains active and Hrk is not upregulated. The importance of this pathway was demonstrated by the fact that Hrk silencing conferred resistance to apoptosis in in vitro and in vivo systems. To evaluate the importance of B55β as a regulator of CD8 T cell survival, we transferred B55β-deficient or sufficient OT-I cells into RIP-mOVA mice. 80% of the mice that received KO cells developed dense islet infiltrates and diabetes (*vs*. 20% of the controls). On the other hand, transferred KO cells controlled the growth of an OVA-expressing melanoma significantly better than WT cells.

These results identify B55β as an essential regulator of cellular lifespan and demonstrate the mechanism through which it controls apoptosis.

W. 36. Systemic Sclerosis is a Disease of a Prematurely Senescent, Inflammatory and Activated Immunome

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Systemic sclerosis (SSc) is an autoimmune disease characterised by excessive fibrosis of skin and internal organs, and vascular dysfunction. Association of T and B cell subsets have been reported in SSc, however there is lack of systematic studies of functional relations between immune cell subsets in this disease. This lack of mechanistic knowledge hampers targeted intervention. In the current study we ought to determine differential immune cell composition and their interactions in peripheral blood of SSc patients and its impact on disease severity and progression. Mononuclear cells from blood of SSc patients (n=20) and healthy controls (n=10) were analysed by mass cytometry using a 36 marker (cell-surface and intracellular) panel. Transcriptome analysis (m-RNA sequencing) was performed on sorted T and B cell subsets. Unsupervised clustering analysis revealed significant differences in the frequencies of T and B cell subsets in patients. Correlation network analysis highlighted an overall dysregulated immune architecture coupled with domination of inflammatory senescent T cell modules in SSc patients. Transcriptome analysis of sorted immune cells revealed an activated phenotype of CD4 and MAIT cells in patients, accompanied with increased expression of inhibitory molecules, reminiscent of phenotype

exhibited by functionally adapted, exhausted T cells in response to chronic stimulation. Overall this study provides an in depth analysis of systemic immunome in SSc, highlighting role of inflammation and chronic stimulation mediated "premature senescence" of immune cells, with implications to delineate mechanism of pathogenesis and identify diagnostic/therapeutic targets.

W. 37. Tissue-Anchored Indoleamine 2,3 Dioxygenase Locally Suppresses Inflammation

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Immunomodulatory biologics for inflammation would greatly benefit from new retention strategies to localize suppression. We have developed a chimeric fusion protein incorporating novel approaches for both retention and suppression to induce potent, confined metabolic programming. Immunosuppressive indoleamine 2,3 dioxygenase (IDO), which depletes tryptophan through the kynurenine pathway, was fused to Galectin 3 (Gal3), which binds extracellular glycans and provides tissue anchoring. Using a luciferase-Gal3 fusion reporter, tissue retention was greatly prolonged for days to weeks, whereas native luciferase is not retained and undetectable by 24 h. IDO-Gal3 injected locally blocked inflammation in multiple models including local LPS-challenge and periodontal disease. Consistent with in vivo results, in vitro studies demonstrate exogenous supply of IDO conditions dendritic cells to a suppressive phenotype and blocks dendritic cell ability to stimulate T cells in antigen specific culture models.

W. 38. Transcriptional correspondence between the activation profiles of human TH1 cells generated in vitro and their counterparts generated in vivo

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Obtaining T_H1 memory cells from human donors at the scale needed for high-throughput functional-genomic and pharmacological screens for identification of new drug targets is a challenge. *In vitro* polarization of naïve human CD4 T cells to IFNγ- and TNFα-producing T_H1 cells allows for the generation of large numbers of cells; however, whether these *in vitro* conditions mimic global transcriptional activation programs of *in-vivo*-generated T_H1 cells is unknown. Using RNA-seq, we have generated global transcriptional profiles of *in-vitro*-generated T_H1 cells and *in-vivo*-generated CD4 T_H1 and T_H2 memory cells from the peripheral blood of healthy human donors (with the latter isolated based on their differential expression of chemokine receptors), under both steady state conditions and upon acute TCR stimulation. For *in-vivo*-generated T_H1 and T_H2 cells, global transcriptional activation profiles were highly consistent and a common activation signature is apparent. We find that there is a shared transcriptional metabolic signature of activation between *in-vivo*-generated T_H1 and T_H2 cells that includes oxidative

phosphorylation, the TCA cycle and multiple amino acid pathways. Furthermore, we demonstrate that the transcriptional activation profiles are strongly positively correlated between T_H1 cells generated *in vivo* and T_H1 cells generated *in vitro*, suggesting that TCR stimulation of *in-vitro*-generated T_H1 cells executes a transcriptional program that mimics that of their *in-vivo*-generated counterparts. Our data support the use of *in vitro* differentiated human T cells as a model system for discovery of novel immunometabolism targets.

W. 39. Ultra-Efficient Short Read Sequencing of Immune Receptor Repertoires

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Sequencing-based analysis of immune receptor repertoires (IRRs) is a powerful approach for enhancing our understanding of adaptive immune responses. However, current methods for quantitative IRR analysis are complex, expensive, or both, limiting routine large-scale IRR studies. We therefore present Framework Region 3 AmplifiKation sequencing ("FR3AK-seq"), an ultra-efficient multiplex PCR-based approach for IRR analysis. FR3AK-seq uses minimal primer sets targeting a conserved region immediately upstream of the hypervariable complementarity determining region 3 (CDR3), allowing for generation of undistorted amplicon that can be analyzed using short read, single-end sequencing. We show that FR3AK-seq is sensitive and quantitative, producing results very similar those of the current industry standard at about 50-fold reduced cost. FR3AK-seq was used to characterize the T cell infiltrates of 146 muscle biopsies from patients with differing subsets of idiopathic inflammatory myopathies and controls. We examined these TCR sequences for disease-specific motifs indicating shared antigen specificity using the open source software GLIPH (grouping of lymphocyte interactions by paratope hotspots).Using this approach, we discovered that TCR sequences from muscle-infiltrating T lymphocytes do indeed contain disease-specific motifs and form disease-specific clusters. Additionally, we found that clones within these clusters are often more expanded than corresponding unclustered clones, as demonstrated by an anti-synthetase syndrome (ASyS) specific cluster (p=0.0001). Sequences in this cluster represent 6/21 (28.6%) ASyS patients in the cohort and suggest a shared antigenic target for these clones in these patients. These findings demonstrate the utility of FR3AK-seq to probe biologically meaningful immune responses using human tissues.

W. 40. Unveiling Novel Biomarkers for Rheumatoid Arthritis Using Machine Learning on Gene Expression Data

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Currently, diagnosis and monitoring the disease progression of Rheumatoid Arthritis (RA) is challenging and there is no a biochemical test for detection early-stage RA. In this study, we aimed to identify putative biomarkers for RA by leveraging gene expression data with machine learning approaches.

We collected publicly available microarray data with 348 synovium (SY) and 2,518 whole blood (WB) samples. The raw data was processed, merged and normalized across studies and treatments. We developed a machine learning pipeline for robust feature selection. We found 16 genes highly associated with RA in both tissues: 6 up-regulated: MARCH1, NMI, LXN, DCP2, IFT20, RPS27L, and 10 down-regulated: DEXI, GPATCH8, BRD2, BMS1, ANKRD11, CBLB, TNPO2, MYC, ZBTB16, PRDX6. The up-regulated genes are involved in immune and inflammatory response, apoptotic processes, and DNA damage response, whereas the down-regulated genes are involved in in tissue homeostasis and bone development, negative regulation of epidermal growth factor-activated receptor activity, and response to oxidative stress, regulation of cell growth and proliferation, oxygen transportation, and regulation of gene expression. Finally, we built a prediction model with selected genes on the WB tissue, obtaining AUC 0.97 with sensitivity 0.85 and specificity 0.95. The validation of results was conducted on an independent synovium RNA-seq data with the model performance of AUC 0.97 with sens. 0.71 and spec. 0.99.

In our comprehensive in-silico search we found novel biomarkers in RA. Identification of extensive proteins secretion in blood could allow precision phenotyping which could have a positive impact on monitoring disease progression and patient treatment.

W. 41. Whole Blood Phenotype and Signaling Analyses Reveal Ethnicity Dependent Dysregulation in SLE Patients with Variable Disease Activity

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Clinical heterogeneity in systemic lupus erythematosus (SLE) is influenced by genetic and non-genetic susceptibility that drive disease expression and severity. The immune pathways that contribute to heightened disease activity in lupus by race are critical to understanding SLE disease mechanisms and outcomes. To assess this, whole blood samples (n=58) of European (EA) or African American (AA) controls and SLE patients with either high (SLEDAI≥4) or low (SLEDAIlo) and double negative B cells (CD27-IgD-), while AA patients with high disease activity had elevated frequencies of memory B cells (CD27+IgD-CD38+). African Americans exhibited greater dysregulation of phospho-signaling pathways following IFNα, TLR4, TLR7/8 and TLR9 pathways with a reduced ability to activate pERK, p38, pSTAT3 and pSTAT5 compared to low disease activity patients and controls, partly contributed by higher basal levels of activation. Further, AA patients with high disease activity had significantly elevated cytokine

production at baseline compared to controls, low disease activity patients and EA SLE patients that included BLyS, IL-12p70, GM-CSF and TNFα. Our results support a model where race influences heightened SLE disease activity mechanisms with alterations in B cell signaling, and greater dysregulation in phospho-signaling and pro-inflammatory soluble mediators observed in AA patients.

W. 42. Dissecting the mechanisms responsible for the generation of regulatory versus pathogenic human CD4+ T cells by TLR9-activated plasmacytoid dendritic cells

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Understanding the mechanisms underlying the balance between regulatory and pathogenic CD4$^+$ T cell generation will accelerate therapeutic target identification in autoimmune diseases such as Systemic Lupus Erythematosus (SLE), where this balance is dysregulated. We recently described that *in vitro* priming of naïve CD4$^+$ T cells with plasmacytoid DCs (pDCs) activated with either CpGA or Oxidized mitochondrial DNA (Ox mtDNA) leads to the generation of Type 1 regulatory T cells (Tr1) or Th10 cells, respectively. Th10 cells are expanded in the blood of SLE patients and accumulate within the tubuloinsterstitial areas of proliferative nephritis (PLN) lesions. These cells produce IL10 and mitochondrial ROS (mtROS) as the result of reverse electron transport (RET) fueled by the tricarboxylic acid (TCA) cycle intermediate succinate. Functionally, Th10 cells are not suppressive, but they provide B cell help through the synergistic effect of IL10 and succinate.

The mechanisms responsible for the acquisition of a regulatory versus helper phenotype upon priming CD4+ T cells with pDCs activated with two different classes of TLR9 ligands remained elusive. We now show that the generation of Th10 cells in response to Ox mtDNA-activated pDCs requires the activation of the Delta Like Canonical Notch Ligand 4 (Dll4)-Notch pathway. Conversely, CpGA-activated pDCs drive the generation of Tr1 through the upregulation and activation of the TLR7-IRF7 pathway on naïve CD4$^+$ T cells. These data carry important therapeutic implications for the identification of therapeutic targets in SLE and beyond.

Bone Marrow or Stem Cell Transplantation

H. 36. Graft GD T-Cell Receptor Sequencing Identifies Public Clonotypes Associated to HSCT Efficacy in AML Patients and Unravels CMV Impact on Repertoire Distribution

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Little is known about the role of intra-graft γδ T-cell receptor (TCR) repertoire on clinical outcome following hematopoietic stem cell transplantation (HSCT). Using next-generation sequencing (NGS), we sought to analyze the TCR δ-chain (TRG) repertoire within donor stem cell grafts and address its potential impact on clinical response. We analyzed twenty peripheral blood stem cell grafts from matched unrelated donors, classified as CMV-positive/negative. γδ T-cells were isolated and the gDNA extracted for NGS (ImmunoSEQ, Adaptive Biotechnologies). The respective acute myeloid leukemia recipients were followed for disease relapse and acute graft-*versus*-host disease (aGvHD) development. Grafts received by non-relapse patients had increased proportion of long CDR3 sequences (54-57 nucleotides) and higher usage of V2-JP1 pairing than relapse patients. Grafts from CMV-positive donors presented lower TCR usage of the pairs V2-J2, V2-JP2, V4-JP2, V9-JP, V9-JP2; significantly reduced diversity; and skewed non-Gaussian distribution of CDR3 sequences, with hyperexpanded clones taking up 2.5 times more space than CMV-negative grafts. We identified twelve unique public clones in addition to four private over-represented sequences exclusively present in grafts given to non-relapse patients, taking from 2.00% to 6.23% of the TRG repertoire and longer than 45 nucleotides. We also identified five private over-represented and one public CDR3 sequence associated to CMV infection. CMV-positive grafts presented the highest percentage or repertoire taken by private over-represented clones, ranging from 13.72% to 41.61%. Our findings show that the TRG composition is not associated to aGvHD incidence, CMV infection reshapes the TRG repertoire and several public sequences are associated to clinical remission.

H. 37. A CD45-Targeted Antibody Drug Conjugate Enables Allogeneic Hematopoietic Stem Cell Transplantation in Mice

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Targeted antibody drug conjugates (ADCs) to mouse antigens have been shown to effectively prepare immunocompetent mice for congenic whole bone marrow (WBM) transplants (Palchaudhuri et al. Nature Biotech 2016 34:738; Czechowicz et al. Blood 2016 128:493). Targeted ablation of host hematopoietic cells via CD45-targeted ADCs has the potential to effectively condition for both autologous and allogeneic transplantation. If this novel approach can be successfully translated to humans, it has the potential to expand the utility of transplantation. We developed a short half-life ADC targeting murine CD45 coupled to saporin (SAP), a ribosome-inhibiting toxin, to model broad hematopoietic depletion as a conditioning agent for allogeneic transplant in immunocompetent mice. Administration of a single dose of the ADC at 1.9 mg/kg effectively depleted murine hematopoietic stem cells (HSCs). To determine if the ADC could enable allogeneic transplant as part of a reduced intensity conditioning regimen, we transplanted recipient DBA-2 mice (H-2d, CD45.2+) with congenically-marked Balb/c (H-2d, CD45.1+) WBM 5 days after single dose administration of a myeloablative dose of our ADC in combination with post-transplant Cytoxan or additional T-cell depletion. CD45-SAP in combination with post-transplant Cytoxan enabled >85% peripheral donor chimerism at 12 weeks post-transplantation with multilineage reconstitution observed in the T-, B- and myeloid cell compartments (>80%, >90% and >90% donor chimerism, respectively). These results demonstrate that anti-CD45 ADCs used in combination with immunosuppression enable

highly efficient allogeneic transplantation in a murine minor mismatch model and suggest that a similar approach may be effective in humans.

H. 38. A longitudinal analysis of TCRB repertoire diversity in immune tolerance of pediatric patients post-UCBT: T cell clonal deletion over clonal anergy

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The mechanisms of long term immune tolerance after successful hematopoietic stem cell transplantation are not fully elucidated. We set up serial experiments to test central and peripheral mechanisms sustaining tolerance after HLA-mismatched unrelated cord blood transplant (UCBT) in the GvH direction. TCRβ ImmunoSEQ® (Adaptive Biotechnology®) was applied for tracking and characterizing host-reactive T cell clones from graft or patient samples.

Seven UCBT recipients with non-malignant diseases were studied. All patients enrolled on the same reduced intensity conditioning trial (NCT01852370). Patients achieved tolerance if discontinued immunosuppression, exhibited immunocompetence without GvHD or graft rejection. Purified T cells responses to host APCs were measured by mixed lymphocyte reaction, cytokine bioplex, and micro CTL assays. T cells clones expanding >0.1% frequency in response to host APC were tracked and characterized with TCRβ ImmunoSEQ®.

ImmunoSEQ® revealed the disappearance of host-reactive clones that expanded from an aliquot of the infused cord blood graft by 7 days of stimulation with purified host APC. Most of these allo-reactive T cell clones were not identifiable even in the presence of IL2 supplementation to break anergy. Serial experiments post-UCBT revealed novel host-reactive clones that were not identifiable within the alloreactive pool of the graft pre-UCBT.

In summary, the acquisition of immune tolerance in the GvH direction post-UCBT is characterized by hypo-reactivity in all assays employed towards host APC. The disappearances of most host-specific clones in the graft indicate that deletion is a major mechanism of long term tolerance with possibly a small degree of anergy in contribution.

H. 39. Anti-CD45RC MAb treatment prevents acute graft-vs.-host disease (aGVHD) induces alloimmune tolerance and preserves protective immune responses.

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aGVHD is a frequent complication of bone marrow transplantation and there is need for new treatments. Transient anti-CD45RC MAb treatment induces tolerance in organ transplantation models through both depletion of CD45RC^{high} cells and activation of CD45RC$^{-/low}$ CD4^{+} and CD8^{+} Treg.

BN-LEW/F1 rats infused with LEW T cells showed a lethal aGVHD. Depletion of CD45RC^{high} cells from the T cell inoculum before infusion resulted in complete prevention of aGVHD (n=4). In vivo treatment after T cell infusion with anti-CD45RC MAb alone (0-30d, n=3) or a suboptimal dose of rapamycine (0-10d, n=3) did not prolong survival while combination of both anti-CD45RC and rapamycine were synergic (65 vs. 33d in controls, n=8) with 50% of the recipients showed indefinite survival. Long-term survivors rejected all third-party Sprague-Dawley but not BN or LEW skin grafts demonstrating donor-specific tolerance and preservation of other immune responses.

Administration of human PBMCs in NSG mice resulted in lethal aGVHD. Treatment with anti-CD45RC MAb alone (0-20d) or a suboptimal dose of rapamycine alone (0-10d) significantly prolonged survival (34d, n=9 and 30d, n=10 vs. 14d in controls, respectively) but all recipients died. In contrast, combination of both anti-CD45RC and rapamycine were synergic (84d, n=10) and 80% had indefinite survival. Human tumor cells implanted in immunodeficient NSG mice were rejected by hPBMCs infused 7d later. Treatment with anti-CD45RC MAb alone or in combination with rapamycine preserved elimination of tumor cells in all animals (n=4) demonstrating preservation of human immune responses.

Thus, treatment with anti-CD45RC in combination with rapamycine has a strong potential to prevent aGVHD.

H. 40. B-cells reconstitution in systemic sclerosis patients after autologous hematopoietic stem cell transplantation

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Introduction: Autologous hematopoietic stem cell transplantation (AHSCT) is an effective and safe approach to treat systemic sclerosis (SSc) patients refractory to conventional therapy. **Aim:** To evaluate the reconstitution of B-cell subsets in SSc patients following AHSCT. **Methods:** Peripheral blood samples

were harvested from 24 SSc patients before transplantation and at 30, 60, 120, 180, 360 days post-AHSCT. The immunophenotyping, regulatory B-cell (Breg) IL-10 production and suppressive assays were assessed by flow cytometry. **Results:** Compared to baseline, naïve B-cells ($CD19^+CD27^-IgD^-$) significantly decreased counts at 30 days post-AHSCT, followed by an increase at 360 days. There was a transient decrease of non-class-switched memory B-cell ($CD19^+CD27^+IgD^+$) frequency at 30 days, followed by an increase at 360 days. Mature class-switched memory-B-cells ($CD19^+CD27^+IgD^-$) and plasma cell ($CD19^+CD27^{high}IgD^-$) decreased respectively at 60 and 360 days post-AHSCT, while the frequency of double-negative B-cell ($CD19+CD27^-IgD^-$) increased at 30 days post-AHSCT. The subset $CD20^+CD43^+CD27^+CD69^-$ showed higher percentage at 30 days, followed by a decrease at 360 days. The Breg $CD19^+CD24^{hi}CD38^{hi}$ significantly increased at 360 days, while $CD19^+CD24^{hi}CD27^+$ Breg transiently decreased from 30 to 180 days post-AHSCT. The frequency of IL-10-producing Breg increased post-transplantation. After AHSCT the Breg $CD19+CD24^{hi}CD38^{hi}$ recover their ability to suppress cytokine production by Th1 CD4+ T-cell in vitro. **Conclusion:** Following transplantation, SSc patients displayed increased naive-B-cell and decreased memory B-cells, which might contribute to self-tolerance reestablishment, disease remission and clinical improvement on these patients. SSc patients showed increases of Breg numbers after AHSCT as well as an increase of IL-10 production, suggesting improvements in immunoregulatory mechanisms.

H. 41. In vitro Glucocorticoid Responsiveness of HSCT Graft cells in Acute Graft-versus-Host Disease in Allogeneic Hematopoietic Stem Cell Transplantation

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Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is potentially curative in several hematological and immunological disorders. It carries the risk graft-versus-host disease (GvHD). Risk factors for GvHD are established and include recipient age, female-to-male transplantation, and Human Leukocyte Antigen (HLA) and Cytomegalovirus (CMV) immunity mismatch. We have developed an assay to determine glucocorticoid (GC) responsiveness (GCR) in peripheral leukocytes. Using this assay, we found that preoperative GCR relates to postoperative recovery in surgical patients. In this study we applied this assay to CD34-depleted HSCT grafts, to investigate if GCR can predict GvHD grade ≥2 in allo-HSCT recipients.

Material and methods

Graft cells, harvested for transplantation to 24 patients (mean age 53 (SD ±13), 8:16 female:male) with hematological malignancies, were incubated overnight in 10^8 M, 10^6 M Dexamethasone (DEX) or DEX-free medium. Relative up- and down-regulation for five GC regulated genes was determined by RTq-PCR. GvHD grade was determined by clinical review. Ten patients developed acute GvHD grade 2-3.

Results
Reduced DEX-induced down-regulation of the GC receptor alpha (GR-alpha) and HLA-DR genes was found in grafts for recipients developing GvHD grade ≥2 ($p < 0.05$). Added to the risk factors for acute GvHD (above) logarithmized relative expression levels of GR-alpha and HLA-DR, at either DEX concentration, improved prediction of GvHD grade ≥2, in logistic regression models ($p < 0.001$).

Conclusion
We conclude that measuring graft GC responsiveness by RTq-PCR of DEX-induced downregulation of GC regulated genes GR-alpha and HLA-DR may add value in the prediction of risk of acute GvHD in adult allo-HSCT recipients.

H. 42. MSCs Interacting with Neutrophils Results in Specific Immunomodulatory Cytokine Signatures – Does it Vary with Sources?

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Background: The immunomodulatory potential of mesenchymal stem cells (MSCs) have been largely discussed while the specificity with the sources have not been enumerated.

Methods: MSCs were isolated from umbilical cord blood (UCB), dental pulp (DP) and liposuction material (LS). Their cytokine profile was enumerated and compared after co-culture with activated neutrophils. Cytokine profiling included IL-1a, IL-2, IL-4, IL-6, IL-8, TNF-a, IFN-g, and TGF-b and added we assessed cellular proliferation, cell death and differentiation to selection pressure.

Results: The results showed a comparable cytokine response among all types in response to activated neutrophil co-culture, while specifically the LSMSCs cytokine response with TNF-a and IFN-g was high owing to its mature cellular phenotype compared with UCBMSCs and DPMSCs. We did not observe any significant change or alterations in cellular viability or proliferation between LP/DP/UCB MSCs when co-cultured with activated neutrophils. Further when the MSCs were directly activated with lipopolysaccharide (LPS), which served as control, however did not induce a rapid proliferation, while cell death was evidenced in all the three types of MSCs. We then induced all the types of neutrophil exposed MSCs for osteogenic, adipogenic and chondrogenic differentiation and observed that, all the MSCs devoid of the sources differentiated, however a significant rapid turnover of DPMSCs positive for osteogenic markers and LSMSCs to adipogenic markers were observed over UCB MSCs.

Conclusion: Taken together these results suggest that though all the MSCs devoid of sources are activated DPMSCs and LSMSCs are niche orientated while UCBMSCs responds equivocally due to its comparative naïve nature.

H. 43. Non-Genotoxic Conditioning with Antibody-Drug Conjugates Targeting CD45 Effectively Deplete Hematopoietic Stem Cells and Lymphocytes

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Hematopoietic stem cell transplant (HSCT) is a validated therapeutic approach able to achieve durable remission in patients with hematologic and autoimmune diseases. However, the side effects of current radiation and chemotherapy based conditioning regimens used to prepare patients for HSCT greatly limit the use of this powerful therapeutic approach. To circumvent these issues, we are developing antibody drug conjugates (ADCs) targeting CD45, a cell surface protein expressed throughout the hematopoietic system to enable simultaneous myelo and lympho-depletion prior to allogeneic transplant for hematological malignancies and autologous transplant for severe autoimmune diseases. Here we report that our CD45-targeted ADCs, conjugated to a non-genotoxic payload, can broadly and effectively deplete human hematopoietic cells in humanized NSG mice. This targeted depletion also confers potent anti-leukemia effects in multiple models of human hematopoietic malignancies, including patient-derived AML xenografts, where median survival times were extended greater than 2-fold. Further, we have established that single-dose administration of CD45-targeting ADCs with a non-genotoxic payload to cynomolgous monkeys achieved >80% depletion of peripheral blood lymphocytes and >85% depletion of both HSCs and lymphocytes in bone marrow at a dose that was well tolerated. The potent efficacy and tolerability of these ADCs support this novel approach for safer conditioning prior to HSCT, with the potential to dramatically increase the number of oncology and autoimmune patients that are eligible for transplant and significantly reduce the side effects associated with current conditioning protocols.

W. 43. Targeting pre-existing anti-transgene T cell response for effective gene therapy of Mucopolysaccharidosis type-I

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Mucopolysaccharidosis type-I (MPS-I) is caused by the deficiency of alpha-L-iduronidase (IDUA) that results in glycosaminoglycan accumulation in tissues. The available treatments are enzyme-replacement therapy (ERT) and allogeneic hematopoietic stem cell (HSC) transplantation. An alternative therapeutic option is *ex vivo* hematopoietic stem cell (HSC) gene therapy and preclinical studies performed in mice demonstrated the efficacy of this approach based on lentiviral vectors in the absence of pre-existing anti-IDUA immunity. However, several MPS-I patients develop anti-IDUA immunity after enzyme replacement therapy (ERT), thus pre-existing immune response may jeopardize *ex vivo* HSC gene therapy efficacy. To study the impact of pre-existing anti-IDUA immunity on gene corrected HSC engraftment in enzyme pre-treated and immunized mice, we optimize an artificial immunization protocol in MPS-I mice to mimic the effect of ERT in patients. We demonstrate that engraftment of IDUA-corrected HSCs is impaired in pre-immunized MPS-I mice and that the rejection of transplanted cells is mediated by IDUA-specific $CD8^+$ T cells. The selective depletion of IDUA-specific $CD8^+$ T cells allows engraftment of IDUA-corrected HSCs

in immunized MPS-I mice. Overall, these data demonstrate, for the first time, the relevance of pre-existing anti-transgene immunity on *ex vivo* HSC gene therapy and suggest the application of tailored immune-depleting treatments, as well as a deeper immunological characterization of patients, to safeguard the therapeutic effects of *ex vivo* HSC gene therapy in immune-competent hosts.

W. 44. Dissecting and Reconstructing the Human Thymus-Designing Roadmaps and Building Blocks for Functional Thymic Organoids

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Adaptive T cell immunity and central tolerance are fundamental functions of the human immune system and constitute the core of anti-infectious host defense, cancer surveillance and self-tolerance. Compromise manifests in infection, autoimmunity, malignancy and transplant complications. Adaptive T cell immunity and central tolerance are developed in the thymus. Untreated congenital absence of the thymus is not compatible with life and acquired thymic injury can lead to infections and autoimmunity. For all these conditions, therapies are lacking, making regenerative thymic tissues a critically unmet clinical need.

Despite promising reports of *in vitro*human thymopoiesis, these efforts have not translated into the production of clinically relevant engineered thymic tissues. We seek to make functional thymic epithelia from induced pluripotent stem cells (iPSCs) and leverages new technologies to address prior limitations. First, we elucidate the cell-cell signals governing thymic ontogeny by dissecting signaling pathways in human fetal thymus using single-cell RNA sequencing. Second, we employ genomic, proteomic, immunohistochemistry, and non-linear imaging methods to map the extracellular matrix (ECM) chemistry and design a customizable, engineered ECM to support 3D culture of TECs. Third, we will recapitulate critical cell-cell and cell-matrix signals necessary to differentiate human iPSCs into functional thymic epithelial cells and mimic the dynamic regulation of endogenous transcription using synthetic biology approaches. These three orthogonal strategies will be pursued in parallel, ultimately converging in the development of an iPSC-derived, functional thymic epithelial organoid embedded within a biologically active 3D matrix for direct transplantation.

W. 45. Potential for NGS based MHC Typing To Reduce Incidence and or Severity of Graft Vs Host Disease in Hematopoietic Stem Cell Transplantation

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Allogeneic Hematopoietic Stem Cell Transplantation [HCT] is the final treatment for hematologic disorders and malignancies. Utmost immunogenetic compatibility, of which recipient-donor Human Leukocyte Antigen (HLA) genes is the most important factor in donor selection and is predictive of Graft vs. Host Disease (GVHD), a life-threatening complication HCT. HLA genes are part of the MHC that initiate T and B cell responses. Despite "matching" of recipient-donor MHC class I and MHC class II the prevalence of GVHD remains 20-70%. The "matching" until the NGS based typing was relative in terms of sequences related to Antigen Recognition sites [ARS] coded by exons 2, 3 for Class I and Exon 2 for Class II. However the amino acid sequences of other coding regions of Class I & II could potentially affect the ARS. So the MHC/HLA matching by Sanger sequencing [SS] probably were not "fully" matched. In a preliminary study we collected data using SS from 26 HCT candidates and the 75 corresponding "potentially matched "NMDP Donors and we found that several mismatched rare alleles were a possibility in either the donors or the patients in question. Recently we resolved following rare alleles without any ambiguities using NGS:

Also, NGS could potentially detect mismatches in the MHC Class III region [gamma block] that in regulate immune responses and therefore GVHD. Our preliminary data using SSP-PCR showed that mismatches among 25 limited SNPs in the gamma region could adversely affect GVHD using 52 recipient/donor pairs of HCT. NGS typing may elucidate more.

W. 46. Regulatory B cell generation from human Induced Pluripotent Stem Cells with a repressible granzyme B expression

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Kidney transplanted patients with long term graft survival harbor a higher frequency of B lymphocytes with regulatory properties. These "regulatory" B lymphocytes prevent effector T cell proliferation through the Granzyme B (GzmB) molecule (Chesneau et al., JASN 2015). Our aim was to generate regulatory B lymphocytes from human Induced Pluripotent Stem (hIPS) cells with a repressible GzmB expression as a tool to better understand their function and ultimately to translate them toward clinical use. Starting with

Embryonic Stem cells (hES) as a proof of concept, and following a well-established protocol (French et al., Stem Cells Dev. 2015), we succeed in generating a small proportion (1,5%) of B cells characterized as pre-B cells. To optimize this protocol we find that both increasing the number of CD34+ cells and adding cytokines for the 42 days of B cell differentiation, increase the amount of differentiated B cells by 2 fold. In order to perform a Knock Out (KO) of the endogenous GzmB expression, we validate our plasmid Cas9/sgRNA targeting GzmB on 293T cells with high cut efficiency (33%) and ready to use on hES cells. An expression cassette composed of the GzmB gene under control of a Tet-Off system is inserted in the AAVS1 locus of hES GzmB KO. In this system, we expect a constitutive expression of GzmB by B cells differentiated from those hES cells, while addition of doxycycline will abrogate its expression. This tool will allow deeply studying GzmB B lymphocytes function and future translation toward clinical uses.

W. 47. Pediatric acute myeloid leukemia blasts are killed by engineered type 1 regulatory cells developed to mitigate graft-vs-host disease

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Acute myeloid leukemia (AML) is the second most common and most difficult to treat pediatric leukemia. Optimal treatment for relapsed and refractory AML is allogeneic hematopoietic stem cell transplantation (allo-HSCT). However, allo-HSCT can result in life-threatening graft-vs-host disease (GvHD), whereby graft-derived T cells attack host tissues.

T regulatory type 1 cells (Tr1) are peripheral regulatory T cells that contribute to antigen-specific tolerance and suppress immune responses through IL-10. Tr1 can also lyse myeloid cells via perforin and granzyme B. We are developing engineered Tr1 suitable for clinical use by lentiviral transduction of *IL10* into $CD4^+$ T cells. In AML treated with allo-HSCT, a Tr1 administration could both prevent GvHD through IL-10 secretion and mediate an anti-leukemia effect through killing of residual AML, combating the two major causes of mortality.

To determine if primary pediatric AML blasts were lysed by engineered Tr1, we measured the killing of 23 blasts after co-culture with 4 Tr1 lines. Blasts were either efficiently killed, or displayed several levels of resistance. Comparing the transcriptomes of killing-sensitive and resistant blasts revealed that sensitive blasts express a myeloid activation signature, while the resistant blasts upregulated genes associated with leukemic cell "stemness". We also identified genes encoding for surface proteins whose expression was significantly upregulated in resistant blasts and negatively correlated with killing. These proteins could be manipulated *in vitro* to reverse the AML resistance to killing. Altogether, Tr1 can eliminate a subset of pediatric AML blasts, and AML resistance to killing is governed by their underlying molecular signature.

W. 48. CRISPR-based Therapy for IPEX Syndrome as a Model of Genetic Autoimmunity

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CRISPR-based gene correction in hematopoietic stem and progenitor cells (HSPCs) has the potential to treat a wide variety of genetic and acquired diseases. Gene corrected HSPCs can be used for autologous transplantation, circumventing the need for HLA-matched donor transplants. Here we propose a CRISPR-based gene correction strategy to treat IPEX syndrome, the prototype of genetic autoimmunity. IPEX syndrome is caused by mutations in the *FOXP3* gene, which leads to dysfunction of T regulatory cells (Tregs) and T effector (Teff) cells and subsequent autoimmune manifestations. Due to the widespread distribution of *FOXP3* mutations throughout the gene, we designed a strategy to insert a *FOXP3* cDNA into the mutated gene locus. Using the CRISPR system, we achieved efficient and specific targeting of *FOXP3* in HSPCs, Tregs and Teff cells. Gene edited Teff cells maintained physiological regulation of FOXP3 expression and characteristic proliferation potential and cytokine production. Gene edited Tregs displayed partial FOXP3 protein expression and suppressive capacity relative to wild-type controls, and various cDNA constructs were compared in Tregs to optimize FOXP3 expression and function. Next, we showed that gene edited HSPCs retained the potential to differentiate *in vitro* and engraft and differentiate in immunodeficient mice. Lastly, we demonstrated that editing of IPEX cells is a feasible and efficient method for functional *FOXP3* gene correction. This study supports a CRISPR-based strategy to treat IPEX syndrome, and further underscores the potential of gene editing treatments for other genetic immune diseases.

W. 49. Absence of AMP-activated Protein Kinase (AMPK) in Donor T Cells Minimizes Graft-versus-host Disease without Compromising Graft-versus-leukemia Responses or Impairing Immune Reconstitution

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Allogeneic hematopoietic stem cell transplantation (alloHSCT) is a viable treatment option for high-risk leukemia and immunodeficiency, but its use is often limited by graft-versus-host disease (GVHD), where donor T cells attack host tissues in the skin, liver, and gastrointestinal tract. Novel therapies to prevent GVHD, while protecting beneficial immune responses, are critical for the future success of alloHSCT. It is increasingly recognized that metabolic reprogramming plays a prominent role in T cells during activation. Here, we examined the role of AMP-activated protein kinase (AMPK), a cellular energy sensor, in alloreactive T cells during the development of GVHD. AMPK activity increased > 15-fold early post-transplant, while transplantation of T cells doubly deficient in AMPKα1/α2 decreased GVHD severity in three separate models. Importantly, disease severity decreased without compromising anti-leukemia

responses or impairing lymphopenia-driven immune reconstitution. Mechanistically, absence of AMPK decreased effector T cell numbers while increasing regulatory T cell (T_{reg}) percentages, all without impacting fat oxidation, autophagy, or mammalian target of rapamycin signaling. Differences in GVHD severity were directly attributable to perturbations in conventional T cells, as depletion of donor T_{reg} minimally impacted the improvements seen using AMPK knock-out cells. Together, these results dissociate AMPK from three classically-ascribed pathways, demonstrate that T_{reg} development can occur in the absence of AMPK, and indicate a prominent role for AMPK in alloreactive T cells. These studies also suggest that AMPK inhibition may offer a novel way to prevent clinical GVHD while still preserving graft-versus-leukemia responses and allowing for robust immune reconstitution.

W. 50. Ex vivo Generated Lymphoid Progenitors for Immune Reconstitution in the Context of Allogeneic Transplantation and Gene Therapy

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Ex vivo Generated Lymphoid Progenitors for Immune Reconstitution in the Context of Allogeneic Transplantation and Gene Therapy

The thymic microenvironment supports T-cell commitment. An *in vitro* feeder-free cell culture based on Notch ligand DL-4 and cytokines has been developed in our laboratory leading to the generation in 7 days of T-cells progenitors from both human cord blood and adult $CD34^+$ hematopoietic stem and progenitor cells (HSPCs).

Since TNFα is constitutively synthesized in the thymus, we explored its role during human early T-cell development. TNFα accelerated early T-cell differentiation and greatly increased the number of $CD34^-$ $CD7^+CD5^-$ T-cell precursors generated in the *in vitro* DL-4 culture both for cord blood and adult $CD34^+$ HSPCs. TNFα improved the production of $CD34^-CD7^+CD5^{-/lo}$ cells at the expense of myeloid and NK progenitors leading to a highly purified population of $CD7^+$ T cell precursors. These T-cell precursors expressed early T-cell commitment markers as shown by extensive RNAseq analysis and had a high T-cell differentiation potential *in vitro* upon differentiation on OP9/DL1 cells and *in vivo* when transplanted to NOD/SCID/γc-/- mice. TNFα increased specifically the rate of proliferation in $CD7^+$ T cell precursors between day 4 and 7 by promoting T-cell progenitors entry into the cell cycle through the activation of NFkB pathway.

We further demonstrated that this improved culture system can be combined with lentiviral gene transfer to produce large numbers of gene-corrected T-cell progenitors, opening new cell and gene therapy approaches for immune deficiencies.

W. 51. Identification of unique stem cell niche-residential Tregs acting as stem cell protectants– promising therapeutic targets for transplantation, tissue injury, and cancer

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Although the stem cell niche has been extensively studied as a site which regulates stem cell fate or functions, immunological attributes of the niche have remained largely unexplored. The locations of germline and embryonic stem cells, testis and placenta, were known to be immunological sanctuaries for stem cells, termed immune privileged (IP) sites. In these tissues, multiple mechanisms conspire to prevent immune attack, even enabling persistence of transplanted allogeneic or xenogeneic grafts without immune suppressive therapy. Little remains known about whether tissue-committed stem cell niches are broadly IP sites. We examined whether the hematopoietic stem cell (HSC) niche within the bone marrow (BM) serves as an IP site. We showed that unique FoxP3+ regulatory T cells (Tregs) with high expression of an HSC marker, CD150, frequently localized adjacent to HSCs. Extracellular adenosine derived from CD150^{high} niche-residential Tregs enabled allo-HSC persistence without immune suppression, promoting engraftment following nonmyeloablative conditioning. Moreover, transfer of niche Tregs improved engraftment to a much greater extent than transfer of other Tregs. In non-transplantation settings, niche Tregs and their product, adenosine, protected endogenous HSCs from oxidative and radiation stresses and maintained HSC quiescence, further mitigating post-irradiation hematopoiesis failure. Finally, transfer of niche Tregs and adenosine receptor agonist treatment rescued lethally-irradiated mice from critical hematopoiesis failure. These results indicate that niche Tregs and adenosine render the HSC niche an immunological sanctuary for transplanted and endogenous HSCs from immune attack and stress. Our work further identifies therapeutic utility of transferring niche-residential Tregs for stem cell transplantation and tissue injury.

Cytokines/Chemokines

H. 44. Finding transcriptional regulators central to RA with transcriptomics of IL-17 dose response, time series, and gene silencing in stromal cells

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In rheumatoid arthritis (RA), tumor necrosis factor alpha (TNFα) and interleukin 17 (IL-17A) are elevated in the synovial fluid. They synergistically activate production of cytokines such as IL-6, a key target of biologic therapies. Since synovial fibroblasts are the major source of IL-6 in the joint, we wanted to understand regulation of IL-6 production. With synovial fibroblasts from 7 patients with osteoarthritis or RA, we used RNA-seq to measure response to TNFα and different dosages of IL-17 (0, 1, and 10 ng/mL) at 8 time points over 24 hours. 409 genes (FDR < 0.01) had expression proportional to IL-17 dose, including genes regulated by Nuclear factor kappa B (NFκB). However, NFκB expression was not proportional to IL-17 dose. We discovered two regulatory mechanisms: one mediated by NFκB inhibitor zeta (NFKBIZ) and the other by Cut Like Homeobox 1 (CUX1). NFKBIZ expression was proportional to IL-17 dose. Silencing NFKBIZ with siRNA caused 82% reduction in protein levels of IL-6, IL-8 (CXCL8), and MMP3 after costimulation by TNFα and IL-17, but only 8% reduction after stimulation with TNFα alone, suggesting NFKBIZ mediates inflammation only after costimulation. In contrast, CUX1 expression was not proportional to IL-17 dose. After costimulation, we found that CUX1 binds NFκB and is recruited to a CUX1-NFκB motif unique to the promoters of CXCL1, CXCL2, and CXCL3. Silencing CUX1 significantly reduced neutrophil recruitment in vitro. In summary, we characterized the transcriptional response to TNFα and IL-17 and identified NFKBIZ and CUX1 as key regulators of fibroblast mediated inflammation.

H. 45. Association of systemic inflammation in COPD patients with and without pulmonary hypertension of Bangladesh

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Background: Although inflammatory markers have an important role in Idiopathic pulmonary hypertension (IPAH), there is limited information about the relationship between inflammatory markers and pulmonary hypertension (PH) occurring secondary to COPD. Our aim was to investigate association of systemic inflammation reflected by circulatory levels of hs-CRP, IL-6 and TNF-α in COPD patient with and without PH.

Methods: The cross-sectional study recruited 68 patients with COPD age above 40 years. Lung function was assessed using spirometry; pulmonary artery pressure (Ppa) levels were measured by color Doppler echocardiography. Serum TNF-α and IL-6 levels were assessed by ELISA, and hs-CRP by Immunonephelometry.

Results: Serum hs-CRP and TNF- α levels were significantly higher in patients with PH compared to those without PH (p=0.034 & p=0.041 respectively). No differences were seen between the two groups in terms of serum IL-6 levels (p=0.0623). A significant linear relationship was observed between log-transformed hs-CRP level and mPAP in the whole group (r= 0.337; p =0.121) and between log-transformed TNF- α and mPAP in the whole group (r =0.413; p= 0.104). No significant relationships were observed between log-transformed IL-6 and mPAP. In multiple linear regression analysis, independent

predictors of mPAP (R^2=0.394) was log hs-CRP (p 0.013) and peripheral oxygen saturation (Spo_2) (p 0.115). Log TNF- α was not an independent predictor of mPAP (p 0.823).

Conclusion: The study suggested that increases in Ppa in patients with COPD are associated with higher serum levels of hs-CRP and TNF-α, raising the possibility of systemic inflammation in the pathogenesis.

H. 46. CD4+ T-cell Phenotypes and Activation of the JAK/STAT Pathway in PBMCs from Newly Diagnosed Parkinson's Disease Patients

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Inflammation is implicated as a major pathogenic factor in the onset and progression of Parkinson's Disease (PD), the most common neurodegenerative movement disorder. Cells of the innate and adaptive immune system are involved in PD, including microglia, infiltrating macrophages and T-cells, with characterization of abundant production of cytokines.In our current studies, we focused on comparing adaptive and innate immune cells in peripheral blood of PD patients and healthy controls (HCs) with assessment by extensive flow cytometry analysis. Our results demonstrate that immunologic abnormalities are mainly in $CD4^+$ T-cell populations in patients with PD, with some observed sex differences. For $CD4^+$ T-cells, we observed a significant decrease of naïve CD4+ T-cells in PD patients, while central memory $CD4^+$ T-cells are increased in male PD patients, suggesting an activational phenotype of $CD4^+$ T-cells. With respect to $CD4^+$ T-cell subsets, there is an increase in Th17 cells producing IL-17A in PD patients, especially males, while there was an increase in Th2 cells producing IL-4, prominently in female PD patients. Surprisingly, IFN-g-induced STAT1 and IL-6-induced STAT3 activation were diminished in CD4+ T-cells from PD patients compared HCs. These changes are related to clinical disease severity, specifically with MDS-UPRS scores and Schwab and England scores. The types and numbers of medication had no influence on immune cell phenotypes. Dysregulated adaptive CD4+ phenotypical profiles and JAK/STAT activation may contribute to the development of PD, and may serve as potential biomarkers and targets for novel immunomodulating therapeutics in PD patients.

H. 47. Development of Chemotaxis Assays for Human-blood Derived Neutrophils and Monocytes Suitable for the Identification and Validation of Potential Drug Targets

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Migration of innate immune cells is an essential mechanism for the resolution of tissue injuries and inflammation. This process is orchestrated by chemokines including IL-8 and SDF-1α that attract neutrophils and monocytes, respectively, to the site of damage. Dysregulation in this process can contribute to the development of conditions, such as asthma, arthritis and psoriasis.

We developed chemotaxis assays using blood-derived human neutrophils or monocytes to test the effects of drug candidates. Neutrophils were isolated from blood donors using Ficoll and dextran-based separation methods, while monocytes were separate with a CD14 magnetic bead-based approach. Isolated cells were seeded in the upper chamber of a 96-well transwell system containing 5.0 µm pore size, and cell migration to the lower chamber was determined by a luminescent-based ATP assay.

Neutrophils stimulated with IL-8 showed consistent and dose-dependent migration reaching a peak at 10 nM of IL-8 after 1 hour. This migratory effect was inhibited by the addition of the CXCR1/2 antagonist Sch527123 in a dose-dependent fashion. Monocytes showed a strong migratory response upon stimulation with SDF-1α after 4 hours. The addition of the CXCR4 antagonist AMD3100, resulted in a dose-dependent inhibition of SDF-1α-induced migration. IC_{50} values were consistent across donors demonstrating strong reproducibility for both assays.

Our data demonstrate a robust and donor independent chemokine-induced migration that is inhibited by selective compounds and provide a rapid method to evaluate the *in vitro* potency therapeutic candidates for the treatment of acute and chronic inflammatory diseases.

H. 48. Evaluation of Novel Adipokines (Omentin-1, Apelin and Chemerin) as Potential Biomarkers of Presence and Severity of Diabetic Retinopathy in Type 2 Diabetes Mellitus Patients

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Background: Diabetic retinopathy (DR) pathophysiology involves insulin resistance, oxidative stress, inflammation but mechanism remains unclear. Also, there is lack of reliable circulatory biomarkers for early diagnosis of DR especially vision threatening retinopathy (VTDR).

Objectives: To study the role of adipokines (omentin-1, apelin & chemerin) in the pathophysiology of DR in T2DM patients and to analyze their potential as the novel circulating biomarkers of DR and VTDR.

Methods: 168 T2DM patients were grouped equally into no DR (NDR), non-proliferative DR (NPDR) and proliferative DR (PDR). Severe NPDR and PDR were taken together as VTDR. Serum omentin-1, apelin and chemerin and related parameters were measured.

Results: Omentin-1 levels in NPDR and PDR groups were low compared to NDR. Chemerin and apelin level in PDR were high compared to NDR. Spearman's correlation showed omentin-1, apelin, chemerin were significantly associated with DR severity. Bivariate analysis showed omentin-1 had a negative correlation with triglycerides, Hs-CRP and triglyceride-glucose index (TyG) but positive correlation with HOMA-beta; while apelin correlated positively with chemerin. ROC curve analyses revealed that omentin-1, apelin, chemerin were associated with DR and VTDR. Serum apelin level was higher in males compared to females.

Conclusion: Serum omentin-1 correlate negatively, while apelin and chemerin correlate positively with DR severity. Omentin-1 correlate negatively with markers of obesity, dyslipidemia, inflammation & insulin resistance. We found sexual dimorphism in the level of apelin. This study suggests that adipokines act as

mediators in the pathophysiology of DR and serum omentin-1 and apelin are potential biomarkers of DR and VTDR, respectively.

H. 49. Highly Aggregated mAb Therapeutics Can Induce Production of Proinflammatory Cytokine Responses

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Aggregation of biotherapeutic products continues to be a significant concern for a biopharmaceutical manufacturer due to the likely impact it can have on product safety. Characterization of protein aggregates, as caused by different stresses, is an essential step towards gaining a deeper understanding of a biotherapeutic product. These stresses may be experienced during manufacturing, formulation, filling, storage and/ or shipping. While there is widespread consensus that protein aggregation can enhance immunogenicity, the underlying immunological and biological mechanisms are not completely understood. All manufacturers rely on controls of particulation so as to ensure the safety and efficacy of protein drug products. Cytokines are an important class of biological molecules that regulate distinct functions of different immunocompetent cells. These are key modulators of inflammation, participating in acute and chronic inflammation via a complex network of interactions. Proinflammatory cytokines are important biomarkers and potent mediators of several biological processes. Chronic uncontrolled levels of such cytokines can initiate and formulate many pathologies, including incidences of autoimmunity and cancer. Therefore, therapies that regulate the activity of inflammatory cytokines, either by supplementation of anti-inflammatory recombinant cytokines or by neutralizing them by using blocking antibodies, have been extensively used over the past decades. This study presents results from an investigation into how aggregates generated by a variety of mechanical and chemical stresses impact pro- and anti- inflammatory cytokine responses. The results affirm that highly aggregated mAb therapeutics can induce the production of pro-inflammatory cytokine responses.

H. 50. Innate Regulation of Tissue-Reparative Human Regulatory T Cells

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Regulatory T cell (Treg) therapy is a promising curative approach for autoimmunity and transplant rejection, in which there is pathological tissue damage. Enabling Tregs to directly promote tissue repair in cell therapy would be attractive for a variety of clinical settings. In mice, Tregs drive tissue repair after infection or injury via production of the growth factor amphiregulin—a process controlled by the alarmins IL-18 or IL-33 and its receptor ST2. We investigated the tissue repair potential of human Tregs via IL-

33/ST2 and amphiregulin. We found that human Tregs in blood and multiple tissue types could produce amphiregulin ex vivo, but this feature was neither specific to Tregs nor upregulated in tissues. Amphiregulin-producing human Tregs were enriched for a naive, non-effector phenotype and were progressively lost upon TCR-mediated proliferation and differentiation. In ex vivo blood Tregs, amphiregulin production was not induced by IL-18, and these cells did not express ST2 and hence did not respond to IL-33. Human ST2+ Tregs were also not detected in tonsil, synovial fluid, colon, or lung tissue. Meanwhile, human Tregs engineered to overexpress ST2 recapitulated canonical IL-33 signalling; in these cells, IL-33 innately upregulated amphiregulin expression but did not affect their TCR-dependent suppressive capacity. Collectively, human tissue-reparative Tregs may function innately and more research is required to understand the role of IL-33 in this process. Future work will investigate other pathways beyond IL-33/ST2 in controlling this function and examine the tissue repair capacity of human Treg-derived amphiregulin in vitro.

H. 51. Interleukin-34 Contributes to Treg Suppressive Function

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Cytokines are powerful tools to regulate immune responses. Among them, IL-34 is a recently discovered cytokine that binds to CSF-1R, CD138 and PTPz and is involved in differentiation, survival of macrophages and regulates immune responses. We showed that IL-34 is expressed by rodent and human $CD8^+$ and $CD4^+$ Treg. Additionally, it induces long-term allograft tolerance in a rat model through M2 differentiation with $CD8^+$ and $CD4^+$ Treg induction.

To understand the function of IL-34 in immune responses, we generated IL-$34^{-/-}$ rats. These rats showed decreased $CD8^+$ and $CD8^+CD45RC^{high}$ T cells in the spleen and $CD8^+$ and $CD8^+Foxp3^+$ T cells in the blood vs. WT animals. Interestingly, CSF-1 (CSF-1R other ligand) serum level was significantly higher in

IL-34$^{-/-}$ animals. The suppressive function of IL-34$^{-/-}$ CD8^{+}CD45RC^{low} Treg was not affected *in vitro* and *in vivo* (in a model of wasting disease in *IL2rg*$^{-/-}$ rats injected with T cells). However, IL-34$^{-/-}$ CD4^{+}CD25^{+}CD127^{low} Treg were not able to control the wasting disease, suggesting that IL-34 is essential for their suppressive function.

Finally, we analyzed the potential of IL-34 in human allogeneic immune responses in a model of GVHD in NSG mice injected with hPBMCs. Treatment with IL-34 and rapamycin at a suboptimal dose reduced the incidence of aGVHD vs. rapamycin or IL-34 alone (median survival: 39, 28 and 14 respectively; n=4-8). The role of Tregs in this model is under investigation.

Altogether, our data demonstrate the implication of IL-34 in CD4^{+} Treg function and the relevance of IL-34 as a regulatory cytokine in aGVHD.

H. 52. Regulation of IL-10-producing T cells by polyunsaturated fatty acids

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The modern day western diet has led to an increase of n-6 polyunsaturated fatty acid (n-6 PUFA) intake and is considered an environmental factor resulting in negative health outcomes in different disease settings. In relation to the immune system, it is not well understood how changes in dietary factors like n-6 PUFAs can influence immune responses, for example during autoimmune disease. PUFAs have a variety of roles within cells such as being used as an energy source, incorporation in cell membranes, and conversion into lipid mediators that influence cell signaling and gene expression. We initially sought to understand how n-6 PUFAs influence the phenotype and function of T cell subsets from the Type 1 Diabetes mouse model (NOD) and non-diabetic control mouse models. By treating splenocytes from these mouse models in vitro with n-6 PUFA linoleic acid we discovered by flow cytometry a dose response inhibition of anti-inflammatory interleukin 10 (IL-10) in both CD4+ and CD8+ T cell subsets. While the inhibition was not strain-specific, further studies by gene expression analysis displayed a decrease of IL-10 mRNA expression and of transcription factors involved in regulation of IL-10 (c-maf, IRF4, and Bhlhe40) after linoleic acid treatment. These results demonstrate that dietary n-6 PUFAs regulate T cell cytokine production and can influence the balance of pro- and anti-inflammatory cytokines. Further studies will provide insight into the regulation of T cell subsets by fatty acids present in the organismal environment, with potential implications for the development of new therapeutics to modulate autoimmune responses.

H. 53. Serum Interleukin-27 (IL-27) Levels in Patients with Psoriatic Arthritis

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Introduction: There are lots of evidence supporting the interplay of genetic, immunologic, and environmental factors which promote pathological bone remodeling and joint damage in PsA. IL-27 was

identified as a pro-inflammatory cytokine promoting the differentiation of T helper 1 cells, through STAT-1 signalling. IL-27 is a lymphokine that controls the survival, proliferation and effector characteristics of T and B cells. Moreover, IL-27 plays important roles in bone remodelling, where an imbalance between bone resorption and formation contributes to bone destruction in inflammatory arthritis. We analyzed the possible role of serum IL-27 levels in the pathogenesis of psoriatic arthritis.

Methods: 48 patients with PsA (32 male, 16 female) and 26 healthy controls were enrolled in this study. Duration of psoriasis ranged between 8 and 96 months and mean duration of arthritis at presentation was 12.9±10.4 months. Oligoarthritis was the commonest presentation. Serum IL-27 levels were determined by ELISA.

Results: The mean serum IL-27 levels were 28,3±9.1 pg/ml in healthy controls, 63,5±27.1 pg/ml in patients with PsA. Serum IL-27 levels in patients with PsA were significantly higher than in healthy controls ($p<0.001$). Serum IL-27 levels in patients with PsA were similar in all groups. In patients with PsA, there are statistically significant correlatiıon between serum IL-27 and serum ESR and CRP levels ($r=0,504$, $p<0.01$ and $r=0.522$, $p<0.01$ respectively).

Conclusions: We demonstrated that serum IL-27 levels were significantly elevated in patients with PsA. This result suggest that IL-27 may play a significant role of in the pathogenesis of PsA.

H. 54. Suppression of inflammatory cytokine production and cell proliferation by salt-inducible kinase inhibition in human myeloid cells is isoform-dependent

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Salt-inducible kinase pan-inhibitors suppress production of TLR ligand-induced pro-inflammatory cytokines in myeloid cells and potentiate production of IL-10. To define the role of specific SIK isoforms (SIK1, SIK2, and SIK3) in induced cytokine production, we created SIK isoform single knockout (KO) THP-1 cell lines. SIK isoform expression in THP-1s is similar to that of primary human macrophages and dendritic cells with SIK2 and SIK3 expressed ~10-fold higher than SIK1. Upon TLR ligand stimulation SIK3 KO cells displayed a significant reduction in TNFα production, with SIK2 KO cells having a modest reduction, and SIK1 KO cells having no reduction in TNFα compared to controls. SIK2/1 or SIK3/1 double KOs showed similar TNFα production to their parental SIK2 or SIK3 single KO, respectively, confirming that SIK1 is dispensable. SIK2/3 double KO cells, however, lead to a significant reduction in TNFα production compared to their parental single KO lines indicating some overlapping function. In addition, we observed that SIK3-deficient THP-1 cells proliferated more slowly than SIK1 or SIK2 KO clones and that SIK2/3 double KOs led to a complete arrest of cell proliferation and induction of a macrophage-like morphology after 12-days in culture. We conclude that SIK3 and SIK2 are critical regulators of TLR-induced TNFα production and proliferation in THP-1 cells, with SIK3 having a more dominant role than SIK2 and no apparent role for SIK1.

H. 55. The transcription factor IKZF3 is associated with, but not sufficient for, IL-10 expression in human CD4+ T cells

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IL-10 expression by CD4+ T cells is an important mechanism to control immune responses. We previously showed that anti-TNF therapy increases the frequency of human IL-10+ CD4+ T cells in vivo and in vitro and identified IKZF3 as a putative transcriptional regulator. Here, we examined the expression of IL-10 and IKZF3 through flow cytometry and quantitative-PCR and manipulated IKZF3 expression using lentiviral overexpression and pharmacological inhibition.

IL-10 expression was increased in CD4+ T cells upon stimulation and was maintained at higher levels (mRNA and protein) upon culture with anti-TNF after 3 days. IKZF3 was expressed at higher levels in IL-10+ CD4+ T cells compared to other cytokine-producing cells both *ex vivo* and after CD3/CD28 activation. Pharmacological inhibition of IKZF3 using the drug lenalidomide significantly reduced the frequencies of cells expressing IL-10 after CD3/CD28 activation but was unable to affect levels of IL-10 expression *ex vivo*. Lentiviral over-expression of IKZF3 was not sufficient to induce IL-10 mRNA or protein expression. Furthermore, luciferase reporter assays using putative regulatory regions of the *IL10* locus indicated that, unlike cMAF, IKZF3 was unable to drive reporter gene expression in isolation. Finally, we show that CD4+ T cells cultured with anti-CD3/CD28 in the presence or absence of the TNF inhibitor adalimumab have increased IL-10 expression, but no increase in the expression of IKZF3.

Our findings indicate that whilst there is an association between IKZF3 and IL-10 expression in CD4+ T cells, IKZF3 is insufficient to drive IL-10 expression.

Funded by Versus Arthritis (#21139)

Diabetes and Other Autoimmune Endocrine Diseases

H. 58. Altered composition and proinflammatory function of neutrophil extracellular traps in type 1 diabetes

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Neutrophil extracellular traps (NETs) have been shown to be powerful initiators of inflammation, which lead to exploration of their role in the pathogenesis of numerous autoimmune diseases, including type 1 diabetes (T1D). Netting neutrophils infiltrate the pancreas prior to T1D onset; however, the precise nature

of their contribution to disease pathology remains poorly defined. To examine how NETs may contribute to the development of T1D, we investigated NET composition and their effect on dendritic cells (DCs), monocytes and T lymphocytes in T1D children. We showed that patient NET composition differs substantially from that of healthy controls, in particular by containing more mitochondrial DNA, more histone-associated DNA and fewer antimicrobial proteins. Additionally, the presence of NETs in a mixed PBMC culture caused a strong shift towards IFNγ-producing T lymphocytes in T1D patients, but not in healthy controls. The NET-induced activation of innate immune cells in a PBMC demonstrated by the upregulation of costimulatory molecules on myeloid and plasmacytoid DCs as well as on monocytes was observed in both healthy and T1D. NETs induced cytokine production was detectable solely on monocytes in healthy controles, whereas T1D patients displayed strong cytokine production by both monocytes and dendritic cells. Importantly, in a targeted model of monocyte-derived DCs culture, NETs induced cytokine production, phenotype change, glycolysis activation and T cell polarization towards IFNγ-producing T cells in T1D patients, but not in healthy controls. In summary, NETs composition differ and promote a distinct proinflammatory response in T1D subjects and healthy controls.

H. 59. TSHR isoforms differential expression in thyroid and thymus: implications for the autoimmune response in Graves' Disease

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Failure of tolerance to the thyrotropin receptor (TSHR) is the crucial event in Graves' disease (GD) pathogenesis. TSHR gene is associated to GD through non-coding SNPs rs179247 and rs12101255 SNPs. Two non-mutually excluding mechanisms have been proposed to explain the association: 1) Differential TSHR soluble isoforms production and 2) allele specific reduction of TSHR expression in thymus leading to peripheral and central tolerance failure respectively. To discern among them, we measured:

1) The effect of the above SNPs in the expression of the full-length transcripts (flTSHR) and isoform transcripts (ST4 and ST5) by qPCR in 39 thymus and 49 thyroid samples. There was no significant effect of GD-associated SNPs on isoform expression neither in thymic nor in thyroid. Interestingly, the expression level of flTSHR in thymus was much above the predicted, being approximately 20% of thyroid expression; ST4 expression was similar.

2) Allele-specific TSHR expression by NGS of gDNA and cDNA obtained from 19 thymi and 8 thyroids heterozygous for rs179247 that confirmed thymic unbalanced transcription and that the *TSHR* protective G allele is preferentially transcribed as reported (Colobran, 2011).

These results argue against the effect of rs179247 and rs12101255 allele on TSHR soluble isoform production by the thyroid but confirm their effect on TSHR thymic expression. The unexpected finding of

abundant TSHR short isoforms in the thymus, and more precisely, in DP thymocytes rather than in thymic epithelial cells, suggests new mechanisms by which tolerance to TSHR may be lost.

H. 60. Type 1 Diabetes Alters Key Immune Circadian Patterns

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Adaptive and innate peripheral immune populations exhibit regular circadian patterns in healthy human subjects. We sought to determine whether these rhythmic patterns are preserved in type 1 diabetes (T1D). We enrolled 10 T1D young adults (mean age 27y, 6 female) diagnosed for at least 12 mo. (mean time post-diagnosis 11y) and free of other diseases. Whole blood samples were collected every 4 hr. over 24 hr. and subjected to FACS analysis. Circadian rhythmicity patterns were determined using "COSINOR" analysis. Data were compared to a group of 10 non-T1D adult historical controls (HC) (mean age 32y, 4 female).
Daily variation within T1D subjects could be large (e.g., one subject had a 43.30% difference in the relative frequency of Granulocytic Leukocytes), but varied between cell populations from 0.34% in DC to 20.42% in unidentified lymphocytes, similar to HC. However, as a group, B-cells, DC, NKT, Treg, CD4 and CD8 naïve and effector memory populations in T1D patients all exhibited significant ($p<0.05$) circadian rhythmicity. Interestingly, compared to HC, B-cells peaked 3.0 hours later in the T1D subjects; CD4 Naïve peaked 2.6 hours earlier; CD4+CD8+ 2.1 hours later and IL-6 9.2 hours earlier. Importantly, cortisol levels did not differ between the two groups. Immune cell circadian patterns are altered in T1D. Further research is needed to investigate the etiology. Studies measuring immune cell populations in T1D must be designed with sample collection times that are appropriate given these underlying circadian patterns with collection times that optimize the likelihood of observing relevant treatment responses.

H. 61. Personalized Immune Mice Model Genetically-Predetermined Immune Dysregulation of Patients with T1D

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To assess the influence of genetic predisposition on type 1 diabetes (T1D)-associated immune abnormalities, we developed a Personalized Immune (PI) humanized mouse model, in which immune systems of T1D or healthy control (HC) donors develop *de novo* following transplantation of their bone marrow hematopoietic stem cells (HSC) and a partially HLA-matched fetal thymus into NOD.SCID.IL2Rgamma$^{-/-}$ (NSG) mice. T1D-derived mice produced fewer total thymocytes in human thymus grafts, lower numbers of single-positive CD4$^+$ cells and Tregs compared to HC-derived mice, suggesting abnormal thymopoiesis. Consistently, T1D-derived mice showed lower levels of human CD4$^+$ cell reconstitution in the periphery. Deep TCRβ sequencing of thymic and peripheral T cells from a T1D compared to a concurrent HC-derived mouse showed reduced diversity of selected thymocyte subsets and peripheral T cells in the T1D-derived animal. Peripheral T cells of T1D-derived mice showed increased proportions of activated/memory cells compared to HC-derived ones, suggesting possible HSC-intrinsic differences in T cell homeostasis. Despite decreased number of Tregs in the fetal thymic graft of T1D-derived mice, peripheral Treg frequency was similar to that in HC-derived animals and Tregs from the thymic graft had normal suppressive function. Comparison of CDR3β sequences from different thymic and peripheral T cell subsets of the PI mice to a database of T1D-reactive CDR3βs revealed fewer T1D-reactive Treg sequences in the thymus and periphery of the T1D-derived mouse compared to the HC-derived one. These data suggest that the genetic factors in T1D HSCs promote fundamental abnormalities in thymopoiesis, Treg selection and peripheral T cell homeostasis.

H. 62. Expansion of Insulin-Specific Regulatory T Cells Restricted to HLA-DQ6

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The major genetic determinant in susceptibility or protection from many autoimmune diseases, including type 1 diabetes (T1D), resides in the human leukocyte antigen (HLA) region. Particular class II alleles (e.g., HLA-DQ8) increase the risk for developing T1D, whereas others (e.g., HLA-DQ6) lead to dominant protection from T1D. HLA class II genes encode major histocompatibility (MHC) molecules that present peptides to T cells. We hypothesized that the protective DQ6 (DQB*0602) allele presents insulin peptides to regulatory CD4 T cells (Tregs), resulting in downstream anti-inflammatory responses. We expanded insulin-specific Tregs from peripheral blood mononuclear cells of DQ6$^+$ non-diabetic individuals (n=6) using an insulin B chain mimotope, known to be a strong T cell agonist in murine models and human T1D. After 7 days in culture, there were increased CD4$^+$CD25$^+$Foxp3$^+$ cells compared to no antigen (5.9% vs. 4.0%, p=0.03). In separate assays, insulin-expanded Tregs were isolated as CD4$^+$CD25^{hi}CD127^{lo}CTV^{lo} cells for T-cell receptor sequencing, performed separately by single-cell TCR sequencing and paired single-cell TCR/RNA-seq. There was good concordance between the two methodologies. Of the 122 expanded Tregs with paired αβTCR sequences from one individual, there were 61 TCRs (50%) that were present ≥2 times, indicating a skewed repertoire. The transcriptomes of the expanded Tregs were consistent with memory cells expressing Foxp3. Using a cytokine ELISPOT assay, insulin-proliferated Tregs secreted IL-10 upon repeat antigen stimulation. A TCR hybridoma was generated and confirmed response to native insulin peptides presented by DQ6. Our findings provide a mechanistic basis for understanding HLA-linked protection from autoimmune disease development.

H. 63. SUSD4 Affects Interleukin-1β Regulation of Glucose Stimulated Insulin Secretion

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Sushi domain containing protein 4 (SUSD4) is a newly discovered, but poorly characterized transmembrane protein. The structure of SUSD4 shows a high degree of homology among species, including 91% identity between mouse and man. SUSD4 is broadly expressed in human and mouse tissues including pancreatic islets. Therefore, in this study, we investigate the possible role of SUSD4 in the major function of pancreatic islets, which is insulin secretion. We found that SUSD4 KO mice displayed significantly impaired blood glucose clearance, which was aggravated with age and by a high fat diet. This defect was not due to increased insulin resistance of SUSD4 KO mice. Isolated islets from SUSD4 KO mice secreted less insulin upon stimulation with glucose and high KCl. No developmental malformation or morphological defect of pancreas or islets in SUSD4 KO mice was detected via Optical Projection Tomography (OPT), which allows the three-dimensional imaging of the spatial and quantitative distribution of the islets in the whole pancreas. RNA sequencing analysis of islets isolated from SUSD4 KO mice revealed significantly increased expression of IL-1R2, a decoy receptor for IL-1, which was confirmed by qPCR. Accordingly, blood glucose clearance was improved in WT but not SUSD4 KO mice by treatment with low levels of IL-1β that augments glucose induce insulin secretion. We propose that IL-1β signaling is impaired in SUSD4 KO mice due to increased expression of the IL-1R2 decoy receptor, which therefore prevents postprandial insulin secretion induced by low level of IL-1β.

H. 64. Use of CART Cells to Selectively Target Autoantigen-Specific T Cells for the Treatment of Autoimmune Diabetes

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Previous clinical trials using biologics-based broad-spectrum T cell- and B cell-depleting molecules for the treatment of autoimmune diabetes have shown promising, yet mixed, results. Their varied extent of success may be due to their non-specific action and failure to permanently and completely remove the pathogenic subpopulations.

As $CD8^+$ T cells, the most dominant cell type in human insulitis, are thought to be the primary mediator of b-cells damage, we thus designed a strategy by adapting chimeric antigen receptor engineered T (CART) technology to directly target these pathogenic T cells. The newly generated CAR construct maintains original transmembrane and intracellular components, while the extracellular scFv antigen-

binding domain was replaced with HLA-A2/b2-microglobulin(B_2M) complex that is linked with either diabetes-associated immunodominant antigenic peptides zinc transporter 8(ZnT8)$_{186-194}$ or negative control peptide HIV Gag$_{77-85}$. 70-80% of lentivirus-transduced Jurkat cells were stained positive with anti-HLA-A2 antibody, suggesting HLA-A2/B_2M complexes were correctly folded and expressed. Antigenic ZnT8 and HIV peptide sequences were also verified as accurate by N-terminal sequencing. To determine whether CAR signaling is sustained in transduced Jurkat cells, we co-cultured Jurkat cells together with ZnT8$_{186-194}$-reactive or non-reactive $CD8^+$ T-cells isolated from patients with T1D. As expected, expression of CD69 was significantly elevated in transduced Jurkat cells presenting ZnT8$_{186-194}$ peptide and cultured with ZnT8$_{186-194}$-reactive T-cells. Since CAR-transduced Jurkat cells can selectively recognize T cells in an antigen-specific manner, we are now expressing CAR in primary T cells to test their ability to deplete autoreactive T-cells both *in vitro* and *in vivo*.

T. 21. A New Multiplex Immunoassay Panel for Simultaneous Quantification of Major Metabolic Mediators

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Diabesity, a term for diabetes occurring in the context of obesity, continues to be one of the major human health concerns due to its global rising prevalence. Etiology of diabesity involves insulin resistance, hyperinsulinemia, metabolic dysregulation, and tissue inflammation. Simultaneous quantification of the mediators involved in glucose utilization, adipose tissue growth and development, hormonal control of homeostasis, and immune regulation is required to study the complicated interactions among complex biological processes associated with the metabolic control, homeostasis and development of diabesity. Existing methodologies for detection of metabolic protein regulators require expensive reagents and dedicated instrument. We developed inexpensive bead-based multiplex assay panels for simultaneous quantification of these metabolic mediators using regular flow cytometers commonly available in most biological research and clinical laboratories. The robust quantification panel uses highly specific antibodies and is validated to detect PAI-1, GLP-1 (Total), Insulin, C-peptide, TNF-α, Glucagon, Leptin, Cortisol, IL-1β, IL-6, and GLP-1 (Active) simultaneously in biological samples (serum, plasma, supernatant of cultured cells) with acceptable assay sensitivities, analytical accuracy and reproducibility. Pre- and post-feeding samples collected from normal and diabetic subjects showed expected profile changes for the target proteins. The multiplex assays are customizable for random combinations of targets within the panel and offer an economic and useful tool for obesity and diabetes researchers.

T. 22. A Soluble Antigen Array Vaccine Prevents Autoimmune Diabetes

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Antigen-specific immunotherapies (ASIT) are among the safest approaches to treat/prevent Type 1 diabetes (T1D) but so far have shown poor efficacy when tested in clinical trials. It is becoming evident that neoepitopes play an important role in driving T1D and may also be clinically relevant as they are often not present in healthy tissues. However, whether they have utility as part of ASITs remains to be determined. In this study, we evaluated a Soluble Antigen Array (SAgA) therapy that bundles ~10 copies of a peptide on a single hyaluronic acid (HA) backbone for late stage prevention of diabetes in the NOD mouse model of T1D. A mix of two SAgAs carrying a hybrid peptide of insulin and chromogranin A, and the p79 mimotope efficiently prevented the onset of autoimmune diabetes (p=0.0003) while each single SAgA failed to provide any significant delay or protection on its own. SAgAs elicited more robust antigen-specific T cell responses to SAgA-derived peptides as compared to equimolar doses of their corresponding free peptides. Mechanistically, SAgAs induced the upregulation of IL-10 and anergy / exhaustion markers (PD-1+, FR4+ CD73+) in antigen-specific T cells. Interestingly, this phenotype was enhanced when the linker used to graft peptides on HA was non-hydrolysable. The HA carrier itself, an FDA-approved biopolymer, also slightly reduced the incidence of diabetes (p=0.03) as compared to control mice, possibly as a result of an anti-inflammatory effect of HA. In sum, SAgAs constitute a novel and promising ASIT platform for the prevention of T1D.

T. 23. Anti-CD45RC mAb Immunotherapy Controls the Development of Auto-Immune Symptoms in an APECED Rat Model of AIRE Deficiency

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Auto-immune regulator (AIRE) is a key transcription regulator that allows negative selection by promoting the expression of tissue restricted antigens in the thymus. In human, AIRE-deficiency results in the development of autoimmune-polyendocrinopathy-candidiasis-ectodermal-dystrophy (APECED), a lethal

autoimmune disease characterized by lesions of multiple peripheral organs and production of many autoantibodies. To date, no treatments are available.

Recently, our team generated the first *Aire*$^{-/-}$ rat model. These animals harbor several key features of APECED such as alopecia, vitiligo, anti-IFNα and anti-IL-17a autoantibodies.

We previously showed that targeting CD45RC, an isoform of CD45, enables a selective depletion of effector T cells (Teffs) while preserving and boosting regulatory T cells (Tregs). Moreover, short-term anti-CD45RC mAb therapy was protective in transplantation. To address the potential of anti-CD45RC mAb immunotherapy to control APECED autoimmune symptoms, we tested this treatment in our model in a preventive setting.

First, we demonstrated that anti-CD45RC mAbs efficiently reduced alopecia and vitiligo and preserved the growth of the *Aire*$^{-/-}$ animals compared to those treated with isotype control mAbs. Besides, isotype-treated *Aire*$^{-/-}$ rats showed complete destruction of the exocrine pancreas and loss of thymus structure at only 4 months whereas these organs were totally preserved by anti-CD45RC mAbs therapy. Interestingly, this treatment also decreased the production of autoantibodies and modified Tregs' transcriptome as shown by western blot, immunofluorescence and DGE-RNAseq. Finally, analysis of PBMCs from APECED patients confirmed that the expression of CD45RC was similar to the one observed in *Aire*$^{-/-}$ rats underlying the clinical potential of CD45RC targeting in this disease.

T. 24. Antigen-specificities of Expanded clones of Islet Antigen-Reactive CD4+ T cells from Type 1 Diabetes versus Healthy Subjects

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Self-reactive T-cells can be found in both healthy individuals and individuals with autoimmune disease. To identify unique characteristics and clonality of islet antigen reactive memory CD4 T cells that relate to type 1 diabetes (T1D), we coupled CD154 enrichment of islet antigen specific T cells with novel single-cell RNA-seq and recombinant T cell receptor (TCR) expression methods. We detected predominantly private TCR clonotypes (α/β pairs) with increased clonal expansion in islet antigen reactive CD4 memory T cells in established (stage 4) T1D subjects compared with healthy controls matched for HLA, age and gender. To assess patterns of islet antigen recognition between individuals we developed viral expression of recombinant TCR sequences and performed functional assays to identify the specific islet peptides recognized by individual TCRs. Re-expression of expanded TCRs from multiple T1D subjects identified IGRP, and GAD as antigenic target in T1D subjects after diagnosis, implicating these molecules as trigger for islet antigen-reactive CD4+ T cell expansion. Current expanded studies involve comparing binding properties of islet-antigen specific TCRs to TCRs specific for foreign antigens. Characterization of recombinant TCR specificities will enable us to understand how the level of islet antigen-reactive T cells and their antigenic specificities change during disease progression and therapeutic intervention, and how to utilize these T cells as biomarkers and therapeutic targets.

T. 26. Co-Stimulation Blockade Uncouples the Relationship Between C-Peptide Decline and Changes in CD4+ and CD8+ T-Cell Subpopulations

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We previously reported that co-stimulation blockade by Abatacept, over a two-year period, significantly reduces the decline of beta-cell function and the frequency of peripheral blood $CD4^+$ central memory ($CD45RO^+/CD62L^+$) T (T_{CM}) cells in individuals with recent onset type 1 diabetes (T1D). In the placebo group of the clinical trial, we found a significant association between the increase in $CD4^+$ T_{CM} cells at a preceding visit and the decline of beta-cell function at a subsequent visit.

To extend and refine these findings, we used cryopreserved PBMCs from the original TrialNet (TN009) study and polychromatic flow and mass cytometry to characterise $CD4^+$ and $CD8^+$ naïve and memory T-cell subpopulations at greater resolution.

In the placebo group we successfully reproduced our original finding of a significant association between alterations in T_{CM} and changes in beta-cell function, and extended this to several other T-cell subpopulations. Furthermore, we found that Abatacept treatment significantly alters the frequencies of a majority of $CD4^+$, $CD4^+$ regulatory and $CD8^+$ T-cell subpopulations; in general, populations with antigen-inexperience increase, and those with antigen-experience decrease. Importantly, Abatacept also uncouples the relationship between changes in T-cell subpopulations and beta-cell function: in every case, rendering it no longer statistically significant.

These data are indicative of an immunological marker (monitoring of specific naïve/memory T-cell subpopulations) for predicting change in beta-cell function during the natural progression of T1D. Abatacept blunts this relationship, pointing to a novel mechanism of action for this successful immunotherapy that may guide other disease-modifying approaches for T1D.

T. 27. DNA demethylase TET2 in the life and death of pancreatic beta-cells

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The responses of tissues to autoimmune attack may play a role in the susceptibility and control of disease by controlling differentiation, survival mechanisms, or even cell death. The development of beta-cells is highly regulated by epigenetic mechanisms. However, there is little known about the epigenetic responses of beta-cells to the chronic inflammation leading to disease. Oxidative conversion of 5mC to 5-

hydroxymethylcytosine (5hmC) by Ten Eleven Translocation (Tet) enzymes constitutes a key step towards active DNA demethylation.

We provide evidence that TET2 protein is a key component of diabetes pathogenesis. During progression of diabetes in NOD mice, there is increased expression of *Tet2* in beta-cells that are infiltrated with immunocytes. NOD mice lacking *Tet2* have normal glucose tolerance but beta-cells are resistant to killing when the mice receive transfer of diabetogenic splenocytes. In the pancreata from humans with autoimmune or chronic pancreatitis, TET2 expression is increased in beta-cells but is reduced overall in the exocrine tissue. Further work revealed that due to lack TET2, beta-cells are desensitized to inflammation and autoimmunity, potentially through reduced IL-6 receptors and thus are insensitive to a feedforward mechanism of killing by IL-6 that can be released by inflamed beta-cells and infiltrating immune cells.

Together, our data show the importance of TET2 pattern in maintaining the beta-cells homeostasis, and the epigenetic responses of the cells to immune attack. Our studies highlight the potential remodeling of beta-cell epigenetic landscape by TET2 protein as a key component of diabetes pathogenesis.

T. 28. Effect of human probiotic bacteria on development of type 1 diabetes (T1D) in SPF and gnotobiotic NOD mice; mechanisms of mucosal immunity

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Environmental factors play a substantial role in the pathogenesis/prevention of type 1 diabetes (T1D) and its increased incidence in developed countries. Studies in spontaneous animal model of T1D, the NOD mouse, documented that the quality of SPF housing conditions and instestinal microbiom (e.g. re-derivation of breeding nucleus) modify penetrance of the diabetes incidence. In this study we tested the effect of three human probiotic bacteria *Lactobacillus plantarum, Lactobacillus casei,* and *E. coli Nissle* on the development of spontaneous diabetes incidence in the NOD mice both in the context of instestinal microbiom in SPF conditions as well as in ex-germ-free conditions. Three-weekly intragastric applications of 10^9 CFU for period of 3 weeks at age of 4 weeks led to slightly delayed and decreased diabetes incidence in SPF NOD mice, being highest for the *Lactobacillus casei* species. Parallel results were obtained in germ-free NOD mice colonized at age of 4 weeks. Changes in proportions of $CD4^+Foxp3^+$ Tregs, $CD3^+CD4^+CD45RBlow$, $CD3^+CD4^+CD62L^+$ and gamma/delta T cells were monitored by flow cytometry. Flow cytometry analysis revealed increased proportion of $CD4^+Foxp3^+$ Tregs in mesenteric lymph nodes and draining pancreatic lymph nodes, but not within the non-mucosal lymphoid compartments. We think that probiotic bacteria and immune mechanisms by which they modify development of T1D may represent a promising and inexpensive approach for a primary prevention of type 1 diabetes and/or should be also tested in combinatorial therapies. *This work was in part supported by grant AZV 16-27994A from the Ministry of Health of the Czech Republic.*

T. 29. Engineering Erythrocytes with the SQZ Cell Therapy Platform to Induce Immune Tolerance

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Current treatments for autoimmune disorders typically rely on the use of broadly immunosuppressive agents that increase the risk of adverse events in patients, such as cancer and opportunistic infections. Antigen (Ag)-specific immunotherapies have the potential to minimize these risks and improve efficacy by generating Ag-specific suppression to maintain immunohomeostasis. Several studies have established Ag-specific tolerance using red blood cells (RBCs) as a platform for delivery and Ag presentation in the context of eryptosis, a mechanism of RBC clearance. Here, we used the SQZ cell therapy platform to create Ag-loaded, pro-eryptotic RBCs to induce tolerance. In this approach, cells pass through constricted channels that cause transient permeation of the cell membrane and permit diffusion of cargo before the membrane reseals. In mice, we demonstrated that these highly delivered SQZ cells are rapidly cleared from circulation by splenic and liver-resident macrophages. The administration of ovalbumin (OVA)-SQZ cells led to a reduction of naïve OVA-specific T cell (OT-I/OT-II) proliferation and cytokine production. With OT-II cells that were activated *ex vivo*, SQZ cell treatment caused a significant decrease in T cells, particularly FOXP3-negative cells. Interestingly, FOXP3-positive OT-II cells were spared. In an autoimmune diabetes BDC2.5 T cell transfer model, mice treated with cells SQZ'd with 1040-p31 peptide delayed the onset of hyperglycemia. Additionally, SQZ cells induced tolerance in mouse models of adeno-associated virus (AAV)-gene therapy and anti-drug antibody (ADA). In summary, SQZ cells are a potentially exciting allogeneic cell therapy strategy to induce Ag-specific tolerance across a range of disease mechanisms and indications.

T. 30. Follicular Regulatory T Cells are Increased in the Peripheral Blood of Subjects with Multiple Islet-Autoantibodies but are Reduced in the Pancreatic Lymph Nodes and Spleen of Patients with Type 1 Diabetes

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Follicular regulatory T cells (T_{FR}) share many features with follicular helper T cells (T_{FH}) and conventional T_{REG}, i.e. expression of FOXP3, CXCR5 and PD-1. T_{FR} have the role to restrain the B cell helper activity of T_{FH} in germinal centers (GC) preventing autoantibodies (AAbs) production. Islet-specific AAbs are a hallmark of T1D and because islet-specific AAbs are high-affinity and class-switched, they possibly derive from GCs. This implicates T_{FH} and T_{FR} in their development. We investigated the frequency and activation phenotype of circulating T_{FR} from adult subjects with clinical and preclinical T1D (TrialNet). While the frequencies of highly activated T_{FH} (PD-1^{++}) and T_{FR} were higher in pre-symptomatic subjects with high risk for developing T1D as compared to HCs, patients with T1D had a reduced frequency of T_{FR}. Consequently, the T_{FH}/T_{FR} ratio was reduced in pre-symptomatic subjects but increased in T1D patients. Analyses of T_{FH} and T_{FR} in the spleen and pancreatic lymph nodes (PLN) of T1D patients (nPOD and San Raffaele Hospital), identified a reduction in the frequency of T_{FR} as compared to non-diabetic controls. Moreover, spleen-resident T_{FR} from T1D could not suppress B-cell helper responses *in vitro*. In summary, our findings indicate that while the T_{FH}/T_{FR} equilibrium shifts toward T_{FR} in the blood of pre-symptomatic subjects, it tilts towards T_{FH} in symptomatic individuals. They also suggest that T_{FR} are reduced from a quantitative and qualitative view in the spleen and PLN of T1D patients, suggesting that adoptive transfer of T_{FR} might provide a novel strategy to prevent T1D progression.

T. 31. Genetically Engineered Tolerogenic Dendritic Cells for Antigen-specific Immunotherapy of Type 1 Diabetes

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The development of novel approaches to selectively control antigen(Ag)-specific effector T (Teff) cell responses and to restore tolerance represents an ambitious goal for the management of type 1 diabetes (T1D). Compelling evidences support the prominent role of dendritic cells (DC) in promoting T-cell

tolerance. Methods to generate clinical grade products have been developed, leading to the application of tolerogenic (tol) DC-based therapy in clinical trials with promising results. With the aim of generating genetically engineered tolDC suitable for cell-based therapy to restore tolerance to islet antigens (Ag) in T1D, we designed lentiviral vectors (LV) which enforce expression of HLA-class-II restricted epitopes in the absence ($DC_{LV\text{-}Ag}$) or presence of tolerogenic molecules (i.e., IL-10 or IDO, $tolDC_{LV\text{-}Ag}$), thanks to the fusion of human invariant chain (Iip33) to known immunodominant peptides of T1D Ag. HLADQ8$^+$ human monocytes engineered with LVs encoding for InsB9-23 were differentiated *in vitro* in DC. When IL-10 was co-expressed, the resulting cell population acquired a tolDC phenotype, (DCs were CD14+ILT4hiCD141+CD163+), and constitutively produced high amounts of IL-10. Overexpression of IDO resulted in immature DCs able to degrade tryptophan and produce L-kynurenine in cell culture supernatants. $DC_{LV\text{-}Ag}$ were able to induce Ag-specific autologous CD4$^+$ T cell proliferation *in vitro*, while DC co-encoding for IL-10 induced a hypo-proliferative response and promoted the expansion of Ag-specific CD49b$^+$LAG-3$^+$ Tr1-like cells. Our preliminary data indicate that human tol-$DC_{LV\text{-}Ag}$ modulate autoAg-specific T cell responses *in vitro*. The success of our strategies will help designing a safe and efficient DC-based cell therapy.

T. 32. IL-17A-IL-17R signaling in photoreceptors enhances retinal oxidative stress and the onset of diabetic retinopathy

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Diabetes induces chronic-low-grade retinal inflammation that elicits vascular impairment and the development of diabetic retinopathy, which is one of the leading causes of blindness worldwide. Although diabetic retinopathy is predominantly attributed to vascular alterations, recent studies have established a novel role for inflammation and the neural retina in the development of diabetic retinopathy. Here we identified constitutive expression of the IL-17A receptor and the Act1 adaptor molecule on photoreceptors. Further, IL-17A enhanced oxidative stress through a TRAF4 signaling cascade that induced Steap4 expression and reactive oxygen species (ROS) production. Enhanced ROS production leads to capillary degeneration and vascular permeability, which are clinically meaningful abnormalities that characterize the early onset of diabetic retinopathy. These findings identify a novel IL-17A signaling cascade, and potential therapeutic targets that could delay the onset of diabetic retinopathy and vision loss.

T. 33. Increased Type 1 Diabetes Incidence Follows the Loss of the Sarco/Endoplasmic Reticulum Ca2+ ATPase in the β cell

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Activation of β-cell endoplasmic reticulum (ER) stress has been implicated in the progression of Type 1 Diabetes (T1D). Markers of β-cell ER stress were increased in mouse models of T1D and in islets from humans with T1D. ER stress is increased by the loss of ER Ca^{2+}, leading to decreased β-cell function and increased apoptosis. Intraluminal ER Ca^{2+} stores are maintained by the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA2) pump. Our group has found that SERCA2 expression is reduced in islets from mouse models of T1D prior to disease onset and in human islets from donors with T1D. Thus, we hypothesized that SERCA2-mediated ER Ca^{2+} dyshomeostasis could be a major contributor to T1D development. To test this, we generated a mouse model haploinsufficient for SERCA2 on the NOD background (NOD-$S2^{+/-}$). Compared to wild type littermates (NOD-WT), NOD-$S2^{+/-}$ mice have higher incidence of diabetes ($p<0.0001$) and incidence occurs earlier on average (14.5 wks vs 19 wks, $p<0.0001$). NOD-$S2^{+/-}$ islets also had increased MHC-I expression. To test whether SERCA2 activation may have beneficial therapeutic effects, we mimicked T1D β-cell loss with multiple-low-dose streptozotocin (MLD-STZ) in WT and SERCA2 haploinsufficent ($S2^{+/-}$) C57BL/6 mice. Following MLD-STZ treatment, $S2^{+/-}$ mice exhibited impaired glucose tolerance and higher fasting glucose levels versus WT mice. MLD-STZ mice treated with CDN1163, an allosteric activator of SERCA2, exhibited improved glucose tolerance and fasting glucose levels. Taken together, our data suggests loss of SERCA2 exacerbates T1D development and SERCA2 is a novel therapeutic target for diabetes reversal.

T. 34. Induced Pluripotent Stem Cells for Type 1 Diabetes In Vitro Modeling and Immunoprotection of β cells

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Our understanding of the causes and possible treatments for Type 1 Diabetes (T1D) has been profoundly limited by a lack of models that recapitulate human T1D. As the foundation for such a model, we have differentiated human induced pluripotent stem cells into functional β cells (hiPSC-β cells) that can reverse diabetes in mice. Herein, we propose an *in vitro* platform can model human T1D using hiPSC-β cells. We then sought to discover the genetic modifications of the T1D hiPSC-β that protect them from an immune response.

A cell-type specific, donor-matched response against the hiPSC-β but not the hiPSC-α cells was achieved *in vitro.* Specifically, co-culturing donor-matched hiPSC-β cells and PBMCs resulted in activation of the effector cells, as assayed through cytokine secretion, T cell activation and specific killing of the hiPSC-β cells. We sought to determine whether the cell killing was mediated by T cell receptor (TCR) engagement. First, we incubated the targets cells with an HLA-class I blocking antibody and second, we used a transwell co-culture system where there was no contact between the immune and hiPSC-β cells. Both experiments prevented T cell activation, confirming the TCR engagement hypothesis. Having confirmed that we are able to recapitulate aspects of T1D immune attack *in vitro*, we are interested in the ability of protecting hiPSC-β cells to avoid recurrent autoimmunity. We have generated gene modified hiPSC-β's that trigger a reduced immune response *in vitro*. Collectively this data suggests that we can immunoprotect and test hiPSC-β cells *in vitro* before autologous transplantation.

T. 35. Inhibiting IL-7 Signaling Modulates T-cell Metabolism in NOD Mice

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Type 1 Diabetes (T1D) is an autoimmune disease where insulin-releasing pancreatic β-cells are destroyed by islet-antigen-specific autoreactive T-cells. One of the marquee unknowns in T1D research is the mechanism by which autoreactive T-cells survive and activate in the periphery. Our published research has shown that blocking IL-7 signaling using an anti-IL-7Rα antibody (aIL-7Ra) prevents the onset of T1D and reverses early cases of T1D in Non-Obese Diabetic (NOD) mice; however, the precise mechanisms by which IL-7 signaling blockade inhibits T1D development has not been fully elucidated. We hypothesized that inhibition of IL-7 signaling causes changes in Nuclear Factor of Activated T-cells (NFAT) expression and mitochondrial metabolism in autoreactive T-cells, compromising their function through cell stress responses.

To test this idea, NOD mice were injected with anti-IL-7Ra antibody or rat IgG2a isotype control antibody twice during one week. Spleens were harvested from the mice and CD4+/CD8+ T cells were isolated. NFAT expression was quantified by qPCR and flow cytometry. Mitochondrial respiration was measured using the Agilent Seahorse platform and T-cell functionality was assessed by cytokine production with ELISA assays.

Blockade of IL-7 signaling in CD4+ and CD8+ T cells resulted in increased expression of NFAT mRNA and protein and decreased mitochondrial respiration due to decreases in oxygen used to produce ATP and proton leak. Furthermore, the blockade resulted in a metabolic shift away from Oxidative Phosphorylation (OXPHOS) towards glycolysis. Our results support our hypothesis that blocking IL-7 signaling delays the onset of T1D by exhausting autoreactive T-cells and decreasing OXPHOS.

T. 36. Interleukin-17A transcellular signaling induces diabetic retinopathy

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Diabetes is one of the most prevalent and growing health issues worldwide. Further, diabetes leads to progressive complications such as diabetic retinopathy, which is the leading cause of blindness within the working-age population of the Western world. Interleukin (IL)-17A is an important cytokine in the promotion and progression of diabetes. However, the mechanism by which IL-17A contributes to diabetes-mediated capillary non-perfusion and the onset of diabetic retinopathy has yet to be determined. In the current study, IL-17A was detected in the retina and Th17 cells were adhered to the retinal vasculature in STZ-induced diabetic mice, while the IL-17A receptor (IL-17R) was expressed on multiple retina cells. Diabetes-induced retinal endothelial cell death and capillary degeneration were significantly lower in IL-17A$^{-/-}$ mice. Through ex vivo studies of human cells, it was determined that retinal endothelial cell death occurs through an IL-17A/IL-17R --> Act1/FADD (Fas-associated protein with death domain) signaling cascade, which causes caspase-mediated apoptosis. These findings establish a novel pathologic role for Th17 cells in the early vasoregressive process of retinal capillary degeneration, and also identify an IL-17A-dependent apoptotic mechanism that leads to the early onset of non-proliferative diabetic retinopathy.

T. 37. LV.InsulinB9-23/Anti-CD3 Combined Therapy Inhibits Recurrence Of Autoimmunity In NOD Mice After Allogeneic Pancreatic Islets Transplant

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The induction of antigen (Ag)-specific tolerance represents a therapeutic option for Type-1 diabetes (T1D). We showed that a single administration of lentiviral vector (LV), enabling expression of insulin B chain 9-23 (InsB9-23) in hepatocytes, arrests beta-cell destruction in NOD mice at advanced pre-diabetic stage, by generating InsB9-23-specific FoxP3$^+$ T regulatory cells (Tregs). Here we show that LV.InsB in combination with a suboptimal dose (1X 5µg) of anti-CD3 mAb (combined therapy, CT5) reverts overt diabetes, prevents recurrence of autoimmunity and maintains insulin independence when provided the day after syngeneic or allogeneic pancreatic islet transplantation in 50% and 40% of treated mice, respectively. Therefore, we investigated whether CT could be optimized (LV.InsB+anti-CD3 1X25µg, CT25) to be more efficient in arresting recurrence of autoimmunity and possibly suppress allo-response to transplanted islets. To establish the CT-driven tolerogenic program prior to islet transplantation, we transplanted allogeneic islets 7 days after CT25. Results indicate that 100% of diabetic NOD mice treated with CT25 and transplanted with Balb-c islets (at glycemia <500mg/dL) remained normoglycemic for 100 days, displayed a reduced T cell responsiveness to InsB9-23 stimulation and an increased frequency of Tregs in pancreatic infiltrates and lymph-nodes. Histological analysis showed that the transplant was lost and cured mice displayed a reduced insulitis compatible with their glycemic levels.

Overall, results indicate that optimized CT25, via induction of InsB9-23-specific FoxP3$^+$ Tregs and control InsB9-23-specific effector T cells, represents a curative treatment for T1D when associated with allogeneic islets transplantation to restore activity of the endogenous beta-cell mass.

W. 52. Molecular Basis for Autoreactive T Effector Memory Differentiation in Type 1 Diabetes

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Type 1 diabetes is an autoimmune disease in which autoreactive cytotoxic CD8 T cells are found in the pancreatic islets, and are actively implicated in the destruction of insulin-secreting β cells. Our group recently tracked multiple, circulating, β cell–reactive CD8 T cells longitudinally in patients with new-onset type 1 diabetes and showed that changes in effector memory (EM) CD8 T cells expressing CD57 were positively correlated with (ie mirrored) changes in β cell function. This suggested that analysis of circulating β cell-reactive CD8 EMs provides a window into understanding ongoing islet immunopathology.

To better understand the molecular basis for these observations, in the present study, we further characterised the transcriptomic profile, chromatin accessibility, and performed single cell TCR and RNA sequencing on CD8 EMs divided according to CD57 expression. Gene signatures of cytolytic/effector function, and expression of transcription factors and receptors known to regulate CD8 differentiation are significantly enhanced in CD57+ EM cells compared with CD57-, suggesting a distinct programme for acquisition of the effector from the memory phenotype. In addition, we simultaneously tracked clonotypes and gene expression at the single cell, epitope-specific level, extending our previous understanding of clonality of CD57+/- EM cells. Multiple identical TCRA/B clones reside in these closely-related but differentially programmed populations.

In summary, we report that CD57 expression is an important distinguishing feature of a subgroup of β cell–specific EM CD8 T cells, with enhanced effector function, that could have therapeutic and biomarker potential in the context of type 1 diabetes.

W. 53. Oral Vaccination with Fc-coupled Preproinsulin Prevents Type 1 Diabetes

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Tolerogenic vaccinations with beta-cell antigens are attractive for type 1 diabetes (T1D) prevention, but have not hold their promise in clinical trials. This is probably because they have been implemented too late, i.e. in auto-antibody positive individuals with an autoimmune reaction already ongoing. We therefore devised a strategy to introduce the triggering antigen preproinsulin (PPI) during neonatal life, when autoimmunity is still silent and central tolerance mechanisms, which remain therapeutically unexploited, are more active. This strategy relies on an oral vaccine with Fc-fused PPI (PPI-Fc), which can cross the intestinal epithelium through the neonatal Fc receptor (FcRn) that physiologically delivers maternal antibodies to the offspring.
One single oral PPI-Fc dose was administered to 1-day-old G9C8 NOD mice transgenic for a PPI-specific TCR, resulting in superior diabetes prevention compared to Fc-devoid PPI. In vivo imaging, confocal microscopy and flow cytometry documented that PPI-Fc was efficiently transferred through the gut epithelium via the FcRn pathway. It was taken up by macrophages and migratory dendritic cells in the gut *lamina propria*, but it was also measurable in the serum. PPI-Fc reached the thymus, where it colocalized with dendritic cells. This systemic bio-distribution was associated with a decrease in PPI-reactive effector CD8+ T-cells and with an increase in thymic-derived Foxp3+ regulatory T cells 4 weeks after treatment. Oral PPI-Fc proved superior to Fc-devoid PPI at all these steps. It may thus provide a novel strategy for T1D prevention in genetically at-risk individuals.

W. 54. PD1 and PD-L1 in Graves' disease: new clues for pathogenesis

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At FOCIS 2018 we reported expression of PD-L1 by human thyroid follicular cells (TFCs) in glands from patients with autoimmune thyroid diseases (AITDs): Hashimoto thyroiditis (HT) and Graves' disease (GD). PDL1 expression has been recently reported in islet cells from human and mouse diabetic pancreas (Osum KC 2018; Colli ML, 2018). We have further investigated PD-1 expression in PBMCs and intrathyroidal lymphocytes (ITL) from AITD as well as the relationship of TFC PD-L1 expression with IFNs.

The proportion of PD-1+ CD4 T cells, but not CD8+, was moderately increased in peripheral CD4 T cells from GD patients compared to HC (16.9±5.4 vs. 9.8±5.4, $p<0.05$). More interestingly, in IFL stained cryostat sections from 9 GD and 5 HT thyroid glands we found that 58,2 and 59,1% of CD4 and CD8 respectively, expressed PD-1. Of them, approximately one third were PD1 bright. The phenotype corresponded to central (CD45RA-CCR7+) and effector (CD45RA-CCR7-) memory T cells. On the other hand, we further assessed PD-L1 expression by TFCs and found that it correlated with IFNG gene expression by qPCR, but not with IFNA1, IFNA4 nor IFNB1.

The finding that of PDL1+ TFC and PD1+ T cells coexist in close proximity in AITD thyroid glands suggests that autoreactive T cells may be actively inhibited by PD-L1+ TFC. This could be a physiological mechanism to reduce the risk autoimmunity in inflamed tissue. Once the autoimmune disease is established, it may slow its progression and, in the case of AITD, explain its very protracted clinical course.

W. 55. Redirecting TCR specificity in regulatory T cells toward class I HLA-restricted islet antigens in type 1 diabetes

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CD8+ effector T cells largely contribute in the destruction of human pancreatic beta cells suggesting that MHC class I is abundant in the inflamed islets and can be employed to redirect regulatory T cells (Tregs) specificities to islets.

We developed novel methods allowing: (1) efficient Tregs expansion in which the endogenous TCR is removed using a non-viral CRISPR-based approach combined with lentiviral transduction for the insertion of an engineered TCR, (2) non-viral substitution of the CD4 co-receptor with CD8α chain, (3) human islets transplantation into the spleen of NSG mice to increase the interaction with transferred human T cells.

Nine day after electroporation, mean efficiency of TCR knockout (KO) in Tregs was 74% for TRAC (n=3) and 79% for TRBC (n=5), which significantly impaired TCR-stimulated suppression of Tregs *in vitro.* An HLA-A2 restricted TCR specific for preproinsulin (PPI_{15-24}) was cloned into lentivirus and transduced in TCRKO T cells restoring up to 100% TCR expression. Importantly PPI_{15-24} tetramer staining was

detected only on CD8 T cells demonstrating that antigen engagement by this TCR is CD8 co-receptor dependent. Using a non-viral approach, we inserted the CD8α chain into the CD4 locus with up to 25% efficiency. Finally, we transplanted 4000 human islets equivalent into spleens of NSG mice which reverted streptozotocin-induced diabetes in 6/9 mice up to 100 days.

Altogether, we generated the tools to engineer Tregs with HLA-class I restricted specificity. Preclinical testing of Tregs suppression in humanized mouse model for type 1 diabetes is currently under investigation.

W. 56. Single cell characterization of CD8 T-cells and their role in disease progression in Type 1 Diabetes

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Type 1 Diabetes is a disease caused by immune mediated destruction of beta cell mass, resulting in the loss of glycemic control. Following disease onset, subjects with Type 1 Diabetes exhibit diverse amounts of residual c-peptide, indicating varying levels of endogenous insulin production and beta cell function. Persistence of residual c-peptide is correlated with improved glycemic control and reduced risk of complications and its preservation has been used as a clinical endpoint in clinical trials. However, the immunologic factors that differentiate subjects who maintain measurable levels of c-peptide (slow progressors) from those whose levels continue to decline (rapid progressors) are not well characterized. Given their established relevance, we characterized the number, phenotype and transcriptional profiles of beta cell specific CD8+ T-cells in a cohort of subjects, stratified into slow progressors (those with c-peptide > 0.1ng/ml) and rapid progressors (those with undetectable c-peptide). Using a pool of tetramers corresponding to established HLA-A2 epitopes, we enumerated beta cell specific CD8+ T-cells in peripheral blood and sorted a portion of these cells for characterization by single cell RNA-Seq. Differential gene expression between slow and rapid progressors indicated differences in effector, exhaustion, and regulatory pathways. Assembly of autoantigen specific CD8 TCRs allowed analysis of diversity, revealing differences in clonal expansion between rapid and slow progressors. Cumulatively, our analysis uncovered aspects of T cell function and repertoire that correlate with disease progression.

W. 57. The Mechanism by which IL-17A contributes to MMP-9 production during diabetic retinopathy

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Diabetic retinopathy is a diabetes-mediated retinal microvascular disease, and is the leading cause of blindness in the working-age population in the Western World. Interleukin-17A (IL-17A) and matrix metallopeptidase 9 (MMP-9) have both been identified as key inflammatory processes that induce retinal

pathogenesis and the onset of diabetic retinopathy. Here the role of IL-17A in the production and activation of MMP-9 in retina, as wells as the signaling mechanism that enhances MMP-9 activity was investigated. It was discovered that diabetes-mediated MMP-9 production and activity was significantly decreased in IL17A$^{-/-}$ STZ-diabetic mice. Also through *ex vivo* studies of human retina cells, it was determined that IL-17A induced IL-17-receptor-expressing Muller glia to produce MMP-9 by activating the IL-17R-Act1-TRAF6 pathway. These findings not only establish a novel IL-17A signaling mechanism in the retina, but also identify potential therapeutic targets that could inhibit retinal pathogenesis and the development of diabetic retinopathy.

W. 58. The PTPN22 R620W SNP is associated with alterations in the frequency and phenotypes of islet antigen-reactive CD4 T cells in T1D.

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Risk for type 1 diabetes (T1D) is associated with a non-synonymous variant in the *PTPN22* gene, R620W. PTPN22 is a tyrosine phosphatase which regulates TCR signaling, leading to changes in T cell activation and effector responses. We hypothesized that the *PTPN22* R620W risk allele may alter the activation and differentiation of islet antigen reactive CD4 T cells in T1D, impacting their frequency and/or effector phenotypes. To assess this, we recruited 36 HLA matched T1D subjects, with equal numbers of non-risk and heterozygous risk individuals, and analyzed their islet and viral antigen-reactive CD4 T cells in peripheral blood using a CD154 activation assay coupled with multicolor flow cytometry. We calculated the frequency of islet-reactive CD4 T cells and analyzed their phenotypic profile using unbiased multidimensional flow cytometry analysis with flowSOM. We found that T1D patients carrying the *PTPN22* risk allele had more islet-reactive CD4 T cells earlier in disease compared to non-risk subjects. Overall the islet-reactive CD4 T cells were less mature (predominantly naive, stem cell memory (TSCM)) than viral-reactive cells (effector memory, central memory). Further, the phenotypes of antigen-specific T cells differed between subjects based on *PTPN22* genotype; PTPN22 risk patients had more TSCM phenotypes amongst islet-reactive T cells, with expression of activation markers and inhibitory receptors, while non-risk patients had more CD4 T effector memory/Th1 phenotypes amongst viral-reactive cells. Our findings demonstrate that the *PTPN22* risk allele influences the frequency and phenotypes of islet-reactive CD4 T cells in T1D favoring activation and expansion of TSCM-like cells.

W. 59. The Role of Aire in the selection of Regulatory T cells in Diabetes

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(1) In autoimmune diseases like T1D, it is proposed that failure in central tolerance mechanisms towards pancreatic islet self-antigens leads to the escape of pathogenic clones and loss of critical immunosuppressive antigen-specific Tregs. The importance of Aire in maintaining central tolerance against self-reactive antigens is evidenced by the multiorgan autoimmune disease seen in individuals with mutation the Aire gene. When Aire-expression is specifically ablated in mouse mTECs, there is a dramatic increase in the number of self-reactive T cells that escape negative selection, in addition, a dramatic loss of FoxP3+ Tregs. Utilizing novel high-throughput platforms and single-cell DNA barcoding technology has allowed us to assess several thousand $CD4^+$ T-cell clones. The use of FoxP3-GFP reporter mice permits us to identify individual $FoxP3^+$ Tregs that have undergone Aire-dependent selection in the thymus and compare the TCR repertoire to mice that have had Aire specifically ablated from the mTECs. Identification of unique clones and gene signatures within our large datasets has allowed us to start to qualitatively assess the functional role of the identified TCRs within the model of T1D. In addition, the peptide specificities of the identified TCRs are currently being evaluated using *Baculovirus* MHC-II peptide libraries. Our proposed study aims to expand our knowledge on TCR repertoire of Aire-dependent $FoxP3^+$ Tregs and the antigen-specificity in T1D, improving our understanding of the respective function of thymic-derived antigen-specific Treg suppression in peripheral tissues associated with autoimmune T1D.

W. 60. Pancreatic and β cell responses to checkpoint inhibition

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Therapeutic agents targeting cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed death-1 (PD-1), known as checkpoint inhibitors (CPIs), have revolutionized cancer treatment by enhancing anti-tumor immune responses. While CPIs increase tumor destruction, they can cause a breakdown in self-tolerance resulting in autoimmune complications such as diabetes. The underlying mechanisms of CPI diabetes remain unknown. To understand β cell killing in patients treated with CPIs, we analyzed clinical and β cell changes in the setting of CPI. We observed elevated lipase and decreased pancreatic volume in patients with CPI diabetes consistent with exocrine pancreas inflammation. IFNy was produced by immune cells in response to human β cells in mixed lymphocyte reactions with anti-PD-1. We cultured human islets with IFNy and analyzed β cell responses by single cell RNAseq. RNAseq revealed higher expression of immune inhibitory ligands, PD-L1 and indoleamine 2,3-dioxygenase-1 (IDO1) in IFNγ treated cells, which was confirmed in cultured islets. PD-L1 expression was strongly correlated with STAT1 and inhibition of STAT1 eliminated upregulation of PD-L1 in β cells. We identified differentially expressed genes between PD-L1+ and PD-L1- β cells including those involved in regulation of apoptosis. Fas expression was higher in PD-L1+ versus PD-L1- β cells and increased apoptosis was observed in PD-L1+ cells. PD-L1 and IDO1 expression were observed in β cells in pancreatic tissue with known inflammation including a patient with CPI diabetes, suggesting a role for these molecules *in vivo*. Expression of immune inhibitor molecules seem to identify β cells under cellular stress that are susceptible to killing.

W. 61. Exhaustion of Autoreactive CD8+ T Cells Distinguishes Slow Progression of Type 1 Diabetes

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Although most type 1 diabetes (T1D) subjects retain some functional insulin-producing islet beta cells at the time of diagnosis, the rate of further beta cell loss varies across individuals. It is not clear what drives this differential progression rate. CD8+ T cells are implicated in the autoimmune destruction of beta cells. Here, we addressed whether the phenotype and function of autoreactive CD8+ T cells influence disease progression. We identified and characterized islet-specific CD8+ T cells using high-content single-cell mass cytometry in combination with peptide-loaded MHC tetramer staining, and applied a new analytical method, DISCOV-R, for phenotyping these rare subsets. We found that islet-specific CD8+ T cells were phenotypically heterogeneous within an individual, yet several dominant phenotypes were shared across T1D subjects. One shared phenotype resembled activated memory cells and was significantly more frequent among rapid progressors. In contrast, slow disease progression was associated with an exhaustion-like profile, with expression of multiple inhibitory receptors, limited cytokine production, and reduced proliferative capacity. Increased exhaustion was not solely driven by disease duration, indicating that additional mechanisms influence exhaustion of autoreactive T cells. Thus, we linked the exhaustion of islet-specific CD8+ T cells with the rate of T1D disease progression, suggesting potential benefit of therapeutics which augment T cell exhaustion.

General Autoimmunity

H. 66. Single-cell analysis of autoreactive CD4 T cells reveals an atypical B helper signature in autoimmune hepatitis

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In autoimmune disorders, CD4 T cell-dependent chronic B cell activation towards multiple self-antigens promotes the production of pathogenic autoantibodies. However, the molecular signature and precise phenotype of autoreactive CD4 T cells in human autoimmunity have been elusive, even more so for rare diseases like autoimmune hepatitis (AIH). Here, we combined brief *ex vivo* restimulation with pools of overlapping antigen-derived peptides and integrative single-cell RNAseq (scRNAseq) to characterize the circulating CD4 T cell response against Soluble Liver Antigen (SLA or SepSecs) in seropositive (defined as positive for anti-SLA auto-antibodies) and seronegative AIH patients. Only seropositive AIH patients had detectable amounts of SLA-specific CD4 T cells, which had a conserved memory CXCR5^{neg} PD-1^{high} CCR6^{neg} phenotype, and an atypical B helper molecular profile with high expression of *IL21*, *IFNG*, *TIGIT* and *CTLA4* among other genes. Strikingly, the expanded SLA-specific CD4 TCR clonotypes identified by scRNAseq were only found in the circulating CXCR5^{neg} PD-1^{high} CD4 T cell population, which was significantly increased in the blood of AIH patients and shared B helper capacity with the classical CXCR5^{high} PD-1^{high} CD4 T_{FH} population. Altogether, our results provide for the first time a fine and comprehensive characterization of autoreactive CD4 T cells in the rare autoimmune disorder AIH, thereby identifying the phenotypic niche and specific molecular signature of pathogenic B helper self-reactive CD4 T cells.

H. 68. Role of C4A and C4B in SLE susceptibility and development

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SLE is a systemic autoimmune disease notably characterized by the production of pathologic autoantibodies by B cells. Deficiencies in proteins implicated in the clearance pathway, such as the complement C4, have been rapidly associated with SLE susceptibility. The human complement C4 locus encodes two paralogous genes, C4A and C4B that are 99% identical but differ in the isotypic region, which defines covalent binding chemical specificity. Interestingly, human genetic studies showed that C4A allele is protective against SLE development while C4B is not.

In order to understand the functional differences of the two isoforms *in vivo*, we used CRISPR/Cas9 technology to convert the murine isotypic region into either human C4A or C4B sequence. These newly generated mice were then crossed with the lupus mouse strain 564Igi to compare C4A and C4B functions in autoimmunity.

Our results showed that despite similar C4 expression levels in both strains, C4B.564Igi mice exhibited a higher percentage of autoreactive B cells in secondary lymphoid organs compared to C4A.564Ig. Besides, germinal center number and size in spleen were decreased in C4A.564Igi mice compared to C4B.564Igi mice, reflecting a dampening of immune responses. By using the mouse model AicdaCre^{ERT2}-EYFP, we were able to identify a strong decrease of the memory B cell population in C4A.564Igi compared to C4B.564Igi mice. Finally we showed that compared to C4B, C4A is more efficient to induce apoptotic bodies uptake by macrophages *in vivo*. Thus, this study demonstrates that

C4A binds immune complexes more efficiently than C4B and therefore help maintain tolerance in periphery.

H. 69. Study of Allele-Specific Expression Reveals Subset- and State- dependent Regulation of Select Genes involved in TNF Signaling by Autoimmunity-Associated Variants

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In diploid organisms, transcription of most genes is bi-allelic with expected equal transcripts originating from each the maternal and paternal alleles. However, this could be altered in a heterozygous individual when the genomic region harbors a variant that regulates transcription, resulting in allelic imbalance. The increased power that is inherent in using the other allele from the same subject has made Allele Specific Expression (ASE) a useful tool to study regulatory non-coding variants. In this study, we used Allele Specific Expression to guide our investigation of four autoimmunity-associated Single Nucleotide Polymorphisms (SNPs) that are intronic in - or proximal to - TNFSF14/LIGHT, its receptor TNFRSF14/HVEM, TNFRSF5/CD40 as well as TRAF3 (TNF receptor associated factor 3) which had previously been shown to interact with both TNFSF14 and CD40. One of the main challenges in translating findings from Genome Wide Association Studies (GWAS) to functional understanding is the lack of the cellular context in which these variants may promote disease. To address this, we derived/differentiated more than 30 cell populations under different activation states from peripheral blood mononuclear cells (PBMC) of five healthy volunteers who are heterozygous for all four SNPs recruited through Cambridge Bioresource. We then used a targeted Next Generation Sequencing (NGS) approach to survey ASE in these cell subsets/activation conditions. We show that while some variants affected expression across multiple subsets, some are associated with a statistically significant ASE only in a specific cell type and in some cases only following activation or in-vitro differentiation.

H. 70. Study of genetic variants of the MTHFR and FGG systems and their association with adipokynes in Pediatric Lupus Nephritis-pLN-. In search of risk factors associated with atheromatosis

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Background: SLE is a chronic autoimmune disease of unknown etiology. pNL is the most frequent complication with the worst prognosis. A chronic systemic inflammatory cascade with high atherogenic and procoagulant potential occurs in SLE. Atheromatosis is a multifactorial inflammatory subclinical process, which is the basis of the plaques that lead to cardiovascular disease in pSLE. Adipokines play a role in atherosclerosis and it is a risk for stroke associated with SLE. The aim of this study was to evaluate the association of serum Leptin and Adiponectin and SNP polymorphisms of the MTHFR (A1298C) and FGG (C10034T) genes, which are associated with metabolic pathways of Atheromatosis in pLN.

Methodology: This was a cross-sectional analytical observational study of pLN. Cases (n = 98) and controls (n = 100), chosen by simple random sampling from a nested cohort of one our previous pLN project. Serum concentrations of Leptin and Adiponectin were performed by ELISA. Polymorphisms of the MTHFR (A1298C) and FGG (C10034T) genes were performed by qPCR. The statistical significance was interpreted as $p<0.05$.

Results and conclusions: In the present study, a statistically significant difference was found between the serum Leptin and Adiponectin pNL subjects levels when those were compared with the controls (p = 0.00). No association was found between the SNPs polymorphisms of the FGG and MTHFR genes with NLp subjects, however, when analyzing the influence of these polymorphisms on the serum adipokines, a relationship was found between the genotypes of the MTHFR gene and FGG with these adipokines.

H. 71. The IL6RAsp358Ala autoimmunity risk genotype is associated with alterations in the transcriptional signature of T cells in response to IL-6

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IL-6 is a pleiotropic cytokine with multiple roles in the adaptive immune response resulting in the promotion of inflammation. Elevated IL-6 and membrane-bound IL-6R expression is associated with autoimmune disease, and therapeutics that target the IL-6 signaling pathway are effective in rheumatoid arthritis, yet little is known about how the increased expression of IL-6R affects the development of autoimmune disease. A genetic variant, *IL6Rasp358Ala*, is associated with T1D and RA and alters mbIL6RA expression on T cells. To better understand how differences in mbIL6RA may influence T cells, we performed bulk RNA-sequencing to assess the IL-6 response of CD4 and CD8 T cells from a cohort of 31 healthy individuals with *IL6R*Asp358Ala genotypes associated with either high (A/A; n=16) or low (C/C; n=15) mbIL-6R expression. T cell activation and differentiation pathways were enriched in the A/A but not C/C CD4 transcript signature after 4 hours of stimulation, while activation pathways were enriched in the C/C but not A/A CD4 signature after 24 hours, suggesting that higher mbIL6RA expression may affect IL-6 signaling kinetics. Genes differentially regulated by IL-6 in this experiment showed little overlap with gene sets previously identified under other activating conditions, indicating distinct gene regulation by IL-6 stimulation alone. These results suggest that increased mbIL-6R expression is associated with an enhanced, distinct response to IL-6. Future studies will determine the functional impact of signaling response differences in healthy and autoimmune subjects and investigate potential mechanisms by which enhanced mbIL6RA expression contributes to development of autoimmune disease.

H. 72. The Vm24 Scorpion Toxin Blocks Kv1.3 Potassium Channels and Attenuates the Effector Memory T Cell Response

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T effector memory (T_{EM}) cells have a critical role in the secondary immune response and in the pathogenesis of different autoimmune diseases. Following activation, the number of $K_v1.3$ channels on the T_{EM} cell membrane dramatically increases. Blockade of $K_v1.3$ channels results in inhibition of Ca^{2+} signaling in T_{EM} cells, thus exerting an immunomodulatory effect. Since we observed that the peptide toxin (Vm24) isolated from the Mexican scorpion *Vaejovis mexicanus* completely and selectively blocked $K_v1.3$ channels currents, without impairing T_{EM} cell viability, we decided to use it to investigate the molecular events that follow $K_v1.3$ blockade in human $CD4^+$ T_{EM} lymphocytes. We found that under TCR stimulation, Vm24 inhibited the expression of the activation markers CD25 and CD40L (but not that of CD69), the secretion of the pro-inflammatory cytokines IFN-γ, GM-CSF and TNF, as well as the release of the Th2 cytokines IL-4, IL-5, IL-9, IL-10, and IL-13. A similar inhibitory pattern was exerted by Vm24 on T cells isolated from patients with rheumatoid arthritis. On the other hand, a proteomic analysis of TCR-activated T_{EM} cells indicated that the biological processes mainly affected by the blockade of $K_v1.3$ channels were cytokine-cytokine receptor interactions, mRNA processing via spliceosome, the response to unfolded proteins and intracellular vesicle transport, targeting the cell protein synthesis machinery. Altogether, these results underscore the role of $K_v1.3$ channels in regulating T_{EM} lymphocyte function and highlight the potential use of the Vm24 peptide as an immunomodulatory agent for the therapy of conditions mediated by Th1 and Th2 lymphocytes.

H. 75. Patients with PIK3CD gain-of-function mutations show PI3K controls B cell self-tolerance outside the germinal center

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Antibody-mediated autoimmune diseases are a major health burden. However, our understanding of how self-reactive B cells escape self-tolerance checkpoints to secrete pathogenic autoantibodies remains incomplete.Inborn errors of immunity resulting from single gene mutations that disrupt immune function can cause immune dysregulation resulting in both immune deficiency and autoimmunity. These "experiments of nature" provide key insights into the critical pathways that control appropriate immune

function. Gain of function (GOF) mutations in *PIK3CD*, encoding the p110δ subunit of PI3-kinase, cause a primary immunodeficiency characterized by recurrent respiratory infections, lymphoproliferation, increased susceptibility to herpes viruses, poor Ab responses to polysaccharide Ags, and altered serum immunoglobulin levels. Interestingly, many of these patients also suffer from a range of autoimmune conditions such as autoimmune cytopenias, glomerulonephritis and autoimmune thyroiditis. Here we demonstrate that patients with*PIK3CD*GOF mutations have highly penetrant secretion of autoreactive IgM antibodies. In mice with the corresponding *Pik3cd*activating mutation, self-reactive B cells exhibit a cell-autonomous subversion of their response to self-antigen: instead of becoming tolerized and repressed from secreting autoantibody, *Pik3cd*gain-of-function B cells are activated by self-Ag to form plasmablasts that secrete high titers of germline encoded IgM autoantibody and hypermutating germinal center B cells. These data show that PI3K is a pre-germinal center gatekeeper of B cell self-tolerance and thus represents an attractive druggable pathway to treat antibody-mediated autoimmunity.

W. 62. Targeting Highly Differentiated KLRG1+ Cytotoxic T cells in Autoimmunity and Large Granular Lymphocytic Leukemia

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A number of specific autoimmune diseases, including inclusion body myositis (IBM), primary biliary cholangitis (PBC), and Sjogren's syndrome (SS), and T cell large granular lymphocytic leukemia (T-LGLL) are highly refractory to immunotherapies and appear to involve highly differentiated cytotoxic T cell mediated tissue injury. Using microarray technology applied to human tissue disease samples, we identified a signature of T cell cytotoxicity coupled with a signature of highly differentiated CD8+ T cell effector memory (TEM) and terminally differentiated effector (TEMRA) cells, and confirmed these signatures through immunohistochemistry and flow cytometry of patient samples. We then applied bioinformatics to identify a therapeutic strategy of targeting these populations of cells for depletion through their surface expression of killer cell lectin-like receptor G1 (KLRG1), demonstrated the correlation of KLRG1 gene expression with T cell cytotoxicity across 28,870 human tissue samples, and validated the presence of KLRG1 on patient tissue infiltrating and blood circulating pathogenic T cells. We furthermore demonstrate that human regulatory T cells lack KLRG1 expression, so that targeted depletion of KLRG1+ cells, unlike broader T cell depletions that deplete regulatory cells using siplizumab, alemtuzumab, daclizumab, or anti-thymocyte globulin, is an improved strategy for targeting cytotoxic T cell mediated autoimmune diseases. We then developed a humanized afucosylated anti-KLRG1 antibody (ABC-008) with potent antibody-dependent cell-mediated cytotoxicity (ADCC) and demonstrated its ability to deplete cytotoxic T cells in non-human primates. Anti-KLRG1 depletion therapy with ABC-008 is a rational strategy for diseases characterized by chronic, refractory expansions of cytotoxic lymphocytes, including IBM, PBC, and T-LGLL.

W. 63. Altered T cell Activation and Apoptotic Pathways Differentiate European American Autoantibody-Positive Healthy Individuals

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Anti-nuclear antibodies (ANA) are a hallmark feature of autoimmunity, but are found in ~20% of healthy women, most that will never develop pathogenic autoimmunity. Further, African American (AA) women are more likely to develop SLE compared to women of European (EA) background. Understanding changes in immune pathways between ANA+ healthy individuals and SLE subjects remains a crucial goal in understanding benign and pathogenic autoimmunity. To assess immune alterations in ANA+ healthy individuals, phenotyping by mass cytometry, plasma cytokine measurement by luminex and altered TCR and BCR phopho-specific signaling were evaluated in EA and AA individuals matched in 24 groups (n=72) as ANA- healthy, ANA+ healthy or SLE patients. Further, whole genome RNA-sequencing was performed on sorted T cells, B cells and monocytes. EA ANA+ controls exhibited greater immune regulation with reduced T and NK cell numbers, and increased expression of CD85j compared to ANA- controls. Further, modules associated with hematopoiesis, T cell activation, intrinsic apoptosis, and autophagy signaling pathways are expressed at higher levels in T cells of EA ANA+ individuals. In contrast, AA ANA+ controls had modules associated with the Th1 pathway and IFN signaling present that were expressed at the highest levels in SLE patient T cells. Following TCR stimulation, EA ANA+ T cells exhibited greater ERK phosphorylation that was absent in SLE patients suggesting early dysregulation leading to exhaustion. These results suggest that mechanisms of preclinical autoimmunity vary by ethnicity, and ANA+ European Americans may have more effective regulatory mechanisms in place to prevent transition to SLE.

W. 64. Analysis of Lupus Nephritis (LN) Gene Expression Reveals Dysregulation of Pathogenic Pathways Activated within Infiltrating Cells.

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Much is still to be learned about immune & inflammatory pathways in LN[1]. A bioinformatic approach (LIMMA-DE & WGCNA) analyzed gene expression of LN biopsies microdissected for glomerulus and tubulointerstitum. Genes differing between LN & healthy individuals were interrogated for cell type specific gene signatures using GSVA validation of I- or T- Scope® analysis of immune or non-immune subsets. Podocytes are in WGCNA modules negatively correlated with WHO class. Genes were functionally characterized using BIG-C® and pathways elucidated using IPA®. LN has an immune cell signature in WGCNA modules positively correlated with WHO class (granulocytes, pDC, DC, myeloid cells, $CD4^+$, & $CD8^+$ Ts, Bs as well as pre- and post-switch PCs as indicated by IgM, IgD, and IgG1 HC genes). The presence of both Ig -κ & -λ as well as VL genes suggests polyclonal activation. Chemokines that mediate lymphocyte organization and/or recruitment into lupus kidney are present. Cytokine (TNF, CD40L, IL1β, IL2, IL6, IL12, IL17, IL23, & IL27) & signaling (PI3K,NF-κB,NF-AT, and p70S6K) pathways as well as

proliferation and HDAC activity are evident. IPA® UPR analysis indicated ongoing signaling by cytokines such as TNF, IFNγ, IFNα, CD40L, IL1β, IL2, IL6, & IL17. Interestingly, connectivity analysis using LINCS/CLUE elucidated high priority drug targets such as IFNb (PF-06823859), IL12 (Ustekinumab) and S1PR(Fingolimod) that may prove to be good options for therapeutic intervention.

[1] J.Immunol.189:988(2012).

W. 65. Association of FokI polymorphism in the Vitamin D Receptor Gene with Adults systemic lupus erythematosus (SLE). A Case-Control Study in Colombian Caribbean patients.

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Background: SLE is a complex autoimmune disease whose severity is associated with different factors. Vitamin-D and polymorphisms in VDR have been associated with chronic inflammatory diseases included SLE in different ethnic groups around of the world.

Objectives: To analyze the genetic association of the Vitamin-D-Receptor (VDR) SNPs: TaqI, ApaI, BsmI and FokI with susceptibility to SLE as the association with Vitamin-D serum levels.

Methods: This case-control study included 133 SLE adult patients and 100 healthy controls. SNPs were genotyped by qPCR and Taqman® probes. Allelic, genotypic and haplotypic association were estimated. Serum levels vitamin-D were quantified by ELISA, normal reference values for this study: 30 to 100 ng/ml. p-values <0.05 was considered statistically significant.

Results: Our results showed that female gender had a higher prevalence of SLE (94%) unlike controls (59%) (pG] was associated with SLE (41%) compared to healthy controls (30.5%) behaving like a risk factor for SLE [OR:1.58; 95%IC: 1.05 - 2.36]. Haplotype A/C/C/A [TaqI / ApaI / BsmI / FokI] was found to be a risk factor for SLE [OR=2.28, 95%CI=1.12-4.66, psim<0.01]. Regarding serum levels of vitamin-D, 11.3% of patients showing insufficient levels. In addition, 88.7% of our SLE patients showed sufficient levels of vitamin D.

Conclusion: This study analyzed the association of the VDR SNPs with SLE conducted in Colombian adult patients. Our results demonstrate that FokI and haplotypes in VDR were associated with SLE. No deficiency of vitamin-D was observed.

W. 66. Autoimmunity Is Promoted By Dysregulated RasGRP1 Expression

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RasGRP1 is a Ras guanine nucleotide exchange factor (GEF), and an essential regulator of lymphocyte receptor signaling. Aberrant expression of RasGRP1 results in defective positive thymocyte selection in mice. Moreover, recent reports describe RasGRP1 deficient patients that suffer from recurrent infections and autoimmunity. It is unknown how RasGRP1 levels are regulated and how aberrant expression contributes to autoimmunity.

We demonstrated that T cell positive selection was mildly impaired in rasgrp1$^{+/-}$ C57/B6 mice and severely impaired in rasgrp1$^{-/-}$ C57/B6 mice, which resulted in elevated levels of serum antinuclear antibodies, indicating that loss of RasGRP1 expression causes inflammatory disease and the severity of the phenotype is inversely correlated with RasGRP1 expression levels.

In patients with autoimmunity, we only detected decreased RASGRP1 mRNA levels in peripheral CD4$^+$ T cells during active inflammation.
Next, by analyzing H3K27 acetylation profiles, we identified two 'autoimmunity hotspots' in the RasGRP1 gene, that showed distinct H3K27 acetylation in CD4$^+$ T cells, and contained autoimmunity-associated SNPs. CRISPR-Cas9 editing of the 'autoimmunity hotspot' upstream of the RasGRP1 promoter, resulted in lower RasGRP1 expression and Ras-MAPK signaling, proving its enhancer function.
Furthermore, with affinity-purification mass spectrometry, we showed that mutation of this hotspot led to decreased binding of transcription factors Runx1, and ZBTB9.

In conclusion, we show for the first time how expression of RasGRP1 is regulated, and that proper regulation of RASGRP1 expression is vital to prevent inflammatory disease.

W. 67. Autoreactivity Supports the Maintenance and Active Participation of Wild-type B cells in the Germinal Center.

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Introduction. The 564Igi mouse is a murine model of SLE, generated by knock-in of a B cell receptor of an autoreactive B-cell clone. Characterization of mixed BM chimeras (of 564Igi and wild-type (WT) mice) demonstrate that WT B-cells acquired new targets of auto-reactivity, became self-sustained and gained independence from the initial trigger (ARTEMIS model). We now further progress on these findings by utilizing the parabiosis model, in which 564Igi and WT mice are surgically joined and lymphocytes can subsequently freely exchange between the mice without the initial lymphopenia and need for irradiation

as in the ARTEMIS model.
Results. An exchange of B- and T cells could already be noticed as early as 6 days post-surgery. After 2 weeks half of the B- and T-cells in the 564Igi mouse were of WT origin (n=6). At this point the mice were separated but circulating WT B-cells remained at a stable level for several weeks post-separation while WT T-cells gradually decreased (n=4). In the germinal center (GC), the majority (~80%) of B-cells were WT, while ~20% of T-cells. To test whether WT B-cells are actively engaged in the GC process, we transferred splenocytes of AID-Cre-Ert2-eYFP mice into 564Igi and WT mice. YFP expression in the 564Igi mouse was evident in WT GC B-cells (15%, n=3), but almost absent in B6 (0.6%, n=4) mice, indicating active GC participation of WT B-cells in 564Igi mice.
Conclusion. The 564Igi murine model of SLE supports the maintenance and active participation of WT B-cells in the germinal center.

W. 68. CD45 Antibody Drug Conjugate Safely Conditions for Transplant and Evaluation in Murine Models of Autoimmune Disease

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Autologous hematopoietic stem cell transplant (autoHSCT) is a highly effective treatment for multiple autoimmune diseases. AutoHSCT can induce long-term remission (up to 15 years) with 70-80% progression free survival in patients with relapsed refractory and secondary progressive MS subtypes (Muraro 2017). Likewise, patients with systemic scleroderma achieved 74% event-free survival at 72 months following autoHSCT (Sullivan, 2018). These results are achieved by eradication of autoreactive immune cells and re-establishment of a self-tolerant immune system. However, only a small fraction of eligible patients elect to undergo autoHSCT, in part due to complications associated with current conditioning. To address these issues, we are developing antibody drug conjugates (ADCs) targeting CD45, a target expressed throughout the hematopoietic system to enable simultaneous myelo- and lympho-depletion prior to autologous transplant in autoimmune diseases. To model this strategy, we created an anti-mouse CD45-ADC that achieved full myeloablation with single dose administration in mice. Following CD45-ADC administration, transplantation of whole bone marrow (BMT) cells enabled full donor chimerism in a congenic model. We are evaluating this CD45-ADC in the murine experimental autoimmune encephalomyelitis (EAE) and sclerodermatous Graft-vs-Host-Disease (scGVHD) models of autoimmune disease. In EAE, conditioning with irradiation (9 Gy) followed by congenic transplant resulted in transient amelioration of disease. The impact of CD45-ADC conditioning and BMT on disease in these models will be presented. This novel approach for conditioning prior to autoHSCT could increase the number of autoimmune patients eligible for transplant and significantly reduce the side effects associated with current conditioning protocols.

W. 69. Circulating follicular helper T cells are numerically reduced and exhibit a CXCR3+ phenotype in children with Down Syndrome

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Down syndrome (DS) patients usually present B-cell deficiency and immune dysregulation characterized by early occurrence of autoimmune disorders, susceptibility to recurrent respiratory tract infections and predisposition to haematological malignancies. Here, we addressed whether the frequency and function of follicular helper (Tfh) and follicular regulatory (Tfr) T cells are altered in DS. Blood was collected from 24 children with DS, nine of which had autoimmunity. Thirty age- and sex-matched healthy control donors (HD) were included as controls. Blood Tfh cell frequencies in DS children were similar to that observed in age-matched HD but were reduced in absolute number. The proportions of $CXCR3^+$ (Tfh1 and Tfh1/17) subsets were significantly increased in DS children as compared to HD, while that of $CXCR3^-$ (Tfh17 and Tfh2) were greatly reduced. The expression of PD-1, a molecule that associates with activation status of Tfh, was similar between DS and HD. However, the proportion of $CXCR3^+PD\text{-}1^+$ Tfh was increased in the patients, while that of $CXCR3^-PD\text{-}1^+$ was reduced. While no differences in the percentage of Tfr were seen, the ratio of Tfh1, Tfh1/17 and $CXCR3^+PD\text{-}1^+$ subsets to Tfr was significantly increased in DS patients. Importantly, plasma CXCL13 levels were significantly elevated in DS as compared to HD and positively associated with the proportion of the $CXCR3^+PD\text{-}1^+$ Tfh cells. Finally, Tfh isolated from DS patients had the capacity to drive the differentiation of B cells into antibody-producing cells. Our findings suggest that alterations in Tfh phenotype, subset distribution and ratio to Tfr might underlie immune dysregulation in DS.

W. 70. Conserved human effector regulatory T cell signature is reflected in super-enhancer landscape

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Regulatory T cells (Treg) are critical regulators of immune homeostasis. Increasing evidence demonstrates that environment-driven Treg differentiation into effector Treg is crucial for optimal functioning. However, programming of human Treg under inflammatory conditions remains poorly understood. Here, we combine transcriptional and epigenetic profiling to identify the human effector Treg core signature. Autoimmune inflammation-derived Treg demonstrated normal suppressive function and enhanced IL-2 signaling. Transcriptome analysis revealed a unique transcriptional profile characterized

by upregulation of both a core Treg (FOXP3, CTLA-4, TIGIT) and effector program (ICOS, GITR, BLIMP-1, BATF, T-bet), indicating effector Treg differentiation and adaptation to the inflammatory environment. We identified specific human effector Treg markers including VDR and IL12Rβ2. H3K27ac occupancy revealed large changes in the (super-)enhancer landscape, including enrichment of the binding motif for VDR and BATF. The observed Treg profile showed striking overlap with tumor-infiltrating Treg. Our data demonstrate that human inflammation-derived Treg acquire a specific effector Treg profile guided by epigenetic changes. The core effector Treg profile is strongly conserved, and fine-tuned by environment specific adaptations.

W. 71. Crossreactive public TCR sequences undergo positive selection in the human thymic repertoire

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We investigated human T-cell repertoire formation using high throughput TCRβ CDR3 sequencing in immunodeficient mice receiving human hematopoietic stem cells (HSCs) and human thymus grafts. Replicate humanized mice generated diverse and highly divergent repertoires. Repertoire narrowing and increased CDR3β sharing was observed during thymocyte selection. While hydrophobicity analysis implicated self-peptides in positive selection of the overall repertoire, positive selection favored shorter shared sequences that had reduced hydrophobicity at positions 6 and 7 of CDR3βs, suggesting weaker interactions with self-peptides than unshared sequences, possibly allowing escape from negative selection. Sharing was similar between autologous and allogeneic thymi and occurred between different cell subsets. Shared sequences were enriched for allo-crossreactive CDR3βs and for Type 1 diabetes-associated autoreactive CDR3βs. Single-cell TCR-sequencing showed increased sharing of CDR3αs compared to CDR3βs between mice. Our data collectively implicate preferential positive selection for shared human CDR3βs that are highly cross-reactive. While previous studies suggested a role for recombination bias in producing “public” sequences in mice, our study is the first to demonstrate a role for thymic selection. Our results implicate positive selection for promiscuous TCRβ sequences that likely evade negative selection, due to their low affinity for self-ligands, in the abundance of “public” human TCRβ sequences.

W. 73. Discovery of Checkpoint Agonist Antibodies for Autoimmune/Inflammatory Disease

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Immune checkpoint receptor-ligand interactions are essential for down-regulating immune responses and maintaining self-tolerance. Functional antagonist antibodies to PD-1 and CTLA-4 enhance existing immune responses and are approved therapies in multiple oncology indications. We hypothesize that many human autoimmune diseases occur due to dysregulated checkpoint signaling, leading to uncontrolled T cell responses. Agonist antibodies to checkpoint receptors that mimic the function of natural ligands have the potential to suppress human autoimmune/inflammatory disease and reinstate tolerance. Two functional anti-checkpoint receptor antibodies that down-regulate human immune responses were discovered and optimized. Antibody #1 is a humanized hybridoma-derived antibody that was optimized for affinity and functional activity using mutations identified from deep sequencing of the splenic repertoire. To avoid potential antagonist activity Antibody #1 was non-blocking for checkpoint ligand binding. Antibody #1 was highly efficacious in a human PBMC-NOD-*scid IL2rγ*^{null} (NSG) graft versus host disease model, and efficacy in the model was not dependent on Fc effector function. Antibody #2 is a humanized anti-checkpoint receptor antibody derived from single antigen-specific B cell sorting and PCR. It was increased in affinity by incorporating mutations identified from sequences of 3 related single B cell clone antibodies. Optimized Antibody #2 demonstrated improved functional activity in inhibiting human T cell activation *in vitro* and potently inhibited tetanus toxoid recall responses in human whole blood. These results indicate that functional anti-checkpoint antibodies that down-modulate immune responses and lack antagonist activity can be discovered and optimized and may have the potential to restore immune balance in autoimmune and inflammatory diseases.

W. 74. An optimized method for expansion and retroviral transduction of mouse regulatory T cells

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Regulatory T cell (Treg) therapy has shown therapeutic potential for treating inflammatory bowel disease (IBD). Animal studies have also shown that intestinal antigen-specific Tregs are more potent at inhibiting colitis than polyclonal Tregs. Chimeric antigen receptor (CAR) technology offers a novel approach to generate Tregs recognising IBD-associated antigens in an MHC-independent manner. We optimised several expansion methods for *ex vivo* isolated mouse polyclonal and *in vitro* generated CAR Tregs for preclinical applications. We compared Tregs sorted as Foxp3^{eGFP+} cells versus CD25^{+}Foxp3^{eGFP+} cells, finding that there were no significant differences in phenotype or function, but that the latter method was optimal in terms of yield and expansion potential. We also compared expansion in the presence/absence of the mTORC1 inhibitor rapamycin, finding that inclusion of rapamycin during expansion resulted in optimal preservation of the expected Treg phenotype, with high Foxp3 expression, low production of inflammatory cytokines and preserved suppressive function. Mouse Tregs transduced with a CAR-encoding retrovirus and expanded *in vitro* using this optimized method maintained their phenotype and suppressive capacity. *In vivo*, adoptive transfer of expanded untransduced or CAR-transduced Tregs

protected mice from colitis in a T cell transfer model. Remarkably, a Treg-mediated suppressive effect was observed even at a 1:100 ratio (Treg:T effector cell), suggesting that *in vitro* expanded Tregs have a heightened suppressive function compared to *ex vivo* cells. These optimized methods can now be applied to test the biological effects of CAR-Tregs in a variety of pre-clinical models of disease.

Genetics

H. 76. Annotation of single cell populations using high-dimensional epigenomic profiles

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Single cell epigenomic assays, such as scATAC-seq, provide genome-wide high-resolution maps of cellular activity and identity. Annotating single cells via comparison to ensemble populations is limited to broader cell types and susceptible to inherent noise and sparsity of single cell data. We hypothesized that via quantitative epigenomic annotation of single cells, using over 500 ENCODE assays, we will identify cellular subpopulations with distinct epigenomic profiles.

To this end, we obtained public scATAC-seq on sorted populations of naïve CD4+ T, memory CD4+ T, CD4+ CD26- Sezary, and CD4+ Th17 cells. We also obtained public epigenomic annotations, including histone modification ChIP-seq, HiC chromatin looping, and open chromatin from DNase-seq and ATAC-seq. We then annotated scATAC-seq peaks with their absolute distance to each epigenomic feature. Next, we employed an unsupervised graph-based clustering algorithm based on canonical correlation analysis (Zhang et al 2018, bioRxiv) to leverage the correlated information between the scATAC-seq and high-dimensional epigenomic features.

As a result, we identified heterogeneity within sorted healthy and Sezary syndrome T cell populations. First, we found Th1, Th2, and Th17 precursors among the naïve CD4+ T cells. Second, we found naïve T effectors, effector memory, and central memory cells among the memory CD4+ T cells. Lastly, we separated naive Th17s from mature Th17s and naïve Sezary cells from mature ones.

In conclusion, with this strategy, we may perform better quality control on sorted populations and potentially identify pathogenic populations with large effect sizes in polygenic traits, which may lead to better diagnostics and potentially subtype-targeting treatments.

H. 77. Assessing Power for Single Cell RNA-sequencing Association Studies

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Recent advances in single-cell sequencing technologies provide the opportunity to perform large-scale association studies, such as case-control studies to define disease associated cell phenotypes, stimulus

response studies where cell phenotypes may be altered by a stimulus, or QTL studies where genotype may be associated with cell type abundances. However, the best practices for properly designing these experiments with sufficient statistical power remains understudied. We simulated single cell RNA sequencing datasets under various conditions and used MASC - a method for performing association testing with single cell data while controlling for interindividual differences and technical effects that can confound analysis - to detect differentially abundant populations in simulated datasets. For example, we considered an scRNA-seq study containing 100,000 cells from equal numbers of cases and controls, where a 5% frequency cell population was on average 20% more abundant in cases than controls. A 100 sample dataset with 1000 cells collected per sample was powered to detect this differential abundance in 49% of simulations (at $p < 0.05$), while a 50 sample dataset with 2000 cells collected per sample only reached 26% power. Similarly, we consistently found that sample size affected power to detect differential abundance significantly more than than the number of cells collected per sample across a range of potential effect sizes. We believe that our findings will allow researchers to design properly powered single-cell RNA sequencing experiments given the frequency and magnitude of differential abundance between cases and controls, which will be key to defining disease-linked immunological subpopulations.

H. 78. Dynamic Allelic Expression during T Cell Activation Uncovers cis Regulatory Effects in HLA-DQB1 and Autoimmune Disease Genes

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Most risk variants for autoimmune diseases affect $CD4^+$ T cell gene regulation. Additionally, human activated $CD4^+$ T cells express the major risk genes: HLA class II genes. Hence, understanding the genetic regulatory effects acting at **$CD4^+$ T cell states** is critical to define autoimmune mechanisms. Since allele specific expression is largely driven by *cis* genetic regulatory variation, it can be exploited to define regulatory effects in many cell states without the need of a large sample size. We hypothesized that studying allelic expression at multiple time-points upon T cell activation, may identify genes with context-dependent *cis* regulatory variation.

We queried $CD4^+$ memory T cells with RNA-seq after non-antigenic stimulation at 8 time-points over 72 hours in 24 individuals. We looked for cell-state specific *cis* regulatory effects by identifying **dynamic allelic expression in 191 genes** (5% FDR), 36 of which are in autoimmune disease loci (e.g. UBASH3A, IL10). We discovered a dynamic ***cis* regulatory effect for a major autoimmune disease gene: HLA-DQB1**. Using a novel HLA-personalized genome pipeline, we found that HLA-DQB1 allelic profiles can be classified into three dynamic *cis* regulatory programs. We have shown the late activation allelic effects translate to the protein level with flow cytometry. Fine-mapping putative regulatory variants, and functional validations with EMSA and **CRISPR/Cas9 nucleotide conversion uncovered a causal SNP**

(P=0.0003). This SNP drives expression changes in T cells but not B cells or macrophages. Our results define the dynamics of regulatory variation in multiple T cell states and offer potential for understanding autoimmune mechanisms.

H. 79. Genetic drivers of stimulation response in immune cells exhibit disease specificity

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Expression quantitative trait loci (eQTL) data have emerged as a reliable source for functional follow-up of disease associations identified from genome-wide association studies (GWAS). Cell type specific data have recently allowed a refined mapping of several GWAS loci that was unattainable with tissue level eQTL studies. However, a significant part of GWAS loci with a putative regulatory potential have not been functionally characterized yet. Activation of cells and data derived from patients, instead of healthy controls, could allow a further refinement of GWAS functional mapping. To address these questions, we generated eQTL data for three peripheral immune cells – naïve CD4+ T cells, memory CD4+ T cells, and classical monocytes – from multiple sclerosis (MS) subjects, untreated (UNT; n=55) or treated with glatiramer acetate (GA; n=84), and healthy controls (HC; n=38). Transcriptomic data were generated both for baseline - unstimulated - and stimulated, i.e. activated, state of the three immune cells. Joint analysis of all subjects, MS and HC, identified an extended number of transcripts (eGenes) that were genetically controlled: 4,016 in naïve CD4+ T cells, 3,843 in memory CD4+ T cells, and 3,652 in monocytes. Stimulation resulted in a reduced number of eGenes across all three cells. A strict formal statistical comparison of the response to stimulation identified 196 response eGenes (reGenes) in naïve CD4+ T cells, 165 reGenes in memory CD4+ T cells, and 338 reGenes in monocytes. We further observed differences in eGene and reGenes that were driven by the MS patient samples and were seemingly disease-specific.

H. 80. Harmony enables the simultaneous single cell RNAseq analysis of circulating and infiltrating immune cells across donors and tissues

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Chronic inflammation is the common pathological phenotype of multiple autoimmune diseases. Characterizing the components of inflammation across diseases is critical to defining and treating diseases based on shared etiology. Recently, single cell RNAseq has made it possible to assay gene expression in thousands of individual cells across diverse conditions. The hope is then to identify common expanded populations across diseases. This goal is hindered by a major analytical challenge: cells from different datasets often group by technical factors, such as platform and read depth, and biological factors, such as source tissue and donor, instead of by cell type. These covariates, though important, confound the identification of shared cell types. We present Harmony, a computational tool to enable common cell type identification across experimental conditions. Harmony projects cells into a shared space in which cells group by cell type rather than dataset specific conditions. Unlike other methods, Harmony can simultaneously account for multiple factors. Moreover, it is the only available algorithm efficient enough to make the integration of ~1 million cells feasible on a personal computer. We use Harmony to power a joint analysis of infiltrating immune cells from inflamed human kidney, synovium, and colon samples. For comparison, we include cells from cord blood and bone marrow, courtesy of the Human Cell Atlas. Through the analysis, we characterize shared and tissue-specific gene expression signatures of immune populations. Harmony is a fast and flexible tool that enables joint analysis of cells across multiple experimental and biological conditions.

H. 81. Higher Native American ancestry proportion is associated with increased TB progression risk in the Peruvian population

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There is a longstanding debate on the role of genetic ancestry in tuberculosis (TB) risk. While associations have been reported in the past, drawing convincing conclusions has been difficult since most of the previous studies could not adequately control for socioeconomic and environmental variables. Using genotyping data from 2,160 admixed Peruvians with active TB and 1,820 household contacts with latent TB, we inferred the level of Native American, European, African, and Asian ancestry per individual and investigated the relationship between TB progression risk and ancestry proportions. We observed a strong association between higher Native American ancestry proportion and increased risk of TB progression in the Peruvian population (p-value=4.2×10^{-15}, OR=12.4, CI=11.8-13.1). This effect could not be explained by environmental or socioeconomic variables even after stringent permutation analysis within each household. We used admixture mapping to search for specific genomic regions that might explain some of this association. No genomic region reached the genome-wide significance threshold (p-value<5.7×10^{-5}, lowest observed p-value=2×10^{-4}). Regions with lower p-values were enriched for genes involved in T-cell (GO:0002456, enrichment p-values: 0.2, 0.02, and 0.007 for bins with p-value <0.01, <0.001, and <0.0005 respectively) and B-cell mediated immunity (GO:0019724, enrichment p-values: 0.2, 0.03, and 0.01) but not for the genes involved in innate immune responses (GO:0045087, enrichment p-values: 0.4, 0.2, and 0.1). Our results bring convincing evidence on the role of Native American ancestry

in TB progression and suggest that this effect follows a polygenic architecture, including many genes that are involved in adaptive immune regulation of TB progression.

H. 82. Mechanistic Characterization of RASGRP1 Variants Identifies an hnRNP K-regulated Transcriptional Enhancer Contributing to SLE Susceptibility

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Systemic lupus erythematosus (SLE) is an autoimmune disease with a strong genetic component. We recently identified a novel SLE susceptibility locus near *RASGRP1,* which governs the ERK/MAPK kinase cascade and B-/T-cell differentiation and development. However, precise causal *RASGRP1* functional variant(s) and their mechanisms of action in SLE pathogenesis remain undefined.We performed a meta-analysis across six Asian and European cohorts (9,529 cases; 22,462 controls), followed by *in silico* bioinformatic and epigenetic analyses to prioritize potentially functional SNPs. We experimentally validated the functional significance and mechanism of action of three SNPs in cultured T-cells.Meta-analysis identified 18 genome-wide significant (p-8) SNPs, mostly concentrated in two haplotype blocks, one intronic and the other intergenic. Epigenetic fine-mapping and allelic imbalance and eQTL analyses predicted three transcriptional regulatory regions with four SNPs (rs7170151, rs11631591-rs7173565, and rs9920715) prioritized for functional validation. Luciferase reporter assays indicated significant allele-specific enhancer activity for intronic rs7170151 and rs11631591-rs7173565 in T lymphoid (Jurkat) cells, but not in HEK293 cells. Using EMSA, mass spectrometry and ChIP-qPCR analysis, we detected allele-dependent interactions between heterogeneous nuclear ribonucleoprotein K (hnRNP-K) and rs11631591. Furthermore, inhibition of hnRNP-K in Jurkat cells downregulated *RASGRP1* and ERK/MAPK signaling.Comprehensive association, bioinformatics, epigenetic analyses together with experimental validation including CRISPR/Cas9 editing, intronic rs11631591 located in a cell type-specific enhancer sequence, where its risk allele binds to the hnRNP-K protein and modulates *RASGRP1* expression in Jurkat cells. As risk allele dosage of rs11631591 correlates with increased *RASGRP1* expression and ERK activity, we suggest that this SNP may underlie SLE risk of this locus.

Immune Monitoring

H. 84. Biomarker harmonization to measure immunological effects of ustekinumab in type 1 diabetes

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Type 1 diabetes (T1D) is caused by T-cell-mediated destruction of pancreatic beta cells. It is likely that blockade of pathogenic T-cells in individuals with recent-onset T1D would halt the destruction of beta cells and may allow restoration of endogenous insulin secretion. Ustekinumab inhibits IL-12/23 p40 and thereby limits the function of IL-17 and/or IFN-γ secreting T-cells, both of which have been implicated in the pathogenesis of T1D.
In 2016-2017, a phase IIa trial was undertaken to test the safety of ustekinumab administration to 20 young adults (18-25yrs) with recent-onset T1D. Biomarker assays were used to measure immune cell populations before and after treatment revealing a dose-dependent increase in the frequency of memory regulatory T-cells ($p<0.01$), changes in the Treg signature, and a reduction in the frequency of IL-17$^+$IFN-γ$^+$ Th17.1-cells ($p<0.05$). Moreover, patients treated with 90mg of ustekinumab had a higher clinical response compared to those treated with 45mg. Results from this trial indicated that changes in immune cell populations may predict a clinical response to ustekinumab therapy.
Two randomized, placebo-controlled clinical trials to test the efficacy of ustekinumab in new-onset T1D are planned in Canada and the UK. We have harmonized sample collection timing, processing and storage conditions, and undertook a cross-lab training process to standardize a series of bioassays to measure the effects of ustekinumab on different T-cell populations. By using standardized assays we will increase the statistical power of these independent trials and provide a platform for adoption of harmonized biomarkers in future immunotherapy trials in T1D.

H. 85. CCA-based Integration of Multimodal Single-Cell Data Identifies Higher-Resolution Immune Cell States

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Currently, single-cell measurements like scRNA-seq are used to define cell state heterogeneity: for example, to classify T cells into states like Th1, Th2, Th17, Tregs, etc., which in turn may underlie complex immune traits (like autoimmunity). While scRNA-seq has led to a broad range of insights, it lacks the resolution of surface markers, particularly for immune cells, and doesn't offer a means to precisely sort these populations. But now, recent technological advancements have enabled multimodal measurements; for example, REAP-seq and CITE-seq measure RNA and surface protein expression in

single cells. Surface marker and RNA data together may permit more robust, fine-grained definition of cell states. Here, we present one such integration approach: a canonical correlation analysis (CCA)-based method that aligns features along canonical variates—axes of variation shared between the measurements—and projects cells into that space. When applied to REAP-seq data, for example, we can cluster cells in CCA space to identify cell states defined by correlated gene and protein markers. This approach also identifies surface markers to isolate these subsets. We applied this method to a single-cell REAP-seq data set measuring gene and surface protein expression in whole blood cultured with tetanus toxoid and an anti-PD-1 inhibitor. While we could identify broad cell-types (such as monocytes and T cells) with RNA-seq alone, by incorporating protein data with CCA we were able to identify subsets that were not identified when clustering on gene expression alone—e.g., T cells expressing TIGIT or PD-1 surface markers—and their correlated gene expression profiles.

H. 86. CD4+ CD25+ CD127hi cells correlate with disease progression in type 1 diabetes: Validated in a multi-site study

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We have previously shown that the frequency of $CD4^+$ $CD25^+$ $CD127^{hi}$ cells correlate with length of partial remission (LoR) in children with Type 1 Diabetes (T1D). In healthy adults these cells have a strong Th2 bias and a dominant central memory (CM) phenotype. The purpose of this study was to validate the correlation between $CD25^+$ $CD127^{hi}$ cells and LoR in a larger cohort of patients, assess the mechanistic potential for these cells in T1D, and in response to Alefacept in the TIDAL study. In a cohort of 91 T1D patients, a significant correlation is observed between the frequency of $CD25^+$ $CD127^{hi}$ cells at diagnosis and LoR. As previously shown for healthy people, $CD25^+$ $CD127^{hi}$ cells in children with T1D have a Th2 bias, express CD2 and are a mix of CM and effector memory (EM) cells, suggesting that they might be a target for Alefacept. Consistent with this notion, the relative frequency of $CD25^+$ $CD127^{hi}$ cells is significantly lower at all time points post-baseline in Alefacept-treated patients compared to the Placebo group following similar kinetics to that previously reported for CM and EM cells in the TIDAL study. However, the frequency of baseline $CD25^+$ $CD127^{hi}$ cells maintained a positive association with LoR in the Alefacept-treated group, suggesting a role in protection. Possible explanations for the apparent paradox between the capacity of Alefacept to deplete $CD25^+$ $CD127^{hi}$ cells, while maintaining a positive association between $CD25^+$ $CD127^{hi}$ cell frequency and a longer LoR will be discussed.

H. 87. Clustering and Visualization of Influenza-specific CD4+ T Cell Responses

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Influenza is a serious health concern, with an urgent need for improvement in vaccine efficacy. Classically, neutralizing antibodies are the driving force of antiviral immunity to influenza. While it is known that $CD4^+$ T cells are also important, their precise role remains poorly characterized. To address this, we used high-dimensional immune cell profiling by mass cytometry (CyTOF®) on peripheral blood mononuclear cells from 46 healthy donors, at Day 0 and Day 7 after influenza vaccination. By applying a novel clustering algorithm called Denoised Ragged Pruning, we identified nine $CD4^+$ T cell clusters, three of which (Clusters 2, 4, and 7) were responsive to influenza peptide stimulation. Since the differences between clusters are subtle, we applied a new visualization tool called Penalized Supervised Starplots to quickly and intuitively visualize the salient features driving cluster separation. We found that the three influenza-responsive clusters expressed markers of central memory (Cluster 2), effector memory (Cluster 4) and TEMRA (Cluster 7) phenotypes. Clusters 2 and 7 were responsive to vaccination, being significantly above background at Day 7 post-vaccination but not at Day 0. Cluster 4 was significantly above background at both Day 0 and Day 7, indicating that it is a pre-existing influenza-specific cluster. All three clusters showed distinct combinations of cytokines, activation markers and cytotoxic markers. Interestingly, the study cohort could be divided into three subgroups, based on its patterns in cluster abundance. Our findings provide valuable insights into $CD4^+$ T cell subsets, and can aid vaccine improvement and baseline prediction of vaccine response.

H. 88. Comprehensive Epigenetic Immune Cell Monitoring in Diverse Clinical Applications

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We describe the application of epigenetic quantification of CD3+, CD8+, CD4+, including T regulatory (Treg) cells, B cells, NK cells, monocytes and neutrophils from fresh or stored blood. This method yields identical results to flow cytometry from blood samples of healthy donors and allows precise quantification of cell subsets such as Treg cells that are challenging to quantify. It is also suitable for less than 100 µl blood or for samples with low cell number such as from patients after hematopoietic stem cell transplantation (HSCT) or with primary or acquired immune deficiencies.

Patients with Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-Linked (IPEX) and IPEX-like were evaluated by analyzing Tregs relative to CD3+ T cells. Despite the dysfunctional FOXP3 mutated protein, IPEX patients exhibited elevated Treg/CD3+ cell ratios which seemed to correlate with disease severity. In contrast, most of the patients with IPEX-like symptoms without FOXP3 mutations exhibited decreased Treg/CD3+ cell ratios.

When applied to sequential blood samples during reconstitution post-HSCT, the epigenetic method allowed for identification of the immune subsets, including Tregs, at earlier time points than flow cytometry according to current clinical practice. This paves the way to a better understanding of the correlation between immune reconstitution and HSCT-related complications.

These results demonstrate suitability of epigenetic immune method to quantify highly specialized subpopulations where established methods lack standardization. To further extend the utility of this method in immune-mediated diseases of different origin we are extending the epigenetic immune cell panel to follicular and IL-17 producing T helper cells.

T. 38. Dynamic gene regulatory network of IFNbeta response in human T cells

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There is emerging evidence that IFNβ, which has been discovered as a major effector cytokine of the immune response against viral infections, can exert multiple functions in host defense and homeostasis. T cells are the major component of adaptive immunity and respond to IFNβ released by innate immune cells, resulted in regulating host immune response against viral infection and cancer. However, the molecular mechanisms by which IFNβ regulates T cell response in human are largely elusive. Answering this question is critical to better understand T cell response to IFNβ and to identify therapeutic target for individual patients who are suffering with infectious diseases, autoimmunity, and cancer. Here, we performed unbiased high-resolution transcriptional profiling of human T cells and constructed an IFNβ-inducible transcriptional regulatory network in human T cells. Our unbiased approach to investigate the kinetics of global gene expression allow us to identify novel IFNβ-responsible genes that have been overlooked by single snapshot-based exploration. We found some of the genes are important to induce expression of exhaustion markers, suggesting that our method could dissect the IFNβ-induced exhaustion program. This high-resolution gene expression profiling will provide a platform that enables us to connect each “dots”, which are already known as individual IFNβ responses, providing the systematic understanding of the molecular network of immune reaction to viral infection, autoimmune and cancer.

T. 39. Extended Poly-dimensional Immunome Characterization (EPIC): A Web-based Immune Reference Atlas of the Healthy Human Immunome and a Tool for Translational Medicine

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We have created a high dimensionality atlas of the healthy human Immunome (EPIC: Extended Poly-dimensional Immunome Characterization) by interrogating the peripheral blood mononuclear cells of over 200 healthy subjects, ranging from cord blood to adult age, with 63 unique mechanistic and phenotypic markers per cell by mass cytometry (CyTOF). EPIC provides a detailed depiction of the architecture of the human healthy Immunome. EPIC analytical and visualization pipeline is based on an open source, web-based R Shiny bioinformatics toolkit. EPIC can be mined in various ways, for instance to follow developmental changes of any given cell subset or to depict the architecture of the Immunome at any given age range. Importantly, we have built and will keep developing datasets from various immune mediated diseases using the same approach. Consequently, by providing the healthy standard, EPIC enables the depiction and dissection of disease-dependent perturbations of the Immunome architecture. EPIC provides a transformational conceptual advance in Translational Immunology from individual subset focused to immune architecture based approach for the understanding of the physiology and pathogenesis of immune mediated mechanisms. We intend to make EPIC available to the entire community in its full capacity.

T. 40. Flu Antigen Recall Responses using TruCulture® with OptiMAP Analysis

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TruCulture® is a whole blood collection and culture system that is designed to be used at clinical sites to investigate human immune responses under specific stimulation conditions. OptiMAP is a Multi-Analyte Profile (MAP) bead based immunoassay panel that measures 13 analytes that span the major immune responses (T_H1, T_H2, T_H17, neutrophil, and monocyte/macrophage activation). The recall response of flu hemagglutinin (HA) antigens was examined in healthy adults vaccinated with the 2018-2019 annual flu vaccine. Whole blood was drawn into TruCulture tubes: null (media only), staphylococcal enterotoxin B (SEB), and null tubes spiked with 1.25mg each of 4 different recombinant HA proteins. All samples were incubated for 48 hours at 37ºC. Supernatant were collected and analyzed using the OptiMAP panel. No inflammatory cytokines were detected in the null tubes from either the baseline or 2 weeks post-vaccination samples. There were no differences in cytokine production in the SEB stimulated samples between the baseline and 2 weeks post-flu vaccination time lines. For flu HA stimulated samples, there was a significant increase in production of GM-CSF, IFN-γ, IL-1β, IL-6, IL-12p70, IL-23, and TNF-α at 2 weeks post-flu vaccination compared to samples collected at baseline. These data demonstrate that TruCulture in conjunction with OptiMAP are useful tools for investigating antigen specific responses.

T. 42. Immunomonitoring of HLA-DR expression and ratio nCD64/mHLA-DR as predictive biomarkers of infection in critical patients at the Intensive Care Unit

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Introduction

Sepsis is characterized by a simultaneous imbalance of hyperinflammation and immunosuppression.The expression of HLA-DR in monocytes (mHLA-DR) and CD64 expression in neutrophils (nCD64) are considered, respectively, predictive and diagnostic biomarkers of infection. The ratio nCD64/mHLA-DR has been described as a prognostic biomarker of sepsis.

Objective

To evaluate mHLA-DR expression and ratio nCD64/mHLA-DR in patients admitted to the Intensive Care Unit (ICU) and their relationship with the development of infection.

Methods

Prospective study of 77 patients admitted to the ICU from our hospital (HGTiP) due to stroke or severe traumatic brain injury. The mHLA-DR and nCD64 expression were analyzed in whole blood samples at baseline, +3, +6, +9, +12 and +15 days after admission, using a standardized flow cytometry protocol.

Results

During the follow-up, 71% of patients became infected (infection without sepsis, sepsis or septic shock). Infected patients showed – already after three days of admission- a lower percentage of mHLA-DR+ (85.8 ± 16.22% *vs*. 92.5 ± 12.13%, p <0.001). Interestingly, on day +3, infected patients also had a higher ratio nCD64/mHLA-DR (0.12 ± 0.19 vs. 0.04 ± 0.08, p<0.001) than the non-infected ones.

Conclusion

The immumonitoring of mHLA-DR expression and ratio nCD64/mHLA-DR may help to evaluate those patients with susceptibility to develop infection and sepsis at the ICU.

T. 43. Inclusion of exploratory PD biomarkers in First in human clinical trials adds value by providing information to confirm the mode of action of a candidate therapeutic.

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Traditionally, in First in Human clinical trials the primary/secondary endpoints are concerned with safety. By expanding these studies to include exploratory biomarkers it is possible to gain added value, as such biomarkers can indicate efficacy and confirm mode of action of the therapeutic. Incorporating this information from an early stage can assist in go/no-go decisions and can greatly increase the likelihood of success. We show here how we currently use this strategy to monitor biomarkers in a translation manner using LPS induced immune responses as an example. In vitro assays using human whole blood and PBMCs are routinely used to screen test compounds and determine their influence at the gene and protein level, using luminex (cytokines) and nanostring platforms. This can be followed by in vivo pharmacology PD models (small rodent LPS challenge) to further refine the compound selection and develop suitable readouts such as Multiplex cytokine analysis. When a candidate compound then progresses to First In Human clinical trials, we can support the inclusion of exploratory biomarkers using 'uplifted' Good Clinical Laboratory Practice (GCLP) level assay validation, to ensure reliable clinical data. At this stage, a more focussed panel of cytokines can be selected and measured either directly in plasma or secondary to ex vivo stimulation. In addition, the use of flow cytometry and ELISPOT support investigations of T cell modulating therapies. Such a strategy provides early indications on whether a therapeutic is hitting it's expected target in man.

T. 44. LPS induced neuroinflammation assay combination for development of new treatment strategies

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Neuroinflammation is linked to the progression of neurodegenerative disorders. Activated inflammatory cells of the myeloid lineage, in particular microglia, play a key role in the pathogenesis of these chronic disorders. Activated microglial cells enhance the production of inflammatory cytokines and chemokines like interleukin-1, interleukin-6, tumor necrosis factor α and make microglia more susceptible to secondary stimuli, promoting microglial activation.

In the described assays a common used bacterial endotoxin (lipopolysaccharide, LPS) is used as a proinflammatory stimulus for microglia cells.

The in-vitro screening assay described uses cortical glial or pure microglial cultures. LPS induces inflammatory reactions in glial cells after 24 hour exposure time. It is possible to determine inflammatory cytokines from the culture medium or cell lysate.

The first in-vivo assay described is the use of microdialysis to continuously sample interstitial fluid (ISF) for example in the hippocampus or the prefrontal cortex to determine cytokine levels after microglia activation by priming with LPS.

The second in-vivo assay is PET imaging to measure neurological changes in various pathological states. However, quantification of PET tracers where reference tissue models cannot be applied, has been challenging in rodents. The method used here is to obtain metabolite corrected arterial input function (AIF) from rats, which allows longitudinal PET scanning individuals and weekly follow-up of neuroinflammation.

The combination of existing in-vitro and in-vivo assays gives us the ability to delineate the underlying molecular mechanisms, thus reinforcing the development of new treatment strategies and biomarkers for neurodegenerative disorders beyond the existing conventional approaches.

T. 45. MHC II dCODE Dextramer reagents allow characterization of antigen-specificity, TCR clonotype and gene expression of single CD4+ T cells

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T-cell mediated adaptive immunity to pathogens, tumor- and self-antigens in autoimmunity, is dependent on the specific recognition by a unique TCR of the MHC-peptide (pMHC) antigen on the target cell. The identification of specific T cells, their functional potential and clonotypic TCR sequence is essential to understand the complexity of an immune response to be able to manipulate it for therapeutic benefit.

We have developed an MHC multimer technology, the dCODE Dextramer reagents, that are comprised of a dextran backbone displaying multiple pMHCs and a unique DNA barcode coding for the displayed pMHC specificity. The dCode Dextramer is compatible with 10x Genomics Chromium platform for single cell analysis.

In this study we have exploited how a panel of MHC II dCODE Dextramer reagents can be used for the characterization of antigen-specificity, TCR clonotype and gene expression of single CD4+ T cells from human peripheral blood.

Initially, the integrity of each dCODE Dextramer was demonstrated by specific labelling of CD4+ T-cells in flowcytometry. Subsequently, using the MHC II dCODE Dextramer panel we were able to identify and correlate antigen specific CD4+ T cells, with their unique TCR clonotypes and phenotypic gene expression profiles.

In conclusion the MHC II dCODE Dextramer can reveal 1) antigen specificity, 2) TCR clonotype and 3) functional gene expression profile of single CD4+ T cells. This allows a new understanding of cellular immunology that can progress immunological research and immunotherapeutic development.

T. 46. Monitoring of antigen-specific, stimulation-induced mediator secretion, in clinical trials of infection, transplantaion, and immuno oncology using PepSup(TM) peptide pool strategy

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Background:The use of clinical immunotherapies targeting T-cells in an ever increasing number of patients, in particular cancer patients, begs the question how the success of such therapies can be monitored in future in a reliable way and at reasonable cost. Typically, antigen-specific T-cells are detected by ELISPOT or flow-cytometry (intracellular cytokines), however these methods are relatively difficult to standardize and costly. PepSup^{TM}is a simple, highly standardized, peptide-based cell stimulation assay, which was designed to produce short-term culture supernatants from whole blood.
Method: Sample processing only required a programmable heat block and a simple bench-top centrifuge. Following 16 hours of overnight stimulation of whole blood with a range of different PepMix^{TM}peptide pools dissolved in optimized PepSup^{TM}media, samples were centrifuged and supernatants collected. Samples were analyzed with the MSD V-Plex platform or the BD Cytometric Bead Array. Cytokines originating from T-cells but also other cells were measured.
Results: PepSup^{TM}provided very similar results as incubation of samples in a standard incubator using CO_2-dependent media, however, offered significant advantages in regards to hands-on time and sample handling.
Conclusions: PepSup^{TM}is the ideal basis for monitoring antigen-specific, stimulation-induced mediator secretion in a range of situations, for example in the study of infection, transplantation, or tumour-specific immunity. Because of its high degree of standardization and hence reproducibility, it is particularly well-suited to longitudinal patient monitoring.

T. 48. Revisiting the initial diagnosis and blood staging of Mycosis Fungoides and Sézary Syndrome with the KIR3DL2 marker

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Aims: Cutaneous T-cell lymphomas (CTCL) are a group of lymphomas comprising Mycosis Fungoides (MF) and Sézary syndrome (SS) which is characterized by erythroderma and high numbers of atypical lymphocytes. A blood classification was added to TNM staging and flow cytometry (FCM) became a promising method. We have shown the reliability of KIR3DL2 as a positive marker for SCs. We report our 4 years expertise for initial diagnosis in 219 patients.

Methods: CD7, CD26, and KIR3DL2 labelling was performed. Patients were diagnosed according to the ISCL/EORTC classification for CTCL. We gathered the samples at diagnosis for 31 SS, 5 pre-SS, 45 MF, 12 EMF and 126 patients with inflammatory skin diseases (ISD). 2 groups of patients were defined, according to their CD4+T-cell absolute counts: group A (≥1000/mm3) and group B (<1000/mm3).

Results: A strong positive correlation between KIR3DL2+ and CD4+CD26-T-cell count was found in group A (r=.81, p<.0001). A value of KIR3DL2+T-cells ≥200/mm3 allowed a specificity of 90% and a sensitivity of 86% for SS diagnosis. In group B, all patients with KIR3DL2+ T-cells ≥200/mm3 were SS. Five patients initially diagnosed as MF or pre-SS developed SS within 2-7 months. These patients already displayed >200/mm3 KIR3DL2+ at initial time point.

Conclusion: the detection of KIR3DL2+T-cells improves the biological diagnosis of SS. A threshold value of KIR3DL2+ cells ≥200/mm3 in MF and pre-SS patients with CD4 lymphopenia may be predictive for SS evolution. We therefore recommend to use this marker for the initial diagnosis and staging of CTCL, in association with CD26.

T. 49. TCRB Convergence in Peripheral Blood: Studies in Chronic Viral Infection and Cancer

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T cell receptor (TCR) convergence refers to the phenomenon whereby antigen-driven selection enriches for TCRs having shared antigen specificity but different nucleotide sequences. The extent to which convergence arises owing to chronic viral infection is not yet established. Here we sought to identify features of chronic cytomegalovirus (CMV) infection using TCRB profiling of peripheral blood (PBL) total RNA. Total RNA from PBL was obtained from 35 blood donors of known CMV status, then used for TCRB sequencing. Data were used to identify TCRB repertoire features correlated with CMV status. CMV-related convergence values are compared to convergent T cell responses in individuals with cancer treated with checkpoint inhibitors (CPI). T cell clone evenness was reduced in CMV positive individuals, predictive of CMV status (AUC=.86, p=2E-4, Wilcoxon), and strongly correlated between assays (Spearman cor=.96). TCR convergence was elevated in CMV positive individuals and uncorrelated with evenness (Spearman cor =-.03). Combination of convergence and evenness improved the performance of a logistic regression classifier (AUC= .93). CMV infection appears to significantly alter the T cell repertoire, suggesting that CMV status may be required for proper interpretation of T cell expansion in the context of CPI for cancer. TCR convergence may detect T cell responses to viral and tumor neoantigens and may serve as a useful biomarker for the identification of immunogenic tumors having few genetic alterations.

T. 50. Th17-derived Cytokines Might be Involved in the Allergic Contact Dermatitis Induced by Poison Ivy Active Ingredient, Urushiol.

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Severe cases of poison ivy, oak, or sumac-induced allergic contact dermatitis (ACD) is a major health hazard especially when affecting the lungs, eyes, or large proportions of skin. Urushiol is the major active

ingredient of poison ivy which is believed to mediate the intense allergic skin reactions. However, no robust preclinical models exist to assess the effectiveness of new agents in ACD. In the present experiment, we established a protocol in mice for reproducible induction of dermatitis by the poison ivy active ingredient, urushiol. Also, using flow-cytometry and qRT-PCR, we identified that Th17-derived cytokines underlie poison ivy-induced skin reactions. Animals were first sensitized with urushiol by application on shaved abdominal skin. Afterwards, animals received several challenges with urushiol by application on both ears. Ear thickness was measured with a micrometer and redness was scored visually.

Concentration-response experiments using different dose levels of urushiol for sensitization and subsequent challenges showed that sensitization with 2% urushiol and at least 5 subsequent challenges with 1% urushiol are required for optimal induction of the ACD. In flow-cytometry experiments, Th17 cells were found to be the major subset that is increased following ACD induction. In qRT-PCR experiments, we found that genes for IL-17A, IL-17F and IL-22 were increased in the ear following urushiol ACD induction. Steroidal drugs clobetasol and dexamethasone alleviated urushiol-induced ACD in the mice.

In conclusion, we validated a protocol for the urushiol-induced ACD in mice and also showed that Th17-derived cytokines might be involved in the urushiol-induced ACD.

T. 51. Validation of a Gliadin α-I/α-II DQ2 Tetramer Flow Cytometric Assay for use as a Biomarker to Assess Gluten Specific T cells in Celiac Disease

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Gluten-challenge in subjects with celiac disease, results in a transient upregulation of gliadin-specific CD4+ T cells in the blood. Despite the increase, these cells are quite rare requiring a selective and sensitive assay for detection. We have developed a 12-color tetramer flow assay to enable detection and immunophenotyping of the gliadin α-I/α-II CD4+ T cells in the blood.

The assay was validated using healthy cryopreserved AccuCell™ PBMCs and CD4+ T cells spiked with T cells specific for gliadin α-I (QLQPFPQPELPY) or gliadin α-II (PQPELPYPQPE). The assay conditions were optimized for sensitivity, optimal signal:noise ratio, and detection of the gliadin α-I/α-II tetramers. The assay identifies gluten-specific (tetramer+) T cells and immunophenotypes the cells as either naïve or memory (CD4/CD3/CD45RA/CD62L/CCR7), activated (CD38), regulatory (CD39) and gut-homing (b7/a4). For validation, pre-specified criteria were used to assess inter-assay, intra-assay, inter-operator precision and post-staining stability. Technical validation was successfully met; and the assay performs within acceptable precision parameters.

Tetramer positive T cells in the blood are rare events; therefore, this assay was developed and validated to be sensitive and selective. This assay will enable characterization of the gliadin α-I/α-II CD4+ T cells in the blood for celiac patients, providing a more comprehensive evaluation of response to new therapies and may reduce invasive biopsy-based measurements.

Immunity & Infection

H. 90. Humoral responses to common dietary antigens leads to accumulation of food antigen-specific plasma cells in the human infant thymus

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We have recently reported on the accumulation of plasma cells (PCs) in the human thymus, starting several months after birth. This thymic PC niche includes clones specific to common viruses presumably generated through peripheral responses. Humans acquire humoral immunity to dietary antigens during the first 3 years of life. Here we investigated whether such immunity also resulted in the homing of specific PC to the thymus. Using ELISPOT assays, we tested for the presence of antibody-producing cells specific to known immunogenic food antigens among B cells isolated from discarded thymus specimens from 1 day to 3 years patients. Results revealed the presence of PC specific to cow's milk, egg and wheat derived antigens in 2 out of 10 patients. Furthermore, the presence of thymic food antigen-specific PC was associated with high serum IgG titers to same antigens, indicative of an ongoing immune response. Our study demonstrates for the first time the presence of antibody-secreting cells specific to antigen towards which tolerance is usually established and maintained, in the thymic of human infants. Their role and possible contribution to T cell education warrant further investigation.

H. 91. The role of the mitochondrial protein TCAIM in controlling CD8+ T cell functions

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Mitochondria are critical regulators for intracellular signalling, metabolism and apoptosis during T cell activation, differentiation and function. Upon activation, T cells undergo a metabolic shift from oxidative phosphorylation towards aerobic glycolysis, which is obligatory for effector T cell differentiation. We previously showed that the mitochondrial protein TCAIM (T cell activation inhibitor, mitochondrial) controls $CD4^+$ T cell activation and function and demonstrated that *Tcaim* overexpression inhibits effector/ effector-memory differentiation of conventional $CD4^+$ T cells, thereby, promoting acceptance of allogenic transplants.

We now investigated the role of mitochondria and especially TCAIM for the activation and function of $CD8^+$ T cells isolated from mice with a T cell specific *Tcaim* overexpression or deletion. Flow cytometry analysis of polyclonal stimulated naïve $CD8^+$ T cells confirmed an inhibitory role of *Tcaim* expression for $CD8^+$ T cell effector/ effector-memory differentiation and IFN-g production as observed for $CD4^+$ T cells. Interestingly, electron microscopy of naïve and activated $CD8^+$ T cells showed that *Tcaim* overexpression induced tight cristae formation and prevented mitochondrial fission upon activation whereas deletion of *Tcaim* caused highly fragmented mitochondria even in naïve cells. This is of particular interest as mitochondrial fission has recently been shown to be crucial for the metabolic shift towards glycolysis, which indeed was abrogated in *Tcaim* overexpressing cells. Furthermore, co-immunoprecipitation

analysis indicated that TCAIM interacts with proteins involved in cristae formation and mitochondrial fusion.
Taken together, our data indicate that the mitochondrial protein TCAIM controls T cell effector/ effector-memory differentiation and function by promoting mitochondrial fusion and cristae formation.

H. 92. Tuberculosis and sarcoidosis hold very distinct CD4 T cell transcriptomic profiles that can be used to improve pathology understanding and diagnostic tools

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Tuberculosis (TB) is the leading cause of death by infectious diseases worldwide. Sarcoidosis is a lung inflammatory disease with unknown etiology that is often misdiagnosed as TB. It also forms granulomas, and shares the same type I interferon whole blood transcriptomic signature widely described in TB. Interestingly, TB-specific CD4 T cell immune responses have been reported in sarcoidosis patients, and linked to pathology. We elected to compare transcriptomic signatures of circulating CD4 T cells in patients with sarcoidosis vs individuals with active or latent TB. TB antigen-specific CD4 T cell responses were interrogated using a pool of 300 TB-specific peptides known to elicit significant reactivity in TB infected individuals. Surprisingly, reactivity to the peptide pool was lower in the sarcoidosis cohort compared to the healthy, TB uninfected cohort. Conversely, sarcoidosis patients were associated with higher reactivity to polyclonal stimulation compared to both TB infected and uninfected cohorts. Both lack of TB-specific reactivity and higher polyclonal reactivity in sarcoidosis were associated with the expression of type I IFN genes, suggesting a dysregulation of this pathway in CD4 T cells in the context of sarcoidosis. We also found that the CD4 T cell compartment in sarcoidosis patients was enriched for effector T cells, which correlated with the expression of genes clusters associated with specific biological functions and cell types such as cytotoxicity and Tregs. Finally, we also identified some promising gene candidates that could be used for differential diagnostic between sarcoidosis and TB, and thus translated into new diagnostic tools.

H. 93. V delta 1 and V delta 2 gamma delta T cells express different marker signatures and are distinctly linked to plasma markers of systemic inflammation with ART-suppressed HIV infection and normal aging.

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Our previous work implicates γδ T cells as an inflammatory driver in ART-suppressed HIV infection and suggests that this unique T cell population serves distinct roles in the 'inflamm-aging' that occurs with age with and without aviremic HIV. We sought to determine the precise γδ T cell subsets that are driving inflammation with age +/- HIV infection. We performed in depth immunophenotyping of circulating γδ T cells via multi-color flow cytometry and analysis of plasma markers of inflammation, coagulation, and intestinal permeability from subjects of our HIV and Aging cohort, which includes ART-suppressed HIV+ individuals and matched uninfected controls stratified by age into younger (≤35yo) and older (≥50yo) groups. We found discrete differences in the combinational expression the inhibitory receptors (IRs) TIGIT, PD-1, and CD160 between the Vδ1+ and the Vδ2+ γδ T cell subsets with HIV infection and healthy aging. Also, we found that the specific IR signatures of Vδ1+ and Vδ2+ γδ T cells correlated with plasma inflammatory markers; however, different connections between the plasma analytes were found for each of the two γδ T cell subsets. Taken together, these data indicate that Vδ1+ and Vδ2+ γδ T cells exist in distinct states and possess divergent roles in the 'inflamm-aging' found in aviremic HIV+ infection and with normal aging. Further investigation into the anatomical locations, *ex vivo* functions, and additional surface markers that define γδ T cell subsets may reveal novel therapeutic targets to abrogate the aberrant inflammation found with HIV infection, aging, and other inflammatory conditions.

H. 95. Neutrophil subsets and their contribution to immune modulation during pneumococcal pneumonia

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Neutrophils are known for their role in bacterial clearance and implication in pathogenesis of infectious diseases. However, recent reports have described that neutrophils have the ability to induce an anti-inflammatory response. Data obtained in our laboratory showed that during pneumonia caused by *Streptococcus pneumoniae*, neutrophils are able to produce IL-10. However the characteristics of these IL-10-producing neutrophils population in pneumococcal pneumonia remain unknown. In this study we have characterized the neutrophils populations present during the first 48 h post-infection, providing evidence about the existence of at least two predominant populations, where the larger is the one producing IL-10. Furthermore, we performed neutrophil transfer assays to C57BL/6 wild type (WT) and IL-$10^{-/-}$ mice (the latter highly susceptible to *S. pneumoniae* infection) to determine the role of IL-10–producing neutrophils during pneumococcal pneumonia. We transferred WT and IL-$10^{-/-}$ cells to each group and then we infected them with *S. pneumoniae*. A 10-days survival assay during was performed. Likewise, we evaluated lung infiltration of pro-inflammatory cells, as well as bacterial burden in lungs and other organs after 24 h post-infection. These results shown that transferred IL-$10^{-/-}$ mice were less susceptible to *S. pneumoniae* infection than untreated IL-$10^{-/-}$ mice. In contrast, we observed that WT mice transferred with WT cells showed an increased clinical score, decreased survival and a reduced bacterial clearance. Our results strongly suggest that neutrophils able to produce IL-10 might be modulating the lung immune response, playing a critical role during the first 48 h of *S. pneumoniae* infection.

T. 56. Crossreactive influenza A T-cell Receptor Repertoire Diversity and Selection in Acute through Persistent Epstein-Barr Virus (EBV) Infection

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Acute infectious mononucleosis (AIM) is associated with a massive expansion of CD8+ T-cells which is directed at two HLA-A2:01-restricted EBV epitopes: BMLF1$_{280\text{-}288}$ and BRLF1$_{109\text{-}118}$. During AIM unique cross-reactive CD8+ T-cells between IAV-M1 and EBV-BMLF1 expend and modulate the immune response to EBV correlating with disease severity. Using TCR deep sequencing we showed that EBV-BMLF1 and EBV-BRLF1 TCR-repertoires have a persistent component from AIM to convalescence (CONV), composed of 10% of unique clonotypes, that form 50% of total response, and a non-persistent component, composed of 90% of unique clonotypes, forming 50% of total response, replaced with "de novo" clonotypes in CONV. Here, we postulate that as crossreactive antigen is driving expansion of the IAV-M1 TCR-repertoire during AIM it may follow a similar pattern. TCR deep sequencing *ex vivo* of cross-reactive IAV-M1 memory from three AIM patients showed that TCR-repertoire differ from healthy donors persistently infected with EBV by using longer CDR3 lengths, highly polyclonal TRAV family with enhanced runs of glycines, and perturbed TRBV-repertoire with reduced use of TCR families, TRAV27 and TRBV19. The IAV-M1 TCR-repertoire mimicked the EBV-specific responses in 2/3 donors with highly individual dominant clonotypes, unusual VA and VB and CDR3 motifs being maintained from AIM to CONV. One donor maintained the CDRb motif "SARD", a highly public motif found in EBV-BMLF1 T-cell responses. These data would suggest that EBV infection can activate crossreactive influenza A-specific memory cells and influence outcome of infection, but also alter IAV-M1 TCR-repertoire for an extended period of time.

T. 57. Cross-reactivity between influenza A and Epstein Barr Virus CD8 T cell responses influences TCRVβ repertoire evolution with age

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CD8+ memory T cells are generated during primary infection with intracellular pathogens, such as viruses. These cells play an important role in the protection of the host upon re-infection with the same pathogen.In this study, we compare CD8+ memory T cell responses to both influenza A virus (IAV), a recurrent virus and Epstein Barr Virus (EBV), a persistent virus. Using EBV seropositive, HLA-A2+, young adult (18-20 years) and elderly (>65 years) donors, this study demonstrates that CD8+ memory T cell responses to both recurrent and persistent viruses co-evolve as an individual ages. Tetramer-staining was used to study both cross-reactive and antigen-specific cells that were present in peripheral blood and proliferated in response to stimulation with immuno-dominant epitopes of IAV and EBV. There were significant differences in IAV-M1$_{58}$, EBV-BMLF1$_{280}$, and EBV-BRLF1$_{190}$ Vβ usage as individuals

age.Young and elderly adults had different cross-reactive patterns. Young adults had increased opportunity for cross-reactivity due to increased TCR diversity resulting in increased numbers of shared Vb families amongst all 3 epitopes.Elderly donors had evidence of increased focusing on cross-reactivity with a more narrow repertoire.Tracking 3 donors over 10-20 years showed that the changes in TcR repertoire of IAV-M1, EBV-BMLF1 and -BRLF1-specific responses during acute IAV infection may be mediated by TcR crossreactivity and may lead to altered T cell activation and function.This study further emphasizes the complexity of human T cell responses to viruses and the need for a better understanding of human T cell responses in order to design successful T cell inducing vaccines.

T. 58. Dramatically decreased and delayed CD8 T cell responses during asymptomatic EBV seroconversion

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EBV infection can induce acute infectious mononucleosis (AIM), with symptoms of mild to severe lymphadenopathy, sore throat, splenomegaly and prolonged fatigue. These symptoms are associated with a massive expansion of CD8+ T cells, which are mostly directed to two EBV immunodominant epitopes: $BMLF1_{280}$ and $BRLF1_{190}$ in HLA-A2:01+ patients. We have shown that during AIM the frequency of unique crossreactive CD8+ T cells between influenza A (IAV)-$M1_{58}$ and EBV-BMLF1 not only directly correlate with but predict disease severity. However, little is known about EBV-specific and crossreactive CD8 T cell responses in the vast majority of individuals, who asymptomatically seroconvert and become persistently infected. Here, we hypothesized that asymptomatic donors would have decreased EBV-specific and crossreactive CD8 T cell responses near the time of seroconversion as compared to AIM patients. By tracking seronegative donors at the time of college admission every 3-4 months for their 4 years in college we have identified
20 asymptomatic seroconverters who had surprisingly low or non-existent EBV lytic epitope specific responses in the first year after seroconversion with no evidence of cross-reactive IAV-M1/EBV-BMLF1 memory CD8 T cell activation ex vivo. In the presence of these much lower and delayed CD8 T cell responses, their viral loads were equivalent to AIM patients. There was also a great deal of individual variation in the kinetics of their CD8 T cell responses. These results are consistent with CD8 T cell activation mediating the symptomatology of AIM, and strongly suggest that in the asymptomatic donor EBV is silently infecting the host.

T. 59. Epitope selection for DQ2 presentation: implications for celiac disease and viral defense

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We have reported that the major histocompatibility molecule HLA-DQ2 (DQA1*05:01/DQB1*02:01) is relatively resistant to HLA-DM (DM), a peptide exchange catalyst for MHC class II. Here, we analyzed the role of DQ2/DM interaction in the generation of DQ2-restricted gliadin epitopes, relevant to celiac disease, or DQ2-restricted viral epitopes, relevant to host defense. We used paired antigen presenting cells (APC), differing in DM expression (DM^{null} vs DM^{high}) or differing by expression of wild type DQ2 versus a DM-susceptible, DQ2 point mutant DQ2a+53G. The APC pairs were compared for their ability to stimulate $CD4^+$ T cell clones. Despite higher DQ2 levels, DM^{high} APC attenuated T cell responses compared to DM^{null} APC after intracellular generation of 4 tested gliadin epitopes. DM^{high} APC expressing the DQ2a+53G mutant further suppressed these gliadin-mediated responses. The gliadin epitopes were found to have moderate affinity for DQ2, and even lower affinity for the DQ2 mutant, consistent with DM suppression of their presentation. In contrast, DM^{high} APC significantly promoted the presentation of DQ2-restricted epitopes derived intracellularly from inactivated herpes simplex virus type 2 (HSV-2), influenza hemagglutinin and human papillomavirus (HPV) E7 protein. When extracellular peptide epitopes were used as antigen, the DQ2 surface levels and peptide affinity were the major regulators of T cell responses. The differential effect of DM on stimulation of the 2 groups of T cell clones implies differences in DQ2 presentation pathways associated with non-pathogen and pathogen-derived antigens *in vivo*.

T. 61. Generation of functional human T-cells in NOD/SCID/IL2rγnull (NSG) humanized mice without the use of fetal tissue

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Humanized mice engrafted only with human hematopoietic stem cell (HSC) do not develop fully functional T-cells. The BLT mouse model, which consists of co-implanting HSC from fetal liver with autologous fetal

thymic tissue, was developed to promote an optimal T-cell reconstitution and maturation. However, access to human fetal tissues is challenging both from the ethical point of view and logistic. The goal of our study was to find an alternative to the use of fetal tissues to create a humanized mouse model with functional T-cells. **Methods**. We used pediatric thymus excised during cardiac surgeries (CS thymus) combined with umbilical cord blood CD34+ cells (CCST mice). CS thymuses pieces were implanted in the quadriceps of an NSG mouse, after being put in culture **Results.** CCST mice exhibited a significant engraftment of T-cells, compared to humanized mice without thymus ($p<0.0001$). T-cells from both CCST and BLT mice showed a similar function as evaluated by proliferation assays upon PHA stimulation *ex vivo* and rejection of allogeneic leukemic cells lines *in vivo.* CCST mice were susceptible to HIV-1 infection via mucosal or intraperitoneal route, as shown by detectable viral load, HIV DNA and p24+ cells, at similar levels to those of BLT mice. Importantly, CCST mice displayed more effective ex vivo HIV-1-specific T-cell responses compared to BLT ($p<0.0001$ for $CD8^+$cells, $p<0.01$ for $CD4^+$cells). **Conclusions.** CCST mice represent an alternative to the regular BLT mouse model. Those easy-to-access thymuses can be used to generate a large number of mice compared to fetal thymuses.

T. 62. High Interleukin-7 serum concentrations in chronic infectious lung diseases as biomarkers of T-cell dysfunction

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Interleukin (IL)-7 is constantly expressed and IL-7 serum levels are largely influenced by T-cell mediated consumption. Lymphopenia is so far the only described pathology marked by increased IL-7 serum levels.

We found increased IL-7 serum levels in tuberculosis and cystic fibrosis patients who suffer from repeated pulmonary infections. IL-7 levels normalized during therapy of tuberculosis patients and were associated with worsened lung function of cystic fibrosis patients. These findings indicated a novel role of high IL-7 serum levels in immune pathogenesis of infectious diseases. In tuberculosis patients, lower soluble IL-7 receptor concentrations and impaired IL-7 sensitivity of T cells due to decreased IL-7 receptor expression were found as possible triggers of high IL-7 serum levels. In addition, we identified an SNP (i.e. rs1494558) that was associated with protection against tuberculosis and affected soluble and membrane IL-7 receptor a-chain expression. This SNP was previously described to increase the risk for type-1 diabetes and we confirmed lower soluble IL-7 receptor serum levels of type-1 diabetes patients carrying rs1494558. Notably, higher serum IL-7 levels in rs1494558 carriers were found in type-1 diabetes patients as compared to carriers of a protection associated SNP (i.e. rs6897932), who also had lower soluble IL-7 receptor serum levels. These findings argued against an exclusive role of soluble IL-7 receptor in IL-7 regulation. Combined ectopic expression of IL-7 receptor variants in cell lines confirmed that the type-1 diabetes protective SNP markedly enhanced membrane IL-7 receptor a-chain suggesting that IL-7 serum effects were caused by membrane IL-7 receptor function.

T. 63. High levels of CD38 expression in SLE CD8 T cells dictate decreased cytotoxicity

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[Background/Purpose]

CD38 is an ectonucleotidase which degrades nicotinamide adenine dinucleotide (NAD). We found a negative correlation between CD38 expression on CD8 T cells and infections in patients with SLE. We sought to determine whether CD38 is responsible for the decreased cytotoxicity of CD8 T cells in SLE.

[Methods]

We used sorted human primary CD8 T cells, TALL-104 and CRISPR-generated CD38-deficient Jurkat cells. Cells were stimulated with anti-CD3/CD28 or P815 cells. Degranulation and cytotoxicity were assayed by flow cytometry (FCM). Expression of cytolytic molecules (granzymeB, perforin and IFN-g) and the transcription factors Eomes, T-bet and EZH2 were measured by FCM and qPCR. NAD production and protein acetylation were measured using colorimetric or Western blot techniques.

[Results]

Compared with $CD38^{low}$, $CD38^{high}$ CD8 T cells displayed lower cytotoxicity as determined by the expression of CD107a, granzyme B, perforin and IFNg. Eomes and T-bet, which regulate cytotoxic function of CD8 T cells, were decreased in $CD38^{high}$CD8 T cells. CD38 suppressed the activity of the NAD-dependent deacetylase Sirt1. $CD38^{high}$ CD8 cells had lower NAD and higher acetylation level on lysine residues and stabilized expression of EZH2 which is known to repress Eomes and T-bet. Finally, overexpression of CD38 in $CD38^{low}$CD8 T cells or TALL-104 cells increased acetylated protein and decreased cytotoxicity against P815 cells.

[Conclusions]

We demonstrated that CD38 reprograms cytotoxicity in CD8 T cells and controlls the cytotoxic response of CD8 T cells. This newly identified molecular pathway in T cells from patients with SLE may explain their increased susceptibility to infections.

T. 64. HVEM Signaling Promotes Protective Antibody-Dependent Cellular Cytotoxicity (ADCC) Vaccine Responses to Herpes Simplex Viruses (HSV)

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HSV glycoprotein D (gD) is an immunodominant envelope glycoprotein required for entry, but also competitively binds the costimulatory molecule, HVEM (TNFRSF14), which is expressed on immune cells. Natural infection elicits neutralizing antibodies (nAbs) targeting gD, but vaccines designed to elicit gD-specific nAbs have failed clinically. We constructed a single-cycle HSV-2 vaccine deleted in gD, DgD-

2. This vaccine induces non-neutralizing antibodies that activate the Fc gamma receptor (FcgRIV) and mediate ADCC. Active or passive immunization protects in mice and prevents the establishment of latency. We hypothesize that gD blocks the generation of ADCC through interactions with HVEM. Wild-type mice were immunized with DgD-2; dl5-29, a replication-defective HSV-2 strain that expresses gD, or adjuvanted gD protein (rgD-Alum/MPL) prior to challenge with HSV. ΔgD-2 protected 100% of mice from disease and latency following challenge with clinical isolates of HSV, while dl5-29 and rgD-2/AS04 provided incomplete protection. Protection correlated with ADCC, not nAb titer. Immunization of HVEM-/- or LIGHT-/- (an HVEM ligand) mice resulted in a significant reduction in ADCC and loss of protection. Surprisingly, transfer of immune serum from wild-type mice also failed to protect HVEM-/- mice, suggesting a role for HVEM in generating and mediating ADCC responses. Immune cells isolated from HVEM-/- mice were impaired in mediating cell killing. Addition of gD protein or anti-HVEM antibodies inhibited FcgR activation with mouse or human immune serum. These findings uncover a previously unrecognized function of HVEM in generating and mediating ADCC and suggest that gD blocks HVEM signaling as an immune evasion strategy.

T. 65. Identification of a common transcriptional signature for regulatory B cells in Humans and Mice

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Regulatory B cells (Bregs) have been described in mice and humans for their ability to regulate inflammation through a variety of mechanisms in different pathological situations. Up to date, no consensual and common Breg phenotype has been described, and whether there is a Breg lineage commitment or if they acquire their function under certain environmental conditions remains unknown. To address these points, we performed a sample size weighted meta-analysis of publicly available transcriptomic data from 4 different Bregs studies in humans and 6 Bregs studies in mice. In humans, 1265 genes were differentially expressed with a false discovery rate <5%. This pattern was cross-validated to highlight a core Breg signature of 61 unique genes. A similar analysis in mice revealed a core signature of 174 differentially expressed genes with a false discovery rate <5%. The comparison between humans and mice datasets identified a unique common signature of 11 genes. While we observed high levels of expected genes, such as *IL10*, in Bregs, we also highlighted additional genes related to regulatory function in humans and mice, including *GZMB* and *CD9*. This transcriptional profile is under validation within different Breg populations by qPCR and flow-cytometry. Identification of a unique and common transcriptional Breg signature as well as extracellular markers will allow identifying, characterizing and sorting Breg cells and will offer new options for future cell therapy.

T. 66. Identification of a Novel NK Cell Immune Evasion Strategy Based on a Secreted Viral CD48 Decoy Receptor

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Cytomegaloviruses (CMVs) have evolved multiple mechanisms to subvert host immunity and establish successful long-term infections. To accomplish it, they encode a large repertoire of immune modulator genes, some of which derive from their host genomes after being captured at different points during host-virus co-evolution. CD48is a GPI-anchored protein that contains an ectodomain composed by 2 immunoglobulin (Ig) domains. Via its N-terminal Ig domain, CD48 recognizes the cell surface receptor 2B4. Engagement of 2B4 by CD48 results in the regulation of cytotoxic T lymphocyte and NK cell functions.We have recently reported the presence of a number of CD48 homologs (vCD48) encoded by different CMVs. Here, we have characterized the three vCD48 of owl monkey CMV, showing that they are highly glycosylated transmembrane proteins which display very distinctive structural and biochemical properties. Among them, only A43, the viral CD48 that exhibits the highest amino acid identity with host CD48 is able to bind 2B4, with the two other vCD48s having diverged to perform 2B4-independent functions. Interestingly, A43 is a soluble protein, released from the cell after being proteolitically processed through its stalk region. Kinetic studies revealed that A43:2B4 interactions are of exceptional affinity and highly stable. We demonstrate that purified soluble A43 efficiently blocks CD48:2B4 interactions. Furthermore, this viral protein is capable to impair immune-cell conjugate formation, interfering with the establishment of the mature NK cell synapse, and severely abrogating 2B4 mediated-NK cell cytotoxicity and interferon production. Thus, A43 acts as a functional virally-encoded CD48 decoy receptor.

T. 67. IL-1 and IL-33 differentially regulate the functional specialization of mucosal Foxp3+ regulatory T cells

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$CD4^+$ regulatory T (T_{REG}) cells are critical mediators of peripheral immune tolerance and homeostasis and express the forkhead box p3 (Foxp3) transcription factor. T_{REG} cells are abundant at mucosal surfaces, where they respond to local signals in order to adapt their transcriptional program. The consequences of these modifications on T_{REG} cells remain largely unknown. To understand the processes involved, we compared the mRNA signature of $Foxp3^+$ and $exFoxp3^+$ T cells isolated from a model developed to study the reprogramming of T_{REG} cells into Th1/Th17 effector T cells. We uncovered that the IL-33 receptor (IL-33R, ST2) was prominently expressed by T cells that maintained Foxp3 expression, while the IL-1 receptor (IL1R1) was expressed on cells that ultimately lost Foxp3. Interestingly, both $ST2^+$ and $IL1R1^+$ T_{REG} cells populations compete for expansion under inflammatory

conditions: the absence of IL1R1 expression (IL1R1$^{-/-}$) leads to the accumulation of ST2^{+} T$_{REG}$ cells at mucosal surfaces, while IL-33 injections facilitate the accumulation of ST2^{+} T$_{REG}$ cells and reduce the onset of exFoxp3 T cells. Using lung infection models, we demonstrate that ST2-expressing T$_{REG}$ cells resist production of inflammatory cytokines, whereas IL1R1-expressing T$_{REG}$ cells express RORγT and proinflammatory cytokines. While IL-1 signalling impairs T$_{REG}$ cell suppressive function, IL-33 is required for the successful prevention of T-cell dependent colitis. Thus, ST2^{+} and IL1R1^{+} T$_{REG}$ represent two distinct populations of reprogrammed T$_{REG}$ cells. These observations demonstrate that IL-1 and IL-33 produced during immune challenge exert distinct roles on the functional adaptation of Foxp3^{+} T$_{REG}$ cells at mucosal surfaces during infections.

T. 68. Isolation and Characterization of an HIV-1 Broadly Neutralizing Antibody From a Clade C Infected Pediatric Elite Neutralizer

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Till date there is no effective vaccine against HIV-1. Human trials and primate challenge studies have shown the protective role of broadly neutralizing antibodies (bNAbs) against HIV-1. Elite-neutralizers are potential candidates for isolation of HIV-1 bNAbs. Disease progression is faster in HIV-1 infected children than adults. Plasma bNAbs with multiple epitope specificities are developed in HIV-1 chronically infected children with more potency and breadth than in adults. Therefore, we evaluated the specificity of plasma neutralizing antibodies of an antiretroviral naïve HIV-1 clade C chronically infected pediatric elite neutralizer AIIMS_330. The plasma antibodies showed broad and potent HIV-1 neutralizing activity with >87% (29/33) breadth, median inhibitory dilution (ID50) value of 1246 and presence of N160 and N332-supersite dependent HIV-1 bNAbs. The sorting of BG505.SOSIP.664.C2 T332N gp140 HIV-1 antigen-specific single B cells of AIIMS_330 resulted in the isolation of an HIV-1 N332-supersite dependent bNAb AIIMS-P01. The AIIMS-P01 neutralized 67% of HIV-1 cross-clade viruses; exhibited substantial indels despite limited somatic hypermutations; interacted with native-like HIV-1 trimer as observed in negative stain electron microscopy and demonstrated high binding affinity. Deletion of the 5 amino acid insertion in the FR3 region of the bNAb led to reduction in neutralizing acitivy of the bNAb. In addition, AIIMS-P01 potently neutralized the coexisting and evolving autologous viruses suggesting the coexistence of vulnerable autologous viruses and HIV-1 bNAbs in AIIMS_330 pediatric elite neutralizer. Characterization of such bNAbs and , screeing the infected plasma for presence of similar bNAbs will provide information towards immunogen design.

T. 69. Laboratory diagnosis and monitoring of treatment efficacy in Lyme disease using an IFNgamma release assay

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The laboratory diagnosis of Lyme disease is currently based on the detection of antibodies against *Borrelia burgdorferi.* Antibody assays are typically not useful indicators of treatment response, as antibody can remain elevated for months to years after infection. We previously demonstrated that IFNγ secretion is detectable in antigen-stimulated whole blood collected from patients at their initial presentation with erythema migrans (EM). The IFNγ response was reduced in paired blood samples two months after treatment, portending that monitoring cellular immunity correlates with treatment efficacy. Expanding on this initial study, we enrolled an additional 44 adults and 26 children in New York and Wisconsin in 2016-18. We incubated whole blood (1CC) overnight with mixtures of *B. burgdorferi* peptide antigens, and quantified plasma levels of IFNγ by ELISA. 33 adults and 19 children received a physician diagnosis of Lyme disease. 25/33 (75.7%) adults and 15/19 (78.9%) children produced significant levels of IFNγ (>3SD above the mean of controls). IFNγ production was reduced at 4 weeks and/or 6 months post-treatment in 16/25 (64%) adults and 12/13 (92.3%) children that were initially positive. By contrast, anti-*B. burgdorferi* antibody remained elevated at follow-up in all patients that seroconverted. Only one control patient had detectable IFNγ, and was also serologically positive by C6 and WCS ELISA. These results support the hypothesis that monitoring *Borrelia*-specific T cell activation through IFNγ release may be an effective method for the laboratory diagnosis of Lyme disease, and for monitoring treatment efficacy.

T. 70. Long-Term Durability of Protective Memory in Recurrent Staphylococcus aureus Skin Infection

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Staphylococcus aureus is the leading cause of skin and skin structure infection (SSSI), a primary portal of entry for invasive infection. Our prior studies discovered a role for innate immune memory in protection against recurrent methicillin-resistant *S. aureus* (MRSA) SSSI. Priming infection 6 weeks earlier resulted in protective memory upon recurrent infection in wild-type and *rag1*$^{-/-}$ mice. To test durability of this memory, we infected wild-type mice one year prior to secondary challenge. Eight-week old mice were infected with MRSA subcutaneously, allowed to resolve for one year, and infected again with the identical MRSA strain. Lesion sizes were measured on days 1, 3, 5 & 7 post-secondary challenge and MRSA were enumerated in skin abscesses, kidney, spleen and liver on day 7. Remarkably, primed mice exhibited protective memory in skin, manifest as reduced lesion sizes and MRSA burden in abscesses during secondary MRSA challenge. However, there was no protection against disseminated infection, as similar MRSA burden was observed in the kidney, spleen and liver. Cellular signatures of protection were represented by increased numbers of monocytes and NK cells in abscesses, and monocyte intensification in lymph nodes. As expected, cell populations in the spleen were similar. In summary, present findings indicate that protective immunity to *S. aureus* infection is tissue-targeted, involves monocytes and NK cells, and durable. These insights enhance understanding of mechanisms of immune

protection vs. *S. aureus*, and may hold novel targets for vaccine and immunotherapeutic development against MRSA.

T. 71. Micro-RNA 29 regulates age-related differences in the CD8+ T cell response

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Neonates are highly susceptible to infection and respond poorly to vaccination for reasons that are not well understood. Based on our published data, we believe neonates are particularly vulnerable to repeated infections because their naïve CD8+ T cells are intrinsically defective at differentiating into memory CD8+ T cells. To understand the underlying basis for these age-related differences, we performed smallRNA sequencing and found that one miRNA particularly (miR-29) was selectively upregulated in adult CD8+ T cells (mice and humans) and acting on their target genes in a predictable manner. We also performed adoptive transfer experiments and compared the ability of WT and miR29KO donor CD8+ T cells to respond to infection. Interestingly, we found that donor mir29KO CD8+ T cells secreted more effector molecules (IFNg, gzmB), expressed higher levels of transcription factors (Tbet, Eomes) associated with effector cell differentiation, and failed to form certain subsets of memory CD8+ T cells (central memory, tissue resident memory), similar to neonatal CD8+ T cells. To translate these findings to humans, we manipulated mir-29 expression in naïve human CD8+ T cells. By introducing mir-29 mimics and antagomirs, we were able to age-adjust mir-29 expression and target genes in neonatal (cord) and adult CD8+ T cells (PBMCs) and are currently comparing the ability of these cells to respond to stimulation. Our research on miR-29 has the potential to uncover novel therapeutic strategies for enhancing the development of neonatal memory CD8+ T cells and identify biomarkers for predicting how individuals respond to vaccination.

T. 72. Modeling CD8+ T Cell Responses Against Liver Infection with 3D Organoids

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Despite the advent of direct-acting antiviral therapies, infection rates for Hepatitis C Virus (HCV) remain high and a vaccine remains necessary to reduce infections rates. Although it is known that a diverse CD8+ T cell response is needed to clear acute HCV infection in the liver, human studies at the HCV-infected liver:T cell interface remain challenging. Here, we present a method to coculture human HLA-matched HCV-specific T cells with human liver organoids infected with HCV. We show by light sheet microscopy that class I HLA is expressed on the external surface of liver organoids, at comparable levels between HCV+ and HCV- donors. While liver organoids are exquisitely sensitive to media containing FBS, we find that T cell clones retain their viability and cytolytic capacity in organoid media for up to five days. To monitor T cell:organoid interactions, the two cell systems were co-cultured in a specific

microfluidic chip (Roger Kamm) that allows for tractable migration of T cells into the organoid-containing matrigel. The system was then analyzed within the chip by quantitative microscopy or after removal by flow cytometry. We find that loading surface HLA with specific HCV peptides induced T cells to express cytolytic cytokines and caused highly reproducible cytolysis of the organoids. We are currently performing experiments with HCV-infected organoids and autologous T cells, the results of which will be discussed. We propose that this coculture system presents a new way to study HCV antigenicity, T cell specificity and the immunotolerant liver environment in a highly tractable manner.

T. 73. Phlebotomus papatasi Yellow-Related and Apyrase Salivary Proteins Are Candidates for Vaccination against Human Cutaneous Leishmaniasis

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Nowadays, there is no available vaccine for human leishmaniasis. Animal experiments demonstrate that pre-exposure to sand fly saliva confers protection against leishmaniasis. Our preceding work in humans indicates that Phlebotomus papatasi saliva induces the production of IL-10 by CD8+ T lymphocytes. The neutralization of IL-10 enhanced the activation of a T-cell CD4+ population-producing IFN-γ. Herein, we used a biochemical and functional genomics approach to identify the sand fly salivary components that are responsible for the activation of the T helper type 1 immune response in humans, therefore constituting potential vaccine candidates against leishmaniasis. Fractionated P. papatasi salivary extracts were first tested on T lymphocytes of immune donors. We confirmed that the CD4+ lymphocytes proliferate and produce IFN-γ in response to stimulation with the proteins of molecular weight >30 kDa. Peripheral blood mononuclear cells from immune donors were transfected with plasmids coding for the most abundant proteins from the P. papatasi salivary gland cDNA library. Our result showed that the "yellow related proteins," PPTSP42 and PPTSP44, and "apyrase," PPTSP36, are the proteins responsible for the aforementioned cellular immune response and IFN-γ production. Strikingly, PPTSP44 triggered the highest level of lymphocyte proliferation and IFN-γ production. Multiplex cytokine analysis confirmed the T helper type 1-polarized response induced by these proteins. Importantly, recombinant PPTSP44 validated the results observed with the DNA plasmid, further supporting that PPTSP44 constitutes a promising vaccine candidate against human leishmaniasis.

T. 74. Prevalence of Rotavirus and Levels of Immunoglobulin (IgA, IgG, IgM) in Children with Gastroenteritis in Nnewi, Anambra State, Nigeria.

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Rotavirus remains the principal cause of severe diarrhoea among infants worldwide. Study aimed at determining the prevalence of rotavirus infection and blood levels of immunoglobulins among children with acute gastroenteritis in Nnewi. The subjects comprised of 43 symptomatic rotavirus positive, 33

symptomatic rotavirus negative, 14 asymptomatic rotavirus positive, 31 asymptomatic rotavirus negative. They were 1 – 59 months of age. Blood samples were collected Rotavirus screening was determined by enzyme-linked immunosorbent assay, immunoglobulin levels by turbidimetric technique. The prevalence rate of rotavirus was 47.16%. Rotavirus gastroenteritis cases occurred highest (40.4%) in children aged 10-18 months. Seropositivity of rotavirus recorded highest among children whose parents had tertiary school education (28.4%). But level of education had no significant impact on rotavirus status χ^2 (1, N = 121) = 3.18, p = 0.08. Rotavirus seropositivity compared between the exclusive and non exclusive breast fed babies, showed no significant difference (P=0.880). Mean IgM mg/dl levels compared among, groups 1 (323.52±76.89), 2 (308.48±79.618), 3 (318.83±67.41) and 4 (299.31±64.58) showed no significant difference (f value=0.718, p=0.543). Immunoglobulin G mg/dl mean levels in group1 (1755.16±229.55), group 2 (1751.12±236.07), group 3 (1770.66±216.02) and group 4 (1730.428±234.93) were not significant (f =0.118, p=0.949). Immunoglobulin A mg/dl mean levels; groups 1 (110.175±64.09), 2 (103.09±58.27), 3 (132.965±55.06) and 4 (105.437±60.31) were not significantly different (f =0.871, p=0.459). In conclusion, the prevalence of rotavirus was 47.16 in Nnewi. There was no significant deference in immunoglobulin levels among the groups.

T. 75. Pro-inflammatory cytokines as potential prognosis biomarkers in respiratory diseases caused by virus

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Background. Lower respiratory tract infections (LRTIs) are considered as problem global public health in the pediatric population. Respiratory virus causes up to 60% of all LRTIs in children. When respiratory virus arrival to the airway, stimulate the production of several pro-inflammatory cytokines in the respiratory epithelium and generate an excessive immune reaction. New evidence has shown that some these cytokines taken in infants undergoing viral LRTIs may be used to predict severe acute illness. ***Aim.*** To identify nasopharyngeal biomarkers for prediction of disease severity in LRTI infants. ***Methodology.*** Nasopharyngeal swabs from 87 patients *Results.* 45 were diagnosed with LRTI (52%) and 42 were diagnosed as ARIs (48%). We observed that the levels of IL-3, IL-8 and IL-33 increased more in LRTI patients positive for virus. Finally, we found that patients with high IL-33 and IL-8 levels at the beginning of study were more susceptible to cough and ARIs to 1 month and 6 months after first infection, respectively. ***Conclusions.*** In this study we observed that high levels of IL-33 and IL-8 are associated with complications in infected patients with virus and they could be potential prognosis biomarker in virus infections.

T. 76. Reprogramming of Exhausted T Cells Following Cure of Chronic Viral Infection

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Exhausted T cells (**Tex**) are a hallmark of chronic infection and cancer. Blocking inhibitory receptors such as PD-1 reinvigorates Tex, but many patients fail to achieve durable disease control. Thus, deeper understanding of reversal of T cell exhaustion is needed. Little is known about "reprogramming" of Tex into recovered T cells (**Trecov**) following cure of chronic disease. Here, we aim to determine molecular mechanisms of recovery from exhaustion. To "cure" chronic infection, we adoptively transferred Tex from LCMV-clone13 infected mice into antigen-free mice. Our results reveal recovery of some phenotypic markers of memory T cells (**Tmem**) and partial recovery from dysfunction, but some "scars" of Tex persisted. For example, CD127/IL-7R expression increased and PD-1 decreased on Trecov, suggesting differentiation toward memory, but frequency of IL-7R+ cells was still lower and PD-1 expression higher when compared to bona fide Tmem. Additionally, single-cell RNAseq indicated that Trecov gene-expression profile resembles Tmem in some respects, but similar to Tex cells in other features. We tested how these changes impacted Trecov recall response, and our challenge experiments revealed that although Trecov recall capacity was better than Tex, it was still inferior to Tmem on a per cell basis. We are currently investigating whether the transcriptional and functional scars have epigenetic roots. Together, these results suggest that following elimination of persistent antigenic stimulation previously-exhausted T cells recover some Tmem properties, while other aspects remain "scarred" from their exhaustion history. These studies will enhance our understanding of mechanisms of Tex recovery, and identify novel immunotherapeutic strategies.

T. 77. Role of Ly6G+ cells during Salmonella enterica serovar Typhimurium infection

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Background: *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium) is Gram-negative bacterium and an important is a worldwide problem that cause of bacterial foodborne and the leading cause of invasive non-typhoid disease, mainly in children. To infect the host, *S.* Typhimurium has several virulence factors encoded in chromosomal cluster known as *Salmonella* Pathogenicity Islands (SPI). The most important are SPI-1 and SPI-2, which allow the infection of intestinal epithelial cells and the survival inside phagocytic cells, respectively. The initial innate immune response is the migration of neutrophils to the site of infection playing an important role by restricting the bacterial growth and dissemination. These cells attack the bacteria using different mechanisms, however, these are not enough to avoid *S*. Typhimurium dissemination. For this reason, it is possible that *S*. Typhimurium evade the function of neutrophils. **Objectives:** To evaluate the neutrophil response against *S.* Typhimurium. **Methods:** We isolate bone marrow derived neutrophils from male and female C57BL/6 wild type mice and evaluate the infection of the neutrophils, intracellular bacterial survival and replication, ROS and cytokine production and NETs release against *S.* Typhimurium infection. We evaluate these parameters with the *wild type* strain, and two mutant strain: D*spi-1* and D*spi-2*, in order to evaluate the immune response mediated by this type of cell and evaluate if the virulence factor encoded in this SPI are relevant during the infection of neutrophils. **Conclusion**: The immune response of $Ly6G^+$ against *S.* Typhimurium infection depends on the SPI that the bacterium possess and the sex of the mice.

T. 78. Scalable and comprehensive characterization of antigen-specific CD8 T cells using multi-omics single cell analysis

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Understanding the antigen binding specificities of lymphocytes is key to the development of effective therapeutics for cancers and infectious diseases. Recent technological advancements have enabled the integration of simultaneous cell-surface protein, transcriptome, immune repertoire and antigen specificity measurements at single cell resolution, providing comprehensive, scalable, high-throughput characterization of immune cells.

Using the 10x Genomics Single Cell Immune Profiling Solution with Feature Barcoding technology along with oligo-conjugated antibodies and peptide-MHC (pMHC) Dextramer reagents, we performed multi-omic characterization of PBMCs from cytomegalovirus (CMV) seronegative and seropositive patients. Full length, paired TCRα/β sequences with specificity to known CMV antigens were identified in the seropositive donor, but not in the seronegative donor. A large Epstein Barr Virus (EBV) pMHC specific T cell expansion was identified in the CMV seronegative donor, suggesting an active EBV response. Moreover, the combination of transcriptomic and cell surface protein information resulted in an increase in resolution of cell type identification. This workflow allowed the identification of enriched amino acid motifs within the TCR sequences that contained novel and known CDR3 amino acid sequences specific to CMV.

We scaled this technology with 14 oligo-conjugated antibodies and 50 pMHC Dextramer reagents spanning different CMV, EBV, Influenza, HIV, and Cancer antigens and performed a comprehensive characterization of ~200,000 CD8+ T cells from four MHC-matched donors. In addition to identifying novel and known TCR–pMHC specificity, we also observed TCR alloreactivity. These technological advancements provide new biological insights that are critical for progress in the field.

T. 79. Signaling via the TLR adaptor MyD88 produces transient immune unresponsiveness during infection of mice with pathogenic Salmonella

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Infection of mice with pathogenic *Salmonella* activates innate immune receptors including Toll-like receptors (TLRs) and Nod-like receptors (NLRs) leading to secretion of inflammatory cytokines and chemokines. These inflammatory responses while contributing to innate immunity also bring about splenomegaly largely through expansion of immature reticulocytes. Whether these changes in splenic cellularity influence immune responses to non-*Salmonella* antigens that an infected host might encounter during this period has not been studied. Here, we show that infection of mice with live but not antibiotic -treated *Salmonella* Typhi produced an early inflammatory response that led to splenomegaly accompanied by increased numbers of TER119+ reticulocytes and F4/80+ macrophages, and reduced

numbers of T and B lymphocytes. These changes, mediated through signaling from the TLR adaptor MyD88, did not affect antibody response to *Salmonella,* and both WT and MyD88 deficient mice produced comparable levels of antibodies. However, mice infected with *Salmonella* responded poorly to tetanus toxoid (TT) and ovalbumin administered at the time of splenomegaly. The ability of mice to elicit antibodies to non-*Salmonella* antigens was restored only after reversion of splenic cellularity back to normal state. These results suggest that changes in splenic cellularity brought about by infection with *Salmonella* might have previously unappreciated impact on immune response to non-*Salmonella* antigens. These findings have significant implications for host defence against other pathogens during typhoid fever and vaccine development against pathogenic *Salmonella*.

T. 80. Temperospatial Evolution of Cellular Immune Memory During MRSA Infection

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Staphylococcus aureus is the leading cause of skin and skin structure infection (SSSI), a primary portal of entry for invasion. Our previous studies discovered a role for protective immune memory against methicillin-resistant *S. aureus* (MRSA) SSSI. Prior infection (priming) protected against localized and disseminated infection at day 7 post-infection (late) in SSSI, but not at day 2 (early). Protective cytokines in skin included increased IL-17, IL-6, MIG and RANTES, while increased IP-10 correlated with protection from dissemination. Here, we characterized evolution of cellular immunity over time (early vs. late) and space (skin abscess; draining inguinal lymph node [iLN]; spleen) in naïve versus primed mice during MRSA SSSI. Early CD4+ Th1 and Th17, and CD8+ T cell subset infiltration in skin, relative to CD4+ subset egress from iLN, corresponded to protection in abscesses of primed mice. Early and late M1 macrophage (Mf) accumulation in skin and iLN correlated with protection in abscesses. In the spleen, priming resulted in gd-T, NK, ILC1 and dendritic cell accumulation during early SSSI. However, Th17, ILC2 and ILC3 populations dominated during late infection, corresponding to protection in the spleen. Together, these findings indicate that priming induces sustained T cell and M1 Mf responses in skin, but late ILC responses in spleen correlating with protective immune memory. This pattern of results reveals coordinated temperospatial evolution of cellular immune memory during MRSA infection. Such immune cell signatures likely synergize with other immune effectors in respective tissues. These insights provide new targets for vaccine and immunotherapeutic strategies against MRSA.

W. 75. USP11 facilitates TGF beta signalling to augment the Treg and Th17 differentiation axis in CD4+ T cells

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We have shown that distinct mRNA translational signatures distinguish Foxp3+ regulatory (T_{REG}) from conventional $CD4^+$ effector T (T_{EFF}) cells through genome-wide analysis of cytosolic and polyribosome-associated mRNA levels in $CD4^+$ T cell subsets. mRNA encoding Ubiquitin Specific Peptidase 11 (USP11) was preferentially translated in TCR-activated T_{REG} cells. USP11 is known to modulate TGF-β signals but its function in T cells remains uncharacterized. Given the preferential translation of USP11 in T_{REG} cells and the importance of TGF-β in T_{REG} cell development, we examined whether this differential translation of USP11 mRNA could affect T_{REG} cell differentiation and function. Herein, we employ viral transduction to ectopically express or knock down USP11 in primary $CD4^+$ T cells, along with pharmacologic inhibition of USP11 to determine how altered USP11 expression affects $CD4^+$ T cell subset differentiation, lineage commitment and function. In a lymphopenia model, USP11 expression correlated with T_{REG} cells that maintained Foxp3 expression and kept a T_{REG} phenotype. Ectopic USP11 expression in T_{REG} cells *in vitro* enhanced lineage commitment and suppressive function. Additionally, ectopic USP11 expression in T_{EFF} cells facilitated TGF-β signalling. This led to enhanced Foxp3 induction both *in vitro* and *in vivo*. Conversely, shRNA knockdown of USP11 reduced Foxp3 induction both *in vitro* and *in vivo*. Furthermore, ectopic USP11 expression in T_{EFF} cells drove T_H17 differentiation in polarizing conditions whereas inhibition of USP11 enzymatic activity reduced Th17 differentiation and Foxp3 induction *in vitro*. In conclusion, we identified a novel mechanism regulating the T_{REG} and Th17 differentiation axis in $CD4^+$ T cells.

W. 76. Development and clinical evaluation of a recombinant vaccine for hRSV

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The human respiratory syncytial virus (hRSV) is among the leading pathogens that cause acute respiratory tract infections, resulting in bronchiolitis and pneumonia in the children, elderly and immunocompromised populations. Efforts for the licensing of a vaccine both protective and cost/effective against this virus, have been unsuccessful up to date. Our laboratory developed a recombinant *Mycobacterium bovis* BCG that expresses the Nucleoprotein of hRSV (rBCG-N) with promising protective capacities. Pre-clinical studies showed that disease parameters -such as weight loss, PMN cells infiltration and viral loads- are reduced in immunized, as compared to non-immunized mice. Immunization was also able to induce a Th1-like immune response, especially suited for the clearance of this virus, with the secretion of IFN-g by $CD4^+$ and activation of cytotoxic $CD8^+$ T cells, with both cell types required for the protection, as transfer of only one of them into naïve mice does not protect upon challenge. Higher antibodies titers against the virus -and several of its proteins- are elicited upon immunization and challenge, as compared to non-immunized mice. Remarkably, these antibodies are capable of protecting challenged naïve mice when sera transfers are performed. This vaccine was manufactured under GMP conditions, exhibiting the same protective capacities seen for the non-GMP vaccine in mice. Recently, a Phase 1 clinical trial was performed with this GMP vaccine, exhibiting significant safety and

immunogenicity in healthy adults. These data support the notion that this vaccine is a promising candidate to protect the human population against hRSV.

W. 77. A novel peripheral T helper subset drives extrafollicular antibody response in dengue

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Antibodies play crucial role in dengue virus pathogenesis and disease severity. Understanding the factors decisive of optimal antibody responses is essential for the rational development of dengue vaccine. In this study, we sought to determine the traits and function of CD4 T helper cell subsets in formulating antibody response to dengue virus. Here, in longitudinal and antigen-specific analyses we demonstrate the existence of a novel T helper (Th)-subset that displays follicular T helper (Tfh)-like characteristics and expands robustly in the critical phase of dengue, together with conventional Tfh cells. Moreover, the novel Th subset harbors the potential of B-cell help in terms of expressing key *help* factors; IL21, CD40L, IL10 and IFNγ. We further demonstrate that IgG responses to NS1 are independent of germinal center (GC) reaction and that novel Th subset is superior to Tfh subset in inducing plasma cell differentiation and NS1 specific IgG in autologous T-B co-cultures. Our work describes the existence of a unique CD4 T helper subset as an underlying determinant of excessive antibody responses in dengue, which has implication for the rational development of vaccine and therapy.

W. 78. Age, Biological Sex and Race Affect Adaptive Immune Response to Vaccination

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The goal of our study was to investigate whether demographic variables contribute to inter-individual heterogeneity in immune responses to influenza, smallpox, and measles-mumps-rubella vaccines in a diverse population of heathy children and adults.
For the influenza vaccine (50-74y/o, 62% females, 99% Caucasian), we found a negative correlation between TREC and age (p=0.001). Memory B-cell-Elispot responses were higher in females (p=0.02). Females had higher TREC levels (p=0.0003), NK-cells (p=0.005), CD8+T-cells (p=0.005), and an increased CD4+/CD8+ ratio (p=0.04).
For the smallpox vaccine (18-40y/o, 26% females, 54% Caucasian), females had higher neutralizing antibodies (159 vs 124, p<0.0001) and higher secretion of IL2 (p<0.001)/IL10 (p=0.02). Males has higher IFNγ-Elispot responses compared to females (55 vs 41 SFU, p<0.001). Caucasians had higher CD8+IFNγ-Elispot (p<0.001), IL2 (p=0.003)/IFNa (p<0.001) responses compared to African-Americans.
For the measles vaccine (11-41y/o, 27% females, 77% Caucasian), no age/sex-based differences in adaptive immunity were observed, but significantly higher neutralizing antibody (p=1.4x10^11) and IFNγ-Elispot (p=0.001) responses were found in African-Americans compared to Caucasians.

For the mumps vaccine (12-18y/o, 47% females, 93% Caucasian), females demonstrated significantly higher antibodies than males (876 vs 677 IU/mL, p=0.003). Higher levels of CD4+T-cells were found in males than in females (p=0.03).
For the rubella vaccine (11-22y/o, 45% females, 85% Caucasian), African-Americans had significantly higher rubella-specific neutralizing antibodies compared to Caucasians (p=0.0007); females had higher IL6 (p=0.03)/IFNγ (p=0.08) secretion than males.
Our data illustrate that viral vaccine-induced immune responses are significantly influenced by age, sex, and race, which highlights the importance of these variables in inter-individual variations following vaccination.

W. 79. An inflammatory status in the brain causes behavioral alterations after infection by the human respiratory syncytial virus

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The human respiratory syncytial virus (hRSV) is the most common infectious agent that affects children before two years of age. Outbreaks due to hRSV cause a significant increase in hospitalizations during the winter season associated with bronchiolitis and pneumonia. Recently, neurologic alterations have been associated with hRSV infection in children, which include seizures, central apnea, and encephalopathy. Also, hRSV RNA has been detected in cerebrospinal fluids (CSF) from patients with neurological symptoms after hRSV infection. Furthermore, previous work demonstrated that hRSV can be detected in the lungs and brains of mice exposed to the virus. The effects of hRSV infection within the central nervous system (CNS) are unknown. In this work using a murine model of hRSV-infected mice, we show that hRSV infection causes an alteration in the permeability of the blood-brain barrier (BBB), which allows the infiltration whether immune cells and the expression of pro-inflammatory cytokines in the CNS. Additionally, we show that the virus infects murine astrocytes both *in vivo* and *in vitro*. Murine astrocytes hRSV-infected presented an increased production of nitric oxide (NO) and TNF-α. hRSV infection caused an acute and chronic behavior impairment (up to two months), as well as altered expression of cytokines, such as IL-4, IL-10, and CCL2. Our results suggest that hRSV infection can impair the proper CNS function and induce local inflammation. Furthermore, this study provides a better understanding of the neuropathy caused by hRSV in humans and the possible detrimental effects on behavior.

W. 80. Bacteriophage Trigger Anti-Viral Immunity and Prevent Clearance of Bacterial Infection

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Bacteriophage are abundant at sites of bacterial infection, but their effect on mammalian hosts is unclear. We have identified novel, pathogenic roles for filamentous Pf bacteriophage produced by *Pseudomonas aeruginosa*(*Pa*) in suppression of immunity against bacterial infection. Pf promote *Pa* wound infection in mice and are associated with chronic human *Pa* wound infections. Murine and human leukocytes endocytose Pf. This results in phage RNA production which triggers TLR3- and TRIF-dependent type-I interferon production, inhibition of TNF, and suppression of phagocytosis. Conversely, immunization of mice against Pf prevents *Pa*wound infection. Together, these data indicate that Pf triggers maladaptive innate viral pattern-recognition responses that impair bacterial clearance. Vaccination against phage virions represents a novel strategy to prevent bacterial infection.

W. 82. CD4+ T Cells Specific for C. Difficile Toxins are a Marker of Patients with Active Disease

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Clostridium difficile infection (CDI) is a leading cause of infectious diarrhea, with approximately 25% of patients relapsing after treatment. *C.difficile* pathogenicity requires the activities of its toxins, TcdA and TcdB, but the T cell-mediated response to these toxins remains uncharacterized. We enrolled a cohort of patients with newly acquired CDI, a cohort with relapsing CDI, and healthy volunteers with no history of CDI. Toxin-specific $CD4^+$ T cell responses were measured using a whole blood flow cytometry assay that measures induced co-expression of CD25 and OX40 following 44h incubation with antigen. In patients with recurring CDI, $CD4^+$ T cell responses to TcdB, but not TcdA, were significantly higher than for healthy controls and newly acquired CDI. In both patient cohorts, TcdB-specific $CD4^+$ T cells were functionally heterogeneous; with a 1:1 ratio of Tregs to T effectors, and T effectors containing Th1, Th2 and Th17 cells at a 1.5:1:3 ratio. Interestingly, TcdB-specific Th1 and Th17 cells were significantly reduced in recurring, compared to newly acquired, CDI. Analysis of sorted TcdB-specific $CD4^+$ T cells confirmed antigen specificity and polarization towards Th17 cells, important for intestinal anti-pathogen immunity. Levels of anti-TcdA/TcdB IgG antibodies were not different between patients and controls. This is the first investigation of T cell immunity to *C. difficile* toxins, and identifies anti-TcdB $CD4^+$ T cells as a marker of active disease. Analysing toxin-specific $CD4^+$ T cell responses has the potential to predict relapse and provides insight into how $CD4^+$ T cell memory develops in response to a prevalent bacterial pathogen.

W. 84. The role of CD47 in the battlefield of Borrelia-host immune modulation in Lyme disease

Michal Tal, Maia Shoham, Balyn Zaro, Maxim Markovic and Irving Weissman

Stanford University, Stanford, CA

The role of CD47 in the battlefield of Borrelia-host immune modulation in Lyme disease

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CD47 is a broadly expressed cell surface molecule that transmits a ("don't eat me") phagocytic inhibitory signal via the SIRPα receptor on macrophages. CD47 blockade in cancer therapy has received a lot of attention, but few studies have investigated the role of CD47 in infectious disease. We find that CD47 upregulation occurs during a broad range of viral and bacterial infections, and is part of the host response to pathogen recognition. Furthermore, SIRPα, the receptor for CD47 is a highly polymorphic immune protein. As all Poxviruses encode their own CD47 mimic, it has been postulated that SIRPα has been under positive selection to distinguish host and mimic CD47. We found and characterized additional CD47 mimic proteins encoded by bacteria and fungal pathogens which can modulate immune responses. Correspondingly, we identified critical SIRPα polymorphisms that have been under balancing selection which impact SIRPα expression levels and responsiveness. The CD47 mimic protein encoded by the bacterial pathogen, *Borrelia burgdorferi (Bb)*, that causes Lyme disease can skew immune responses from protective to pathological. We are characterizing the immunomodulatory mechanisms employed by Bb in order to understand how this differentially impacts a polymorphic population. We hope that this will enable targeted treatment of very diverse symptomatic presentations of Lyme disease.

Immunodeficiency: Primary or Acquired

H. 96. Loss of IL6R Causes Immunodeficiency, Atopy, and Abnormal Inflammatory Responses

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The pleiotropic cytokine IL-6 plays a central role in the pathogenesis of multiple inflammatory diseases. In clinical practice it is targeted with the monoclonal antibody tocilizumab, which blocks the IL-6 receptor (IL-6R) encoded by *IL6R*. In classical IL-6 signalling, presentation of IL-6 to gp130 by IL-6R triggers a potent intracellular signal transduction cascade, mediated by the phosphorylation of STAT3. Loss of function mutations in gp130 and STAT3 cause multisystem disorders encompassing IgE elevation, host defence defects and connective tissue abnormalities, however the specific contribution of poor IL-6 signalling itself in those disorders is not yet established. We report here the first patient with a homozygous loss of function mutation in *IL6R*, who presented with recurrent infections, abolished acute phase response, eczema, atopy, elevated IgE and eosinophilia. For the first time we have used whole-genome sequencing to identify uniparental disomy of chromosome 1 as the mode of inheritance. T-cells isolated from our patient were found to have absent expression of IL-6R, and absent STAT3 phosphorylation despite stimulation with supraphysiological doses of IL-6, which was restored following transfection with wild-type IL6R. Patient T-cells demonstrated abnormal Th17 differentiation, and an increased population of pathological effector Th2 cells was found. This rare patient defines a novel immunodeficiency, helps us to understand the contribution of IL-6 signalling to the phenotypes of patients with mutations in IL6ST, STAT3 and ZNF341, genes encoding components of the IL-6 signalling pathway, and alerts us to the possible toxicities associated with therapies targeting IL-6R.

H. 97. Chronic mucocutaneous candidiasis and connective tissue disorder in humans with impaired JNK1-dependent responses to IL-17A/F and TGF-β

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Genetic etiologies of chronic mucocutaneous candidiasis (CMC) disrupt human IL-17A/F-dependent immunity at mucosal surfaces, whereas those of connective tissue disorders (CTD) often impair the TGF-β-dependent homeostasis of connective tissues. The signaling pathways involved are incompletely understood. We report a three-generation family with an autosomal dominant (AD) combination of CMC without other overt immunological phenotypes, and a novel and complex CTD, which clinically overlaps with Ehlers-Danlos syndrome (EDS). The patients are heterozygous for a private splice-site variant of *MAPK8*, the gene encoding c-Jun N-terminal kinase 1 (JNK1), a component of the MAPK signaling

pathway. This loss-of-expression variant results in low levels of JNK1 protein in the patients' fibroblasts. These cells display impaired, but not abolished, responses to IL-17A and IL-17F, accounting for the patients' CMC. This phenotype is rescued by the wild-type JNK1β1 isoform, whereas the mutant isoforms do not display negative dominance in control fibroblasts, consistent with AD JNK1 deficiency by haploinsufficiency. Additionally, the development of patients' T_H17 cells was slightly impaired *ex vivo* and *in vitro*, probably due to the involvement of JNK1 in the TGF-β-responsive pathway. Consistently, the patients' fibroblasts displayed impaired JNK1- and c-Jun/ATF2-dependent, but SMAD2/3-independent induction of key extracellular matrix (ECM) components and regulators, but not of EDS-causing gene products, in response to TGF-β, probably accounting for the patients' novel and complex CTD phenotype. This experiment of Nature indicates that the integrity of the human JNK1-dependent MAPK signaling pathway is essential for IL-17A- and IL-17F-dependent mucocutaneous immunity to *Candida,*and for the TGF-β-dependent homeostasis of connective tissues.

H. 98. The characteristics of CD8+ T lymphocytes in a family with a novel CD40 ligand mutation

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CD40 ligand (CD40L) deficiency is an X-linked primary immunodeficiency, associated with the opportunistic infection and increased rate of malignancy. CD40L is expressed primarily on the activated $CD4^+$ T lymphocytes which interacts with CD40 expressed on various immune cells. CD40-CD40L engagement regulates the innate, cellular and humoral immunities including recall memory function of $CD8^+$ T lymphocytes. Alternations in T cell immunity occur with aging, likely contributing to increased infections and malignancies. Aging increases the frequency of memory $CD8^+$ T lymphocytes, especially those expressing high and low levels of CD57 and IL-7Rα, respectively. Here we addressed the hypothesis that early $CD8^+$ T lymphocytes senescence occurs in CD40L deficiency by analyzing the frequency, phenotypic and functional characteristics of peripheral $CD8^+$ T lymphocytes subsets in a family with a novel CD40L mutation and healthy controls (HCs) using flow cytometry and high-dimensional mass cytometry (CyTOF). The five-year-old CD40L deficiency male patient and his twenty-one-year-old mother with the heterozygous mutation had an increased frequency of effector memory (EM) CD8+ T lymphocytes expressing high and low levels of CD57 and IL-7Rα, respectively, and higher levels of IFN-γ, IL-13 and granzyme B production in IL-7Rα low EM $CD8^+$ T lymphocytes compared to age-matched and middle age HCs (age 40-65). The patient had persistently increased frequency of $IL7R\alpha^{low}$ EM $CD8^+$ T lymphocytes even in the absence of active infection and on stable IVIG therapy. Our findings suggest the development of early CD8 senescence in CD40L deficiency despite IVIG therapy restoring humoral immunity.

H. 99. A New PIK3CD Gene Variant in APDS

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Common Variable Immunodeficiency (CVID) affects about 1 in 25,000 people and causes impaired B cell differentiation, hypogammaglobulinemia, poor vaccine response, recurrent infections, lung/GI disease, autoimmunity, and susceptibility to lymphoma. Activated PI3Kδ Syndrome (APDS) is one of the most common forms of monogenic CVID. It is caused by heterozygous, gain-of-function mutations in the leukocyte-restricted p110δ PI3K (*PIK3CD*) or its binding partner p85α (*PIK3R1*) and leads to hyperactivation of the PI3K pathway. This causes increased effector cell differentiation, lymphoproliferative burst, CD8 T cell senescence, decreased memory T cell function and decreased control of EBV/CMV. Patients have recurrent bacterial and persistent viral infections, lymphoproliferative/autoimmune disease, and pathologic inflammation. We describe an APDS patient with a new mutation in the helical domain of p110δ. Activating mutations in the homologous residue of the ubiquitously expressed *PIK3CA* are described in cancer. The patient is a 14-year-old female who has recurrent infections, autoimmune vasculitis, asthma, and allergies. She has the classic APDS phenotype involving loss of switched memory B cells, as well as eosinophilia and hyper-IgE, which have not been previously described in this disease. We show this mutation leads to hyperactivation of the PI3K pathway. E522K in *PIK3CD* is functionally relevant given the association of the mutation in *PIK3CA* with cancer and hyperactivation of the PI3K pathway. Future directions involve finding more patients with this mutation to see if the allergy phenotype is shared and measuring the *in vitro* kinase activity.

T. 81. Different Clinical Manifestations in A Large Cohort of Predominantly Antibody Deficiency Patients with Monogenic Defects

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BACKGROUND: Predominantly antibody deficiencies (PADs) are the most common primary immunodeficiencies, characterized by hypogammaglobulinemia and inability to generate effective antibody responses.

OBJECTIVE: We intended to report most common monogenic PADs and to investigate how PAD patients who were primarily diagnosed as agammaglobulinemia, hyper IgM syndrome (HIgM) and common variable immunodeficiency (CVID) have different clinical and immunological findings.

METHODS: Stepwise next generation sequencing and Sanger sequencing were performed for confirmation of the mutations in the patients clinically diagnosed as agammaglobulinemia, HIgM and CVID.

RESULTS: Among 550 registered patients, the predominant genetic defects associated with agammaglobulinemia (48 BTK and 6 μ heavy chain deficiencies), HIgM (21 CD40L and 7 AID

deficiencies) and CVID (17 LRBA deficiency and 12 atypical ICF syndromes) were identified. Clinical disease severity was significantly higher in patients with μ heavy chain and CD40L compared to patients with BTK and AICDA mutations. Paralysis following live polio vaccination was considerably higher in patients with μ heavy chain deficiency compared with BTK deficiency. We found a genotype-phenotype correlation among patients with BTK mutations regarding clinical manifestation of meningitis and chronic diarrhea. Surprisingly, we noticed that first presentations in the majority of ICF patients were respiratory complications, while first presentations in LRBA patients were non-respiratory complications.

CONCLUSION: This study highlights similarities and differences in clinical and genetic spectrum of the most common PAD-associated gene defects. This comprehensive comparison will facilitate clinical decision making, and improve prognosis and targeted treatment.

T. 82. Gene polymorphisms in IFNγ and TNFα genes associate with faster disease progression in HIV-1 Subtype-C infected individuals from North India

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Host genetic factors may be one of the possible reasons for variable HIV-1 disease progression. The aimwas to determine the association of functional IFNγ gene and TNF-a SNPs and its associated parameters related to apoptosis that may influence the rate of HIV-1 disease progression. We planned to evaluate the association of certain SNPs in IFNγ gene and promotor region of TNFa gene with the susceptibility to or rate of progression of HIV disease.Therapy naive, 100 HIV slow progressors, 100 fast progressors, 50 HIV exposed seronegative individuals and 260 healthy controls from same ethnic origin were recruited. Genotyping of IFNγ (+874 T/A) and TNF-a variants (2863C/A, -308G/A and -238G/A) was done using PCR-RFLP. Genotype and allele frequency of IFN-γ+874 variants were observed to be 0.61% for 'A' allele and 0.39% for T-allele. Frequency of AA genotype (associated with low production of IFN-γ) was found to be significantly higher in fast progressors in comparison to slow progressors (p=0.017, OR= 2.76). While that of TNF-a -238G/A and -863C/A was not significantly different in HIV-1-infected patients when compared to controls, while that of TNF-a -308G/A variant (high TNF-a producer) was significantly higher in FPs compared to SPs (p,0.01, OR = 3.43). Haplotype analyses also showed that carriers of high TNF-a producing haplotype CAG was significantly more common among FPs compared to SPs (p,0.01, OR = 3). The lymphocyte mitochondrial membrane potential of FPs having CAG haplotype was significantly low as compared to wild type (CGG) haplotype (417622 vs 571628, p,0.01).

T. 83. GM-CSF signalling pathway activates human GATA-2 transcription factor trough STAT5 phosphorylation.

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Patients with GM-CSF receptor deficiency and patients with anti-GM-CSF autoantibodies commonly suffer from alveolar proteinosis. This rare disorder is also observed in GATA-2 haploinsufficiency. We therefore hypothesized a link between GATA-2 activity and GM-CSF signaling. Confirming our hypothesis, we observed that GATA-2 transcription was increased in response to GM-CSF treatment in human peripheral blood mononuclear cells. By luciferase reporter assay we observed that GM-CSF as well as IL3 pathway activation were able to increase the transcriptional activity of GATA-2. Treatment with specific inhibitors revealed that the signal transduction was mediated by Akt and STAT5. An increase in GATA-2 transcriptional activity was also induced by a constitutively active form of STAT5 in absence of exogenous stimulation. To our knowledge, this is the first demonstration of the link between GATA-2 activity and GM-CSF/IL3/IL5 common beta chain signalling pathway in human immune cells. In addition, our observations likely explain the alveolar proteinosis observed in GATA-2 haploinsufficiency as well as its pleiotropic phenotype.

T. 84. Homozygosity for a novel CARD11 mutation causes profound combined immunodeficiency (CID), inflammatory gastrointestinal disease, and complete abrogation of MALT1 activity

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Background: The CARD11–BCL10–MALT1 (CBM) complex is a critical signalling adaptor that regulates lymphocyte activation, proliferation, and survival. Primary immunodeficiencies (PIDs) affecting each component result in broad clinical manifestations ranging from combined immunodeficiency (CID) to lymphoproliferation. We present the laboratory and clinical findings of two Canadian First Nations patients found to be homozygous for the same novel *CARD11* mutation (c.2509C>T; p.R837*).

Results: We have identified an 8-month-old boy who presented with a severe case of entero/rhinovirus bronchiolitis with interstitial lung disease and a 17-year-old boy with a history of severe pulmonary infections, chronic sinusitis, candidiasis, invasive bacteremia, and severe ileo-colitis and oral ulceration requiring total colectomy. Both patients lacked Tregs and memory B cells, and possessed hypogammaglobulinemia. Sequencing both patients revealed homozygosity for the same novel variant of *CARD11* (c.2509C>T; p.R837*), which rendered CARD11 protein undetectable by immunoblot. To confirm CARD11 deficiency, we stimulated patient B cells with phorbol 12-myristate 13 acetate (PMA) and ionomycin across a time-course and immunoblotted for various signalling proteins in both the NF-κB and MAPK pathways and cleavage substrates of the MALT1 paracaspase. NF-κB and JNK activation were completely absent, MALT paracapase activity was lost, and the CBM complex could not be assembled.

Conclusions: These two cases highlight the crucial role of CARD11 in regulating lymphocyte development, function, and humoral responses. In addition, we have identified the oldest known living individual with CARD11 deficiency and he presented uniquely with inflammatory gastrointestinal disease in addition to CID, thus further broadening the spectrum of phenotypes associated with CARD11-related PIDs.

T. 85. Low Dose Azithromycin Prophilaxys Reduces Respiratory Exacerbations in Primary Antibody Ddeficiencies: a Multicenter, Double-Blind, Placebo-Controlled Randomized Clinical Trial

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Background. Lacking protective antibodies, patients with Primary Antibody Deficiencies (PAD) suffer from frequent respiratory infections leading chronic pulmonary damage. Macrolides prophylaxis has been proven effective in chronic respiratory diseases.

Objective: We aimed to test the efficacy and safety of orally administered low-dose azithromycin prophylaxis in PAD patients.

Methods. We designed a three-year, double-blind, placebo-controlled, randomized clinical trial to test whether oral azithromycin (250 mg once daily three-times a week for 2 years) would reduce respiratory exacerbations in PAD with chronic infection-related pulmonary diseases. The primary endpoint was the number of annual respiratory exacerbations. Secondary endpoints included time to first exacerbation, antibiotic additional courses, number of hospitalizations and safety.

Results. Eighty-nine patients received azithromycin (n=44) or placebo (n=45). The number of exacerbations was 3.6 per patient-year (95%CI 2.5-4.7) in the azithromycin arm, and 5.2 (95%CI 4.1-6.4) in the placebo arm (p=0.02). In the azithromycin group the HR for having an acute exacerbation was 0.5 (95%CI 0.3-0.9, p=0.03) and the HR for hospitalization was 0.5 (95%CI 0.2-1.1, p=0.04). The rate of additional antibiotic treatment per patient-year was 2.3 (95%CI 2.1-3.4) in the intervention and 3.6 (95%CI 2.9-4.3) in placebo groups (p=0.004). *H. influenzae* and *S. pneumoniae* were the prevalent isolates and

they were non-susceptible to macrolides in 25% of patients of both arms. Azithromycin's safety profile was comparable with placebo.

Conclusion. The study reached the main outcome centered in the reduction of exacerbation episodes per patient-year with a consequent reduction of additional courses of antibiotics, and of risk of hospitalization.

T. 86. Men' immune system age faster and more severely than women'

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Aging has a profound impact on immune cell functions. Similarly, sex affects immune cell responses, disease susceptibility, severity, and symptoms. However, it is not well understood how sex and age together impact human immune system. To systematically study this, we generated flow cytometry, ATAC-seq, and RNA-seq maps from peripheral blood mononuclear cells (PBMCs) of healthy individuals (58 women, 50 men) across the adult lifespan (ages 22-93). Epigenomes/transcriptomes of PBMCs went through significant changes with aging. Some of these changes were shared between men and women (e.g., declines in T cell-associated loci), whereas others were sex-specific (male-specific decline in B cell-associated loci). Furthermore, trajectory and differential analyses revealed that male immune system age faster and more severely (e.g., magnitude of changes) and sooner than female immune system. Finally, unexpectedly, genomes of male and female PBMCs diverged over time, where old women had more active epigenomes/transcriptomes for adaptive cells compared to more activity for innate cells in older men, suggesting a more severe aging signature for men. Together these multi-faceted data provide a precise description of how aging affect immune system of men and women.

T. 87. Primary immunodeficiency with severe multi-organ immune dysregulation

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Introduction:

Autoimune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) syndrome is a rare autosomal recessive disorder caused by a mutation in the *AIRE* gene that is responsible for immune regulation. We present a patient with clinical APECED and a single pathogenic AIRE mutation. Whole exome sequencing identified a mutation in *BTNL2* gene that we propose may have also contributed to his disease.

Case Description:

15-year-old male patient presents with multiple autoimmune disorders including adrenal and pancreatic insufficiency, vitiligo, Sjogren's syndrome, celiac disease, as well as hypothyroidism, hypoparathyroidism, candida onychomycosis, recurrent nasal sinus and ear polyps. He developed acute autoimmune

hepatitis requiring systemic corticosteroids and tacrolimus. Genetic sequencing identified a monoallelic pathogenic variant p.Arg257Ter in the *AIRE* gene at the c.769 C>T coding DNA. An IL7 receptor heterozygous mutation was also reported but the patient did not have laboratory findings suggestive of its associated disease. Whole exome sequencing identified a frameshift deletion mutation at the promoter/enhancer region of the *BTNL2* gene at the 32370969,TG>T site.

Discussion:

AIRE is critical for self-antigen expression in the thymus, allowing for destruction of self-reactive T cells by negative selection. Butyrophilin-like 2 (*BTNL2*) is a butyrophilin family member with immunoregulatory properties. It is found in gut tissue and Peyer's patches and is thought to be involved in immune surveillance, serving as a negative T-cell regulator. We suggest that the unique combination of mutations in each of these genes contributed to the development of severe autoimmune disease in our patient.

T. 89. The adenosine salvage pathway is induced in late human transitional 3 B cells and facilitates their metabolic reprograming toward a mature follicular B cell fate

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Human B cell maturation is organized through a series of highly controlled developmental checkpoints. After leaving the bone marrow, the BCR repertoire progressively contracts as the pool of transitional B cells undergoes selection and acquires a mature follicular B cell phenotype. The triggers controlling the final steps in B cell maturation are largely unknown in humans. Using bulk RNA-seq on FACS-sorted B cells, we identified a dramatic metabolic switch, orchestrated by mTORC1 downregulation, that takes place as B cells progress from the transitional to the follicular B cell stage. This reprogramming encompasses ribosome and protein biogenesis, as well as aerobic respiration. Reinforcing the fundamental role of the reduction of mTORC1 signaling in B cell maturation, CVID patients with gain-of-function PI3Kδ mutations, which cause increased tonic mTORC1 signaling, exhibit a profound loss of

follicular B cells. The attenuation of mTORC1 activity in follicular B cells was associated with a coordinated upregulation of the ABCB1 transporter, promoting the accumulation of extracellular ATP, which is broken down to adenosine by the conjugated action of extracellular exonuclease, CD39 and CD73, fostering the activation of the mTORC1 inhibitor, AMPK. Taken as a whole, these data suggest that mTORC1 governs a novel checkpoint in the maturation of human B cells. To pass this checkpoint, developing B cells require the attenuation of mTORC1. Consequently, late transitional B cells limit mTORC1 signaling by activating AMPK through the adenosine salvage pathway.

W. 85. A Cell-traceable Model of Reticular Dysgenesis in Human Hematopoietic Stem Cells Linking Metabolism and Differentiation

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It is an emerging paradigm that mitochondrial metabolism impacts the fate of hematopoietic stem and progenitor cells (HSPCs), however, the underlying mechanisms remain poorly understood. Biallelic mutations of a mitochondrial enzyme, adenylate kinase 2 (AK2), cause Reticular Dysgenesis (RD), one of the most severe forms of severe combined immunodeficiency, characterized by almost complete absence of neutrophils and lymphocytes, and sensorineural hearing loss. AK2 catalyzes the interconversion between adenine nucleotides (AMP, ADP, and ATP) and controls the availability of ADP for oxidative phosphorylation. We hypothesize that AK2 drives HSPC fate decisions through regulation of metabolites that lead to lineage-specific epigenetic modifications. To test the hypothesis, we have developed a novel AK2-depleted cell model system in primary human HSPCs by CRISPR/Cas9 genome editing. Using homologous recombination-mediated GFP and BFP reporters, we are able to track and enrich biallelic AK2-edited HSPCs. Compared to AAVS1-edited controls, $AK2^{-/-}$ HSPCs showed severely decreased potential to form colonies of myeloid and erythroid lineages. An in vitro neutrophil differentiation assay showed $AK2^{-/-}$ maturation arrest at the $CD117^{+}$, $HLA\text{-}DR^{-}$ stage. These defects are consistent with observations in RD patients. Currently, using a targeted metabolomics approach and global methylation/acetylation quantifications, we are analyzing AK2-dependent metabolite changes and their impact on the epigenome. We are also examining AK2-dependent changes in gene expression and chromatin accessibility by RNA-seq and ATAC-seq. Understanding how metabolism governs differentiation and self-renewal of human HSPCs will advance our understanding of many immune disorders and has important translational implications to improve stem cell products and transplantation outcomes.

W. 86. Compound Heterozygous Mutations in Forkhead Box N1 (FOXN1) and 22q11.2 Deletion Syndrome (DiGeorge) Cause a Thymic Hypoplasia through Distinct Molecular Mechanisms

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Patients with 22q11.2 deletion syndrome (DiGeorge syndrome) and individuals with mutations in the *Forkhead Box N1*(*FOXN1*) transcription factor (Nude/SCID) can both present with a thymic hypoplasia contributing to a severe T cell lymphopenia. In each clinical condition, the thymic anlage fails to develop properly within the 3rdpharyngeal pouch during embryogenesis. We analyzed the development of the thymus in mouse models of 22q11.2 deletion syndrome (22q11.2del) and a new set of mice with mutations in *Foxn1*that genocopied a SCID patient with novel compound heterozygous mutations in *FOXN1*. The two distinct mouse models exhibit a phenotypically similar hypoplasia of the thymus. An analysis of thymopoiesis in the fetal thymii reveals distinct development problems. The hypoplastic thymii from the 22q11.2del mice are primarily sized restricted, with normal percentages of the distinct thymocyte subsets. This phenocopied human thymii from patients with 22q11.2del. In contrast, the compound heterozygous mutations in *Foxn1*result in a severe block in early T cell development. Comparative gene expression analyses of e13.5 fetal thymii revealed differentially regulated transcripts that define the basis of the hypoplasia. A dysregulated mesenchymal cell signature was apparent in the 22q11.2del model, which contrasted a thymic epithelial dysregulation in the presence of the *Foxn1*mutations. The data suggest different strategies are necessary to correct the thymic tissue abnormalities in patients depending on their genetic mutations.

W. 87. CRISPR/Cas9-based gene editing for IL-10/IL10RA immunodeficiency

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Homozygous loss-of-function mutations in IL-10 and IL-10-receptors (IL-10R) cause severe infantile inflammatory bowel disease. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only cure. However, limited donor availability and morbidity/mortality prevent the wide use of allo-HSCT. An approach based on gene-correction of patients derived hematopoietic stem and progenitor cells (HSPCs) will allow us to use an autologous HSCT. We have developed CRISPR/Cas9-based strategy to target the *IL-10* locus. We selected 3 small guide RNAs (sgRNAs). To test the targeted integration mediated by those guides, we designed recombinant AAV6 (rAAV6) DNA donor templates. We observed for IL10-3, -5 and -6 an average integration of 20%, 45% and 55% in $CD34^+$; while 17%, 28% and 30% in $CD4^+$ T cells. Based on these data, we selected the IL10-6 guide for further studies. We designed rAAV6 carrying a codon optimized *IL-10* cDNA. Digital droplet PCR showed 38% of integration at *IL-10* locus in $CD34^+$ cells. We are currently testing sgRNAs targeting *IL-10RA* locus.

As the regulation of *IL-10* is still poorly understood, we will use this technology to define molecular signals sustaining normal IL-10 production by Tr1 cells. To this goal, we generated Tr1 clones which produce high level of IL-10 and inhibit proliferation of $CD4^+$ responder T cells.

In conclusion, we identified a sgRNA mediating high targeted integration at the *IL-10* locus in both human HSPCs and T cells. These tools will be used to gene correct IL-10 deficient cells and for studying IL-10 regulation and the molecular mechanisms underlying IL-10 deficiency.

W. 88. Diagnostic Tool to Assist Clinical Decisions for Unexplained Congenital T Cell Immunodeficiencies

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We improved the classical OP9-DeltaLigand culture system for *in vitro* differentiation of CD34+ hematopoietic stem and progenitor cells (HSPC) into the mature T cell lineage so as to render it robust and amenable for smaller quantities of HSPC (minimum of 300 CD34+ cells) that can be isolated from as few as 1-5 ml of peripheral blood. In turn, we applied this functional test in order to demonstrate that it could be successfully used to distinguish cell-autonomous from non-cell-autonomous defects in T cell differentiation of HSPC taken from SCID patients. Particularly, we confirmed that $CD34^+$ cells obtained from peripheral blood of one *IL2Rgc* SCID patient were stalled early in their differentiation, as they could not produce any CD34-CD7+CD1a+ double-negative (DN), CD4+CD8+CD3+/- double-positive (DP) or CD3+ single-positive (SP) cells after three weeks of culture, although CD34+CD7+ proT cells were abundantly present. On the other hand, $CD34^+$ cells obtained from peripheral blood of patients with primary thymic defects (22q11 deletion and *TBX1* SCID) differentiated normally up to the CD3 single-positive stage, with the major progenitor intermediates (proT, DN, DP) being present in similar fashion as to controls. Therefore, such tools could be used to facilitate clinical decisions facing unknown SCID, where doubt exists as to whether bone marrow or thymus transplantation is appropriate.

Immuno-dermatology

H. 100. Analysis of Discoid Lupus Erythematosus (DLE) Gene Expression Reveals Dysregulation of Pathogenic Pathways Activated within Infiltrating Cells

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Immune & inflammatory pathways in DLE skin are poorly understood[1, 2, 3]. A bioinformatic approach (LIMMA-DE & WGCNA) analyzed skin biopsy gene expression to gain insight into precise pathogenic mechanisms involved. Genes differing between DLE & healthy individuals were interrogated for cell type specific gene signatures using GSVA validation of I- or T- Scope® analysis of immune or non-immune subsets. Non-immune subsets (fibroblasts, keratinocytes, melanocytes and Langerhans cells) are in WGCNA modules negatively correlated with disease. Genes were functionally characterized using BIG-C® and pathways elucidated using IPA®. DLE has an immune cell signature in WGCNA modules positively correlated with CLASI-A (DCs, myeloid cells, $CD4^+$ & $CD8^+$ Ts, gdTs, NKs, Bs as well as pre- and post-switch PCs as indicated by IgM, IgD, and IgG1 HC genes). The presence of both Ig -κ & -λ as well as VL genes suggests polyclonal activation. Chemokines that mediate lymphocyte organization and/or recruitment into lupus skin are present. Cytokine (TNF, IFNγ, IFNα, CD40L, IL1β, IL2, IL6, IL12, IL17, IL23 & IL27) & signaling (PI3K,NF-κB,NF-AT, and mTOR) pathways as well as proliferation and HDAC activity are evident. IPA® UPR analysis indicated ongoing signaling by TNF, IFNγ, IFNα, CD40L, IL1β, IL2, IL6, IL12, IL17, IL23 and IL27. Statistically significant WGCNA module preservation was observed between all three DLE datasets. Interestingly, connectivity analysis using LINCS/CLUE demonstrated high priority drug targets such as IKZF1/3 (lenlidomide) as well as CC-220, JAK1/2 (ruxolitinib) and HDAC6 (Ricolinostat) may prove to be good options for therapeutic intervention.

[1] J.Invest.Dermatol.134:87(2014).

[2] ArthritisRes.Ther.17:324(2015).

[3]ArthritisRheumatol.69:1840(2017).

H. 102. Targeting CXCR3-mediated T cell recruitment to the skin in Cutaneous Lupus Erythematosus

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Cutaneous Lupus Erythematosus (CLE) encompasses a spectrum of skin manifestations of lupus, all of which are characterized by interface dermatitis and autoantibody deposition. Understanding the pathogenesis is critical to developing new treatments and for determining which patients are at risk for developing systemic disease. Prior studies from our laboratories using archived CLE skin biopsies and our novel CLE mouse model revealed significant upregulation of IFN signatures including the CXCR3 chemokine system in whole tissue. Here, we used a novel blister biopsy technique to sample interstitial skin fluid from CLE patients to perform single cell RNA sequencing (scRNAseq) and high parameter flow cytometry (Cytek Aurora) to confirm expression of key molecules. Single cell RNAseq analysis of skin from human CLE patients revealed upregulation of the CXCR3 ligands CXCL9 and CXCL10 in lesional skin by keratinocytes, macrophages and T cells themselves. In concert, we found high expression of

CXCR3 on both human and mouse T cells and other immune cells in lesional CLE by flow cytometry. Treating CLE mice with CXCR3 blocking antibodies did not significantly impact the frequency of antigen-specific T cells in the skin, but did prevent worsening of skin disease in mice. Surprisingly, CXCR3 antibody treatment also reduced spleen weight and autoantibody titers. Taken together, our data indicate that the CXCR3 chemokine axis contributes to both skin and lymphoid pathogenesis in the CLE mouse model, and indicate that similar pathways of migration exist in CLE patients. Future studies will focus on translating related therapies for CLE patients.

H. 103. PD-1H (VISTA)-mediated Suppression of Autoimmunity in Systemic and Cutaneous Lupus Erythematosus and its Implication as a Therapeutic Target

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Systemic lupus erythematosus (SLE) and discoid lupus erythematosus (DLE) of the skin are autoimmune diseases characterized by inappropriate immune responses against self-proteins whereas the key elements that determine disease pathogenesis and progression are largely unknown. Recently, we and others described a novel immune checkpoint molecule belonging to the B7/CD28 gene family – named programmed death-1 homolog (PD-1H) or V-domain Ig-containing Suppressor of T cell activation (VISTA) – that functions to control T cell activation. Here we show that mice lacking immune inhibitory receptor PD-1H on spontaneously develop cutaneous and systemic autoimmune diseases resembling lupus. Furthermore, cutaneous lupus lesions of PD-1H KO mice share a similar skin-infiltrate to both the canonical murine model of lupus, MRL/*lpr* mice, as well as human DLE. Using mass cytometry, we identified neutrophils as critical early immune infiltrating cells within cutaneous lupus lesions of PD-1H KO mice that exhibit proinflammatory phenotypes. We also found that PD-1H is highly expressed on immune cells in human SLE, DLE lesions and cutaneous lesions of MRL/*lpr* mice. PD-1H agonistic monoclonal antibody in MRL/*lpr* mice reduces cutaneous disease, autoantibodies, inflammatory cytokines, chemokines and pathogenic T cells. Furthermore, PD-1H on both T cells and myeloid cells could transmit inhibitory signals resulting in reduced activation and function, establishing PD-1H as an inhibitory receptor on T cells and myeloid cells. On the basis of these findings, we propose that PD-1H is a critical element in the pathogenesis and progression of lupus and PD-1H agonist could be effective for treatment of systemic and cutaneous lupus.

Immunology of the Eye

T. 90. Application of the Anti-Inflammatory Ocular Neuropeptide Alpha-Melanocyte Stimulating Hormone (α-MSH) Suppresses Damage in Retinas with Ischemia/Reperfusion

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Inflammation has a potential role in the initial stages of diabetic retinopathy involving ischemia/reperfusion (I/R)-induced retinal damage. We studied the possibility that therapeutic use of α-MSH, a potent anti-inflammatory neuropeptide, could reduce retinal damage caused by I/R. Ischemia was induced in C57BL/6 mice via anterior chamber cannulation and elevating intraocular pressure to 90-100 mmHg for 90 minutes. The treatment group was immediately given 5μg of α-MSH intraperitoneally, and on day 2 and 7 the eyes were enucleated and prepared for histological analysis. Retinal survivability was gauged via retinal ganglion cell (RGC) count density, and clinical histological scoring for the severity of inflammation and retinal damage. Visual analysis demonstrated evident increases in infiltration, retinal folding, and retinal layer loss. The damage in untreated mice worsened with time and was ameliorated by α-MSH treatment with significantly lower clinical scores in the α-MSH treated mice on day 2 (0.9 ± 0.65, $P < 0.05$), but not on day 7. Untreated mice had significantly fewer RGCs on day 2 (43 ± 9 cells/mm) and day 7 (28 ± 4 cells/mm) compared to retinas of healthy mice (78 ± 5 cells/mm). RGC survival was significantly higher in the α-MSH treated mice on day 2 (63 ± 14 cells/mm, $P = 0.02$), but not on day 7 (40 ± 14 cells/mm) compared to the untreated mice. The results demonstrate that α-MSH-therapy has the ability to reduce I/R-induced retinal damage. The results suggest that suppressing inflammation early in diabetic retinopathy can minimize damage to the retina.

T. 91. Enhanced memory CD8 T cells with immunosenescent-like phenotype in uveitis patients

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Uveitis is characterized by an inflammation of the uveal tract of the eye and is responsible for approximately 15% of blindness in the US. Uveitis is considered to be mediated primarily by $CD4^+$ T cells, with little information on the role of $CD8^+$ T cells. Here, we identified significant differences in $CD8^+$ T cell memory subsets in non-infectious uveitis patients using an unbiased flow cytometry based approach. To validate these results, we studied a larger cohort of patients and analyzed their $CD8^+$ T cell memory subsets. Consistent with our initial findings, $CD8^+$ T Effector Memory (TEM) cells were increased in uveitis patients as compared to healthy controls, with a concomitant decrease in $CD8^+$ naïve T (TN) cells. Majority of the study participants had uveitis without any systemic autoimmune disease however these differences were evident regardless of whether uveitis was associated with a systemic immune mediated disease. The TEM subset in patients also expressed increased levels of CD57 and KLRG1 with decreased expression of CD28, exhibiting a senescent-like phenotype. Additionally, these senescent-like $CD8^+$ TEM cells corelated with disease activity. Functionally, these cells were metabolically active and produced more TNFα upon stimulation with CD3/2/28 beads compared to healthy controls. These results shed a new light on the involvement of $CD8^+$ T cells in uveitis and suggests $CD8^+$ TEM cells could serve as a potential biomarker for disease activity.

T. 92. Prolonged antibiotic treatment reverses the protective effect of gut microbiota depletion on autoimmune disease

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Human autoimmune uveitis is a major cause of blindness. Commensals may be involved as a trigger and/or modulator of the disease. In the model of experimental autoimmune uveitis (EAU) induced by immunization with the retinal antigen IRBP, conflicting findings were published by us (PMID:26287682) and by others (PMID:27415793) concerning disease amelioration by antibiotic treatment (ABX). Since the main variable between the studies appeared to be duration of ABX, we set out to examine whether short-term vs. long-term ABX differentially affected EAU development, gut microbiota and host immune responses.
EAU-susceptible B10.RIII mice were given continuous ABX starting 12 weeks (long-term) or 1 week (short-term) before EAU challenge. Short-term ABX ameliorated EAU, whereas long-term ABX had no effect on disease onset or severity vs. untreated controls. Metagenomic analyses revealed ABX-duration-dependent depletion of gut microbial communities, including differential effects on *Lactobacillus* and other taxa. Interestingly, ABX duration was also associated with progressive disappearanceof CD4+ and CD4+CD8+ intraepithelial lymphocytes (IELs). Notably, these IELs, which are thought to regulate gut barrier integrity, exhibited cytotoxic activity *in vitro* against autologous immune cells and were modulaed at the transcriptomic level by ABX.
We propose that microbiota play a dual role in uveitis development: they provide a stimulus for uveitogenic effector cells, but also maintain a “regulatory” IEL population, whose progressive loss reverses the protective effect of short-term ABX. Our findings may have implications for extended use of antibiotics in clinical situations and may lead to a better understanding of the role of IELs in the microbiota-gut-eye axis.

T. 93. Retinal Pigment Epithelial cells do not suppress the phagocytic antigen processing pathways in antigen presenting cells during Experimental Autoimmune Uveitis

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Retinal Pigment Epithelium (RPE) produce the neuropeptide alpha-melanocytes stimulating hormone (α-MSH) that regulates the phagocytic pathway in monocytes. This regulation has the potential to alter antigen processing and presentation that would activate effector T cells. The expression of α-MSH is greatly diminished during Experimental Autoimmune Uveitis (EAU). This suggests the possibility that RPE regulation of the phagocytic pathways in antigen presenting cells (APC) is diminished in EAU permitting the activation of effector T cells. We assayed for changes in RPE regulation of the phagocytic pathway by collecting the conditioned-media of cultured RPE-eyecups from eyes of healthy and EAU mice. Resting macrophages (RAW 264.7 cells) were treated with conditioned-media and fed opsonized-Ovalbumin (OVA)-coated magnetic beads for 24 hours. The magnetic bead containing intracellular vesicles were

isolated, and assayed by immunoblotting for Rab5 and Lamp1. APC activity was assayed by feeding resting peritoneal macrophages with opsonized-OVA-antigen while treated with conditioned-media overnight, washed, OVA-specific T cells were added, and proliferation was measured 72 hours later. The isolated phagosomes from EAU conditioned-media treated macrophages matured showing a significantly higher ratio of Lamp1 to Rab5 (1.2±0.13) than the healthy conditioned-media treated macrophages (0.8±0.23). The EAU conditioned-media treated OVA presenting APC were not suppressed in their ability to stimulate OVA-specific T cell proliferation (0.9±0.04%) in contrast to the suppressed stimulation of proliferation (0.45±0.06%) by APC treated with healthy conditioned-media. The results demonstrate that EAU RPE cannot suppress phagosome maturation in potential retinal APC, and may contribute to uveitis by permitting antigen presentation to effector T cells.

T. 94. Single-cell RNA sequencing coupled with T-cell-receptor (TCR) sequencing provides unprecedented insight into granulomatous uveitis.

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Methods We performed RNA and TCR sequencing on aqueous fluid and peripheral blood obtained from a 67-year-old female with active chronic granulomatous anterior uveitis, suspected to be sarcoidosis.

Results The ocular inflammatory cells included 4 major cell types. CD4+ T cells comprised 48% of the sample, CD8+ T cells 18%, B cells 26% and monocytes 8%. The CD4:CD8 T cell ratio was 2.7. Pathway analysis revealed upregulation of types I and II interferon (IFN) as well as TNFa, but not of IL-17 signaling.

Clonal TCR sequences were found among CD4+ but not CD8+ T cells. The five most frequent clones represented 25% of all TCRs. Notably, these clones were not detected in the patient's peripheral blood.

While a subset of the ocular CD4+ T cells had a similar phenotype to circulating peripheral memory T cells, most had increased expression of effector molecules including IFN g, granzyme and perforin molecules. Notably, the dominant TCR clones were found amongst these effector cells.

Most ocular B cells had a class-switched memory phenotype, without evidence of monoclonality and expressed MHC II, T cell costimulatory CD40 and CD86, and toll-like-receptors (TLRs). Additionally, 19% of the B cells were plasmablasts.

Conclusion These data suggest a local antigen-driven immune response. Furthermore, the apparent lack of common CD4+ T cells clones in the peripheral blood suggests that the local immune response may be self-perpetuating and may not require distal priming/activation of inflammatory cells. This insight provides the groundwork for enhanced prediction and monitoring of therapeutic responses in uveitis.

Immuno-oncology

H. 89. A New Specific Promoter to Control Chimeric Antigen Receptor Expression Allow Long Lasting CAR Therapy using Hematopoietic Stem Cells

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The loss of engineered cells and the cytokine release syndrome represent two major drawbacks of Chimeric Antigen Receptor (CAR)-T cell therapy. The engineering of hematopoietic stem cells (HSCs), by providing a continuous replenishment of CAR-T cells, could circumvent these issues. To avoid a potentially dangerous pan-hematopoietic CAR expression, **we designed a T-cell specific specific synthetic promoter** to restrict the CAR expression only to T cell progeny issued from CAR-modified HSCs. **Methods**: Potential sequences for T-cell specific expression were designed *in silico* and cloned in GFP or CD22-CAR vectors. Specific expression was assessed in cell lines or primary peripheral blood cells. We then transduced $CD34^+$ cell and humanized NSG mice or differentiated them on OP9-(DL4)-culture system. **Results**: Upon transfection with GFP under the control of our synthetic promoter, only Jurkat cell line (T cells) or primary T cells expressed GFP. When transduced HSCs were co-cultured with on OP9-DL4 cells or injected into NSG mice, the transgene was expressed only in T cell lineage. We also validated that CAR expression under our promoter was detectable and functional in *in vitro* cytotoxicity assays. Indeed, primary T cells transduced with CAR under our promoter showed the same lysis activity against leukemic cell lines as a classic strong promoter . **Conclusion**: Our results show that we could generate a new synthetic promoter with a lineage specificity. This new strategy could help overcome side effects while improving CAR-T cells persistence and could be used for both hematological malignancies and solid tumors after autologous transplantation.

H. 104. STING Activation Cell-Intrinsically Modulates CD4 T Cell Differentiation and Enhances the Antitumor Activity of Th1 and Th9 Cells

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Stimulator of Interferon Genes (STING), a critical player in cytosolic DNA sensing, has been shown to be required for anti-tumor responses, illustrating the potential of STING-targeting for cancer immunotherapy. The cyclic dinucleotide Cyclic guanosine monophosphate–adenosine monophosphate (2'3'-cGAMP) is a STING agonist which induces TBK1/IRF3-dependent production of Type I IFN and leads to potent antitumor properties in mouse models of cancers by triggering innate immune response. While recent

studies evidenced that STING ligands can activate STING signaling in T cells, their effects on CD4 T cell differentiation and anti-tumor functions remain to be determined. Here, we show that the transfection of 2'3'-cGAMP enhances IL-9 and IFN-γ secretion from differentiating Th9 and Th1 effector cells, respectivelly, in a STING-dependent manner. We found that P65 NF-KB appears involved in both 2'3'-cGAMP-driven production of IFN-γ and IL-9 while IRF3 is only required for 2'3'-cGAMP-driven production of IFN-γ. Because Th1 and Th9 cells exert antitumor functions, we investigated their involvement in the antitumor activity of 2'3'-cGAMP *in vivo*. Treatment of tumor-bearing mice with antibodies neutralizing IFN-γ and IL-9 strongly impairs the capacity of 2'3'-cGAMP to reduce tumor growth. We also demonstrate that 2'3'-cGAMP treatment enhances Th9 anticancer functions when adoptively transferred into mice. Overall, this study uncovers a novel cell-intrinsic role for STING in modulating CD4 T cell differentiation and shaping adaptive antitumor immune responses. The contribution of CD4 T cells in 2'3'-cGAMP antitumor activity brings important insights for the design of immunotherapies involving STING activation.

H. 105. Uncovering heterogeneity and dynamics of human tumor infiltrating T cells to identify orthogonal mechanisms and potential novel targets for cancer immunotherapy

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Elicitation of tumor cell killing by $CD8^+$ T cells is an effective therapeutic approach for cancer. A widely utilized strategy to boost anti-tumor immunity is to reinvigorate existing but unresponsive tumor-specific T cells by blocking immune checkpoint receptors such as PD1. Alternative therapeutic approaches also have been developed to overcome the potential limitations of immune checkpoint inhibitory therapy in patients with low pre-existing anti-tumor immunity, including the stimulation of polyclonal T cell cytolytic activity against tumors using Bi-specific T cell engager (BiTE®) antibody constructs that simultaneously engage the T cell receptor (TCR) complex and a tumor-associated antigen. Given that tumour-infiltrating T cells are highly heterogeneous regarding subset composition, gene expression and functional properties, which might contribute to diverse responses to different cancer immunotherapies, an integrated approach, STARTRAC, was developed to utilize TCR sequences as specific markers to quantitatively track the dynamic relationships among T cell subsets identified inside colorectal carcinoma, adjacent normal mucosa and peripheral blood. Using STARTRAC method, we found that there were two distinct activation and expansion routes for tumour-resident effector memory $CD8^+$ Tcells, with one leading to effector $CD8^+$ T cells and the other to exhausted $CD8^+$ T cells, depending on their different TCR usages. These CD8 T cell subsets express distinct checkpoint inhibitors and thus their anti-tumor function might be regulated via distinct pathways. Moreover, our data identified potential novel regulatory molecules such as *IGFLR1* for several T cell subsets inside colorectal tumours. These discoveries will shed light on the development of effective immunotherapeutic strategies.

H. 106. Neuropilin-1 is a T cell memory checkpoint limiting long-term anti-tumor Immunity

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CD8$^+$ T cell memory is pivotal for long-term protective immunity, but often impaired in the tumor setting, partially due to extensive T cell exhaustion and loss of memory precursors, which is not reversed by checkpoint blockade immunotherapy. It is, however, incompletely understood how T cell exhaustion is maintained, which in turn impedes functional CD8 memory development. Here we report that mice with CD8$^+$ T cell-restricted deficiency of Neuropilin 1 (NRP1) showed significantly enhanced protection from re-challenged B16.F10 tumors, despite unchanged primary tumor growth. NRP1 acted on multiple inhibitory receptors (IRs)-expressing CD8$^+$ T cells to reinforce their exhaustion status and restrain the potential of memory differentiation, by repressing the Id3-dependent transcription program. These data reveal NRP1 as a unique "immune checkpoint" contributing to the lineage stability and blocking the memory conversion in the exhausted CD8$^+$ T cells, a mechanism of action that is distinct from that of well-known immune checkpoints (PD1, CTLA4, LAG3). Blockade of checkpoint inhibitors of T cell memory may be necessary to achieve durable anti-tumor immunity.

H. 108. IL-35+ B cells establish immunosuppressive network in pancreatic ductal adenocarcinoma.

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Despite advances in our understanding of the mutational landscape in pancreatic ductal adenocarcinoma (PDAC), this devastating disease is now the third-leading cause of U.S. cancer-related deaths. While recent successes of cancer immunotherapy have generated considerable excitement, this form of treatment has been largely ineffective in patients with pancreatic cancer. A major barrier for immunotherapeutic approaches is marked immunosuppression within the PDAC milieu. We have previously identified a novel role for IL-35 producing B cells in the pathogenesis of pancreatic cancer. However, little is known about the mechanisms behind IL-35 activity in cancer. Here, we set out to elucidate molecular and cellular mechanisms by which IL-35 facilitates the emergence of pancreatic cancer. Our results demonstrate that IL-35, but not IL-10, potentiates PDAC growth. This correlates with induction of regulatory T cells and suppression of effector T cell activity, suggesting that IL-35 controls endogenous anti-tumor immune responses in PDAC. Furthermore, while IL-35 is expressed by several immune cell types in PDAC, we show that its expression specifically in B cells is essential for suppression of anti-tumor T cell responses. Importantly, while PDAC is typically resistant to anti-PD-1 immunotherapy, we demonstrate robust synergistic reduction in tumor growth when IL-35 deficiency is combined with anti-

PD-1 treatment. Insights gleaned from these and further mechanistic studies of IL-35 in PDAC may be expeditiously translated into IL-35 targeted combination immunotherapy.

H. 109. Antigen Cross-Presentation Promotes Development of ICB non-Responsive CD8 T Cells

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Tumor microenvironment (TME) represents a unique immunological niche that contributes to the observed resistance to immune checkpoint blockade (ICB) therapy. Here we describe our efforts to understand the role of TME during early differentiation of CD8 T cells in response to tumor antigens. For this, we stably expressed the dominant MHCI-restricted LCMV-derived epitope GP33, in MC38 tumor cells. Co-expression of a reporter gene encoding for mCherry allows detection of antigen presentation. Subcutaneous inoculation of MC38-GP33 cells into immunocompetent syngeneic hosts resulted in a massive expansion of highly functional CD8 effector cells in peripheral lymphoid tissues. In contrast, tumor-infiltrating GP33-reactive CD8 cells showed a striking impairment in their ability to produce effector cytokines (IFNγ/TNFα/IL-2). This tumor-associated suppression is mediated by the PD1/PDL1 axis since PDL1 blockade partially recovered the immunomodulatory properties. Genetic ablation of B2m gene in tumor cells resulted in a decreased expansion of GP33-specific CD8 T cells. The absence of direct antigen-presentation by tumor cells had no impact on the tumor-induced dysfunction of T cells. Importantly, PDL1 blockade also failed to recover the functionality of CD8 TILs in the B2mKO tumors, indicating that antigen cross-presentation by myeloid cells in TME skews differentiation of CD8 T cells to an ICB non-responsive state. Aligned with these findings, analysis of immune infiltrates from pediatric solid tumors revealed a strong correlation between expression of PDL1 on myeloid cells and enrichment of CD8 TILs with an exhaustion phenotype. Collectively our findings identify tumor-associated monocytes/macrophages as a potential driver of antigen-induced CD8 T cell dysfunction.

H. 110. The Anti-tumor Effect of Trastuzumab in HER2+ Breast Cancer is Principally Mediated by Antibody-Dependent Phagocytosis of Tumor-Associated Macrophages and can be Significantly Enhanced by CD47 Innate Immune Blockade

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Background: HER2 overexpression define ~20% of breast cancers (BC) that are currently treated using HER2-specific monoclonal antibodies (mAb), such as Trastuzumab. While multiple studies have confirmed Trastuzumab's anti-tumor efficacy, its dominant immune mechanism of therapeutic action (MOA) remains unclear. As Trastuzumab efficacy is subverted in advanced immunosuppressive cancers, an understanding of its MOA and strategy to boost its therapeutic effect is of clinical interest and scientific significance for immunologically enhancing targeted mAb treatments.

Results: We found that Trastuzumab significantly suppressed tumor growth and stimulated the infiltration of tumor-associated-macrophages (TAMs). Importantly, this anti-tumor activity did not require adaptive immunity or NK cells, but did require FcγR engagement, implicating macrophages as the dominant

immune effector. Consistently, we demonstrated Trastuzumab activates FcγR4 signaling and elicited significant macrophage-mediated phagocytosis (ADCP) of HER2+ BC. To test if enhanced ADCP could confer more effective anti-tumor immunity, we combined Trastuzumab with blockade of the ADCP checkpoint, CD47 ("don't eat me signal"), and found that this combination significantly increased Trastuzumab-mediated ADCP. Critically, we also found that this combination enhanced TAM infiltration in vivo, as well as stimulated stronger anti-tumor efficacy and prolonged survival in highly immunosuppressive tumor microenvironments.

Conclusion: Our study demonstrates the major MOA of clinically relevant HER2 mAbs requires the engagement with TAMs to elicit ADCP of tumor cells, which could be significantly enhanced by CD47 blockade. We conclude CD47 blockade could unleash the full potential of HER2 mAb therapy by abrogating the innate immunosuppressive signals on macrophages to stimulate immunity and enhance anti-tumor efficacy.

T. 95. A C-terminal fragment of adhesion protein Fibulin7 (Fbln7C) inhibits tumor growth via targeting both cancer cells and tumor associated macrophages.

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Fibulin-7 (Fbln7) is one of the latest members of the Fibulin family of secreted glycoproteins. Previous reports have shown that a C-terminal fragment of Fbln7 (Fbln7-C) have antiangiogenic activity and could modulate migration and functions of inflammatory monocytes and macrophages. In this study, we have investigated the potential anti-cancer activity of Fbln7C. Our *in vitro* studies demonstrate that Fbln7-C could inhibit the proliferation of MDA-MB-231, MCF-7 and PANC-1 cell lines. Similarly, Fbln7-C also reduced the growth of tumors in a 4T1 cell induced murine mammary tumor in a dose dependent manner. Additionally, detailed phenotypic analysis of tumor associated macrophages (TAM) using various surface markers revealed that TAMs were arrested in an anti-tumorigenic M1 type phenotype ($MHC^{hi}Ly6C^{low}CD206^{low}$) in animals treated with Fbln7C compared to control group. In line with the *in vitro* results, Fbln7C inhibited the polarization of human monocyte derived macrophages into tumor supernatant induced TAMs which retained their inflammatory M1 like phenotype even after removal of Fbln7C from culture. The above observation indicates that Fbln7-C could be used as supportive immunomodulatory therapeutic for cancers.

T. 96. A New Conditioned Tumor Lysate Based Melanoma Vaccine Inhibits Tumor Growth and Potentiates anti PD1 Treatment

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Clinical strategies using immune-checkpoint blockers, such as anti-CTLA4, (Ipilimumab) or anti-PD1 antibodies (Nivolumab), have recently demonstrated durable survival benefits in patients with melanoma and other tumors. Nevertheless, an important percentage of treated patients remain refractory,

suggesting that combination with other kind of active immunization is required for increasing responses rate. In this context, cancer vaccines become again a complementary alternative. The optimal delivery of antigens (Ags) and the use of adequate adjuvants are crucial for vaccine success. Here, a prototype of a generic therapeutic vaccine for the treatment of malignant melanoma named TRIMELVax^{TM} was tested in an experimental model. This vaccine is based on heat-shock conditioned melanoma allogeneic tumor lysates combined with specific adjuvants. The vaccine is intended to activate immune responses against tumor in vivo, inhibiting its growth. TRIMELVax was evaluated, in terms of safety and efficacy in C57Bl/6 murine model. In short, immunocompetent mice was vaccinated with three doses of TRIMELVax, and then challenged with B16 murine melanoma cells. Alternatively, immunization was tested therapeutically in tumor bearing mice. Our results showed that only TRIMELVax was capable to reduce the occurrence of tumor. In contrast the use of non-conditioned tumor lysate, or adjuvant alone did not impact tumor growth. Observed response was associated with $CD8^+$ T cells intratumoral accumulation and antibody production in sera. Moreover, anti-PD1 therapy was strongly potentiated by combination with TRIMELVax in immunocompetent mice, which encourage testing of the combined treatment in future clinical trials. Financed by grants FONDECYT 1171213, FONDEF ID16I10148 and MIII P09/016-F.

T. 97. A Translational Platform to Support and Advance Immuno-Oncology Drug Discovery

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Charles River is establishing a powerful translational immuno-oncology platform with the capability of progressing biologics or small molecule modulators of immune response from in vitro to in vivo assays using human and mouse variants of current check-point inhibitors and small molecules.

The platform includes validated (using chemotherapeutics, including anti-CTLA4, anti-PD1 and small molecule inhibitors of targets known to modulate immune responses including IDO inhibitors) primary human immune cell assays which profile T cell activation, cytokine release, T cell mediated cancer cell kill, expansion of T cell populations, T cell invasion and macrophage mediated T cell phagocytosis and is currently being expanded to determine the effect of activated immune cell populations on tumour cell spheroid cultures. We are in the process of developing a range of nuclear-restricted GFP expressing cell lines which will be used to support co-culture experiment.

Syngeneic mouse tumour models have frequently been used to profile immune responses in tumours, CRL have optimized and profiled existing check-point inhibitors to support immuno-oncology drug discovery using mouse and rat antibody variants of anti-CTLA4 and anti-PD1.

To confirm the translational development of our platform CRL have developed and optimized humanized mouse models using sub-cutaneous implanted patient derived xenografts (PDX) with human engraftment via CD34+ haematopoeitic stem cells in NOG mice which were treated with anti-CTLA4 and anti-PD1. Infiltration of human immune cells and PDL-1 expression was detected by flow cytometry (FC) and

immunohistochemistry (IHC) in hematopoietic organs and tumor tissue, supporting the initial in vitro response in primary immune cells.

T. 98. An antigen-specific memory stem T cell (TSCM) expansion system for adoptive T cell immunotherapy

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Cell culture methods that yield high numbers of functional antigen-specific T cells are crucial to translate adoptive T cell immunotherapy into clinics. In pre-clinical models, early differentiated memory T cell subsets showed superior engraftment, survival and function when compared to late differentiated T cell subsets. We aimed at the specific enrichment and preferential expansion of the distinctly early differentiated memory stem T cell subset (T_{SCM}; $CCR7^+CD45RA^+CD95^+$). In an explorative way, we established a Cytomegalovirus (CMV)-specific T cell culture system that investigates the influence of cytokine regimes, the addition of $CD4^+$ T cells and the presence of regulatory T cells (T_{REG}) on T_{SCM}-derived T cell expansion. Here, we found that the expansion of antigen-specific T_{SCM}-cells was diminished when bulk peripheral blood mononuclear cells were used as starting material. Specifically, we found that other memory T cell subsets inhibit the expansion of T_{SCM}-cells. By the same token, we established that T_{SCM}-cells expanded best when derived from pre-enriched $CCR7^+CD45RA^+$ T cells, cultured in the presence of irradiated bulk $CD4^+$ T cells and distinct doses of IL-7 and IL-15. Surprisingly, depletion of T_{REG}-cells significantly diminished T_{SCM}-expansion irrespective of cytokines applied during culture. Further, *post-stimulation* CD45RO-selection owing to proliferation-induced CD45-isoform switch (CD45RA to CD45RO) significantly increased the purity and yield of T_{SCM}-derived cultures. Our findings enable direct access to human antigen-specific T_{SCM}-cells from peripheral blood and pave the way for rapid broad clinical application.

T. 99. Analysis of molecular and cellular responses associated with immune checkpoint inhibition in the MC38 colon carcinoma model

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The clinical success of immune checkpoint therapies such as PD-1, PD-L1, and CTLA-4 inhibitory antibodies has stimulated resurgent interest in a wide range of approaches to cancer immunotherapy. To support the development of these new modalities it is essential to have robust, well characterized

preclinical animal models to evaluate efficacy and identify toxicities. Preclinical efficacy assessments of novel immune-oncology therapies requires a functional immune system which limits the usefulness of traditional xenograft models and drive the use of humanized (human immune cell engrafted animals) and syngeneic model systems. The syngeneic MC38 mouse colon cancer model is popular for efficacy assessment studies due to its responsiveness to typical immune checkpoint inhibitors (ICI). We endeavored to characterize the kinetic immune response to checkpoint inhibitors (anti-PD-1 + anti-CTLA-4) in MC38 tumors using flow cytometry and gene expression analysis. Gene expression analysis provided a signature of gene changes that correlate to immune driven changes in tumor growth and may be used in preclinical pharmacodynamics studies as evidence of mode of action for other novel immune-oncology therapies. In addition, gene overrepresentation analysis highlighted the presence and involvement of B cell populations in the MC38 tumor environment which was supported by IHC data that show distinctive B cell staining in what resemble tumor associated tertiary lymphoid structures. The in depth molecular and cellular characterization associated with ICI responses in the MC38 model serves as a template for evaluating the mechanism of action and therapeutic activity of other novel immuno-oncology therapies.

T. 100. Antagonism of the TNF Superfamily of Receptors: Focus on Novel Functional Antibodies with Preference for the Tumor Microenvironment

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Tumor necrosis factor (TNF) superfamily receptors are often differentially expressed on lymphoid cells and are a primary focus of immunotherapy approaches. Many are linked to death receptors; some are linked to growth pathways, like tumor necrosis factor 2 (TNFR2). In diverse human and murine cancers, TNFR2 is heavily expressed in the tumor microenvironment on regulatory T cells (Tregs) and myeloid-derived suppressor cells. It also serves as an oncogene for direct tumor expansion. Clinical data consistently show TNFR2 overexpression in the tumor microenvironment after checkpoint failures.

Over the past decade, we have worked to create antagonistic antibodies to the TNF superfamily receptors. One major challenge has been that natural ligands such as TNF have a very high affinity and strongly promote agonism.

Using sequential receptor peptide fragment mapping, we have gradually made a new class of TNFR2 antagonistic antibodies that are dominant. A select region of the TNFR2 receptor creates antagonism resistant to TNF. These TNFR2 antagonists kill tumor residing Tregs; they also kill cancer cells expressing the oncogene. Unlike many other therapeutic antibodies, this new antagonist class does not require antibody-dependent cellular cytotoxicity (ADCC), a trait often associated *in vivo* human toxicity. We can now demonstrate by framework class switching experiments that there is no need for Fc region function and the construction of F(ab)2 fragments also still shows efficacy. Remarkably, a known serum biomarker of poor cancer prognosis is high sTNR2 from overactive tumor microenvironment TNFR2 agonism; dominant TNFR2 antagonists rapidly stop sTNFR2 secretion.

T. 101. Application of a novel dimer-avoided multiplex PCR technique in immune repertoire profiling for renal cancer treatment evaluation

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Next generation sequencing of the immune repertoire is a comprehensive immune profiling methodology that allows detailed, sequence-specific insight into the adaptive immune response. While many studies focus solely on the T-cell receptor beta chain, insight into the variable rearrangements of the immune repertoire as a whole through the study of all seven TCR and BCR chains together (i.e., TCR-beta, TCR-alpha, TCR-delta, TCR-gamma, BCR-IgH, and BCR-IgK and IgL) provides a broader view of the immune landscape with potential prognostic value. A major challenge for all-inclusive multiplex PCR development is primer-dimer formation. We have developed a new technique, dimer avoided multiplex PCR (dam-PCR), that effectively avoids dimer formation during PCR and incorporates unique molecular identifiers for direct RNA quantification and error removal. With one sample - either PBMC, FFPE, or tumor tissue - we are able to amplify and obtain sequences of all seven chains in the immune repertoire together. Applying this technique to clinical renal cancer PBMC with treatment, we found that both TCR-alpha and -beta diversity prior to treatment and the expression ratio between B cells and T cells are good predictors of treatment efficacy. Furthermore, differences in treatment protocols can potentially increase BCR-IgH and BCR-IgL expression. Our study suggests that examining multi-chain immune repertoire composition can be valuable for predicting treatment response and evaluating treatment protocols.

T. 102. Association of Tumor-Associated Immune Cell Markers With Tumor Size And Lymph Node Metastasis in Oral Squamous Cell Carcinoma-Gingivo-Buccal (SCC-GB) : A Retrospective Study on Formalin Fixed Paraffin Embedded (FFPE) Tissue

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The composition of immune cells in primary tumors of **GINGIVO-BUCCAL SQUAMOUS CELL CARCINOMA** patients with lymph node metastases (N stage) / without metastases and size (T stage) is currently unknown. Therefore, we studied tumor infiltrating immune cells in archived samples to find a correlation with pathological T and N stages of the disease.
The analysis of the location, density and functional orientation of different immune cell populations was done on 94 retrospective cases. Characterization of immune cells by immunohistochemistry (IHC) was done using the following markers : CD3, CD4, CD8, CD68, Granzyme B, CD14, CD15, HLADR, Arginase1, Neutrophil Elastase, CD56 and CD20. Quantification of immune cells was done at the invasive margins of tumor (IM) and tumor centre (CT) for each of the above markers.

Markers that inversely correlated significantly with tumor size, both at the invasive front and tumor centre, are CD3 (CT p=0.001; IM p=0.005) and CD68 (CT p=0.010; IM p=0.006). Absence of lymph node metastasis correlated significantly with high numbers of CD3 and CD8 positive cells in tumor margins and centre. (CD3 – CT p=0.010, IM p<0.001; CD8-CT p=<0.001, IM p=0.001). Therefore, we proposed that quantification of CD3 and CD8 positive cells in primary tumors may predict disease progression. A deeper understanding of host tumor interactions and tumor immune escape strategies in SCC-GB is required.

T. 103. Attenuated Listeria Vaccine for Cancer Immunotherapy Expands Vγ2Vδ2 T Cells in Rhesus Monkeys that Retain their Central Memory Phenotype and HMBPP Responsiveness

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Human γδ T cells expressing Vγ2Vδ2 TCRs monitor self- and foreign-isoprenoid metabolites to mediate immunity to tumors and microbes. Bisphosphonate treatment of tumors increases isopentenyl pyrophosphate that is sensed by butyrophilin 3A1, allowing Vγ2Vδ2 cells to kill tumors independent of MHC expression or tumor mutational burden. In clinical trials, adoptive immunotherapy with Vγ2Vδ2 cells has few side effects but has resulted in only a few partial and complete remissions. Direct immunization of cancer patients with either a bisphosphonate or bromohydrin pyrophosphate and IL-2, expands Vγ2Vδ2 cells but they rapidly contract, terminally differentiate, and lose responsiveness. Similarly, we find that monkeys immunized with zoledronic acid and IL-2 lose Vγ2Vδ2 cell responsiveness with terminal differentiation. To identify a live bacterial vaccine for Vγ2Vδ2 cells, we tested *Listeria monocytogenes* (*Lm*) attenuated by *actA* deletion with a mutant PrfA protein (G155S) that increases their virulence. Immunization with these attenuated *Lm* expanded Vγ2Vδ2 cells on secondary immunization that persisted after a third immunization. Vγ2Vδ2 cells retained central memory phenotypes, expressed inflammatory chemokine receptors, and produced IFN-γ without induction of anergy. Expression of PrfA (G155S) was critical because immunization with Δ*actA Lm* with wild-type PrfA resulted in minimal Vγ2Vδ2 T cell expansions. Similarly, despite high HMBPP levels, immunization with Δ*lytB* Δ*actA prfA*^{wt} *Lm* resulted in minimal expansions. Thus, *Lm* stimulation of Vγ2Vδ2 T cells is dependent on their virulence rather than their HMBPP levels. Unlike zoledronic acid, immunization with Δ*actA prfA*^{G155S} *Lm* induces large expansions of Vγ2Vδ2 T cells that retain central memory phenotypes and responsiveness.

T. 104. Building a Translational Pathway Using Human Myeloid Cell Assays to Enable Development of Cancer Immune Therapies

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Myeloid cells are a key immune population in the tumor microenvironment (TME) and represent a tractable target. Precursors can differentiate into multiple cell types, including macrophage (Mϕ), which

are heterogeneous. Tumor-associated macrophage (TAM) and myeloid-derived suppressor cells (MDSC) can be found in the TME and may promote tumor progression. We have developed a range of in vitro assays to assess whether immunosuppressive mechanisms can be overcome by immuno-modulators. Monocytes were differentiated into TAM following exposure to tumor cell supernatants alongside classical M1, M2(a) or M2(b) Mϕ. Mϕ phenotype and cytokine/chemokine production were determined and function assessed via phagocytosis and antigen-presentation assays. TAM exhibited a resting phenotype whereas M1 were CD25hi, CD127+, CD80hi, CD68hi and MHCIIhi. M2a were CD25lo, CD80lo, CD163lo, CD68lo and MHCIIint whereas M2b expressed high levels of CD184, CD80 and little CD163 or CD68. TAM produced little IL-12p70 and raised IL-10 and VEGF. M1 produced IL-6, IL-12p70, TNFα and IL-23 whereas M2a produced little detectable cytokine. M2b produced IL-6, IL-10 and TNFα. Mϕ expressed checkpoint inhibitors such as PD1 and Tim-3. Phagocytosis assays were performed using tumor targets and inhibition of the CD47-SIRPα pathway drove enhanced uptake. T cell co-cultures were also performed to assess effects on antigen-presentation. Gene expression analysis was performed using an nCounter® Myeloid Innate Immunity Panel (Nanostring). Our myeloid/macrophage assay platform can be used to test novel myeloid-targeted therapies, and help elucidate the MOA. This has potential for use in immune oncology screening programs and may accelerate progress into the clinic.

T. 105. Building a Translational Pathway Using Pharmacodynamic Models to Enable the Development of Cancer Immune Therapies

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In order to develop therapeutics which drive the immune system to target tumour cells and eliminate tumour growth, sophisticated in vitro and in vivo models are required. We have developed models which enable us to combine data from human immune cell in vitro assays and murine pharmacodynamic (PD) and syngeneic tumour models to evaluate which pathways a therapeutic is hitting and whether it is effective at inhibiting tumour growth in vivo. We show here the powerful combination of using TCR transgenic adoptive transfer models and syngeneic tumour with flow cytometry and Nanostring profiling of gene expression within the tumour microenvironment (TME) to determine the effect of therapeutic interventions. To determine the effect of checkpoint inhibitors, mice bearing a defined population of ovalbumin (OVA)-specific T cells are challenged either with antigen (PD model), or an OVA expressing tumour. In the PD model, activation, proliferation and differentiation into CTL are assessed offering a rapid screening tool. In OVA-expressing tumour models comprehensive TIL analysis, can determine how changes to T cell activation impact on the wider TME. Nanostring analysis of gene expression using the murine immune-oncology 360 panel enables a deeper analysis of the TME and acts as a powerful tool for the identification of potential PD biomarkers. This molecular profiling information can also direct which in vitro assays should be used to map the immunological mechanisms underlying immune modulation. Taken together, the use of in vitro and in vivo PD/efficacy models better enable assessment of novel cancer immune therapeutics.

T. 106. CD45 activity modulation as a novel therapeutic target in B-cell malignancies

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The underlying mechanism of chronic proliferation in B-cell malignancies is unresolved, however, several common characteristics exist. First, signaling through the B-cell receptor (BCR) is crucial, and secondly, T-helper signals from the microenvironment contribute to proliferation of malignant B-cells in diseases such as chronic lymphocytic leukemia (CLL) and multiple myeloma (MM) (Os et al, 2013, Wang et al., 2017).

We discovered a mechanism where T-cell help enhance the ability of B-cells to signal through the BCR (Szodoray et al, 2016). We found that T-helper signals regulate CD45 phosphatase activity in B-cells, which is a key positive regulator of the BCR-signaling and proliferation machinery. Mechanistically, we show that T-cell mediated Galectin-1 surface binding upregulate CD45 phosphatase activity, and thus, T-helper signals enhance the ability of B-cells to respond to antigenic stimulation, leading to proliferation and survival through CD45 activity regulation.

For malignant B-cells, inhibition of CD45 activity significantly downregulated T-cell mediated BCR-signaling and proliferation in CLL. Moreover, inhibiting Galectin-1 binding to malignant B-cells reduced cell proliferation, however, this was more prominent for IKAROS+ CLL-cells. Interestingly, upregulation of Galectin-1 surface expression was mainly restricted to IKAROS+ CLL-cells upon CD40L-stimulation, suggesting a link between IKAROS and CD45 phosphatase activity through Galectin-1 modulation.. These data suggest that expression of the CD45 ligand Galectin-1, and thus CD45 phosphatase activity may be under the control of IKAROS.
We propose that regulation of CD45 phosphatase activity, survival and proliferation capacity of malignant B-cells may be controlled by CD45-ligand(s) such as Galectin-1.

T. 107. Characterisation of Activated T-Cells and Virus Specific T-Cells as a Platform for Chimeric Antigen Receptor Therapy

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The success of Chimeric Antigen Receptor (CAR) T-Cell therapy against non-solid malignancies has not been replicated in solid tumor settings. One contributing factor for the decreased efficacy could be due to the T-Cell expansion methodologies utilized for CAR transduced cells, which largely rely on generic proliferation techniques via the cross-linking of co-stimulatory receptors. These techniques result in the expansion of a T-cell population with mixed phenotype and polarization, which could result in poor trafficking to the tumor sites, poor effector function within the tumor environment and decreased persistence within the patient. The use of virus specific T-Cells (VSTs), which are naturally polarized to a

Th1/Cytotoxic effector function, offer an alternative to improve CAR-T therapy against solid malignancies. Single cell RNASeq analysis of VSTs and conventional expanded T-Cells (ATCs), revealed an increase in transcripts associated with cytotoxic function, IL-12 signaling as well as mitochondrial respiration in VSTs. Phenotypic analysis demonstrated that VSTs are comprised mainly of effector and central memory cells, with a decreased proportion of regulatory, and naïve T-cell phenotypes. Examination of receptors associated with purinergic metabolism, showed a lower expression on VSTs compared to ATCs. Antigen stimulation of VSTs resulted in decreased secretion of Th2 and myeloid stimulating cytokines in contrast to higher amounts from ATC stimulation. Taken together, these results indicate the utility of VSTs as a viable platform for CAR-T therapy.

T. 108. Characterization of the immunogenic determinants of tumor neoantigens improves their identification

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Neoantigens are somatic mutations, that are unique to the tumor and can be presented on major histocompatibility complex (MHC) molecules and recognized by T cells resulting in a protective anti-tumor response. Next generation sequencing and computational methods have been successfully applied to predict neoantigens that may be presented by MHC molecules, leading to a high interest in using these non-self antigens for personalized cancer vaccination. However prioritization remains challenging since only few candidate neoantigens are immunogenic. To further enlighten the determinants driving T cell responses, we assessed immunogenicity of single amino-acid mutations in mouse tumor models.

We identified two kinds of mutations inducing a CD8 T cell response: the ones at a non-anchor residue for which the absolute binding affinity is predictive of immunogenicity, and the ones at an anchor residue for which the relative affinity (compared to the WT counterpart) is a better predictor. These results, validated on human datasets, demonstrate that incorporating these determinants of immunogenicity will help further prioritize MHC-I neoantigens for immunotherapy.

Although neoantigens were selected using an MHC-I prediction algorithm, we mostly observed mutation-induced CD4 T cell responses, as shown by others. We developed a 2D-LCMS peptide exchange assay allowing to reveal I-A^b epitopes, binding affinity and stability measurements. We then confirmed immunogenicity of identified strong I-Ab binders, and identified tetramer+ CD4 T cells from the spleen of vaccinated mice. These new experimental data and tools will help unraveling the determinants of immunogenicity and favors the selection of immunogenic MHC-II neoantigens.

T. 109. Deciphering the role of the tumor infiltrating lymphocytes in anti-angiogenic therapy using a humanized PDX in vivo platform

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In the current study, we evaluated the antitumoral activity of bevacizumab an anti-VEGF monoclonal antibody in two NSCLC PDX in vivo in the presence or absence of human immune cells.

Two NSCLC PDX models were subcutaneously implanted into different mouse strains: NMRI-nude, CD34+-humanized NOG (huNOG), NOG and NOG-EXL both substituted with human monocytes by weekly iv injection. Animals were treated with control vehicle or bevacizumab. Tumor and lymphatic organs were harvested and flow cytometry as well as IHC analysis was performed. The serum levels of 40 human and 23 murine cytokines were determined using a Bioplex system.

In both tumor models, bevacizumab showed in the absence of human immune cells moderate antitumoral activity. The co-injection of human monocytes markedly enhanced the therapeutic effect in both NSCLC PDX. The effect human immune cells on antitumoral activity was similar in NOGs, NOG-EXL and huNOG. The injection of monocytes alone did not affect tumor growth. The TIL infiltrates were enhanced under treatment specifically in the NOG-EXL mice. In all treatment arms receiving bevacizumab the CD11b+ subpopulations were increased. The cytokine analyses revealed an upregulation of human cytokines under treatment with bevacizumab towards a pro-inflammatory micro-environment including IL-6, CCL24 and CXCL-1. Interestingly, also the murine host cells contributed to this pro-inflammatory reaction by upregulation of mouse IL-6, TNF-alpha, Eotaxin and others.

Monocytes have been reported to induce antibody-dependent cytotoxicity of tumor cells in the presence of antibodies like bevacizumab. Our results confirm these observations in a PDX based in vivo model.

T. 111. Evaluation of TNFR2 Antagonism with and without Anti-PD-1 Therapy in Murine Colon Cancer Models

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TNFR2 in humans and mice is central to immune balance control, acting on regulatory T cells (Tregs) and T effectors (Teffs) in opposing ways and serving as an oncogene in select cancers. Indeed, published work suggests TNFR2 is the bidirectional switch for Treg expansion/depletion, and TNFR2 derangements have been identified in diverse human and murine cancers.

We identified a murine-directed surrogate antibody to TNFR2 (TY101) that shares certain traits identified in human-directed antibodies as critical to limiting Treg expansion and activating Teff function.

In MC38 and CT26 murine colon tumor models, we compared: anti-TNFR2 therapy (TY101); a commercially available anti-PD1 therapy; and anti-TNFR2/anti-PD1 combination immunotherapy. Mice were dosed bi-weekly (100ug/mouse antibody) to test the impact on tumor growth. Antigen-specific CD8

and Treg infiltrates were studied within the tumor to evaluate possible tumor microenvironment changes secondary to therapy.

Single-agent anti-TNFR2 (MC38, p=0.04; CT26, p<0.001) and anti-PD1 (MC38, p=0.005; CT26, p=0.002) enhanced murine survival in both models. Combination immunotherapy was the most effective treatment in the MC38 model (p=0.004), but less beneficial than either single-agent in the CT26 model.

In situ tumor flow cytometry showed that single-agent anti-TNFR2 depleted intra-tumor Tregs (both models) and induced infiltrating CD8 Teff expansion (MC38 model), whereas anti-PD1 had no tumor microenvironment effects.

We conclude that anti-TNFR2 immunotherapy provides benefits in two murine colon tumor models, both with and without anti-PD1. Anti-TNFR2 was distinct from anti-PD1 therapy in showing pronounced Treg depletion and enhanced Teff infiltration in the tumor microenvironment.

T. 112. FIGHTING HIGH-RISK ACUTE LEUKEMIA: γδ T CELLS AS AN IMMUNOTHERAPY

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Treatment of children with either relapsed or refractory acute leukemia is still a major challenge. This explains the recent burst of immunotherapies.
In the allogeneic setting, γδ T cells represent a perfect candidate due to their ability to lyse tumor cells in a major histocompatibility complex (MHC)-independent manner, bypassing the occurrence of Graft-versus-Host Disease (GvHD). The majority of circulating γδ T cells are constituted of Vγ9Vδ2 cells, whereas a minor Vδ1 subset resides within the epithelial tissues.

Here, we developed a novel strategy for *ex vivo* expansion of both Vγ9Vδ2 and Vδ1 γδ T cells at a clinical-scale level with the ultimate goal of administering multiple infusions of such a cell product after allogeneic stem cell transplant.

We have previously demonstrated that *in vitro* exposure of γδ T cells to zoledronic acid (ZOL) combined with IL-2, allows robust expansion and activation of the Vδ2 subset alone. Herein, we developed a novel protocol for the expansion of both Vδ1 and Vδ2 by exploiting the mitogenic effect of Concanavalin-A (Con-A), together with ZOL and a cocktail of different cytokines.

Preliminary results obtained from 5 different healthy donors show that 1) we succeeded in expanding γδ T cells up to 50 folds, 2) the use of Con-A is essential for the Vδ1 subset expansion, 3) γδ T cells *ex vivo* expanded acquire an effector memory phenotype and are cytotoxic *in vitro* against pediatric acute leukemia cell lines. Therefore, these encouraging results lay the foundation for advancing the translational use of γδ T cells.

T. 113. High salt blocks tumor growth by enhancing anti-tumor immunity

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Excess salt intake could affect the immune system by shifting the immune cell balance towards a pro-inflammatory state. Since this shift of the immune balance is thought to be beneficial in anti-cancer immunity, we tested the impact of high salt diets on tumor growth in mice. Here we show that high salt significantly inhibited tumor growth in two independent murine tumor transplantation models. High salt fed tumor-bearing mice showed alterations in adaptive and innate immune cell subsets. Of note, depletion of specific innate immune cells significantly reverted the inhibitory effect on tumor growth. In line with this, high salt conditions almost completely altered the functional activity of these cells in vitro. Importantly, similar effects were observed in human cells isolated from cancer patients. Thus, high salt conditions seem to inhibit tumor growth by enabling more pronounced anti-tumor immunity through the functional modulation of this cell type. Our findings might have critical relevance for cancer immunotherapy.

T. 114. β-catenin/TCF-1 signaling in Treg contributes to colon cancer

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Abstract

Regulatory T-cells (Tregs) play a dual role in colon cancer, promoting immune tolerance and suppressing inflammation. We have observed two types of Tregs in patients with colon, lung, and pancreatic cancer. The subset that preferentially expands in these patients has pro-inflammatory properties and express RORgt. Using mouse models, we showed that expression of RORgt is controlled by b-catenin. Tregs and CD4+ T-cells from colon cancer had elevated expression of b-catenin. To test the role of canonical Wnt signaling in the generation of pro-inflammatory Tregs, we stabilized β-catenin or deleted its DNA binding partner TCF-1 in Tregs. In both instances' expression of RORγt was upregulated. While the stabilization of β-catenin led to loss of Treg functions, ablation of TCF-1 reproduced the phenotype and functional characteristics of Tregs in colon cancer. The TCF-1 deficient Tregs were pro-inflammatory, potent T-cell suppressive, and tumor promoting. Therefore, we were able to reproduce the phenotype and functions of Tregs in colon cancer patients. Expansion of this subset of Tregs is responsible for deregulation of tumor promoting Th17 inflammation in colon cancer.

T. 115. Long term time-course monitoring of NK cell-mediated ADCC using the Celigo Image Cytometer

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Antibody-dependent cell-mediated cytotoxicity (ADCC) assay has been widely performed for immuno-oncological research. Traditionally, ADCC assays are conducted by measuring the amount of released Chromium-51 after the target cancer cells are killed by effector/antibody pair. These methods can be inaccurate due to indirect measurement of supernatant at the end point of 4 hour, however, there is a need to characterize the effects of antibody-dependent cytotoxicity for longer than 72 hours. Previously, we have demonstrated an image-based ADCC detection method using the Celigo Image Cytometer, where the target cells are stained with calcein AM and other tracer dyes to monitor cell count or viability. In this work, we demonstrated the time-course monitoring of NK cell-mediated ADCC of A375 cells in the presence or absence of IL2 and antibodies for 76 hours. The image cytometer was able to count individual cells by segmenting highly fluorescent objects in the images. We were able to show time-dependent ADCC killing with decrease in ZsGreen positive cells as the read-out. In addition, the uniformity of cell killing can be observed in the Celigo obtained whole well images. One of the most important findings was that there was regrowth of target cells after the initial 30 hours of cell killing, indicating the activated NK cells without antibodies did not eradicate the cancer cells. Therefore, the ability to perform long term time-course monitoring of ADCC is highly important to better characterize the effects of target antibodies on cell killing function.

W. 89. Measurement of Target Cell-Specific Killing Using a Novel Highly Sensitive Bioluminescent Assay

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Specific target cell killing by immune effector cells is a key functional endpoint for immunotherapy strategies including (i) T cell recruitment using bi-specific molecules, (ii) NK cell-mediated ADCC and (iii) cell therapy using CAR-T and/or CAR-NK cells. Common to these approaches is the need to quantitatively measure target cell killing in mixed cell cultures. Assays that measure *total* cell death are complicated by the presence of effector cells in the system, which can lead to high background and poor sensitivity. Assays that measure *specific* cell death of target cells often require radioactivity or other complex labeling protocols and are relatively low-throughput.

We have developed a highly sensitive bioluminescent assay to measure target cell-specific killing in mixed cell cultures. This assay involves target cell-specific expression of a cytosolic protein fused to HiBiT, an 11 a.a. protein tag. When the target cells are killed, HiBiT is released into the extracellular medium that contains LgBiT. Binding of HiBiT and LgBiT reconstitutes a bright, luminescent enzyme that is measured using a standard benchtop luminometer. The assay is sensitive and linear over a wide range of cell number (e.g. 100-500,000 cells). Here we demonstrate the target cell-specific expression of

HaloTag-HiBiT, its release and binding to LgBiT following cell death, and quantitative measurement of bioluminescence at a single time-point or in real-time over several hours or days. The assay is compatible with a broad range of cell types expressing endogenous or exogenous cellular targets for antibody and/or cell therapies.

W. 90. PD-1H (VISTA) induces immune evasion in acute myeloid leukemia

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Limited response to single agent anti-PD-1 antibody in AML suggests that co-inhibitory molecules other than PD-1 may induce immune evasion in AML. Programmed Death-1 Homolog (PD-1H, VISTA) is a novel co-inhibitory molecule, highly expressed on myeloid cells and T cells. Interestingly, the Cancer Genome Atlas revealed that PD-1H is highly expressed in AML. We performed flow cytometric analyses demonstrating that PD-1H is expressed on AML blasts in addition to immune cells such as myeloid cells and T cells. To determine if AML surface PD-1H induces immune evasion, we transplanted C1498FF cells (murine AML cells, engineered to express luciferase) transduced with PD-1H expression lentivirus (C1498FF-PD-1H) or with control lentivirus (C1498FF-mock) in syngeneic B6 mice to assess AML proliferation *in vivo* using a bioluminescence assay. Interestingly, *in vivo* proliferation of C1498FF-PD-1H cells was significantly faster than C1498FF-mock cells. While AML surface PD-1H induces immune evasion, PD-1H has been reported to be expressed in host immune cells, including T cells and myeloid cells. Thus, we further hypothesized that PD-1H on host immune cells induces immune evasion in AML. C1498FF cells were transplanted in PD-1H knockout (KO) or wild-type B6 mice to assess *in vivo* proliferation using a bioluminescence. The genetic deletion of PD-1H in host immune cells (KO mice), especially myeloid specific PD-1H deletion confers significant anti-leukemic effect, compared with wild-type control. Together, our data suggest that PD-1H is highly expressed in human AML, can induce immune evasion in a murine model of AML and can be an important immunotherapeutic target in AML.

W. 91. Switching CAR Drivers: from T to NK Cells

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Canada, [7]UPMC Paris 6 and CIRI – International Center for Infectiology Research, Team EVIR, Paris, Ile-de-France, France, [8]The University of Chicago, Chicago, IL, [9]CNRS, UMR5308 and Université Côte d'Azur, INSERM, C3M, Lyon, Rhone-Alpes, France, [10]CHU Sainte-Justine Research Center, Department of Microbiology, Infectiology and Immunology, University of Montreal; Department of Pediatrics, University of Montreal, Montreal, PQ, Canada

NK-cells, with their intrinsic ability to recognize and kill tumor cells, represent an interesting tool for immunotherapy. Although infusions of activated NK cells are promising immunotherapy that are safe and well tolerated, the modification of NK-cells with chimeric antigen receptors (CAR) could dramatically improve their functions. The aim of this project was to develop an efficient transduction technique and produce NK cells expressing CARs. **Methods.** Freshly isolated NK (FI-NK) and NK obtained from the NK-cell Activation and Expansion System (NKAES) were transduced with lentiviral vectors pseudotyped with different envelope glycoproteins. Then, NK-cells were re-expanded using the NKAES system for 14-21 days. Viral receptors expression was evaluated by RT-PCR. The effect of CAR expression was tested with cytotoxicity-assay against a NK-resistant pre-B-ALL leukemia cell line. **Results.** VSV-G-LVs and MV-LVs resulted in poor NKAES transduction rate (16% and 14%, respectively) while RD114-LVs performed better (38%). The use of BaEV-LVs outperformed them all with a mean transduction rate of 83% in NKAES (p60%). Transgene expression was sustained for at least 21 days. The expression of both BAEV receptor (ASCT1 and ASCT2) could explain the transduction efficacy with BaEV. The transduction with a third generation anti-CD22 CAR (44%) allowed very efficient killing of pre-B-ALL cells. A dual CAR-expressing vector was also tested and could help prevent tumor evasion. **Conclusions.** This BAEV-LVs will likely be a major tool for the development of NK-based immunotherapy and for the study of NK-cell biology.

W. 93. Teaching an Old Assay New Tricks. A New Automated Cell Population Discovery Method Identifies Common Baseline Predictors of Outcome Across Cancer Immunotherapy Trials.

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A significant challenge facing computational cell population discovery tools for flow and mass cytometry is the cluster matching and annotation problem. The cluster matching problem is the challenge of identifying and labeling biologically identical clusters of cells discovered across independent samples. The annotation problem is the challenge of labeling cell populations with their phenotypes. Most existing methods tackle the former by standardizing and multiplexing data across samples prior to clustering, thereby ensuring each analyzed sample has the same number of clusters and the same cluster labels. This approach often fails in the presence of biological and technical variability and with larger data sets where methods come up against computational limitations. The latter problem usually requires significant user intervention in order to annotate discovered cell populations with their phenotypes. Here we present a new methodology entitled FAUST (Full Annotation Using Shape Constrained Trees) that tackles these problems by working in phenotypic space; standardizing and partitioning the space across samples and using the discovered phenotypes to match discovered cell populations across independent samples. Here we demonstrate how FAUST can be used for biomarker discovery across disparate data sets. We apply FAUST to perform cell population discovery across three cancer immunotherapy trials. We show that FAUST recovers expected biology by identifying PD1 expressing T cells as a correlate of outcome, but

also that it makes new discoveries, by independently identifying cell populations with monocyte & dendritic cell phenotypes across the trials that predict response to therapy at baseline.

W. 94. The Impact of Pharmacological Targeting of Glucose Metabolism with 3-Bromopyruvate on Immunogenic Cell Death in Breast Cancer Cells

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The compound, 3-bromopyruvate (3-BP), has been shown to eradicate cancer in an animal model. Whether the apoptotic fragments resulting from 3-BP treatment result in immunogenic cell death is unknown. We demonstrate that 3-bromopyruvate-induced apoptosis of mouse 4T1 breast cancer cells stimulated immature dendritic cells (DCs) of the immortal JAWS II cell line to produce pro-inflammatory cytokine IL-12 and increase expression of co-stimulatory molecules CD80 and CD86. Increased uptake of fragments from dying tumor cells correlated with increased surface levels of calreticulin and the release of high group motility box 1 (HMGB1) from cancer cells. The anti-phagocytic signal CD47 was reduced by treatment with 3-bromopyruvate. 3-BP treated breast cancer cells were able to activate dendritic cells through TLR4 signaling. Killing by 3-BP was compared to that induced by mitoxantrone and doxorubicin, among the few chemotherapeutics that induce immunogenic cell death. 3-BP killing was likewise compared to camptothecin, a compound that fails to induce immunogenic cell death. Importantly, 3-BP did not markedly decrease the levels of the key peptide presenting molecule MHC I on DCs that were co-cultivated with dying tumor cells. 3-BP treatment of the aggressive triple negative BT-20 human breast cancer cell line also resulted in immunogenic cell death with activation of human dendritic cells *in vitro*.

W. 95. The rational design of synthetic long peptides using a universal helper epitope can improve the therapeutic effects of neoantigen vaccines

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The recent FDA approval of several immunotherapeutic drugs, such as checkpoint blockade, have solidified immunotherapy as a viable means for treating several types of cancer. A major drawback to many of these approved therapies, however, is that they often induce immune responses that cross react with normal healthy cells, resulting in autoimmunity. Personalized cancer vaccines targeting tumor-specific neoantigens that arise from somatic missense mutations represent a promising modality that can mitigate this outcome, but uncertainties remain as to their most effective design. To better understand the compositional requirements of efficacious neoantigen vaccines, we investigated the mechanism of several therapeutic synthetic long peptide (SLP) vaccines targeting mouse-tumor neoantigens. This led to the identification of three generalizable principles governing the effectiveness of neoantigen-targeting SLPs: (1) neoantigen-reactive CD8+ T cells drive direct antitumor benefits; therefore, SLPs must contain a MHC I-restricted neoepitope; (2) to induce potent neoantigen-reactive CD8+ T-cell responses, SLPs must mediate CD40L interactions (i.e. T-cell “help” signal); and (3) CD40L interactions are conferred by a

SLP only when a “helper” epitope is physically linked to a MHC I-restricted neoepitope. These findings prompted us to test rationally-designed neoantigen vaccines comprised of a MHC I-restricted neoepitope linked to the universal “helper” epitope from tetanus toxin, P30. Remarkably, this vaccine design was able to unveil immune and antitumor effects to neoantigens that were otherwise poorly immunogenic. These data are encouraging because they demonstrate a clinically tractable approach with the potential to increase the therapeutic breadth of neoantigen vaccines for a variety of tumor types.

W. 96. TIGIT+TRDV1+ Expressing Tumours have a Transcriptomic Signature with Positive Prognostic Value

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γδT cells are emerging as a potent antitumor innate immune population for cell-based immunotherapy. Although they recognise and kill cancerous cells, γδ T cells also express inhibitory receptors. Unravelling the complement of inhibitory receptors on γδT cell subsets may highlight therapeutic targets complementing their potential for cell-based immunotherapy. We characterised inhibitory receptor expression on human Vδ1, Vδ2 and Vδ3 γδ T cells in cord blood (CB), adult blood (AB) and endometrial tumours by flow cytometry. CB Vδ1 cells constitutively express PD1, whereas, TIGIT expression was low in CB versus AB (n=9, $p<0.0003$) and high at end-stage differentiation in AB. The majority of TIGIT+Vδ1 cells in endometrial tumours are T_{EM}, whereas, in blood are T_{EMRA}. We hypothesised TIGIT engagement may prevent progression to end-stage differentiation. To test this, we engaged TIGIT with an agonist which significantly (n=6, $p<0.0001$) decreased the proliferative capacity of T_{EM} TIGIT+ Vδ1 cells from blood. We used the Cancer Genome Atlas RNA-Seq data of 373 endometrial cancer patients to reveal significantly ($p=0.001$) better overall survival of patients with high tumour gene expression for *TRDV1* (Vδ1) and *TIGIT*. Comparing high and low *TRDV1* and *TIGIT* expression, patient cohorts with high expression had significantly enriched immune KEGG pathways ($p<0.05$); such as NK cell mediated cytotoxicity and antigen processing and presentation. Flow cytometry revealed endometrial tumour TIGIT+Vδ1 cells maintain granzyme B levels versus blood. These results indicate TIGIT+Vδ1+ tumours could be ‘hot’, have a cytotoxic signature and good prognosis, suggesting potential benefits of combined checkpoint blockade and Vδ1 cell-based immunotherapy.

W. 97. Tumor-associated M2 macrophages may adopt mesothelial precursor cell phenotypes to initiate mesothelioma tumorigenesis in mice

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We observed accumulation of macrophages and mesothelial precursor cells (MPC) in peritoneal lavage during mesothelioma development after exposure to asbestos in Nf2 heterozygous mice, suggesting that mesothelioma-associated macrophages (MAM) and MPC are critical to promote tumorigenesis. Depletion of tumor-induced macrophages reduced sphere formation in *ip* murine mesothelioma model. We hypothesize that MAM may adopt MPC phenotypes thus to initiate mesothelioma tumorigenesis in the immunosuppressive microenvironment.

Methods: The MAM and lymphocytes in association with MPC were evaluated at different times after *ip* injection of RN5 mouse malignant mesothelioma cells. The characteristics of MAM and MPC were identified using flow cytometry and immunofluorescence. Lavage cells, spleen and blood were used to determine gene expression by RT-PCR and microarray. Tumorigenesis was assessed by *ip* mesosphere-forming assay and *sc* injection. Sorted M2-polarized macrophages were injected *sc* or *ip* into mice to evaluate tumorigenesis.

Results: MAM and MPC significantly increased in the peritoneal lavage after tumor challenge. Transcriptome analysis showed that TCR- and BCR-associated gene expressions were down-regulated. M2-specific gene was significantly up-regulated while co-stimulatory genes were down-regulated. Gene expression of pluripotency pathways was up-regulated. More strikingly, solid tumors grew in mice at 8wk after *sc* injection, and tumor spheroids were visible at 10wk after *ip* injection of sorted M2 macrophages.

Conclusion: Accumulation of MAM is correlated with MPC in tumor microenvironment, suggesting that both are indispensable for mesothelioma tumorigenesis. A subpopulation of MAM may share MPC property to initiate tumorigenesis in mouse model. This finding may provide a new mechanism explaining why tumorigenesis occurs in immunosuppressive microenvironment.

W. 99. Genetic Risk for Skin Autoimmunity Impacts the Safety and Efficacy of Immune Checkpoint Blockade in Urothelial Carcinoma

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Immune checkpoint inhibitors have made significant advances in metastatic urothelial carcinoma (mUC). Small clinical studies suggested that dermatological immune related adverse events (irAEs) are associated with efficacy. Applying a time-dependent covariate, we associated occurrence of irAEs and overall survival (OS) in patients receiving atezolizumab (anti-PD-L1) monotherapy for treatment of mUC, using data from a randomized controlled trial (IMvigor211), and a single-arm trial (IMvigor210). We collected whole genome germline sequencing data from a subset of patients from IMvigor211 (N=238

received atezolizumab; N=227 received chemotherapy). We constructed patient level polygenic risk scores (PRSs) for dermatological autoimmune diseases and associated them with irAEs, OS, and tumor gene expression, adjusting for baseline covariates and genotype eigenvectors. Individuals that experienced low grade dermatological irAEs had longer OS in IMvigor211 (p=0.024; HR 0.66; 95% CI 0.45-0.95) and IMvigor210 (p=0.0023; HR 0.53; 95% CI 0.35-0.80). Polygenic risk for psoriasis was associated with increased odds of skin irAEs (p=0.002; OR 1.79; 95% CI 1.24-2.40). High vitiligo (p=0.0016; HR 0.58; 95% CI 0.41-0.81), high psoriasis ($p=5.5´10^{-5}$; HR 0.50; 95% CI 0.36-0.70), and low atopic dermatitis (p=0.0008; HR 0.57; 95% CI 0.41-0.79) polygenic risk were predictive of longer OS under anti-PD-L1 monotherapy compared to chemotherapy. The predictive capacity of a psoriasis PRS was associated with tumor gene expression signatures of $CD8^+$T-effector and $CD4^+$Th17 function. Polygenic risk for skin autoimmunity is associated with efficacy of atezolizumab in mUC and supports the role of Th17 driven anti-tumor immunity during PD-1 checkpoint blockade.

Inflammatory Bowel Diseases

H. 111. Pharmacological inhibition of RORγt preserves human Th17-like regulatory T cell stability

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Inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis, are incurable chronic conditions that result from uncontrolled gut inflammation. Pathogenic Th17 cells, characterised by production of IL-17 in the absence of IL-10, are thought to contribute to this inflammation, but in humans, antibody-mediated blockade of IL-17 is an ineffective IBD therapy. We hypothesized that anti-IL-17 antibodies may not completely disable Th17 cells, and moreover could have deleterious effects on Th17-like $FOXP3^+$ regulatory T cells (Th17-like Tregs). We investigated whether pharmacological inhibition of RORγt, the Th17 cell lineage-defining transcription factor, was an alternate approach to inhibit Th17 cells. Addition of BMS-336, a small molecule RORγt inhibitor, to human peripheral Th17 ($CXCR3^-$ $CCR4^+CCR6^+$) and Th17.1 ($CXCR3^+CCR4^+CCR6^+$) cells inhibited expression of RORγt target genes in a dose-dependent manner. Similarly, IL-17 production by laminar propria mononuclear cells, isolated from IBD and non-IBD subjects, was significantly inhibited by BMS-336. BMS-336 also inhibited expression of RORγt-regulated genes in Th17-like Tregs ($CD4^+CD25^{hi}CD127^{lo}CXCR3^-CCR4^+CCR6^+$) without affecting expression of FOXP3 or their suppressive function. Interestingly, RORγt inhibition significantly increased production of IL-10 in Th17-like Tregs. When cultured under proinflammatory conditions, Th17-like Tregs were destabilised, as evidenced by loss of FOXP3 expression and re-methylation of the Treg specific demethylation region; this destabilisation was repressed by BMS-336. Overall, these results demonstrate that inhibition of RORγt is a promising approach to selectively inhibit Th17 cells, and in parallel enhance the function Th17-like Tregs by increasing IL-10 production and restraining their lineage instability in the presence of inflammation.

H. 112. Metabolic targeting of microbiota-reactive CD4 T memory cells as an immunotherapy for inflammatory bowel disease

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Microbiota-reactive CD4 T memory (T_M) cells are generated during intestinal infections and inflammation, and can potentially serve as a reservoir for pathogenic CD4 T effector (T_E) cells, thus driving the progression of inflammatory bowel diseases (IBD). Unlike T_E cells, T_M cells keep a low rate of metabolism unless they are activated by re-encountering cognate antigens. Here we show that microbiota flagellin-specific CD4 T cell activation plus simultaneous metabolic inhibition via mTORC and AMPK resulted in CD4 naïve and memory T cell death and anergy, but greatly enhanced the induction of CD4 regulatory T (Treg) cells with strong suppressive function. This metabolic inhibition treatment successfully prevented colitis development in the CBir1 TCR Tg CD4 T cell transfer model. CBir1 flagellin-specific CD4 T cells, especially the pathogenic T_E subsets, were decreased 10 fold in the intestinal lamina propria. Furthermore, using this metabolic inhibition strategy, we were able to prevent microbiota flagellin-specific T_M cell formation upon initial antigen encounter, and ablate pre-existing T_M cells upon re-activation in mice. In both instances Treg cells were significantly elevated. Human microbiota flagellin-specific CD4 T cells isolated from patients with Crohn's disease, stimulated with flagellin antigens plus metabolic inhibitor rapamycin, were ablated in a similar manner with half of the antigen-specific T cells undergoing apoptosis. These results indicate that metabolic inhibition of activated microbiota-specific CD4 T cells is an effective way to eliminate pathogenic CD4 T_M cells and to induce Treg cells that provide antigen-specific and bystander suppression, serving as a promising immunotherapy for IBD.

H. 113. Analysis of APL expression in an animal model of chronic colitis

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Background & Aim: Apelin (APL) has been reported to be upregulated in the colon during acute colitis induced by dextran sodium sulfate (DSS), but the mechanism and function remain unclear. Here, we analyzed APL expression in another colitis model but with chronic inflammation.
Methods & Results: Colonic epithelia and lamina propria lymphocytes were isolated from wild type C57BL6 mice (WT) to assess APL expression. Semi-quantitative PCR (qPCR) revealed higher $CD4^+$ T cell expression of APL compared to other cell types including colonic epithelium. Next, naïve T cells isolated from WT were adoptively transferred into Rag deficient mice ($Rag^{-/-}$) to induce chronic colitis. Interestingly, qPCR showed downregulation of APL in the colon of the $Rag^{-/-}$ induced colitis compared to those without colitis, which is different from previous report with DSS colitis. Therefore, colonic and splenic $CD4^+$ T cells were isolated from WT and T cell-reconstituted $Rag^{-/-}$. Subsequently, qPCR showed decreased APL expression in T cells from $Rag^{-/-}$ induced colitis compared to that of WT. WT naïve T cells were differentiated into either Th1, Th2 or Th17 in vitro. APL expressions in these differentiated T cells were significantly downregulated compared to that of non-differentiated control. Given these results, Rag^-

$^{/-}$ receiving naïve T cells were administered synthetic APL peptide to rescue the APL downregulation. This resulted in reduced severity of colitis compared to that of control.
Conclusion: APL downregulation in the effecter T cells may result in the development of chronic colitis, and APL may be a therapeutic target for inflammatory bowel diseases.

H. 114. c-Maf-dependent Treg cell control of intestinal TH17 cells and IgA establishes host-microbiota homeostasis

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Foxp3^{+}T regulatory (T_{reg}) cells are crucial to maintain immune homeostasis both in lymphoid and non-lymphoid tissues. Here we demonstrate that the ability of intestinal T_{reg}cells to constrain microbiota-dependent interleukin 17–producing T helper cell (T_H17) and immunoglobulin A (IgA) responses critically required the expression of the transcription factor c-Maf. The terminal differentiation and function of several intestinal T_{reg}cell populations, including RORgt^{+}T_{reg}cells and T follicular regulatory cells, was c-Maf-dependent. c-Maf controlled T_{reg}cell-derived interleukin 10 (IL-10) production and prevented excessive phosphatidylinositol-3-OH kinase (PI(3)K)–kinase Akt–mechanistic target of rapamycin (mTORC1) signaling and inflammatory cytokine expression in intestinal T_{reg}cells. c-Maf-deficiency in T_{reg}cells led to a profound dysbiosis of the intestinal microbiota, which when transferred to germ-free mice, was sufficient to induce exacerbated intestinal T_H17 responses, even in a c-Maf-competent environment. Thus, c-Maf acts to preserve the identity and function of intestinal T_{reg}cells, which is essential for the establishment of host-microbial symbiosis.

H. 115. Combined Analysis of Flagellin-Specific Immune Responses and Microbiome Diversity in IBD Patients

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Inflammatory bowel disease (IBD) is a chronic intestinal inflammation of the gut, consisting of two closely related diseases, Crohn's Disease (CD) and Ulcerative Colitis (UC). Both diseases are partly driven by pathogenic T-cell responses to components of commensal bacteria, with evidence that flagellin is a key antigen driving IBD pathogenesis. Chronic inflammation in IBD leads to microbial imbalance in the gut, resulting in a loss of key beneficial bacteria, further perpetuating the disease. Using stool and peripheral blood samples from IBD patients and healthy controls, we investigated the relationship between the gut microbiome composition and adaptive immune responses to *Lachnospiraceae*-derived A4-Fla2 and *E.*

coli H18 FliC flagellin antigens. We used a flow cytometry-based assay to detect circulating flagellin-specific $CD4^+$ T-cells following antigen-stimulated upregulation of CD25 and OX40, and detected anti-flagellin IgG and IgA by ELISA. We observed that, compared to healthy controls, IBD patients had enriched proportions of Fla2-specific $CD4^+$ T-cells and anti-Fla2 antibodies. Microbiome analysis utilized 16s rDNA sequencing, with IBD patients and healthy controls clustering significantly separately by β-diversity analysis. As expected, we observed a reduced Shannon's diversity in IBD patients, with lower diversity correlating with greater disease severity and higher proportions of circulating flagellin-specific $CD4^+$ T-cells. Differential abundance analyses confirmed enrichment of previously reported bacterial species in IBD patients. Importantly, we show that the relative abundance of these enriched bacteria positively correlated with immune responses to flagellin antigen. These data are the first report of relationships between gut bacteria and flagellin-specific immune responses in IBD patients.

H. 116. CRISPR/Cas9-based platforms for therapeutic target identification and validation

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The immune system protects the body against invaders that cause infection and disease. On the other hand, disorders of the immune system can lead to autoimmunity, inflammatory disease and cancer. The clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) protein 9 system (known as CRISPR/Cas9) is a powerful new technology with a wide range of applications in biomedical research, including the potential to treat human genetic disease. To better understand the immune system and discover novel therapeutics for autoimmune diseases, we have built CRISPR/Cas9-based platforms for new target identification, target exploration and validation, and evaluation of the efficacy and toxicity for early targets. In this work, we will describe the methodologies we have established for delivering the CRISPR/Cas9 system to the cell types of interest, and the in vitro and in vivo model systems we have built using CRISPR/Cas9 to study gene function. With CRISPR/Cas9-based platforms, we aim to build a mechanism to continuously screen new targets from various data sources and to explore novel biology involved in the immunological diseases.

H. 117. Evaluation of Different Lactobacillus Strains on the Treatment of Inflammatory Bowel Disease in a Murine Model

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Ulcerative colitis (UC) is characterized by chronic digestive track inflammation and immune system malfunction. We investigated the efficacy of different strains of bacteria in an *in vivo* model of DSS colitis. Stool and body weight were evaluated and a total disease activity index (DAI) was calculated. Colons were collected for histo-pathological evaluation. Bacterial dosing, individually or as a combination of the

three strains, was performed daily at $1.5x10^{10}$cfu/ml.The same strains were also tested, individually, for their ability to competitively inhibit adhesion and invasion of pathogenic E. coli strains (AIEC and HLMN-1) in a model of gut epithelium (co-culturing Caco-2 and HT29-MTX cells).

When administered separately, lower DAI score was noted for one of the three strains compared to DSS controls during the early phase of the treatment period. No meaningful differences were noted in the length and weight of colon. When administered as a mixture, significantly lower DAI score were noted on Day 5 and lasted until the end of the treatment period. Slight improvements in the length of colon were noted for treated animals compared to DSS controls. Cell culture indicates that all strains competitively inhibited adhesion, invasion and/or translocation of the two pathogenic strains.

Our data clearly indicate treatment of mice with our combination of strains by oral gavage significantly prevents development of DSS-induced colitis in mice with no significant safety concern. These bacterial strains present an alternative approach in treatment of colitis in clinic.

H. 118. IL-12 and mucosal CD14+ monocyte-like cells induce IL-8 in colonic memory CD4+ T cells of patients with Ulcerative colitis but not Crohn's disease

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Background and Aims. $CD14^+$ mononuclear phagocytes (MNPs), neutrophils and T cells infiltrate colon in ulcerative colitis (UC). We here investigated how $CD14^+$MNPs and cytokine they produce, shape colonic effector T cell profile.

Methods. Colonic or mesenteric lymph node (mLNs) $CD4^+$T cells isolated from UC or Crohn's disease (CD) were stimulated with cytokines or autologous $CD14^+$ MNPs. Cytokine expression was assessed by intracytoplasmic staining and multiplex ELISA. Unsupervised phenotypic multicolor analysis of colonic $CD14^+$ MNPs was performed using FlowSOM algorithm.

Results. Among $CD14^+CD64^+$HLA-$DR^+SIRP\alpha^+$MNPs, only the pro-inflammatory cytokine-producing $CD163^-$ subpopulation accumulated in inflamed UC colon and promoted mucosal IL-1β-dependent Th17, Th17/Th1, Th17/Th22 but not Th1 responses. Unsupervised phenotypic analysis of $CD14^+CD64^+$MNPs segregated $CD163^-$ monocyte-like cells and $CD163^+$ macrophages. Unexpectedly, IL-12, IL-1β and $CD163^-$, but not $CD163^+$,cells induced the neutrophil-attracting chemokine IL-8 in colonic $CD4^+$T cells, which co-expressed IFN-γ and/or IL-17 in UC and not CD. The $CD163^-$ monocyte-like cells increased the frequency of $IL\text{-}8^+IL\text{-}17^{+/-}IFN\text{-}\gamma^{+/-}$ T cells through IL-1β and IL-12. Finally, mucosal $IL\text{-}8^+$ T cells co-expressed GM-CSF, TNF-a and IL-6 *ex vivo*, which was promoted by IL-12 in the mucosa and mLNs.

Conclusions. Our findings established a link between monocyte-like CD163^{-}MNPs, IL-12, IL-1β and the detection of colonic memory IL-8-producingCD4^{+}T cells, which might all contribute to UC pathogenesis.

H. 119. Modelling Inflammatory Bowel Disease using a Translational Assay Platform

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Ulcerative Colitis (UC) and Crohn's disease (CD) are the two main forms of inflammatory bowel disease (IBD) and are likely to result from distinct mechanisms. Interplay between the gut microbiota and the immune system in genetically susceptible individuals plays an essential role in disease development and both Th1 and Th17 T cell responses have been shown to play an integral part in driving pathology. First-line treatments for IBD focus on anti-inflammatories and there remains an unmet need to target pathological immune responses, both innate and adaptive, in a specific manner. We use a DSS-colitis inflammatory model to test novel therapeutics and examine their effects on the frequency and phenotype of both infiltrating leukocytes and circulating T cells, and to characterise the composition of intraepithelial lymphocytes (IEL) and gut lamina propria (LP) immune subsets. The relative proportions of Th17, Th1 and Treg were determined for each tissue. To complement this, we have developed a range of human primary T cell *in vitro* assays. Naïve CD4+ T cells from healthy donor blood samples were differentiated into helper T cell subsets or iTreg under polarising conditions. The ability of novel compounds to inhibit Th1/17 differentiation or to drive iTreg generation was then determined. Treg suppression assays were also performed. These models provide a compound screening platform which delivers synergistic information on efficacy and mode of action whilst also enabling biomarker discovery.

H. 120. Molecular Characterization of Colonic MAIT Cells in Crohn's Disease

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Mucosal-associated invariant T (MAIT) cells play a central role in mucosal antimicrobial immunity by recognizing a finite variety of B vitamin precursors produced by bacteria common to the gut microbiome. Dysregulation of mucosal antimicrobial immunity is believed to be central to Crohn's disease pathogenesis, however, little is known about intestinal MAIT cells in this condition. We used flow cytometry to analyze MAIT cells from the blood or colon biopsies of Crohn's disease patients and healthy controls for mRNA transcriptome profiling and T cell receptor (TCR) sequencing. By flow cytometry, colonic MAIT cells in Crohn's disease were not significantly different from healthy controls, regardless of inflammation, but did express less NKG2D, which contradicts what has been described in blood. At the mRNA level, MAIT cells overexpressed several immune-related genes relative to conventional CD8+ T cells. The most significant was the IL-23 receptor, which has been implicated in Crohn's disease pathogenesis by genetic studies and clinical trials. MAIT cells also expressed more TNFRSF25 than conventional CD8+ T cells, where genetic polymorphisms of its ligand TNFSF15 have been associated

with Crohn's disease, particularly in Asia. Paired TCR sequencing was performed on single cell sorted MAIT cells from colon biopsies. In cells with a canonical MAIT TCR alpha chain rearrangement TRAV1-2/TRAJ12/20/33, we observed more TCR beta chain diversity in healthy controls than Crohn's disease, regardless of mucosal inflammation. Additionally, we found considerable heterogeneity in the clonality of MAIT cells among all patients despite having limited antigens which are presented by the non-polymorphic MR1 molecule.

H. 121. Potential contribution of NK cells to the Immunopathogenesis of Crohn disease

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Natural Killer (NK) cells play an important role in protecting the host by killing cancer and virus-infected cells. However, they also have the potential to kill body's own 'altered' cells and contribute towards tissue destruction. Crohn disease is a chronic inflammatory disease affecting gastrointestinal tract especially terminal part of ileum and colon in children and adolescents. In order to investigate whether NK cells may play a role in the immunopathogenesis of this disease, we compared the expression of KIR and non-KIR receptors, activation status and cytotoxic activity of peripheral blood NK cells between Crohn disease patients and age-matched healthy control subjects. The investigations were performed ex vivo directly on whole blood taken from the patients as well as from the control subjects. Our results show that NK cells from CD patients expressed higher levels of activating KIR as well as other non-KIR activating receptors vis-à-vis healthy control donors.. The expression of inhibitory KIR and other non-KIR receptors tended to decrease compared to healthy donors. The NK cells from the patients expressed higher levels of CD69, NKG2D and IL-23R. NK cells from the patients also expressed increased levels of different gut-homing integrin molecules. They also had higher expression of CD107a on their surface. The expression was higher constitutively as well as in response to incubation with NK-sensitive K562 cells. Furthermore, the NK cells from the patients showed higher cytotoxic activity against NK-sensitive target cells. Therefore, they are very likely to play a role in the immunopathogenesis of the disease.

H. 122. Smad7 controls immunoregulatory PDL2/1-PD1 signaling in intestinal inflammation

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Smad7, a negative regulator of TGF-β signaling, has been implicated in the pathogenesis and treatment of inflammatory bowel diseases (IBDs), such as Crohn's disease (CD) and ulcerative colitis (UC). However, the molecular mechanisms underlying Smad7 pathogenic functions in IBDs remain poorly

understood. Here, we uncovered a novel role for Smad7 in mediating intestinal inflammation by limiting the PDL2/1-PD1 axis in dendritic cells (DCs) and $CD4^+$ T cells. Smad7 deficiency in DCs promotes TGF-β responsiveness and expression of the co-inhibitory molecules PDL2/1 on DCs, and further imprints T cell-PD1 signaling to promote Treg differentiation. DC-specific Smad7 deletion mitigates DSS-induced colitis in mice and is mediated by increased $CD103^+$ $PDL2/1^+$ DCs and Tregs. Additionally, Smad7 deficiency in $CD4^+$ T cells promotes PD1 expression and PD1-mediated Treg differentiation *in vitro*. Adoptive transfer of Smad7-deficient $CD4^+$ T cells enhances Treg development *in vivo* and confers striking protection against a T cell-mediated colitis model. Furthermore, antisense Smad7 oligonucleotide treatment ameliorates DSS-induced UC and increases TGF-β and PDL2/1-PD1 signaling. Together,our results identify previously unknown mechanisms by which Smad7 mediates intestinal inflammation and leverages these pathways therapeutically, providing novel strategies for IBD intervention.

Innate Immunity

H. 123. Neutrophil maturation and their response to infectious pathogens are regulated by microbiota

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It has long been considered that a neutrophil's response to various infectious challenges is innately pre-determined. Here, we provide data that demonstrates that neutrophil proteomes are modulated by the microbiota. We found that the proteomic signatures of mature neutrophils derived from germ free (GF) and specific pathogen free (SPF) mice were significantly different. In the absence of microbiota, mature neutrophils lacked GM-CSF-driven priming.

GF-serum exposed neutrophil progenitors did not mature efficiently and had compromised bactericidal properties when compared to progenitors matured in SPF-derived serum. To identify molecular pathways, we set-up an *in vitro* system where neutrophil progenitors were transduced with lenti-guides to knock-down key microbiota-driven gene targets. To identify which of the microbiota-regulated proteins directly impacted bactericidal functions of neutrophils, we knocked out 19 candidates and tested their killing of *P. aeruginosa*. Excitingly, one of the targets demonstrated a superior inhibition of neutrophil bactericidal capacities. This protein had no previously identified function. Namely, the knock down of prenylcysteine oxidase-like 1 (pcyox 1l) protein reduced killing of *P. aeruginosa in vitro* due to diminished ROS release. It is likely that pcyox-1l generates ROS, independent of the NADPH oxidase. Hence, we identified a novel mechanism for microbiota-driven control of innate immunity.

Cumulatively, our data support the concept that microbiota affects neutrophil maturation by defining not only the quantity, but also the quality of mature neutrophils. We predict that neutrophil responses can be specifically tailored to pathogens. In conclusion, neutrophil responses, although innately determined, are adapted and molded by the commensal presence.

H. 124. Association of RIG-I and TLR4 Pathway Suppression with Increased Disease Severity in Critically Ill Children with Influenza Pneumonia

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Host immune response influences development of life-threatening influenza infection in healthy individuals. We previously reported that in children admitted to the intensive care unit (ICU) with influenza, early Toll-like receptor 4 (TLR4) pathway suppression with *ex vivo* lipopolysaccharide (LPS) stimulation (low tumor necrosis factor (TNF)α production), predicted higher mortality. In an independent cohort of 105 influenza infected children enrolled across 24 PICFLU Study ICUs, we evaluated immunosuppression of the viral-specific retinoic acid-inducible gene-I (RIG-I) pathway using polyinosinic:polycytidylic acid (poly(I:C)-LMW/LyoVec) and the TLR4 pathway. In blood collected ≤72 hours of ICU admission, interferon (IFN)α and TNFα were measured in unstimulated controls and after 24-hour poly(I:C) and 4-hour LPS stimulation, respectively. Poly(I:C) stimulation resulted in three response types. "Suppressed" patients had low control IFNα levels which remained low after stimulation, whereas "Peaked" patients produced high control IFNα with no increase after stimulation. "Responders" had at least double the amount of IFNα after stimulation over their control sample. RIG-I Suppressed children had higher organ dysfunction and fewer days alive and off mechanical ventilation. TLR4 suppression (TNFα≤200pg/ml) was again associated with worse outcomes and 60% were also RIG-I suppressed. The 33 patients with early suppression in *both* RIG-I and TLR4 pathways (compared to 70 others) had higher proinflammatory serum cytokines (IL-8, TNFα), more prolonged (≥7 days) multiple organ dysfunction (30.3% vs 8.6%, p=0.004), and prolonged hypoxic respiratory failure (39.4% vs 11.4%, p=0.001). Identification of RIG-I/TLR4 pathway suppression during severe influenza infection may provide an opportunity to modulate the immune response and improve outcomes.

H. 125. Identification and Characterization of Distinct Microglial Subsets in Aging

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Currently few methods exist for identifying subsets of microglia. Microglia are a functionally heterogeneous population in which subsets may be differentially impacted by aging. Using a novel parameter (SB), we sought to characterize two unique subsets of murine microglia: SB-positive and SB-negative. Throughout life, these two subsets were maintained at roughly 2:1 ratio (SB-positive:SB-negative), though the level of SB increased with age only in SB-positive microglia. Correlating with the observed increase of SB, SB-positive microglia expressed higher levels of the lysosomal and phagocytic markers Lamp1 and CD68. Electron microscopy of flow-sorted subsets revealed differences in the organization of subcellular compartments, the magnitude of which was age-dependent. Differences in levels of SB indicated unique functions between the two subsets. In neonatal brain-slice cultures, SB-positive microglia exhibited increased phagocytosis compared to SB-negative microglia. Genetic

mutations disrupting lysosomal or cholesterol transport pathways increased SB only in the SB-positive population and altered the subset ratios. Finally, in aged mice the observed number and ratio of SB-positive microglia decreased significantly compared to the SB-negative population, indicating differences in survival or homeostatic proliferation. Correlating with SB levels, the microglia subsets displayed age-dependent differences in cellular ROS, providing a mechanism whereby aging contributes to microglia dysfunction. SB-positive and SB-negative subsets represent distinct populations of microglia marked by subcellular and functional differences and the increased level of SB in SB-positive microglia negatively impacts their function and survival in aging.

H. 126. Intracellular Calcium Signals and Chemotaxis are Altered in Human Neutrophils with Aging

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Elderly susceptibility to infectious diseases results from a decline in the functions of neutrophils that are regulated by the intracellular calcium concentration $[Ca^{+2}]_i$ and store-operated Ca^{+2} entry (SOCE). In this study, we analyzed the effects of aging on Ca^{+2} mobilization towards fMLP, IL-8, C5a, LPS, and thapsigargin (TG) as well as on chemotaxis of neutrophils. To examine Ca^{+2} mobilization, neutrophils from healthy elderly and young adults were loaded with 1 µM Fluo-4-AM. Baseline $[Ca^{+2}]_i$ level was recorded for 60 seconds before the addition of 100 nM or 1µM fMLP, 300nM IL-8, 10nM C5a, 25µg/ml LPS or 2 µM TG, responses were recorded 10 min. Chemotaxis towards 10 nM fMLP, 1 nM and 10 nM C5a or IL-8 was measured by transwell migration assay for 2 h at 37 C in 5% CO_2. Both assays were analyzed by flow cytometry. We found lower baseline $[Ca^{+2}]_i$ in neutrophils from elderly. C5a, IL-8 and fMLP induced a transient peak of Ca^{+2}followed by a second minor Ca^{+2} wave. However, in neutrophils from elderly, the second Ca^{+2} wave was lower with C5a, and higher with IL-8 and fMLP. We did not find significant difference in Ca^{+2} responses to LPS and TG (SOCE), as well as in the expression of stimulus receptors (CD88, CXCR2, FPLR1 and TRL4). Finally, we found that neutrophils from elderly migrated less towards fMLP, IL-8 and C5a. Our data suggest that the mechanisms that underlie the decrease on neutrophil functions with aging might be alterations in Ca^{+2} signals.

W. 100. Context-Specific Regulation of Monocyte Surface and Soluble IL7R Expression by a Genetic Risk Allele

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Background: Interleukin 7 (IL-7) plays a key role in T cell biology and its effects are modulated by the pro-inflammatory soluble form of the receptor (IL7R). Polymorphisms of IL7R are associated with multiple inflammatory diseases including multiple sclerosis and ankylosing spondylitis and the disease-associated variant leads to increased circulating soluble IL7R (sIL7R). IL7R mRNA is induced in stimulated

monocytes in a genetically determined manner, yet a role for IL7R in monocyte biology remains unexplored.

Methods: Monocyte surface IL7R protein was measured by flow cytometry after LPS stimulation in a cohort of genotyped volunteers(n=84). sIL7R was quantified by ELISA in purified monocyte cultures stimulated with LPS from separate cohort (n=161) of genotyped donors. Bulk and single-cell RNA sequencing was performed on in-vitro stimulated monocytes and synovial monocytes of patients with spondyloarthritis.

Results: Monocyte surface and soluble IL7R protein are markedly expressed in response to LPS and stimulated monocytes are the main cellular source of sIL7R. Alleles of rs6897932 are the key determinant of both surface IL7R and sIL7R in stimulated monocytes. Stimulated monocytes were sensitive to exogenous IL-7, which elicits a defined transcriptional signature. Single-cell RNA sequencing of synovial fluid monocytes from patients with spondyloarthritis showed an distinct subset of IL7R+ monocytes with a unique transcriptional profile that markedly overlapped the in-vitro IL-7 induced geneset.

Conclusions: These data demonstrate disease-associated genetic variants at IL7R specifically impact monocyte surface IL7R and sIL7R following innate immune stimulation, suggesting a previously unappreciated key role for monocytes in IL-7 pathway biology and IL7R-associated diseases.

W. 101. Leukotrienes and resolvins induced by HMGB1 and HMGB1 plus C1q reciprocally regulate IRF5

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Macrophage polarization is critical to inflammation and resolution of inflammation. We previously showed that HMGB1 can engage RAGE to direct monocytes to a pro-inflammatory phenotype characterized by production of type 1 interferon and pro-inflammatory cytokines. In contrast, HMGB1 plus C1q form a tetra-molecular complex cross-linking RAGE and LAIR-1 and directing monocytes to an anti-inflammatory phenotype. Lipid mediators, as well as cytokines, help establish a milieu favoring either inflammation or resolution of inflammation. This study focuses on the induction of lipid mediators by HMGB1 and HMGB1 plus C1q and their regulation of IRF5, a transcription factor critical for the induction of pro-inflammatory macrophages. Here, we show that HMGB1 induces leukotriene production through a RAGE-dependent pathway, while HMGB1 plus C1q induce specialized pro-resolving lipid mediators through a RAGE and LAIR-1 dependent pathway. Leukotriene exposure contributes to induction of IRF5 in a positive feedback loop. Resolvins, in contrast, block IRF5 induction and prevent the differentiation of inflammatory macrophages. Finally, we have generated a molecular mimic of HMGB1 plus C1q which crosslinks RAGE and LAIR-1 and can polarize monocytes to an anti-inflammatory phenotype. These findings may provide a mechanism to control non-resolving inflammation in many pathologic conditions.

W. 102. Myo-/Fibroblast MyD88-Mediated Signaling Regulates Inflammatory Responses in the Colon by Suppressing Influx of Inflammatory Macrophages

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MyD88-dependent signaling in myo-/fibroblasts (MFs) is involved in epithelial barrier restoration and tolerance. However, the role of MyD88-dependent signaling by MFs in the regulation of inflammatory responses by macrophages in the colonic mucosa is poorly understood. Because colonic MFs respond to MyD88 activation with production of molecules involved in the regulation of macrophages (PGE_2, PD-L1, etc.), we hypothesize that MF mediated MyD88 signaling regulates inflammatory responses from macrophages in the colon. Tamoxifen inducible Col1α2Cre Myd88 floxed mice (fibroblast and myofibroblast-specific MyD88 deletion) and α-SMACre MyD88 floxed mice (myo-fibroblast and smooth muscle cell-specific MyD88 deletion) on the same genetic background (C57BL/6) were used in this study. We observed that deletion of MyD88 within MFs resulted in the inflammatory changes and infiltration of lymphocytes within colonic mucosa and moderately aggravated DSS induced acute colitis. Activation of the lymphocyte trafficking and type 1 inflammatory pathways were observed (RNAseq data) in DSS-treated and non-treated mice lacking MyD88 within MFs. This was associated within increased infiltration of $F4/80^+$ $CD11b^+$ macrophages, but not $CD11c^+$ dendritic cells. $CX3CR1^{high}$ $CCR2^+$cells producing TNF-α were predominant within the macrophage population suggesting increased chemotaxis of inflammatory macrophages to the colonic mucosa. Further, overall increased TNF-α production was observed in the mice lacking MyD88 within MFs and depletion of the macrophages resulted in a decrease in TNF-α levels within the colonic mucosa. Therefore, our data suggest that MF- mediated MyD88 signaling contributes to the maintenance of colonic mucosal homeostasis through suppression of the influx of TNF-α producing inflammatory macrophages.

W. 103. Physical Activity at Lower Intensities Produces a Tolerogenic Effect and Reduces Localized IL-1β in a Murine Model of Gout in Circulating Neutrophils

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Gout is a type of inflammatory arthritis characterized by monosodium urate (MSU) crystal-induced inflammation in joints and surrounding tissues. Recent research suggests that regular exercise has anti-inflammatory effects which may help reduce inflammation in chronic inflammatory diseases. Using NF-κB-luc reporter mice, we investigated the effects of exercise intensity on inflammation in an acute model of

MSU-induced gout. Mice were exercised daily at low, medium, or high-intensities on a treadmill for 2 weeks before receiving intra-articular MSU injections. Assessment of NF-κB activity via *in vitro* imaging system (IVIS) revealed significant reductions in NF-κB activity in mice exercised at low and moderate-intensities compared to the high-intensity group or non-exercised control. Immunohistochemistry (IHC) of the macrophage marker F4/80, the granulocyte marker myeloperoxidase (MPO), and the inflammatory cytokine IL-1β similarly revealed significantly reduced macrophages/granulocytes and IL-1β at the site of MSU injection in the low and moderate-intensity groups. Toll-like receptor 2 (TLR2), an important mediator of the MSU response, was significantly decreased on peripheral neutrophils from the low and moderate-intensity groups via flow cytometry. The neutrophil chemokine CXCL1 was also significantly decreased in the exercised groups. An examination of IL-1β production in peripheral monocytes and neutrophils isolated from exercised mice and stimulated by MSU/LPS *in vitro* revealed increased intracellular levels of pro-IL-1β and significantly decreased IL-1β in the supernatant of neutrophils. Cumulatively, these results suggest that low to moderate-intensity exercise produces a tolerogenic effect which makes immune cells less responsive to inflammatory stimuli and may partially explain the underlying anti-inflammatory mechanisms of physical activity.

W. 104. Profiling of 1,600 novel natural product-based compounds in human primary cell-based phenotypic assays

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Cells of the immune system play a key role in the resolution of tissue injuries, inflammation and infections and in regulation of tissue homeostasis. Here we aimed to profile natural product-inspired drug discovery platform, developed by Janssen Incubator, using natural product scaffold-based chemical entities, to aid in identifying new chemical starting points with a disease-relevant mechanisms of action. We used 13 human primary cell-based assays, amongst which several assays target the immune system, to screen approximately 1,600 compounds.

Inflammatory assays utilized for screening include neutrophil migration, activation of B-cells, T-cells, macrophages, dendritic cells (DC) and synovial fibroblasts. Immune cells were isolated from healthy donor buffy coats and synovial fibroblasts were isolated from rheumatoid arthritis patients. Neutrophils were subjected to IL-8-mediated migration in a transwell system. Isolated B-cells were stimulated with CD40L and IL-4 to secrete IL-6. PBMCs were stimulated with anti-CD3/CD28 antibodies to induce IL-2 secretion by T-cells. For macrophage activation, PBMCs were stimulated with LPS to induce TNF-α secretion. Monocytes-derived DCs were stimulated with CD40L+enhancer to produce IL12p40. Synovial fibroblasts were stimulated with platelet-derived micro-particles to induce IL-6 secretion.

Assays were successfully validated using reference compounds and subsequently subjected to screening of the 1,600 compounds resulting in multiple new starting points for a hit-to-lead program. These screens also highlighted which assays could help identify unique, disease-relevant mechanisms of action.

W. 105. Recombinant Fragment of Human Surfactant Protein D (rfhSP-D) Interacts with GRP78: Potential Role in SP-D Induced Anti- Prostate Cancer Activity

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SP-D an innate immune molecule has indispensable role in host defense and regulation of inflammation. We reported a novel anti-cancer role of SP-D in various cancer cell lines. In accordance with our previous finding, rfhSP-D induced intrinsic mitochondrial apoptosis in LNCaP (androgen-dependent) cells, PC3 (androgen-independent) cells, human prostate tissue biopsies and primary cancer cells (PrCEC) isolated from tissue biopsies of metastatic prostate cancer patients, in time and dose-dependent manner. Importantly, viability of primary normal prostate epithelial cells (PrEC) was not affected. In the present study, we observed significantly higher calcium dependent binding of rfhSP-D with the PC3 cells than LNCaP and PrEC cells suggesting the differential expression of interacting proteome. LC-MS/MS analysis of rfhSP-D-PC3 interactome resulted in identification of 672 proteins. Further screening of these proteins based on the abundance (proteins with high Peptide Spectrum Match) and molecular function resulted a list of 25 potential interacting partners. PANTHER analysis suggested that these proteins are part of Apoptosis signaling pathway, Glycolysis, Gonadotropin- releasing hormone receptor pathway and Integrin signaling pathway. We selected GRP78, a chaperone, expressed only on cell surface of cancer cells, and involved in apoptosis signaling pathway for further analysis. The structures of active rfhSP-D (PDB ID: 1PW9) in the trimeric form and GRP78 (PDB ID: 5EVZ) were docked using PatchDock and ZDock server with default parameters. Thus, the proteomics and *insilico* approach confirmed interaction of membrane GRP78 protein with carbohydrate recognition domain of rfhSP-D on prostate cancer cells, and may be involved in anti-prostate cancer role of SP-D.

W. 106. Role of XCR1+ DC in cross-presentation of antigen in XCR1-RYDL mice

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Cross-presenting dendritic cells (DC) play a critical role in regulating immunity and tolerance. A subset of DC expressing the chemokine receptor XCR1 have superior antigen cross-presentation ability and are crucial for eliciting $CD8^+$ T cell responses. Understanding the importance of cross-priming for generating $CD8^+$ T cell response against tumor-derived, viral, or self-antigens will help elucidate therapeutic targets for intervention. To understand the role $XCR1^+$DC play in cross-presentation, we used genetically modified mice to selectively target $XCR1^+$DC subset. We generated XCR1-RYDL (Rosa-YFP-DTR-Luciferase) mice, which express luciferase for detection of $XCR1^+$ cells and Diphtheria Toxin Receptor (DTR) for deletion of $XCR1^+$ cells to deplete $XCR1^+$DC *in vivo*. Injection of Diptheria Toxin (DT) resulted

in deletion of XCR1$^+$DC in XCR1-RYDL mice. DT treatment resulted in up to 80% reduction of XCR1$^+$DC subset in spleen, thymus, and mesenteric lymph node (MLN), corresponding with no change in conventional T cell or Foxp3$^+$ regulatory T cell pool *in vivo*. Furthermore, DT-treated mice were defective in generating antigen-specific CD8$^+$ T cell response, exhibiting ~3-fold reduction in ovalbumin-specific CD8$^+$ T cells in response to immunization with ovalbumin and a Toll-like receptor 3 (TLR3) agonist. Most notably, deletion of XCR1$^+$DC prior to tumor implantation resulted in up to 3-fold increase in tumor burden in DT-treated mice. Thus, XCR1$^+$DC are required for CD8$^+$ T cell activation and play a role in generating anti-tumor responses. These findings suggest that XCR1-RYDL mouse model offers a unique ability to study the requirement and function of XCR1$^+$DC in initiation and regulation of anti-tumor immunity.

W. 107. Single-cell Multiplex Proteomics Identifies Functional Differences between Circulating CD14+ CD16- and CD14+CD16+ Monocytes in Patients with Frontotemporal degeneration, Providing Basis for Understanding Disease Progression

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Circulating monocytes are directly involved in neuroinflammation of neurodegenerative diseases, but their role remains unclear in the pathophysiology of frontotemporal degeneration (FTLD). Single-cell IsoCode chip proteomic technology has delineated the functional heterogeneity of blood monocytes that may provide the potential to serve as biomarkers in patients with FTLD, with further study and larger patient numbers in the future.

Blood monocytes from age-matched healthy subjects (n = 2) or patients with FTLD (n = 8) were enriched with Pan Monocyte Isolation Kit. Cells were stimulated with LPS at 37°C, 5% CO2 for 24 hours, stained with Alex Fluor 647 or PE-conjugated anti-CD14 or CD16 and loaded into an IsoCode chip pre-patterned with a 32-plex antibody array per cellular microchamber. After 16-hour-on-chip incubation, secreted proteins from ~ 1000 single cells were analyzed by fluorescence ELISA-based assay; polyfunctional cells (co-secretion of 2+ proteins per cell) were analyzed by IsoSpeak software.

CD14$^+$CD16$^-$ monocytes exhibited a greater polyfunctionality than CD14$^+$CD16$^+$ subsets, which was observed in both health and disease. The increased polyfunctional CD14$^+$CD16$^-$ monocytes had elevated secretions in IL-6, IL-8, MIP-1α, MIP-1β and TNF-α compared to CD14$^+$CD16$^+$ cells. Interestingly, patients with FTLD showed higher polyfunctional upregulation in both CD14$^+$CD16$^-$ and CD14$^+$CD16$^+$ subsets than healthy donors. Enhanced polyfunctional strength of monocyte subsets in FTLD patients was mainly driven by IL-8, MIP-1α and MIP-1β.

Increased inflammatory polyfunctional monocyte subsets with unique cytokine signatures provide a potential basis for biomarkers for peripheral immune pathology unique to FTLD, which will be further validated in a larger cohort.

W. 108. The calcium response of human monocytes to CCL2 increases in aging

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It is widely accepted that dysregulation of the innate immunity is a contributing factor to chronic low-grade inflammation in aging. Monocytes are critical regulators both in the initiation and in the resolution of inflammation, and undergo significant changes in their function with age. Intracellular calcium (Ca^{2+}) increase and duration are crucial for various biological functions of immune cells. Therefore, here we investigated the effects of aging on Ca^{2+} mobilization in human monocytes. Purified peripheral blood monocytes from healthy elderly and young adults were loaded with 1 μM Fluo-4-AM, a Ca^{2+} indicator, and analyzed by flow cytometry. Baseline Ca^{2+} level was recorded for 60 seconds before the addition of 35 nM CCL2, 25 μg/ml LPS or 2 μM thapsigargin (TG, an sarco/endoplasmic reticulum Ca^{2+}-ATPase inhibitor that triggers store-operated Ca^{2+} entry, SOCE). The expression of TLR4 and CCR2, and monocytes subpopulations, were also assayed by flow cytometry. No significant differences in the baseline Ca^{2+} and Ca^{2+} response towards LPS were observed between monocytes of elderly and young adults. In contrast, an increased response was observed when monocytes of elderly were stimulated with CCL2 or TG. On the other hand, the expression of TLR4 and CCR2, and frequency of monocytes subpopulations, were similar. Taken together, our data suggest that, the inflammatory state in the elderly could be due to an increased response of monocytes to sterile stimuli due to an alteration of the mechanisms regulating the Ca^{2+} signals in these cells

Organ Transplantation

H. 127. Human tolerogenic dendritic cells used in a phase I/II clinical trial regulate immune responses through lactate synthesis

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Cell therapy is a promising strategy to treat patients suffering from autoimmune or inflammatory diseases, or receiving a transplant. Our preclinical studies revealed that treatment with Autologous Tolerogenic

Dendritic Cells (ATDCs) is safe in non-human primates and promotes allograft tolerance in rodents. We have now established a robust procedure for manufacturing human ATDCs, which are currently being tested in a first-in-man phase I/II clinical trial as an adjunct immunosuppressive therapy in patients undergoing kidney transplantation. In the present study, we report human ATDCs properties and their mechanisms of action on T cells.The defining characteristics of ATDCs are their suppression of T cell proliferation and their expansion of regulatory T cells through secreted factors alone. We found that ATDCs produce high levels of lactate which shape T cell responses towards tolerance. Our results showed that T cells take up ATDC-secreted lactate leading to a decreased of their glycolytic metabolism. In a humanized mouse model, ATDCs delay graft-versus-host-disease development by promoting expansion of $CD4^{+}Foxp3^{+}$ regulatory T cells, which correlates with elevated levels of lactate in the blood. The contact-independent, non-specific suppression of T cell immunity through lactate production by ATDCs is a novel mechanism of action that distinguishes ATDCs from other cell-based immunotherapies currently under clinical investigation. In light of their strong tolerogenic potential *in vitro* and *in vivo*, we believe that ATDC therapy should be extended to other clinical trials with the aim of regulating the immune response.

H. 128. Association between cytotoxic T cells CD8+CD28- in peripheral blood and histological diagnosis of renal transplant biopsy

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Chronic rejection is a burden on the long-term survival in renal transplantation. Diagnostic biomarkers would optimize the management of patients at risk for rejection. Blood cell immunophenotyping with flow cytometry is used in clinical routine. Our objectives were to explore an association between blood lymphocytes populations (CD8+CD28- cytotoxic T cells and CD4+CD25highCD127 regulatory T cells (Treg)) and the allograft histology. Between 2008 and 2016, 1097 renal transplant recipients were included involving 1640 biopsies associated with a blood immunophenotyping of CD8+CD28- cytotoxic T cells and CD4+CD25highCD127 Treg. Histological analysis of the biopsies were classified into 9 categories based on Banff 2015 classification : normal (n= 540), subnormal (n=79), antibody mediated rejection (AMR) (n=128), suspected AMR (n=83) cell rejection (n=41), borderline rejection (n=92), interstitial fibrosis with (n=255) and without inflammation (n=264), other diagnoses (n=149). Blood CD8+CD28- rate was higher in AMR (14%, p=0.04) and suspected AMR (19% p=0.003) than in normal histology (11%). Blood Treg was lower in suspected AMR (1.8% vs 2,3%, p=0.01). The multivariate

analysis showed that for a similar patient any 10% increase in peripheral blood CD8+CD28- T cells was significantly more associated with borderline lesions (OR = 1.40, IC95 [1.1-1.8]) and with suspected AMR (OR=1.44, IC95 [1.14-1.82]) compared to normal histology. We did not find any association between histology and blood CD4+CD25highCD127 regulatory T cells. We concluded that the routine phenotype of peripheral CD8+CD28- T cells may be associated with histological diagnosis of borderline or suspected AMR lesions.

H. 129. Corticosteroid-Resistant Severe Acute GVHD Promptly Rescued By Plasma-Transfusion-Mediated Donor HLA-targeted Serotherapy

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***Purpose:** Acute Graft-versus-Host Disease (GvHD) is a rare but frequently lethal complication after solid organ transplantation (SOT), occurring in unduly immunocompromised recipients. Immunosuppression escalation increases the risk of fatal sepsis. We hypothesized that transfusion of plasma with high levels of antibodies targeting at least one donor mismatched HLA antigen could control GvHD in such setting.
***Methods:** We faced a therapeutic dead-end in an immune-deficient child with severe steroid-resistant GvHD after a kidney transplantation. An urgent nationwide search among 3800 registered blood donors with known anti-HLA immunization was coordinated by the French National Blood Service and identified 3 donors. The DSA was measured at nearly 10,000 MFI units in neat plasmas.
***Results:** The patient received a 200 mL plasma infusion (4-fold dilution), twice, three days apart. A rapid DSA adsorption on donor cells was observed. Plasma transfusions were remarkably well tolerated. The patient had been experiencing severe neutropenia and major hyperbilirubinemia for 15 and 6 days, respectively. The day following the infusion, white cell count rose sharply, meanwhile the bilirubin dropped. Within a week, the general status dramatically improved. Diarrhea completely and durably resolved. Steroids doses were progressively tapered down and stopped on POD311. Before the first plasma transfusion, donor-derived T cells were bound to recipient-derived extracellular microvesicles. Strikingly, this T cell subset sharply decreased as early as 3 days after the first infusion and was barely sizeable thereafter.
***Conclusions:** An innovative immunotherapy strategy, coined Donor-Targeted Serotherapy, based on the transfer of anti-HLA DSA, can successfully rescue a refractory SOT-associated GvHD.

W. 110. Preliminary mechanistic studies of regulatory dendritic cell (DCreg) cell infusion in living donor liver transplant recipients

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This is a first-in-human phase I/II safety and preliminary efficacy trial of donor-derived regulatory dendritic cells (DCreg) in living-donor liver transplantation (LTx).

DCreg generated from four separate living-donors' elutriated monocytes were infused iv (2.5-10x10^6/kg) 7 days before LTx in 4 separate recipients. A half dose of MPA was given from d-7 to d0 until LTx, followed by standard-of-care immunosuppression (steroid, MPA, tacrolimus) that is gradually withdrawn at 6 months post-LTx. Pre-, post-infusion, and 1, 3, 6, 12 months post-LTx immunological analyses are conducted on recipient blood to track DCreg, donor-specific antibody (DSA), and anti-donor T cell reactivity.

GMP-grade DCreg generated for infusion were HLA-DR$^+$CD1c$^-$CD11c$^+$CD83$^-$IRF4^{lo}CD141$^+$ with high PD-L1:CD86 ratio. In addition, DCreg were resistant to maturation (LPS stimulation), produced IL-10^{hi}IL-12^{lo}, induced donor-specific hyporesponsiveness of recipient T cells. No infusion reaction or cytokine release responses were observed. DCreg were detected in whole blood of three recipients immediately after infusion, but no longer after 3 days post-infusion. A small population of DC co-expressing both recipient and donor MHC, ie cross-dressed DC, were detected in the circulation. In one examined patient, a post-infusion signal in the host CD8+ T cells was identified at the time of transplant, indicating a decrease in memory cell subsets, down-regulation of T-bet/Eomes, CTLA-4 and PD-1. No DSA has been detected in the circulation of these 4 patients.

DCreg infusion into LTx recipients appears safe. Cross-dressed DC can be detected in peripheral blood within a few days post-infusion and may have signaled CD8+ T cells, suggesting their possible *in vivo* regulatory function.

W. 111. Early CXCR5+PD1+ICOS+ circulating T follicular helper cells are associated with de novo donor-specific anti-HLA antibodies after renal transplantation

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Donor-specific anti-HLA antibodies (DSAs) are a major risk factor associated with renal allograft outcomes. As a trigger of B cell antibody production, T follicular helper cells (Tfhs) promote DSA appearance. We measured circulating Tfh (cTfh) levels on the day of transplantation and one year after transplantation in blood from a prospective cohort of 237 renal transplantation patients without DSA

during the first year post-transplantation. Total cTfh were characterized as $CD4^+CD45RA^-CXCR5^+$, and the three following subsets of activated cTfh were analyzed: $CXCR5^+PD1^+$, $CXCR5^+PD1^+ICOS^+$ and $CXCR5^+PD1^+CXCR3^-$. Immunizing events (previous blood transfusion and/or pregnancy) and the presence of class II anti-HLA antibodies were associated with increased frequencies of activated $CXCR5^+PD1^+$, $CXCR5^+PD1^+ICOS^+$ and $CXCR5^+PD1^+CXCR3^-$ cTfh subsets. By contrast, ATG-depleting induction and calcineurin inhibitor treatments decreased the total level of cTfhs, and activated cTfh subsets were increased at one year post-transplantation. In multivariate survival analysis, we reported a decrease in activated $CXCR5^+PD1^+ICOS^+$ at one year after transplantation in the blood of DSA-free patients and a significant association with the risk of developing dnDSA after the first year ($p=0.018$, HR $=0.39$), independent of HLA mismatches ($p=0.003$, HR $=3.79$). These results highlight the importance of monitoring activated Tfhs in patients early after transplantation and show that current treatments cannot provide early, efficient prevention of Tfh activation and migration. These findings indicate the need to develop innovative treatments to specifically target Tfhs to prevent DSA appearance in renal transplantation.

W. 112. Enhanced myocardiac autophagy induces heart transplant tolerance by blockade of BET signaling pathway

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Graft rejection is a critical cause of graft poor outcome even graft loss after transplantation, which is mainly associated with immune attack. Autophagy plays important roles in the cardiac ischemia/reperfusion process and its implicated in the pathogenesis of cardiac injury, although its role in the process is complicated. JQ1, a potent BET protein inhibitor, was reported to be capable of quenching hyper-inflammatory responses. Whether JQ1 has beneficial effect on transplant tolerance, and if so whether autophagy or immune suppression is involved in the protective effect has not been investigated. Our present study originally revealed that JQ1 treatment evidently prolonged allograft survival and well-preserved histological architecture in a mice model of cardiac transplantation. Intriguingly, we found that JQ1 efficiently induced an elevated protein expression of ATG5, ATG7, and LC3-II in cardiac allografts. Autophagy activation was suppressed by Bafilomycin A1 treatment based on the immunohistochemistry staining and immunoblot analyses of ATG5 and LC3-II. Furthermore, combined use of JQ1 with Bafilomycin A1 partially reversed the tolerogenic effect of JQ1 suggesting the involvement of autophagy signaling pathway in the process. Interestingly, JQ1 also decreased frequency of splenic Th1, Th2, Th17, and regulatory T cells as well and downregulated the expression of inflammatory cytokines such as IL-2, IL-6, IL-1beta, TNF-alpha, IFN-gamma, and IL-17. Taken together, these results indicate that JQ1 could significantly prolong allocardiac graft survival by potentiating myocardiac autophagy and inhibiting subsequent release of inflammatory cytokines. This unveiled mechanism may therefore provide novel insights into transplant tolerance induction.

W. 113. IL-6 Receptor Expression on T Cells Is Needed for the Development of Donor-Specific Antibodies Towards Vascular Allografts

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The inflammatory cytokine IL-6 contributes to immune activation that causes acute and chronic rejection of organ transplants. However, very little is known about the signaling mechanisms by which IL-6 functions in this setting. IL-6 can transduce signals that lead to distinct biological outcomes through mechanisms that are either dependent or independent of IL-6 receptor (IL-6R) expression on the surface of target cells. Using mice that lack IL-6R expression only in T cells (IL-6R-TKO) and a murine aortic interposition model of vascular rejection, we examined the role of IL-6R in T cells on allogeneic immune responses. IL-6R-TKO mice were generated by crossing IL-6R$^{flox/flox}$ mice with CD4-Cre partners. IL-6R expression was absent in T cells from IL-6R-TKO mice but remained abundant on other leukocyte lineages. There was no difference in the accumulation of CD4 and CD8 T cells in allograft arteries from IL-6R-TKO graft recipients as compared to IL-6R$^{flox/flox}$ control counterparts. IL-6R expression on T cells also did not affect the accumulation of macrophages in allograft arteries. When donor-specific antibodies (DSA) were examined, their *de novo* generation was apparent 2 – 3 weeks after transplantation, remained high thereafter, and was markedly and significantly reduced in IL-6R-TKO graft recipients as compared to controls. Overall, our findings indicate that IL-6R expression in T cells is dispensable for the development of peripheral effector T cell responses towards vascular allografts but is needed for the development of DSA.

W. 114. Interleukin-7 receptor blockade by a human anti-CD127 monoclonal antibody in non-human primate kidney transplantation

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IL-7 is an important cytokine for T cell lymphopoiesis. Blockade of the IL-7 signaling pathway has been shown to induce long-term graft survival or graft tolerance in murine transplant models through inhibiting T cell homeostasis and favoring immunoregulation. In human transplantation, it has been suggested that T cell homeostasis following some immunosuppressive therapies might be detrimental to transplant patients. In this study, we assessed for the first time the effects of an anti-human CD127 mAb administered in combination with low-dose tacrolimus (n=4) or thymoglobulin (n=4) in a life-sustaining

kidney allograft model in baboons. Contrary to our expectation, the addition of anti-CD127 mAb to low-dose tacrolimus or thymoglobulin did not prolong graft survival compared to low-dose tacrolimus alone or thymoglobulin alone, respectively. Anti-CD127 mAb administration leaded to full CD127 receptor occupancy during the follow-up period. However, all anti-CD127 mAb-treated animals lost their kidney graft between one and two weeks after transplantation. Pathological study of explanted kidney grafts revealed 2 mixed acute T cell-mediated rejection (TCMR) and acute antibody-mediated rejection (AMR), 3 TCMR, and 3 ischemic necrosis. Donor-specific antibodies were not detected using flow cytometry method, despite prominent peritubular capillary C4d deposition in 2 animals with AMR. Unlike in rodents, anti-CD127 mAb treatment did not decrease the absolute numbers of lymphocyte and lymphocyte subsets and did not effectively inhibit post-depletional T cell proliferation and homeostasis in nonhuman primates. Thus, unlike in mice, IL-7 does not seem to be a limiting factor for T cell homeostasis in primates.

W. 115. Metabolic pathways regulate the migration of TEMRA CD8 to inflammatory site

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Background. Accumulation of TEMRA CD8 ($CD45RA^+CCR7^-CD28^-CD27^-$) in kidney transplant recipients (KTR) with a stable graft function is associated with an increased risk of kidney graft dysfunction. Upon TCR and IL-15 stimulation, TEMRA CD8 activates the endothelium by secreting high amount of IFNg and TNFa.

Aim. Migration of T cells to inflamed tissue is crucial for their immune effector function, especially in the context of transplantation. In this study, we aim to characterize the migratory properties of TEMRA CD8 from KTR by analysing their adhesion and their transmigration across endothelial cell barrier and we investigate the ability of metabolic interferences to prevent the migration of TEMRA CD8 to inflamed site.

Results. With a high expression of VLA-4, LFA-1 and glycosylated PSGL1, we show that TEMRA CD8 from KTR in resting state can adhere and transmigrate across endothelial cell barrier following the CXCL12 gradient. Short term IL-15 stimulation fosters the expression of glycosylated PSGL1 and consequently increases the binding to P-Selectin, the adhesion and the transmigration capacity of TEMRA CD8. Finally, we show that glycolysis and mitochondrial respiration were instrumental for the migration of TEMRA CD8 whereas the inhibition of these 2 processes has only a modest impact on EM trafficking.

Conclusion. Our data demonstrate that TEMRA CD8 from KTR have a high potential to migrate to inflammatory site in response to gradient of CXCL12. Their migration could be blunt by targeting the interaction between glycosylated PSGL1 and P-selectin that can be disrupted either directly at PSGL1 or indirectly via metabolic pathways.

W. 116. Positive Selection of a Human-Restricted TCR in a Swine Thymus in a TCR Transgenic Humanized Mouse Model

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Porcine thymic transplantation is a promising approach for tolerance induction in pig-to-human xenotransplantation. In immunodeficient mice transplanted with human CD34+ cells, porcine thymus grafts support selection of a diverse, pig-tolerant human T cell repertoire. However, human T cells developing in a swine thymus may show reduced human-restricted vaccination responses compared to those from a human thymus. To understand the impact of positive selection in a swine thymus on human-restricted TCRs, we used a TCR transgenic humanized mouse model to study selection of Mart1, a human HLA-A2-restricted TCR, in a pig thymus. Mart1 was positively selected inefficiently in a human HLA-A2- or pig thymus compared to an HLA-A2+ thymus. While proportions of GFP reporter+ thymocytes were similar (A2+:15.0±6.46%; A2-:19.2±5.85%; Sw:11.2±6.73%; n.s. by one-way ANOVA), Mart1+ and CD8SP Mart1+ thymocytes were enriched in A2+ compared to A2- and swine thymi (A2+:3.51±0.553%; A2-:1.25±0.583%; Sw:0.629±0.481%; A2+ vs. A2-, p=0.049; A2+ vs. Sw, p=0.010; and A2+:2.28±0.591; A2-:0.261±0.057; Sw:0.342±0.236, A2+ vs. A2-, p=0.034; A2+ vs. Sw, p=0.029, respectively). However, Mart1+ cells were detected in the periphery at similar frequencies among GFP+ cells in all groups (CD4: A2+:10.3±6.25%; A2-:6.69±2.49%; Sw:4.53±1.69%; CD8: A2+:41.5±11.6%; A2-:45.5±15.6%; Sw:12.1±7.22%; n.s.) and could proliferate when pulsed with peptide *ex vivo*. Possibly, the few T cells with human-restricted TCRs that were positively selected in a swine thymus received adequate postthymic signals for normal homeostasis in transplanted mice. These results suggest that, while positive selection of human-restricted T cells in a transplanted pig thymus may be inefficient, those that are selected may function normally.

W. 117. The role of circular RNA FSCN1 in dendritic cell mediated immunomodulation

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Circular RNAs (circRNAs) that are a new class of endogenously expressed non-coding RNAs produced from back splicing with a covalently closed loop structure have been emerging as an important gene regulator in the physiological and pathological development of cells. It remains unknown about roles of circRNAs in DCs. The objective of the study is to investigate the impact of circular RNA FSCN1 (circFSCN1) on dendritic cell immune function. Bone marrow derived dendritic cells were cultured in vitro and circRNA expression was detected by circRNA microarrays and qRT-PCR. The effect of circFSCN1

on DCs was studied. We found that the expression profiles of circRNAs were significantly different in mature immunogenic DCs v.s immature immunosuppressive DCs. circFSCN1 was the most significantly highly expressed in mature DCs compared with immature DCs. Treatment with immunosuppressive cytokines TGF beta and GDF15 reduced circFSCN1 expression in DCs. Knockdown of circFSCN1 using siRNA reduced the phosphorylation of Rel Ap65, but increased phosphorylated Foxo3. Silencing of circFSCN1 impaired DCs to activate T cells, changed inflammatory cytokine production and enhanced Treg generation whereas circFSCN1 siRNA did not affect DC maturation, suggesting that circFSCN1 siRNA may help induce immunosuppression in organ transplantation. In conclusion, it is a first report to demonstrate that circFSCN1 is essential for DC immune response.

W. 118. Time-dependent lymphocyte count after transplantation is associated with higher risk of graft failure and death

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Introduction : The transplantation field requires specific biomarkers to assess the level of immunosuppression. We aimed to analyze the independent correlation between the number of circulating lymphocytes, simply and routinely monitored by blood cell counts during outpatient visits, and patient and graft survival to explore its role as a potential biomarker of immunosuppression. **Methods :** 3002 kidney or combined kidney-pancreas transplanted patients between January 2000 and December 2016, from two University Hospitals, alive with a functioning graft at 1 year post-transplantation, were enrolled. Clinical and biological information were extracted from the DIVAT data base. We investigated the etiological relationship between time-dependent lymphocyte count after one year of transplantation and patient and graft survivals, viral infection and cancer risks using a time-dependent multivariate Cox model. **Results :** A patient with a lymphocyte count below 750 /mm3 at a given time within the follow-up had a higher risk of graft failure (HR 3.08, $p<0.001$) and death (HR 2.06, $p<0.001$) when compared to a similar patient with a normal lymphocyte count (more than 1500 /mm3) at the same time, independently from other classical confounding factors. Patients with less than 750 /mm3 lymphocytes were more at risk of viral infections than comparable patients with a normal lymphocyte count (HR 1.62, $p<0.001$). **Conclusion :** Deep lymphopenia over time is highly associated with a risk of graft failure, death and occurrence of viral infection. These data suggest that the longitudinal lymphocyte count could be used as a simple routine biomarker of long-term graft and patient outcome.

Other

Autoimmunity in Cardiovascular Disease

H. 65. BTLA Stimulation Protects Against Atherosclerosis by Regulating Follicular B cells

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Aims: The immune system is strongly involved in atherosclerosis and immune regulation generally leads to attenuated atherosclerosis. B and T lymphocyte attenuator (BTLA) is a novel co-receptor that negatively regulates the activation of B and T cells, however, there have been no reports of BTLA and its function in atherosclerosis or cardiovascular disease (CVD). We aimed to assess the dominant BTLA expressing leukocyte in CVD patients and to investigate whether BTLA has a functional role in experimental atherosclerosis.

Methods and results: We show that BTLA is primarily expressed on B cells in CVD patients and follicular B2 cells in low-density lipoprotein receptor-deficient *(Ldlr$^{-/-}$)* mice. We treated *Ldlr$^{-/-}$* mice that were fed a Western-type diet (WTD) with PBS, an isotype antibody or an agonistic BTLA antibody (3C10) for 6 weeks. We report here that the agonistic BTLA antibody significantly attenuated atherosclerosis. This was associated with a strong reduction in follicular B2 cells, while regulatory B and T cells were increased. The BTLA antibody showed similar immunomodulating effects in a progression study in which *Ldlr$^{-/-}$* mice were fed a WTD for 10 weeks before receiving antibody treatment. Most importantly, BTLA stimulation stabilized preexisting lesions.

Conclusion: Stimulation of the BTLA pathway in *Ldlr$^{-/-}$* mice reduces initial lesion development and increases stability of established lesions, presumably by shifting the balance between atherogenic follicular B cells and atheroprotective B cells and directing CD4^{+} T cells towards regulatory T cells. We provide the first evidence that BTLA is a very promising target for the treatment of atherosclerosis.

B cells, Immunology

H. 56. Granzyme B secreting B cell as a potential cell therapeupic target

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B cells secreting granzyme B (GZMB^{+}) with suppressive properties have been evidenced in a growing number of immunological contexts, autoimmunity, chronic infection, neoplasias as well as in healthy

volunteers in physiological conditions. Until now, no phenotype has been proposed for these GZMB$^+$ B cells and their biology as well is still not understood. We found that GZMB$^+$ B cells are mature IgD $^{low/-}$ B cells harboring a CD19^{int} BACE2^{int} CD266^{lo} LAG3^{int} CD307b^{lo} enriched profile. We demonstrate our ability to expand these GZMB$^+$ B cells that express high level of regulatory molecules after expansion and still display suppressive functions, blocking CD4$^+$CD25$^-$ effector T cells proliferation in a GZMB dependent manner, whereas keeping their ability to differentiate, activate and produce immunoglobulins. Accordingly to their BCR usage, we show that GZMB$^+$ and GZMB$^-$ B cells likely share a common B cell progenitor. Finally, we report that GZMB$^+$ B cells expand under the control of GZMB through phosphorylation of ERK1/2 whereas they are more prone to apoptosis compared with their GZMB$^-$ B cell counterpart, suggesting their endocrinous tight control. These data bring new insights into the GZMB$^+$ B cell biology and function that emerge as one component of a complex regulatory network. Success to expand them while keeping their potent immunosuppressive properties provides new clues for novel future cell therapies.

Bioinformatics

T. 53. An open-source web-based analytics platform for the human immune atlas

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Mass cytometry (CyTOF) measures the expression of many proteins (currently up to 40) in single cells. TII applies CyTOF to characterise cellular diversity of the immune system in peripheral blood and other tissues. Our version of the immune atlas, called EPIC (Extended Poly-dimensional Immunome characterisation) is built on a growing collection of CyTOF data acquired from blood of clinically and demographically diverse human subjects. The core components of the atlas are 'immune maps', which comprise CyTOF data of samples labeled with identical antibody panels and grouped according to a common biological theme. Besides protein expression patterns, immune maps contain contain clinical and demographic metadata, as well as phenotypic information inferred from clustering and cell type annotation. To intuitively analyse these complex multi-dimensional data, we developed a Shiny/R web application that has two main objectives. First, clients can explore the immunome at different levels of details using a wide range of interactive visualisation methods, such as bar charts comparing the abundance of all or subsets of immune cell types in different age groups, or tSNE/UMAP scatter plots providing global perspectives of expression domains. Second, users can upload their own CyTOF data and, through pattern matching, obtain instant estimates about the abundance of selected immune cell

populations in their own samples. We tested our system using immune maps constructed from healthy paediatric samples. We will provide updates on the data analytics pipeline and software development.

Data Analysis; Bioinformatics

T. 55. Single cell RNAseq data analysis using an extendable shiny app

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The single cell mRNA sequencing (scRNA-Seq) has become a widely used tool for immune profiling and biomarker discovery. A major bottleneck in the initial steps of scRNA-Seq data analysis is initial processing of data, which is time-consuming since it requires a lot of interaction between the end-user and the bioinformatician. We have developed a graphical user interfaced based on the Shiny/R framework (scShinyHub) that allows immunologists to intuitively and autonomously perform cell and gene selection in the process of scRNA-Seq data analysis.

The tool is open (source) and easily extendable. Oher tools with a graphical user interface mainly focus on post-differential data analysis and use all data, including cells/genes not pertinent to the biological question. scShinyHub focuses on filtering cells and genes based on various criteria and on subsequent reanalysis of the cells of interest. Another feature is the plug-in structure that allows developers to easily add functionalities.

We are presenting the scShinyHub workflow that allows to (1) remove mitochondrial/ribosomal genes and other non-interesting genes; remove cells not relevant to the biological question; (2) perform quality controls (UMI distribution, …); (3) apply dimensionality reduction (tSNE, UMAP, PCA, …) and clustering (hierarchical, self-organizing maps) methods; (4) select/remove cells based on different criteria and re-run analyses; (5) finalize the workflow by running differential expression analysis, or trajectory inference; (6) generate reports.

Avalability: https://github.com/baj12/scShinyHub

Developmental Immunology

T. 116. Building a Roadmap of Early Human Immune System Development Through Single Cell and Bulk RNA Sequencing

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During development in utero, many human fetal immune cells are predisposed towards tolerogenic responses. Compared to adult human counterparts, human fetal naïve CD4 T cells exhibit a higher propensity for differentiation into tolerogenic regulatory T cells (TReg), and human fetal hematopoietic stem and progenitor cells (HSPCs) generate CD4 T cell progeny that are predisposed to TReg differentiation. After birth, naïve T cells must transition toward a more mature state that supports protection against pathogens and cancer. At the time of birth, it is unknown whether adult-associated

protective programs are fully expressed, either universally within most naïve T cells, or heterogeneously within just a subset. Here, through single-cell transcriptional profiling of both naïve T cells and HSPCs, from fetal, full-term newborn umbilical cord blood, and adult human sources, we demonstrate that the fetal to adult transition is incomplete across most cord blood naïve T cells and their hematopoietic stem cell progenitors. Cells expressing a fully adult-like transcriptional signature are rare in cord blood, where most naïve T cells and HSPCs exhibit a distinct, intermediate transcriptional state. Our results provide a mechanism that might explain why many vaccines, which were largely designed around elicitation of immunity in the protection-predisposed adult immune system, show reduced efficacy in neonates. Continued analyses of how expression of specific gene programs vary across ages will be carried out with the translational aim of informing specific ways that targeted, tolerogenic or protective, therapeutic responses might be elicited in both cord blood transplant and neonatal vaccination interventions.

Eosinophilic Esophagitis Pathology

W. 13. Administration of an Anti-Interleukin-13 (IL-13) Antibody Significantly Attenuates Esophageal Eosinophilic Infiltration and Systemic Inflammation in a Mouse Model of Eosinophilic Esophagitis

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Eosinophilic esophagitis (EoE) is a chronic, allergic, inflammatory condition characterized by infiltration of eosinophils into the esophagus and symptoms such as dysphagia, food impaction, and vomiting. EoE is associated with a strongly enhanced mucosal T helper type 2 (Th2) cell response elicited by food allergens and subsequent production of pro-inflammatory cytokines and chemokines in esophageal tissue. IL-33 and IL-13 both contribute to EoE pathology (Travers J, et al. *Sci Rep*. 2017;7:17563; Blanchard C, et al. *J Allergy Clin Immunol*. 2007;120:1292-1300). In the present study, we assessed the effects of anti-IL-13 antibody treatment in a week-long, IL-33-mediated model of EoE in mice by thoroughly characterizing immune cell subsets in the esophagus using flow cytometry. Control mice with challenge developed marked esophageal inflammation and eosinophilic infiltration, similar to that observed in EoE patients. Furthermore, challenge promoted immune cell activation and production of pro-inflammatory cytokines in the esophagus, which was associated with tissue inflammation and remodeling. In addition to a dramatic infiltration of eosinophils, challenge led to a marked influx of ILC2 cells and to a lesser extent, T cells, dendritic cells, and macrophages. Measurements of systemic cytokines and chemokines in challenged animals also revealed enhanced levels of IL-13, IL-5, eotaxin, and periostin, important inflammatory mediators associated with human EoE. Administration of anti-IL-13 monoclonal antibodies to challenged mice significantly prevented esophageal eosinophilia and systemic inflammation. These findings confirm a pivotal role for IL-13 in promoting esophageal inflammation in a relevant model of EoE and the therapeutic potential of anti-IL-13 antibodies in EoE patients.

Food Allergy

H. 7. Gastrointestinal Eosinophilia Is Present in Oral Immunotherapy Subjects With IgE-Mediated Peanut Allergy at Baseline

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Rationale: Oral immunotherapy (OIT) is an emerging treatment for food allergy and many subjects experience gastrointestinal symptoms while few develop eosinophilic gastrointestinal disease. It is unclear whether these subjects have subclinical gastrointestinal eosinophilia (GE) at baseline. We aimed to evaluate the presence of GE in subjects with food allergy before and during peanut OIT.

Methods: We performed esophagogastroduodenoscopies on 21 adults undergoing peanut OIT. Subjects completed detailed gastrointestinal symptom questionnaires. Endoscopic findings were assessed using the Eosinophilic Esophagitis (EoE) Endoscopic Reference Score (EREFS) and biopsies were obtained from the esophagus, gastric antrum, and duodenum. Esophageal biopsies were evaluated using the EoE Histologic Scoring System. Immunohistochemical staining for eosinophil peroxidase (EPX) was also performed. Hematoxylin and eosin and EPX stains of each biopsy were assessed for eosinophil density and EPX/mm^2 was quantified using automated image analysis.

Results: At baseline, all subjects were asymptomatic. Pre-existing esophageal eosinophilia (>5 eosinophils per high-power field [eos/hpf]) was present in five participants (24%), three (14%) of whom had >15 eos/hpf associated with mild endoscopic findings (edema, linear furrowing, or rings; median EREFS = 0, IQR 0–0.25). Increased eosinophils were noted in the gastric antrum (>12 eos/hpf) or

duodenum (>26 eos/hpf) in 9 subjects (43%). EPX/mm^2 correlated strongly with eosinophil counts (r = 0.71, p < 0.0001).

Conclusions: Pre-existing GE is common in adults with IgE-mediated peanut allergy. Eosinophilic inflammation (EI) in these subjects may be accompanied by mild endoscopic and histologic findings. Longitudinal data collection during OIT is ongoing.

H. 8. Diminished Long-Term Outcome in Large Phase 2 Study of Peanut Immunotherapy

R. Sharon Chinthrajah[1], Natasha Purington[1], Sandra Andorf[1], Andrew Long[1], Katherine L. O'Laughlin[1], Shu-Chen Lyu[1], Monali Manohar[1], Scott D. Boyd[2], Robert Tibshirani[3], Holden Maecker[4], Marshall Plaut[5], Kaori Mukai[6], Mindy Tsai[7], Manisha Desai[8], Stephen J. Galli[9] and Kari Nadeau[1]

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BACKGROUND

Dietary avoidance is currently recommended for peanut allergies. We evaluated sustained effects of treating peanut allergy with oral immunotherapy (OIT) in the first phase 2 randomized-controlled long-term study in adults and children.

METHODS

In a double-blind, placebo-controlled, randomized study, 120 peanut-allergic participants (7-53 years) received up to 4 g of peanut protein—about one tablespoon of peanut butter—or placebo daily for 3 years. Participants received placebo (N=25) or peanut protein (N=95) over 104 weeks; 60 then discontinued (peanut-0) while 35 received 300 mg daily—about one peanut kernel—(peanut-300). Double-blind, placebo-controlled food challenges (DBPCFCs) to 4 g peanut protein were conducted at baseline, week 104, and every 13 weeks thereafter for one year.

RESULTS

The primary endpoint was reached at week 117 after 3 months of discontinuation to test sustained unresponsiveness: 21/60 (35%) peanut-0 participants passed the challenge with no reaction versus 1/25 (4%) placebo (primary endpoint, P=0.002). Time to failure was significantly longer in peanut-300 vs.

peanut-0 vs. placebo arms (P<0.0001). The percentage of participants passing DBPCFCs in peanut-300 declined significantly (weeks 104-156; 83% vs. 37%, P<0.001). Adverse allergic reactions decreased over time in all arms. Peanut-specific IgG_4/IgE levels were higher (P<0.001), and Ara h 2-specific IgE (P<0.001) and basophil activation responses (P=0.037) were lower at baseline in those achieving sustained unresponsiveness at week 117.

CONCLUSIONS

Peanut OIT can desensitize most peanut-allergic individuals to 4 g peanut protein but discontinuation, or even a reduction to 300 mg daily, increases the likelihood of regaining clinical reactivity to peanut.

Gene-Therapy / Immunity

H. 131. Monitoring T cell responses toward the Streptococcus pyogenes Cas9 nuclease

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Previous studies showed that there is a pre-existing adaptive immunity toward CRISPR-associated nucleases (Cas) in humans. Clinical studies of viral gene therapy suggest that immune responses toward vectors may decrease the efficacy and safety of these treatments. Thus, clinical translation of CRISPR-Cas-based therapies will require careful immune monitoring as well as strategies to suppress unwanted immune responses in patients. We characterized the T-cell responses toward the whole protein of the CRISPR-associated nuclease derived from *Streptococcus pyogenes* (SpCas9) in the peripheral blood of healthy human donors. SpCas9-reactive T-cells were found in 95% of all the human donors comprising both effector and regulatory subsets with distinct functional properties. SpCas9-reactive regulatory T-cells suppressed the proliferation and cytokine production of their effector counterpart *in vitro*. Through depletion of regulatory T-cells, we established SpCas9-reactive effector T-cell lines that lysed SpCas9-expressing target cells in a dose-dependent manner. Further, we established an efficient and rapid method to identify the entire SpCas9-reactive T-cell repertoire using a customized peptide library covering the complete amino-acid sequence of SpCas9. Our method allows immune monitoring for risk assessment before and during clinical trials employing Cas-derived therapeutic approaches. Moreover, it provides the basis for an unsupervised characterization of the antigenic structures that mount the pre-existing Cas-reactive T-cell response. In addition, SpCas9-reactive effector T-cell lines could be used to evaluate the immunogenicity of autologous Cas-modified cell products before infusion into the patient. Regulatory T-cells with specificity for Cas epitopes may be an attractive option for a targeted immunosuppression strategy during gene therapy.

Immune Aberrations in Human Disease

T. 118. Comparison of two different recombinant mycobacterial vaccine strains for HIV-TB coinfection

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Human immune deficiency virus type I (HIV-1) and *Mycobacterium tuberculosis* (*M.tb*) have become intertwined over a past few decades that exacerbates the morbidity and mortality associated with each pathogen alone. In general, many individuals get infected with *Mycobacterium tuberculosis*, but fails to develop active tuberculosis. the only available vaccine Mycobacterium bovis Bacilli Calmette Guerin (BCG) fails to stop adult TB cases and successful in the pediatric population only. Thus, the development of an effective and improved anti-TB vaccine has become an urgent need for control and elimination of this disease. *M.smegmatis* strains which are nonpathogenic and commensal in humans are used in this study for cloning two immunodominant antigens namely HspX and Mpt51.The HspX is a latency associated immunodominant antigen of *M. tuberculosis*. Mpt 51 has been reported to be highly expressed during the re-activation of tuberculosis particularly in HIV positive individuals.

Characterization of recombinant *M. smegmatis* expressing hspX and mpt51 was carried out by growth curve analysis and induction of the monocytes. Recombinant strain containing hspX was found to play a vital role in the survival of bacterium under stress conditions like acidic and microaerophilic conditions and also changed the morphology of bacterium when compared with the strain containing mpt51. *Recombinant strains with* hspX differentiated monocytes into macrophages efficiently than the strain with mpt51. The Realtime PCR analysis revealed that Th1 cytokines were more upregulated by *M. smegmatis* containing hspX than by *M. smegmatis* containing mpt51. The efficiency of the above vaccine strains will be discussed in detail.

Immune Development

H. 83. Immune Atlas of Second Trimester Human Intestine, Liver and Spleen

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It has been thought that the immune system is immature and naïve at birth. A third of neonatal deaths are attributed to infections and newborns have reduced vaccine responses. Yet, most newborns are healthy. There is limited data on human *in utero* mucosal immunity. We performed deep immunophenotyping with CyTOF and T and B-cell receptor repertoire analysis with Next generation sequencing (NGS) in discarded spleen, liver and small and large intestinal samples from 20 fetuses (16-23 weeks' gestation (GA)) and 5 neonates. We demonstrate that intestinal, hepatic and splenic immunity are unique to the organs, complex and functional at 16 weeks' gestation (GA). B/TCR repertoires are diverse in fetal samples, with an increase in CDR3β/H length and distance-from-germline with advancing GA. Intestinal innate immunity

is dominated by APCs, including CD103+DCs, ILCs and NK cells, whereas the liver and spleen innate compartments are made up mostly of APCs. Follicular and transitional B-cells are enriched in the fetal and CD69+IgM+B-cell in neonatal intestinal tissue. Yet the liver tissue contains few B and T-cells and the spleens contain abundant naïve B-cells. Greater than 50% of the fetal intestinal tissue leukocytes, but not those from the spleen or liver, are T-cells with majority being memory T-cells. Finally, functional tissue resident memory T-cells are abundant in the intestines but not the other tissues. Our data provide the foundation for a second trimester mucosal immune atlas and suggest that intestinal development occurs significantly earlier than previously reported.

Immune System Development in Toddlers

H. 94. A Novel Serum Antibody Profiling Assay Reveals a Stratification of Healthy Toddlers into Low, Intermediate and High IgG Responders towards Autoantigens, Infectious Agents, and Vaccine Antigens

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Theadaptive immune responses are relatively weak in newborns, with protection against infections largely due to maternal antibodies. As their immune response develops during the first two years of life, an infant/toddler begins making antibodies in response to infections, environmental exposures, and vaccinations. Yet, surprisingly little is known about the specificity of these antibodies. We have an IRB approved study for1000 healthy 1- and 2-year old toddlers to characterize their antibody specificities. A new antigen array was developed comprising autoantigens, infectious agents, vaccine antigens, and allergens. Serum profiling of 160 samples to date reveals a stratification of these toddlers into low, intermediate, and high IgG response groups. The separation is based on the normalized aggregate response, with high responders selected at 1 SD beyond the mean. Sixteen % are high responders, with their IgGs recognizing many self-antigens and infectious agents. Interestingly, 26% and 9% of the cohort had moderate and high anti-nuclear antibody (ANA) titers, respectively. Comparing clinical data reveals asignificant correlation with the high IgG responders and a family history of asthma and maternal gestational diabetes. Targeted DNA sequencing in the high responder group revealed a strong genetic association signal at the HLA locus, with genetic polymorphisms at this locus associated with high ANA and IgG titers to many antigens. Findings from our study may support implementation of a new wellness screen to identify toddlers at risk for immune system abnormalities later in life

Immunogenicity Screening

T. 119. Immunogenicity of Peptide Drugs and their Impurities: A Case Study of Taspoglutide and its Impurities

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Cost-effective peptide synthesis methods are contributing to an influx of generic peptide drugs in the pharmaceuticals. Despite the rapidity and simplicity of peptide synthesis, regulatory agencies have raised concern about the potential for impurities introduced during the synthetic process that induce unwanted immune responses. These impurities are derived from the parent drug but may contain amino acid insertions, deletions, truncations and the incorporation of D-stereoisomers and non-natural amino acids. Any of these impurities can create new T cell epitopes within the peptide sequence resulting in unexpected immune responses.

Taspoglutide, used for the treatment on type 2 diabetes, failed during Phase III Clinical Trials due to the development of a hypersensitivity reaction in 38% of patients. Analysis of a typical batch used in the Phase III Trials revealed several manufacturing impurities. When it was determined that drug hypersensitivity was linked to HLA (DRB7 and DRB11), the developer suspected that the allergic reactions might be attributable to new T cell epitopes present only in the impurities. EpiMatrix analysis of impurities resulting from amino acid duplications reveals the creation of several neoepitopes when compared to the baseline sequence, five of which created neoepitopes predicted to bind HLA DR7 and DR11 supertype families that could have contributed to the observed hypersensitivity in subjects with DRB1*0701 and DRB1*1104.

In summary, this Taspoglutide case study illustrates the importance of identifying manufacturing related impurities and assessing their immunogenic potential. Methods for immunogenicity screening using silico and in vitro analysis will be described in this presentation.

Immunology

T. 117. Clinical Immunones Dashboard for Electronic Health Records (CIDEHR): Securely Visualizing and Analyzing Flow Cytometry Data from Electronic Health Records

Thomas Peterson, Benjamin Glicksberg, Zicheng Hu, Sanchita Bhattacharya and Atul Butte

UCSF Bakar Computational Health Sciences Institute, San Francisco, CA

Results from flow cytometry assays on real-world patients are routinely stored in Electronic Health Records (EHRs) but using these data for research is prohibitive due to data privacy concerns. Furthermore, the limited availability of healthy controls in EHRs makes interpretatibility difficult. To leverage the wealth of clinical flow cytometry within EHR systems, we have developed a tool, the Clinical Immunomes Dashboard for EHRs (CIDEHR; pronounced “CIDER”), for mining EHR flow cytometry results and comparing to the 10k Immunomes cohort (http://10kimmunomes.org), a collection of immune measurements from healthy individuals from open-access clinical studies. CIDEHR allows users to build and visualize EHR cohorts of interest with the ability to filter patients by diagnosis and medication records while comparing to the healthy 10k Immunomes. The tool can be employed within a secure environment using OMOP or Epic Caboodle, commonly used EHR formats, and users can utilize the full functionality of the application without the ability to view Protected Health Information (PHI). For example, using the UCSF EHR, CIDEHR has the ability to visualize flow cytometry and other clinical information from 5,330 patients, which will soon grow in population size with the inclusion of multiple University of California institutions. This simple tool enables researchers to study real-world populations in the EHR without the need to relinquish PHI, which could serve as a model for more types of data using more interconnected EHRs in the future.

Immunology and Inflammation

W. 109. Extracellular Cold-inducible RNA-binding Protein Enhances Murine Pulmonary Fibrosis

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Introduction: Pulmonary fibrosis is a class of inflammatory lung diseases with poor prognosis. Recent studies have implicated Toll-like receptor 4 (TLR4) and damage-associated molecular patterns (DAMPs) in its pathogenesis. Extracellular cold-inducible RNA-binding protein (eCIRP) is a novel DAMP which exacerbates inflammation. eCIRP promotes inflammation through its binding to the TLR4/MD2 complex. We hypothesized that eCIRP enhances the fibrotic process in lungs by fibroblast activation.

Methods: Pulmonary fibrosis was induced in male 8-week old C57BL/6 WT and CIRP $^{-/-}$ mice through intratracheal (*i.t.*) instillation or subcutaneous (*s.c.*) injection of bleomycin and lung tissues were obtained and analyzed for hydroxyproline contents. To study the effect of eCIRP on fibroblasts, we preincubated mouse embryonic fibroblasts (MEF) cells with rmCIRP and the effects of transforming growth factor-β (TGF-β) in the expression of profibrotic genes were assessed by quantitative real-time PCR (qRT-PCR).

Results: Exposure of WT mice to bleomycin elicited significant changes in the lungs with substantial collagen accumulation as confirmed by increased hydroxyproline content as compared to the control WT mice (both *i.t* and *s.c and* $p<0.05$). Furthermore, pulmonary fibrosis showed substantial attenuation in CIRP KO mice (ANOVA, $p<0.05$). In MEF cells exposed to TGF-β, pre-incubation with rmCIRP significantly induced the expression of fibrotic genes compared to pre-incubation with PBS (Col1a2, α-SMA, MMP2, and MMP9, $p<0.05$).

Conclusion: eCIRP enhances TGF-β signaling in fibroblasts and induction of fibrotic process pulmonary tissue. The detailed mechanism by which this enhancement occurs is now under investigation. The results suggest a role for eCIRP in the pathogenesis of pulmonary fibrosis.

Immunology and Metabolism

T. 54. Salt modulates cellular metabolism of regulatory T cells

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High salt intake has been associated with shifts in the immune cell balance, mainly by promoting proliferation and activity of pro-inflammatory cells, such as T helper 17 (Th17) and M1 macrophages, and by impairing the functions of anti-inflammatory cells such as regulatory T cells (Tregs) and M2

macrophages. However, the precise molecular mechanisms that lead to this phenotype are still unknown. The role of metabolic regulation in shaping immune responses has gained increasing attention in recent years. Cellular metabolism is a vital process, which is essential for growth, survival and proliferation of every cell type, and can be greatly influenced by environmental factors such as diet. Previous studies have shown that high-salt leads to metabolic changes in M2 macrophages by decreasing their mitochondrial oxidative phosphorylation (OXPHOS) and glycolysis necessary for their activation. Here we analyzed the effect of high-salt on cellular metabolism of human Tregs. Of note, our results show significant salt-induced changes in metabolism of human Tregs. Since these changes are known to alter Treg suppressive function both *in vitro* and *in vivo*, we hypothesize that the observed metabolic alterations might be linked to the loss of suppressive function seen in human Tregs upon high-salt challenge. Thus, the interference with these pathways may have the potential for targeting Tregs in salt-sensitive diseases.

Immunoregulation

H. 57. Role of the intracellular ion channels TMEM176A and TMEM176B in the immune system

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Ion channels represent attractive targets for the development of new therapies but still remain poorly studied in the immune system, in particular intracellular ion channels. In this work, we explored the role of two highly redundant ion channels named TMEM176A and TMEM176B that are intriguingly strongly expressed both in RORγt$^+$ cells (ILC3, Th17) and immature dendritic cells. To investigate the role of these homologs and avoid any compensation effect, we generated a double KO (DKO) mouse using CRISPR-Cas9. Surprisingly, *Tmem176a/b* appeared dispensable for the function of RORγt$^+$ cells and in the protective function of type 17 immunity during chemically-induced or infectious colitis. In contrast, antigen presentation by dendritic cells to CD4$^+$ T cells through MHC II was selectively impaired in DKO mice. Using a real-time fluorescence-based system to analyze intracellular trafficking we found that both channels co-localized in highly dynamic post-Golgi vesicles preferentially interacting with, but not accumulating in, acidic organelles. These results indicate that TMEM176A/B ion channels play a predominant role in adaptive immunity as new intracellular components of the intracellular MHC II machinery.

Immunosenescence

T. 52. Age related declined immunity and inflammation as reflected by the CD4 T-cell landscape

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Age-associated changes in the functionality of CD4 T cells have been linked to declined immunity and chronic inflammation. Nevertheless, a detailed characterization of CD4 T-cell phenotypes, which may better explain the duality in the deterioration of the immune system in aging, is lacking. By profiling thousands of CD4 T cells from young and old mice via single cell RNA-sequencing and multidimensional protein analysis, we revealed a distinct landscape of CD4 T-cell subsets, including exhausted, cytotoxic, and activated regulatory (aTregs) cells. These three cell subsets are rare early in life and gradually accumulate with age. At the transcriptional and functional level, the aTregs and cytotoxic CD4 subsets exhibit enhanced anti- or pro-inflammatory capability, respectively.Our results provide a comprehensive view of the dynamic reorganization of CD4 T cells with age and illuminate dominant cell subsets associated with declined immunity and chronic inflammation. These findings suggest new therapeutic avenues for age-related diseases.

Mathematical Modeling of Immune System Activity

H. 73. System Simulation as an Aid to Immunological Research

Kenneth Cooper

System Dynamics Society, Nashua, NH

Adding computer-based system simulation to medical research processes could enhance the progress of some immunological research, potentially reducing the long time and high cost of successful drug development for treating long-term chronic disorders. Expanding the use of system simulation modeling would help advance the analysis of the elegantly complex, feedback-intensive processes that make up the human immune system and its interaction with disease conditions.

The benefits of doing so include the ability to: (1) examine the performance of long-term chronic conditions (such as autoimmune and neurodegenerative disorders and cancers) in high-speed analyses; (2) test rapidly and systematically each of many different possible points of intervention in the system, to identify those with the most leverage in improving a disease condition; (3) test *combinations* of interventions (treatments and potential cures), because some systemic disorders will likely require combination therapies—some of these could be difficult if not impossible to find with real-time experiments; and (4) test alternative hypotheses of the systems' workings—having a platform to test

explicitly different theories about current unknowns would be a valuable aid to advancing our understanding.

This presentation offfers arguments for expanding system modeling as a weapon in the fight against cancers, autoimmune and neurodegenerative disorders, and more--a productivity-enhancing prelude to clinical research. Examples presented will illustrate simulation models that have been built and used to analyze immune diseases and disorders such as HIV, type 1 diabetes, CTE and others. It is written not by a medically-trained scientist, but rather entirely from a system modeler's viewpoint.

Micrbiota and Auto-Inflammatory Diseases

W. 83. Altered early life exposures due to immigration patterns and autoinflammatory diseases: Evaluation of the T cell immune response to commensal bacteria

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A high prevalence of autoinflammatory diseases (ADs), such as IBD, Multiple Sclerosis, Type 1 Diabetes and Asthma, exists in Canada, with a 3- to 5-fold increase in incidence being observed over the last 30-50 years. A changing environment, as well as inappropriate immune responses to microbiota, may contribute to such a sharp rise in disease incidence. Microbiome disturbances (also known as "dysbiosis") have been associated with ADs.

A previous study by our team showed that South-Asians that have immigrated to Ontario are protected from developing autoimmune disease. However, 2nd generation South-Asians born in Ontario lose this protection (1). To assess what might be driving this increased risk of disease, we have been recruiting first and second generation healthy South Asians from the Toronto area ("The GEMINI study", n=174 collected to date), to assess differences in genetics, diet, the microbiome and immune responses in these two subject groups<./p>

Using a metagenomic sequencing approach, our preliminary data examining a small sub-cohort of 106 stool samples from 1st (GEN1) and 2nd (GEN2) generation subjects show that several bacterial taxa are differentially abundant. My work focuses on measuring immune responses to differentially abundant taxa in both first and second generation South Asians using banked peripheral blood mononuclear cells. In summary, the GEMINI study aims to discover how early life exposures in the Ontarian environment influence immune/microbiome detente in order to gain insights into environmental impacts on AD.

Neuroimmunology

H. 23. Single-cell RNA-sequencing of human microglia reveals subtype-specific association with neurological disorders

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Microglia are the main resident immune cell population in the brain. Recent studies of bulk microglia have provided insights into the role of this immune cell type in central nervous system development, homeostasis and dysfunction. Nonetheless, our understanding of the diversity of human microglial cell states remains limited; microglia are highly plastic and have multiple different roles, making the extent of their phenotypic heterogeneity a central question, especially in light of the development of therapies targeting this cell type. Here, we investigated the population structure of human microglia by single-cell RNA-sequencing. Using surgical- and autopsy-derived brain samples, we identified 9 human microglial subpopulations and noted substantial intra- and inter-individual heterogeneity. These putative subpopulations display divergent associations with Alzheimer's disease, multiple sclerosis, and other neurological and neurodevelopmental diseases and disorders. Several human microglia sub-populations show enrichment for genes found in disease-associated mouse microglial states. Overall, human microglia exist in different functional states with varying levels of association with different brain pathologies.

Pediatric Systemic Lupus Erythematosus

H. 34. All leukocytes contribute specific transcripts to the SLE blood signature

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Patients with Systemic Lupus Erythematosus (SLE) display a complex blood transcriptome whose cellular origin is poorly resolved. Using single-cell RNA-seq, we profiled ~276,000 peripheral blood mononuclear cells (PBMCs) from 33 pediatric SLE, with different disease activity scores, as well as 11 matched healthy controls. Our analysis yielded 20 transcriptionally distinct cell populations, including three monocyte, two B cell, a plasma cell (PC), three CD8+ T cell, and two NK cell clusters. Overall, the SLE signature was comprised of each cell type, although minor populations of PCs and pDCs over contributed. Interferon-stimulated genes (ISGs) were expressed within a restricted SLE $CD14^+$ monocyte population, which correlated with disease activity. While the most prevalent ISGs found in SLE patients were restricted to few clusters, those previously associated with flares spread to every cell type. These results will be

compared and validated using an adult SLE cohort and will lay a foundation for resolving the heterogeneity of SLE.

Skin Cancer

H. 107. Single-cell lymphocyte heterogeneity in advanced Cutaneous T-Cell Lymphoma skin tumors

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The heterogeneity of tumor cells presents a major challenge to cancer diagnosis and therapy. Cutaneous T cell lymphomas (CTCL) are a group of T lymphocyte malignanciesthat primarily affect skin. Lack of highly specific markers for malignant lymphocytes prevents early diagnosis, while only limited treatment options are available for patients with advanced-stage CTCL.Using single-cell RNA-sequencing analysis we profiled the transcriptomes of malignant and reactive T lymphocytes in advanced-stage CTCL skin tumors to identify specific markers for diagnosis and cure of CTCL.Single-cell transcriptional profiles displayed intra- and inter-tumor lymphocyte heterogeneity but also a common gene expression signature among several advanced-stage CTCL skin tumors, likely identifying highly proliferating malignant lymphocytes. Analysis of tumor infiltrating T lymphocytes revealed heterogeneity in effector and exhaustion programs across patient samples. Thus, our single-cell analyses of CTCL skin tumor samples provide new insight into disease pathogenesis and progression by showing patient-specific landscapes of malignant and reactive lymphocytes within the local microenvironment of each tumor, thus opening avenues for tailoring therapy to specific patients.

Theoretical Model of the Immune System

H. 74. What really is the role of the immune system

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Examined through the multiple and diverse specific lines of research, our understanding of the immune system is formed by an attempt to reconcile several fragments of very narrow observations. As much as this approach has been useful, it has also caused harm because, by being very specific, it tries to assemble the puzzle not by trying the pieces together, but by studying them one by one, making it very difficult to grasp the big picture: what is the role of the immune system? Where does it fit in the puzzle of Life? And what is the driving evolutionary directive that commands it.

Analyzing this question through the perspective of the one characteristic every living organism has in common: being alive, and that life is subject to the same physical laws that command the whole universe, we make a case for redefining the role commonly assigned for the immune system (to protect the organism against foreign invasions), to something more comprehensive that brings several implications

for research and therapeutics: safeguard the energy accumulated by an organism, so it can use it for its own interests and not someone else's, or, in essence, keeping that organism's ΔG negative, which represents the basic definition of survival or extinction.

This new context allows for a new, wider and more logical contextualization of life and immunity and, we think, a useful and evolving one.

Reproductive Immunology

W. 119. Tissue Derived Fetal T Cells Secrete Labor Cytokines in Response to Antigen at the Fetal-Maternal Interface

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In healthy pregnancies, labor is marked by the well-defined transition to an inflammatory state in reproductive tissues. It has been postulated that this increase in inflammation is the result of maternal loss of tolerance towards the fetus. However, recent work has discovered central memory T cells in cord blood of preterm but not term infants that are capable of secreting cytokines in response to maternal antigens suggesting that they might play a role in initiating preterm labor. To address directly if fetal T cells can be found at the fetal-maternal interface that might contribute to the initiation of parturition, we performed deep immunophenotyping and functional analysis of cells present at the feto-maternal interface. Using mass cytometry (CyTOF) this study has identified diverse T cell populations in the fetal derived tissues (placental villi and fetal membranes), including effector, central and tissue resident memory T cells in the second trimester (17-23 weeks' gestation) and at full term (38-40 weeks' gestation) of healthy pregnancies. Furthermore, upon stimulation with lysed maternal (decidual) components from the same pregnancy, fetal T cells secrete TNFα but don't secrete TNFα when stimulated with third party PBMCs. This result suggests fetal T cells within placental villi respond to antigens present on the maternal side of the interface, but not to blood antigens from unrelated donors. Collectively these findings illustrate a dynamic and functional arsenal of T cells within fetal tissues as early as the second trimester and insinuate that fetal T cells may contribute to inflammation seen during labor.

W. 120. Intravenous Immunoglobulin modulates blood monocyte subsets in women with recurrent gestational failure of inflammatory cause

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Background: Peripheral blood monocytes (pbMo) are essential mediators of vascular remodeling and inflammation. However, little is known on the role of pbMo subsets in recurrent gestational failure of inflammatory cause (iRGF), namely, recurrent miscarriages and recurrent implantation failure after in vitro fertilization.

Objectives: To analyse the modifications in pbMo subsets in iRRF patients induced by IVIg.

Methods: Eleven consecutive patients with iRRF (with expansion of cytotoxic NK cells) were treated with intravenous gammaglobulin (IVIg) during their next gestation. pbMo subsets were studied before and after IVIg administration (0.4 g/Kg) in 200 uL of fresh whole blood by multiparametric flow cytometry. The expression of CCR2, CCR5 and CX3CR1 was analysed n classical CD14++CD16- , intermediate CD14++CD16+ and non-classical CD14+ CD16++ pbMo after gating for size, granulation and HLA-DR+ expression. Informed consent was obtained for all patients.

Results: The frequency of Intermediate pbMo significantly decreased after the IVIg infusion ($p<0.0186$), while no significant changes of classical and non-classical pbMo were noted. After IVIG, a significant increase of the non-classical CCR2+ ($p<0.0098$) and CCR5+ ($p<0.0059$); and of intermediate CCR2+ ($p<0.0137$) pbMo subsets were detected.

Conclusions: IVIg modulated the phenotype of pbMo in iRRF patients, with decreased Intermediate Mo frequency. A differential increased proportions of CCR2+ and CCR5+ chemokines in non-classical and intermediate functional pbMo subsets might play a role in IVIG effects on pregnancy course.

Therapeutics/Pharmacology

H. 130. Sustained engraftment and protein secretion using gene-edited human B cells in humanized mouse models

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Injectable protein or peptide drugs now constitute ~10% of the pharmaceutical market. Recently, we developed a cell-based method to stably deliver protein drugs. To do this, we coupled CRISPR/Cas9-based nucleases with adeno-associated virus for delivery of donor homology templates to safe-harbor loci in human B cells, which we subsequently differentiate into antibody-secreting cells (ASC). A subset of these cells resembled long-lived plasma B cells (CD38hiCD138+), whereas others exhibited phenotypes (CD38+CD138-) not previously associated with longevity. We show that engineered B cells can engraft in recipient immune deficient, NOD/SCID/gc-null (NSG) mice and stably produce human antibody for >1 year. ASCs engineered to express firefly luciferase primarily migrated to the bone marrow, the endogenous location of human long-lived plasma cells. Upon provisioning NSG mice with human cytokines (IL6, and/or BAFF) that promote survival of long-lived plasma cells, we observed substantial increases in antibody production and durability of B cell grafts. BAFF preferentially promoted class-switched, $CD138^+$ plasma cells. In contrast, IL6 promoted surface IgM-expressing $CD38^+CD138^-$ ASCs, as well as $CD138^+$ plasma cells. Finally, quantification of human ASC in the murine bone marrow and

spleen showed that as few as 20,000 engineered cells/recipient was sufficient to maintain IgG titers of 10 ug/mL, levels that could be of therapeutic valuable if achieved using expression of candidate mAb reagents. Together, these studies show that engineered human B cells have the capacity to engraft long-term and function normally *in vivo,* strongly supporting further studies using this novel cell therapy platform for long-term delivery of protein drugs.

H. 132. Anti-inflammatory effect of honey on pulmonary inflammation associated with neutrophil by lipopolysaccharide (LPS) in mice

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Object: Lipopolysaccharide (LPS) has a variety of immune activity and is contained within air and environmental smoke. LPS inhalation also induces neutrophils into the lung and causes lung inflammation in animals. Honey is used as a traditional medicine for colds, skin inflammation but not edible. However, the mechanism of the anti-inflammation activity of honey on lung inflammation are not fully understood. Therefore, we investigated the mechanism of anti-inflammation activity of honey. **Materials & Methods:** 8-10weeks female C57BL/6 mice were used. Mice were inhaled 600μg of Japanese honey (Kyoto Sangyo University) and following 1 day later, mice were inhaled 60μg of LPS by intranasal administration. Alveolar macrophage (AM) and neutrophil (Neu) were obtained by broncho alveolar lavage (BAL) . The expressions of Gr-1 and hydrogen peroxide production were analyzed by flow cytometry. Chemotactic activity for Neu was measured by EZ-TAXIScan. IL-1β and CXCL1 mRNA expressions of AM were analyzed by RT-PCR. **Results:** The number of New was significantly (pConclusion: These results suggested that the mechanism of anti-inflammatory effects by honey due to inhibit the infiltration of Neu to lung via suppression of CXCL1 production from AM. Honey also inhibited Neu functions. Honey may be a candidate as anti-inflammatory medicine.

H. 133. Anti-TNF Treatment Delays Human CD4+ T-cell Activation, Maturation and Proliferation, but does not Confer an Anergic or Suppressive Phenotype

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Anti-TNF antibodies such as adalimumab are widely used in the treatment of immune-mediated inflammatory diseases. We previously demonstrated that CD4+ T-cells from patients with inflammatory arthritis treated with anti-TNF biologics had increased proportions of IL-10 expressing cells. This effect was recapitulated *in vitro* by stimulating CD4+ T-cells from healthy donors in the presence of anti-TNF. Here we show that stimulation of CD4+ T-cells in the presence of anti-TNF resulted in decreased activation and maturation as shown by decreased frequencies of CD25+ and CD69+ cells, and lower CD45RO+ cell frequency. These phenotypic changes were coupled with reduced cell proliferation. Furthermore, analysis of previously generated gene expression datasets of anti-TNF-treated IL-17+ or

IFNg+ CD4+ T-cells revealed changes in multiple pathways associated with cell proliferation and cell cycle. Kinetics experiments further revealed that anti-TNF treatment led to delayed, rather than impaired T-cell activation and maturation. We investigated whether anti-TNF treated CD4+ T-cells acquired an anergic or suppressive phenotype. Anti-TNF treated CD4+ T-cells displayed some hyporesponsiveness upon restimulation but did not differentially affect responder T-cell proliferation or monocyte phenotype, compared to control-treated cells. Anti-TNF treated cells however displayed a reduced ability to induce IL-6 and IL-8 production by synovial fibroblasts and to a lesser degree by monocytes, compared to control-treated cells. Collectively, our findings demonstrate that anti-TNF treatment can result in delayed activation, maturation and proliferation of CD4+ T-cells. Although these cells did not acquire a global suppressive phenotype, they exerted reduced pro-inflammatory effects on synovial fibroblasts and monocytes. Funded by Versus Arthritis (#21139).

H. 134. Aurora Kinase A signaling alters tumor microenvironment to shape tumor evolution in therapeutic resistance

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EGFR-TKI in *EGFR*-mutant NSCLC has been a major breakthrough. However, EGFR-TKIs leads to acquired resistance, often a lethal event. The emergence of immune checkpoint inhibitors that reverse cancer immunosuppression and enhance antitumor immunity but have yielded disappointing results for patients with an *EGFR* mutation. Mutated tumors generally have lower PD-L1 expression and lower mutation burden. Elucidating the factors that drive the lack of sensitivity to checkpoint inhibitors is a crucial issue, and novel combination approaches are urgently needed in this patient population.

To address this, we have developed the in-vitro models of acquired resistance (AR models). RNA-seq analysis of these AR models revealed lower antigen presentation, alteration PD-L1 expression and EMT gene expression in tumor cells. We further used these AR models to identify therapeutic strategies that could overcome resistance. This drug screen revealed that aurora kinase inhibitors exhibited strong synergy with EGFR-TKI to abrogate cell proliferation and induced potent apoptosis. Moreover, this novel combination altered the expression of certain immunomodulatory genes and hence have a capacity to restore the efficacy of immune response.

In summary, we identified the AURKA axis as a molecular driver behind the evolution of drug resistance and induced an alteration in the immune environment which are hallmarks of cancer. Moreover, abrogation of AURKA can serve as a means to alter tumor environment to prevent the tumor evolution. This preclinical insight dictates the unique way to steer the evolution of resistance towards more targetable manner by upfront use of rational polytherapy in the clinic.

H. 135. CD19+ IL-10+ changes after bacterial suspension treatment in patients with allergic conjunctivitis.

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Bacteriallysates (BL) had been used as immunomodulators in asthma and allergic rhinitis. No data is available for bacterial suspensions (BS) and ocular allergy. Previously, we observed that peripheral blood mononuclear cells (PBMC) from healthy subjects stimulated with BS increase the percentage of CD19 +IL-10 + cells in vitro. This finding is relevant since it has been reported that patients with ocular allergy had low expression of IL-10 in B circulating cells. Thus, the aim of this work was to evaluate the percentage of IL-10+ producing B cells in patients with allergic conjunctivitis treated with a commercial BS formulation (IPI). The clinical study was approved by the local Ethical board, and patients included were treated according to manufacturer instructions. After 3 months of treatment with BS, patients increased 9.8 times the percentage of circulating CD19+CD10+ cells (p=0.01) and decreased 3 times the ocular severity score when compared with the beginning of treatment (p=0.008). Our results suggest that adding BS to the standardized ophthalmological treatment could be a new therapeutic tool to modify pathological immune response seen in ocular allergy by inducing positive changes in the percentage of circulating IL-10+ B cells and favoring a better clinical outcome in patients with allergic conjunctivitis.

T. 121. Chimeric Antigen Receptor-mediated Control of Human Regulatory T Cell Function

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Chimeric antigen receptor (CAR) technology has propelled T cell engineering. CAR T cells now represent the most promising strategy to treat several cancers. CD19 CARs with either a CD28 or a 4-1BB cytoplasmic domain fused to CD3z have been approved in the clinic. Regulatory T cells (Tregs) offer great promise as next-generation adoptive cell therapies for autoimmune disorders, transplant rejection, and graft-versus-host disease (GvHD). Yet, utilizing CARs to redirect Tregs remains largely unexplored. Here, we generated CD19 CAR Tregs featuring different signaling domains. 28z and 4-1BBz CAR Tregs expressed high levels of Treg markers, did not secrete IL-2, displayed low levels of Treg-specific demethylated region (TSDR) methylation, and suppressed T cell proliferation *in vitro*. Unexpectedly, CAR-mediated Treg activation also induced expression of perforin and granzyme B, as well as inflammatory cytokines, namely IFN-g and TNF-a. When coadministered with CD19-expressing leukemia cells and bulk T cells into NSG mice, CD19 CAR Tregs suppressed T cell proliferation and prevented GvHD. Surprisingly, however, CAR Tregs also controlled CD19-expressing tumor growth for several weeks with potencies comparable to bulk CAR T cells. Bulk T cell or recombinant IL-2 infusion did not prevent CAR Treg-mediated tumor control. Yet, this phenomenon was dependent on CAR costimulation, as z CAR Tregs failed to control tumor growth in the presence of bulk T cells. Altogether, these data suggest that CAR activation can activate a cytotoxic program in Tregs, making them effective hematological tumor killers *in vivo*. Experiments dissecting the mechanism of CAR Treg-mediated tumor killing are ongoing.

T. 122. Comparison of C57Bl/6, NSG-PBMC, CD34-NSG, and SGM3 mice models for immune checkpoint receptor studies

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Comparison of C57Bl/6, NSG-PBMC, CD34-NSG, and SGM3 mice models for immune checkpoints.

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Immune checkpoint receptors have become an important class of therapeutic targets. Intensive research has found major differences between mice immune system and human immune system. In order to identify suitable models for particular research purpose, this study compared C57Bl/6 with complete mice immune system, NSG-PBMC that has mostly human T cells engrafted, CD34-NSG that has most human immune cell compartments and SGM3 that introduced human cytokines as transgenes. A model based on original findings from Tasuku Honjo and co demonstrated anti-viral efficacy of commercial immune checkpoint receptor antagonist in C57Bl/6. Similar responses are observed in NSG-PBMC.

We also looked at various immune cell compartments of CD34-NSG and SGM3 by flow cytometry analysis. Major differences between the two models have been identified including the abundance of Treg and pDC. Expression of several checkpoint receptors have been examined in different immune cell compartments of these models. These findings provide useful insights that will expedite immune checkpoint research in both Onco-Immunology and Autoimmunity.

T. 123. Discovery and Characterization of PD-1 Agonist Antibodies

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T cell function is regulated by complex signaling networks of interconnected activators and inhibitors. Blockade of inhibitory receptors such as PD-1 has emerged as a novel treatment for multiple forms of cancer. Rather than block the interaction of PD-1 with its endogenous ligands, we sought to develop antibodies that can activate this pathway to inhibit T cell activation and function.

We conducted our antibody screen against human, cynomolgus monkey, and mouse PD-1 to select for antibodies with species cross-reactivity. We then conducted functional screens for antagonism and agonism using a human PD-1 Jurkat reporter cell-based screen. We identified several classes of PD-1-specific antibodies with a range of functional activities. Approximately one-third of antibodies exhibited antagonist activity when in solution, but agonist activity when immobilized. Less than 1% of antibodies were antagonists in solution with no agonist. The observation that most PD-1 antibodies that are

antagonists in solution also function as agonists when immobilized suggests a fundamental relationship between agonism and antagonism of the PD-1 pathway. Of particular interest to us were antibodies that displayed agonist activity yet did not antagonize PD-1/PD-L1 interactions. We further characterized the ability of this subset of antibodies to inhibit function of primary T cells.

In summary, we identified several classes of PD-1 antibodies that antagonize and/or agonize human and mouse PD-1 when in solution and/or when immobilized. We believe the subset of antibodies that can agonize PD-1 and attenuate T cell activation create an opportunity for developing new therapeutics for autoimmune and inflammatory diseases.

T. 124. Effects of Toll-Like Receptor 4 Protein Expression and Trait Anxiety on Adolescent Mesocorticolimbic Neuroplasticity in Amphetamine-Induced Locomotor Sensitization.

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The central neuroimmune microenvironment plays a regulatory role in substance use disorders. Clinical populations that experience issues with anxiety also show high comorbid rates of substance abuse. We hypothesize that exposure to drugs of abuse initiates a proinflammatory cascade that first activates toll-like receptors which results in increased levels of proinflammatory cytokines and other innate immune factors that are thought to contribute to mesocorticolimbic neuroplasticity. The present study examined differences in toll-like receptor 4 (TLR4) protein levels in adolescent male Long-Evans rats (N=30) phenotyped as showing high (HAn) and low (LAn) anxiety-like behavior after exposure to an amphetamine sensitization regimen. Anxiety phenotypes were screened using the elevated plus maze (EPM), and values on the EPM measured included percent open arm (OA) entries and time spent on the OA. We employed a quartile analysis on the EPM values, with the upper quartile categorized as LAn, and values in the lower quartile categorized as HAn. Male HAn adolescents exhibited the most hyperactivity to the locomotor-activating effects of acute and 4-day amphetamine (4.0 mg/kg IP). Male HAn lines displayed the greatest locomotor response to a low challenge dose of AMPH (1.0 mg/kg IP) after a 7-day withdrawal period. Evaluation of TLR4 protein levels are currently underway, but preliminary analysis provides evidence for greater TLR4 protein levels in the amygdala of HAn adolescent males. HAn profiles map on to amphetamine sensitivity and may be a viable tool for investigations of the role of neuroinflammation in mediating the neuroplasticity important for sensitization and relapse vulnerability.

T. 125. Engineered FVIII specific murine CAR Tregs with optimized signal strength demonstrate enhanced in vivo efficacy

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Hemophilia A (coagulation factor FVIII deficiency), inherited as an X-linked recessive trait, is the most prevalent genetic bleeding disorder worldwide (1 in 5000 male births). Conventional treatment involves intravenous replacement therapy with recombinant or plasma derived clotting factor, which poses a risk for developing anti-drug antibodies (inhibitors) in up to 30% of severe patients. Inhibitors largely neutralize the infused replacement clotting factor, thus limiting availability, increase the risk for morbidity and mortality and may also cause immunotoxicities. Immune tolerance induction to eradicate inhibitors has a significant failure rate. Inhibitor development is $CD4^+$T helper cell dependent and tolerance via recruitment of regulatory T cells (Tregs) represents a potential approach to control inhibitor development. Here we evaluated cellular therapy with antigen-specific Tregs engineered to express a murine 2^{nd}generation chimeric antigen receptor (CAR) specific to human FVIII. FVIII CAR-Tregs were activated and proliferated in response to soluble recombinant hFVIII, secreting key cytokines in vitro. Activation induced cell death (AICD) was observed in a significant percent of stimulated T_{eff} cells, while Tregs were more resistant to AICD. Introducing specific mutations in immunoreceptor tyrosine-based activation motifs (ITAMs) in CD3z was able to improve AICD. Ex vivo expanded FVIII CAR-Tregs, when adoptively transferred into mice with a targeted deletion in exon 16 that rendered them deficient in endogenous FVIII (BALB/$c^{e16-/-}$hemophilia A mice), prevented the formation of inhibitors. Studies are ongoing to test whether cellular therapy with FVIII CAR-Tregs can tolerize mice that have already established inhibitors, which would more closely model clinical disease in patients.

T. 126. Essential Role for the RHO-KINASES in Intestinal Stem Cell Viabilty and Maintenance of Organ Homeostasis

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The Rho-kinases, Rock1 and Rock2, regulate cell shape/cytoskeletal rearrangement downstream of the RhoA small GTPase. These processes are critical to many cellular functions including contraction, proliferation, motility, and viability. As aberrant kinase activity is seen in cardiovascular and autoimmune diseases and cancers, the development of selective inhibitors is an active area of pharmaceutical research. While acute perturbation of this pathway is tolerated, it is unclear whether Rho-kinases have homeostatic functions, precluding blockade for chronic conditions. Here, we used inducible gene targeting to ablate *Rock1* and *Rock2* individually or together in adult mice, and found an obligate requirement for these enzymes in maintaining stem cell viability and proliferative capacity. *Rock1*$^{flox/flox}$, *Rock2* $^{flox/flox}$, or *Rock1* $^{flox/flox}$: *Rock2* $^{flox/flox}$ mice were crossed to the Rosa26-Cre ERT2 line to delete the floxed alleles with tamoxifen. Loss of either allele did not impact survival, indicating functional redundancy between the kinases, however combined deletion caused rapid mortality. Histological evaluation revealed tissue homeostasis was disturbed in organs with rapid cell turnover and renewal, such as the alimentary tract and lymphoid tissues. Particularly, crypt regeneration within the small intestine was severely compromised, impeding nutrient absorption, promoting systemic inflammation and subsequent lethality. Mutant intestinal stem cells displayed mitotic arrest due to defective cytokinesis, signaling cell death. Similarly, cycling of hematopoietic stem cells was blocked, causing severe cytopenia. Thus, given this fundamental role in stem cell renewal and tissue homeostasis, Rho-kinase inhibition is not a viable therapy for chronic diseases.

T. 127. Immunogenicity and Vaccine Potential of Tag-free Recombinant BmHAXT against Brugia malayi Parasite in a Mouse Model

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Lymphatic filariasis (LF) is a profoundly disfiguring human disease caused by filarial parasite and transmitted through infected mosquitoe bite. Currently, there are no licensed vaccine to control LF. The r*Bm*HAXT vaccine protein prepared in our laboratory is expressed as a His-tagged protein. FDA doesn't allow tags in any recombinant vaccine developed for human use. In this study we determined if tag removal has any effect on the protein structure and vaccine efficacy. We expressed r*Bm*HAXT protein with a TEV cleavage site in a bacterial expression system. Using TEV protease, cleaved the His-tag from the protein. Western-blot analysis using anti-His HRP antibodies and anti-r*Bm*HAXT antibodies demonstrated that we were able to generate tag-free r*Bm*HAXT. Circular-Dichroism spectroscopy analysis showed that the structure and folding of tag free r*Bm*HAXT protein is like His-tagged r*Bm*HAXT. We immunized three groups of 10 mice each with AL019 or His-tag r*Bm*HAXT or tag-free protein. Mice were immunized four times at 2 weeks interval. The tag-free and His-tag r*Bm*HAXT immunized mice generated comparable and significant titers of antigen-specific IgG antibodies. There were no significant differences in the levels of anti-r*Bm*HAXT IgG isotype antibodies in the sera of tag-free or His-tag r*Bm*HAXT immunized mice. Antibody dependent cell mediated cytotoxicity assay showed that the protective antibodies in the sera from tag-free r*Bm*HAXT and His-tag r*Bm*HAXT vaccinated mice killed 100% and 97.52 % of *B. malayi* L3 respectively. The findings from this study showed that removal of His-tag did not alter the protein structure, immunogenicity and vaccine potential of r*Bm*HAXT.

T. 128. MAP7 and MUCL1 are Biomarkers of Vitamin D3-Induced Tolerogenic Dendritic Cells in Multiple Sclerosis Patients

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Background: Cell therapy with autologous tolerogenic dendritic cells (tolDC) is a promising alternative, alone or in combination with other drugs, for the treatment of autoimmune diseases, such as multiple sclerosis (MS). The use of vitamin D3 for the generation of tolDC (vitD3-tolDC) constitutes one of the

most widely studied approaches, as it has evidenced significant immune regulatory properties, both *in vitro* and *in vivo*.

Objective: To identify and validate differentially expressed genes (DEG) in vitD3-tolDC from healthy donors and MS patients, as biomarkers for tolerance-induction in these DC.

Methods: Cell cultures of monocyte-derived immature (iDC), mature (mDC) and vitD3-tolDC from 24 healthy donors and 10 MS patients were performed. Subsequently, we selected —from a previous microarray study— and validated by qPCR the expression of several DEG in vitD3-tolDC compared to both iDC and mDC conditions. Finally, we studied the functional relevance of these genes by constructing a network of protein interactions based on the available literature.

Results: Our vitD3-tolDC exhibited a semi-mature phenotype, secreted IL-10 and inhibited allogeneic lymphocyte proliferation. Our results validated *CYP24A1*, *MAP7* and *MUCL1* genes as biomarkers of vitD3-tolDC in samples from both healthy donors and MS patients. Furthermore, the analysis of the functional network evidenced that *MAP7* and *MUCL1* genes are closely connected between them and involved in immune-related functions.

Conclusions: Our study shows that *MAP7* and *MUCL1* constitute robust and potentially functional biomarkers of vitD3-tolDC, opening the window for their use as quality controls in clinical trials for MS.

T. 129. Promising multifunctional peptide isolated from the Mexican tree frog Pachymedusa dacnicolor in the treatment of psoriasis

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Inflammatory and antimicrobial diseases are a major burden for the society today and fighting them is a national and WHO strategic priority. Nowadays, most of the treatments available on the market to fight inflammatory diseases are anti-inflammatory drugs, such as corticosteroids or immunomodulators that lack cellular specificity and lead to numerous side effects. However, in addition to suppressing undesired inflammation and reducing disease progression, these drugs lessen the immune system protective functions. Furthermore, treating infectious diseases is more and more challenging, due to the increase of microbial resistance to antimicrobial drugs.

Psoriasis is a lifelong chronic inflammatory disease with no curative treatments available, where inflammation and angiogenic processes and bacterial infection play a major role in its development. Thus, specifically controlling the inflammatory and angiogenic processes without compromising the ability of the body to combat infections is an essential feature of the treatment of psoriasis.

In this study, we characterized a new peptide isolated from the Mexican tree frog, *Pachymedusa dacnicolor* that exhibits at the same time, anti-inflammatory, anti-angiogenic, and antimicrobial properties. Indeed, *in vitro*, this peptide specifically inhibits the development of angiogenesis, kills immune cells without compromising the integrity of non-immune cells and kills Gram-positive and Gram-negative bacteria. *In vivo*, this peptide reduces disease progression in the preclinical imiquimod-induced murine model of psoriasis, by inhibiting the formation of neo vessels and psoriatic skin. Thus, our peptide could be a promising drug in the treatment of psoriasis.

Funded by *Fronteras de la Ciencia*/CONACYT, Mexico

T. 130. Role for CD45 Phosphatase in Multiple Myeloma Plasma Cell Proliferation and Survival

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Multiple myeloma (MM) is a B-cell malignancy characterized by abnormal growth and proliferation of plasma cells within the bone marrow. While signals originating from the bone marrow microenvironment are crucial for MM cell growth, explorations into the molecular mechanisms governing MM proliferation have been inadequately addressed. However, results from our lab and others demonstrated the importance of T helper signals in B-cell malignancies and in potentiating BCR signaling via upregulation of CD45 phosphatase activity. Thus, we hypothesized CD45-dependent signaling may be an important factor mediating MM plasma cell growth and proliferation.

To test our hypothesis, we used multiplexed phosphoflow to define CD45 regulation in plasma cells obtained from bone marrow aspirates of MM patients. Preliminary results demonstrated malignant plasma cells had increased CD45 activity with paradoxically less CD45 expression relative to control plasma cells. A potential CD45 ligand, galectin-1, was upregulated in MM plasma cells suggesting enhanced CD45 activity resulted from increased galectin-1:CD45 interaction. Confocal microscopy performed on healthy B cells demonstrated colocalization between CD45 and galectin-1, corroborating galectin-1 as a potential CD45 ligand. Recapitulation of T cell help additionally increased CD45 activity in MM cells and correlated with increased proliferation. In a similar set of experiments, inhibition of CD45, or of its potential ligand galectin-1, reduced the proliferative capacity of MM cells.

Together, our results highlight the importance of T helper cell dependent upregulation of CD45 activity via galectin-1 as a potential molecular mechanism governing MM cell survival. The CD45:galectin-1 interaction warrants further investigation as therapeutic targets.

T. 131. Selective Expansion of Regulatory T cells by IL-2 Muteins

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Regulatory T cells (Treg) play a critical role in immune homeostasis and are dysfunctional in many autoimmune diseases. Interleukin 2 (IL-2) via the heterotrimeric IL-2 receptor drives the proliferation and function of Treg and IL-2Ra/CD25 loss-of-function in mice is associated with Treg deficiency and widespread autoimmunity. Low dose IL-2 expands Treg and is being evaluated as a therapy for patients with autoimmune diseases. However, IL-2 can also activate other immune cells including conventional T cells and Natural Killer (NK) cells which express IL-2Rb/CD122 and IL-2g/CD132. To enhance IL-2 selectivity for Treg, mutations can be introduced that increase the affinity for CD25and decrease affinity for CD122/CD132. IL-2 Muteins with these properties are able to selectively activate and expand Tregs. Here we describe the activity of PT101, an IL-2 mutein cytokine Fc fusion protein that selectively induces STAT5 phosphorylation downstream of the IL-2R in human and cynomolgus monkey Tregs in vitro. In humanized NOD-scid IL2Rg-null (NSG) mice, PT101 expands Treg without significant effects on other immune cell types and without inducing pro-inflammatory cytokines. Treg from PT101-dosed humanized mice have increased expression of FOXP3 and CD25, suggesting enhanced function and stability. In cynomolgus monkeys, single dose administration of PT101 dose-dependently and selectively expands Treg.

T. 132. Targeting the Epichaperome as an Effective Precision Medicine Approach in a Novel PML-SYK Fusion Acute Myeloid Leukemia

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The epichaperome is a new cancer target defined by changes in the interaction strength between chaperone and cochaperone proteins to form stable hyperconnected networks that support oncoprotein stability and are vital for tumor. Cancers with this altered chaperone configuration may become susceptible to drugs that target the epichaperome, such as the inhibitor PUH71. We developed a novel flow cytometrybased test, the PUFITC binding assay, to evaluate epichaperome levels at the single cell level and successfully identified a patient who has a potential to respond to PUH71 treatment.
A 61-year-old woman was diagnosed with myeloproliferative neoplasm in 2013. After treatment with hydroxyurea, in 2013 she underwent a MUD transplantation. In 2016, she relapsed with atypial GvHD symptoms and mixed chimerism, and progressed to refractory AML in 2017. Patient-derived cells were found to harbor a novel fusion gene, PML-SYK, generated by translocation (9;15) and were constitutively activated of Syk, Stat5, Erk and ribosomal S6 kinase. Elevated epichaperome levels were found in the cell populations bearing PML-SYK, suggesting sensitivity to PUH71. In vitro, treatment of patient's cells

with PUH71 resulted in cell death and decreased colony formation. Based on laboratory data, the poor prognosis, and lack of effective therapies, the patient was granted compassionate access to this medication by the FDA. After 16 doses of PUH71 over 3 months, the patient has attained complete remission, with normalization of peripheral blood counts.
This refractory AML patient is presently in remission and the assay is being used to screen other potential patients.

T. 133. Two in vitro assays for evaluating the potency and efficacy of prospective drugs in multiple diseases involving both classically and alternative activated macrophages

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Monocytes and macrophages are critical regulators of the innate immune response. Classically activated macrophages (M1) are pro-inflammatory cells, which promote Th1 responses and tumoricidal activity. Alternatively activated macrophages (M2) have anti-inflammatory functions and elicit tissue repair, fibrosis, tumor growth and progression. We have developed off-the-shelf *in vitro* macrophage polarization assays using human primary blood derived cells to assess the translational potential of small molecules as novel therapies.

Blood-derived human CD14+ cells were cultured in presence of M-CSF for 5 days to accommodate differentiation into M0 macrophages, and subsequently treated with LPS or IL-4+IL-10 to induce polarization into M1 and M2. Inhibition of polarization was assessed by adding compounds one hour prior to induction of polarization and subsequent measurement of TNF-α secretion and CD80 expression as markers of M1 polarization and CCL-18 secretion and CD206 expression as markers of M2 polarization.

Exposure of M0 macrophages to LPS resulted in increased CD80 expression and TNF-α secretion. LPS-mediated TNF-α secretion is strongly inhibited by dexamethasone and prednisolone with consistent IC50 values across donors. Exposure of M0 macrophages to IL-4+IL-10 resulted in increased CD206 expression and CCL18 secretion. IL-4/IL-10-mediated CCL18 secretion is fully inhibited by tofacitinib with consistent IC50 value across donors.

Our data show that both the M1 and M2 polarization assays are robust and can serve as reliable tools for evaluating the potency and efficacy of prospective drugs in multiple diseases associated with classically or alternatively activated macrophages, such as fibrosis, rheumatoid arthritis, inflammatory bowel disease and cancer.

Transplantation

W. 121. Early expansion of TEMRA CD8 with innate-like function identifies kidney transplant recipients at high-risk of graft failure

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As CD8 TEMRA cells are associated with higher risk of long-term graft dysfunction, in this study, we evaluate if the monitoring of CD8-related biomarkers could improve the prognostic capacities of a clinical-based scoring system (Kidney Transplant Failure Score; KTFS). We also characterizethe functionality of TEMRA and especially their reactivity upon donor-specific stimulation. 286 kidney-transplant recipients prospectively enrolled were followed for more than 8-years. 51 return in dialysis. We demonstrate that the frequency of early memory CD8 cells (EM) and TEMRA measured at 1-year post-transplantation is correlated with the risk to return in dialysis during time. For patients at high-risk of long-term graft dysfunction (according to KTFS), the use of one-year TEMRA frequency allows the discrimination of patients that will lose their graft from those that will not. Donor-specific reactivities from TEMRA and EM were similar with an early expression of $CD25^{+}CD69^{+}CD107a^{+}$and the high secretion of pro-inflammatory and cytotoxic molecules. Importantly, we identify an innate-like signature of TEMRA, with more than 5-fold higher expression of FCGR3A (CD16) by TEMRA as compared to NAIVE and EM. Cross-linking of CD16 triggers the secretion of TNFa and IFNg by TEMRA and their cytotoxic function and was further enhanced by the provision of IL-15. Finally, we demonstrate TEMRA and not EM display *in vitro*Antibody Dependent Cell Cytotoxicity conferring to TEMRA features of both adaptive and innate-like immunity and showing that anti-HLA antibodies, a major risk factor for long-term allograft outcome, could activate TEMRA in a TCR-independent manner leading to the inflammatory response.

W. 122. Generation of CD8+ and CD4+ Regulatory T Cells from Human Pluripotent Stem Cells

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Organ or cell transplantation is the only therapeutic solution for pathologies causing an irreversible loss of vital organs function. The development of novel specific and non-toxic anti-rejection immunotherapies is a major goal in transplantation. Strategies based on regulatory T cells (Tregs) are promising. However, Tregs cell-based therapies have been hampered by the technical limitation of obtaining large batches of functional Tregs. The project aim is to obtain an unlimited number of Tregs from human pluripotent stem cells (hPSCs). We have developed a new differentiation protocol in two separate steps: the first step is to generate hematopoietic stem cells (HSCs) from hPSCs and the second is to engage HSCs towards the lymphoid lineage. We have generated HSCs thought embryoid bodies (EBs) formation for 9 days. After 9 days, we obtained 50% of cells expressing $CD34^+$, a key marker of HSCs. Then, we dissociated EBs and co-cultured them onto OP9-DLL1 to induce T-lymphoid differentiation for 26 days. At day 10 of co-culture, we transduced our cells with a lentivirus encoding FOXP3, a known master transcription factor of Tregs. FOXP3 transduction at day 10 of co-culture resulted in significant differentiation of $Foxp3^+CD3^+TCR\alpha\beta^+CD8^+$ or $CD4^+$Tregs cells until day 26. To our knowledge, it's the first time that CD4+ and CD8+ Tregs have been differentiated from hPSCs. Moreover, our result support that human $CD4^+$ and $CD8^+$Tregs differentiation would be under the control of Foxp3. Our findings open new possibilities for cell therapy.

W. 123. A TCR Sequencing Approach to Study the Dynamics of Alloreactive and Autoreactive T Cells Following Liver Transplantation for Autoimmune Liver Disease

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Primary sclerosing cholangitis and autoimmune hepatitis are poorly-understood autoimmune liver diseases (ALD). Liver transplantation (Ltx) is the only treatment option once ALD progresses to end-stage liver disease. In ALD Ltx there is overlap in diagnostic findings for recurrent disease, rejection and biliary complications. There is an urgent need for a way to clearly distinguish alloimmune from autoimmune processes so that an appropriate diagnosis/treatment can be provided. We hypothesized that we could use high-throughput sequencing (HTS) of the TCR CDR3 β-chain to distinguish disease

recurrence from allograft rejection after Ltx for ALD. We identified pre-transplant donor-reactive T cell clones (DRTCC) using the CFSE MLR method previously described (STM, Vol. 7, Issue 272, pp. 272ra10) and pre-transplant putative autoreactive T cell clones (ARTCC) by sequencing lymphocytes isolated from native liver. Using tissue resident memory T cell markers, we demonstrated very little contamination of the T cell population extracted from the native liver by circulating T cells. Unexpectedly, we found a predominance of ARTCC compared to DRTCC in post-transplant liver biopsies diagnosed as ACR in 3 ALD patients. In the post-transplant stool/bile for 1 patient there was a predominance of ARTCC. In conclusion, we are able to identify, characterize, and track ARTCC and DRTCC in post-transplant specimens of ALD Ltx recipients. The greater frequency of ARTCC than DRTCC in 3 patient biopsies diagnosed as ACR suggests that an autoimmune attack on the donor graft may be misdiagnosed as rejection in liver transplantation.

W. 124. Blood CD9+ B Cell, a Biomarker of Brochiolitis Obliterens Syndrome After Lung Transplantation

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Background: Bronchiolitis obliterans syndrome is the main limitation for long-term survival after lung transplantation. Recent data suggest that some specific B cell populations are associated with long-term graft acceptance and may serve as biomarkers in other organ transplantation. We aimed to monitor B cell profile during early development of bronchiolitis obliterans syndrome after lung transplantation.

Methods: B cell longitudinal profile was analyzed in peripheral blood mononuclear cells from patients with bronchiolitis obliterans syndrome and patients who remained stable regarding pathology development after lung transplantation.

Results: $CD24^{hi}CD38^{hi}$ transitional B cells were only increased in stable patients to reach a peak 24 months after transplantation, whereas remaining unchanged in patients who developed a bronchiolitis obliterans syndrome. We showed that these $CD24^{hi}CD38^{hi}$ transitional B cells specifically secrete IL-10 and express the CD9marker. Thus, patients with a total $CD9^{+}$ B cell frequency below 6.6% displayed significantly higher incidence of bronchiolitis obliterans syndrome.

Conclusions: These data are the first to associate IL-10-secreting $CD24^{hi}CD38^{hi}$ transitional B cells expressing $CD9^{+}$ with better allograft outcome in lung transplanted patients. The identification of CD9-expressing B cells as a contributor to a favorable environment essential for the maintenance of long-term

stable graft function and as a new predictive biomarker of bronchiolitis obliterans syndrome–free survival provide new strategies for treatment of the pathology and its prediction.

W. 125. BMP signaling pathway modulation promotes tolerance in GvHD and prevents colitis.

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Graft-versus-host disease (GvHD) is a major complication of allogeneic hematopoietic stem cell transplantation (HSCT). Current strategies to prevent GvHD with immunosuppressive drugs carry significant morbidity and may affect the graft-versus-tumor (GVT) effect. We hypothesized that neutralization of BMP signaling using a monoclonal antibody would suppress GvHD and ameliorate immune disorders *in vivo*. RGMb expression was significantly increased in murine small intestine at 24h post total body irradiation. In an HSCT murine model, 3 doses of anti-RGMb antibody but not the isotype control protected the recipient mice against Tcon-induced GvHD improving survival rate (75% versus 30% at 60 days post-transplantation), while the GVT effect remains intact. Allogeneic Tcon transplantation induced cytokine expressions of IFN-gamma, BMP2, and BMP4 in small intestine tissue. The expression was mitigated by anti-RGMb therapy via induction of anti-inflammatory IL-10 expression. Bioluminescence imaging showed that anti-RGMb treatment reduced Tcon proliferation *in vivo*, but also, enhanced $CD4^+$ versus $CD8^+$ T cells polarization. The phenotype was further confirmed *in vitro* where anti-RGMb treatment reduced both naïve $CD4^+$ and naïve $CD8^+$ T cell proliferation in a mixed lymphocyte reaction assay. We also evaluated blocking anti-RGMb antibody therapy in Inflammatory Bowel Disease (IBD). Anti-RGMb antibody in dextran sulfate sodium (DSS) treated-mice prevented body weight loss for a least 8 days, improved survival, and also normalized the colon length unlike with isotype control antibody treated mice. Therefore, blocking anti-RGMb antibody therapy can prevent GvHD following HSCT but also reduce autoimmunity.

W. 126. Cellular Desialation Promotes Xenogeneic Neutrophil-Endothelial Adhesion via Galectin-3

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Xenotransplantation is a rapidly growing scientific field whose goal is to solve the shortage of organs available for transplantation by using pig organs in place of human organs. However, acute neutrophil sequestration is consistently associated with xenograft lung injury, despite genetic modifications of the donor organ. Sialic acid levels regulate cellular adhesive mechanisms in inflammatory or tumor

environments. Here we asked if sialic acid removal promotes xenogeneic neutrophil adhesion and examined the role of galectin-3.

The adhesion of human neutrophils to genetically modified (GTKO.hCD46) pig aortic endothelial cells (pAECs) was measured in static and microfluidic flow chamber conditions (Bioflux, 1dyne/cm^2) in presence of various treatments.

Desialation of pAECs or calcein^{AM} labelled neutrophils with *Clostridium perfringens* neuraminidase (NA, 25mU/mL) increased neutrophil adhesion (36% vs. 22%, $p<0.0001$). In contrast, pan-neuraminidase inhibition decreased neutrophil adhesion: treatment of pAECs with 2-deoxy-NANA (DANA, 1mM) almost completely prevented adhesion, whereas treatment of neutrophils with DANA reduced adhesion by ~60%. N-acetyllactosamine, which inhibits galectin binding, decreased neutrophil adhesion to NA-pretreated pAECs (14% vs. 36%, $p<0.0001$). Pig galectin-3 mRNA levels were increased after lung perfusions. Incubating both pAECs and neutrophils with anti-human galectin-3 antibody completely abrogated NA-dependent adhesion to human tumor necrosis factor-treated (25ng/mL) pAECs (NA/gal-3: 22% vs. NA: 66%, no NA: 30%, $p<0.0001$).

Modulation of cellular sialation regulates human neutrophil adhesion to pAECs, such that inhibiting sialidase activity may prevent neutrophil-mediated inflammation and tissue injury in xenogeneic models. This work further identifies galectin-3 as a candidate for therapeutic interventions to limit neutrophil adhesion in xenotransplantation models.

W. 127. Expansion of Highly Purified Functional Alloantigen-specific Tr1 Cells for Therapeutic use in Kidney Transplanted patients

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The use of longterm immunosuppression leads to downside side effects for transplanted patients, including drug toxicity, increased susceptibility to infection and development of neoplasies. Recent reports have supported the use of Tr1 cells, a subset of Foxp3- regulatory Tregs, as one of the best candidates for use in new therapeutic protocols, based on their high production of IL-10. Tr1 cells can be identified by the co-expression of CD49b and LAG-3, which facilitates their purification. A recent protocol based on the co-culture of naïve T cells with allogeneic DC-10, which coexpress the co-inhibitory molecules ILT4 and HLA-G and allows efficient Tr1 differentiation in vitro, has been approved for clinical trials. However, recent data indicate that this subpopulation is heterogeneous and that only a proportion of CD49b+ LAG3+ cells actually express IL-10. Here, we show an improved method to increase the purity of functional allospecific Tr1 cells which maintain their phenotype and function. Monocyte derived dendritic cells were differentiated in the presence of GM-CSF, IL-4 and IL-10 for 8 days, obtaining more than 80%

of ILT4+/HLA-G+ DC (DC10). Co-cultures of DC10 with sorted CD4+ CD45RA+ CD25- allogeneic T naïve cells (1:10) resulted in 30% of CD4+CD49b+LAG-3+ cells after 9 days in culture, which were sorted by FACS and polyclonally expanded, leading to an enrichment of 90% of CD49b+LAG-3+ cells. These cells were able to suppress 40% of the proliferation of allogeneic CD4+T cells, but not of polyclonal T cells. We are currently investigating the stability of these Tr1 under different inflammatory conditions.

W. 129. Immunologic Impact of Tocilizumab Treatment in Kidney Transplant Recipients with Allograft Inflammation

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Background: Proinflammatory cytokine IL-6 has an important role in regulating the balance between Th17 cells and regulatory T cells (Tregs). We studied the immunological impact of tocilizumab (TCZ), a monoclonal antibody to IL-6R, in kidney transplant recipients with subclinical graft inflammation.
Methods: Patients were prospectively enrolled in a randomized controlled clinical trial (2014-2018). PBMCs were collected at baseline, 3, 6, 9 and 12 months from stable kidney transplant recipients on tacrolimus/MMF/±prednisone with subclinical inflammation receiving TCZ (8 mg/kg IV q4 weeks X6) or no treatment (controls). PBMCs (N=28, 14 in each arm) were analyzed with respect to the Treg population, T cell activation, and cytokine (IFN-γ and IL-17) production after *ex vivo* PMA/Ionomycin stimulation.
Results: Mean frequency of CD4+CD25+Foxp3+ Tregs was similar in the 2 groups at enrollment (4.3% ± 0.83 vs 5.1% ± 0.63, p= 0.37). At 6 months, the Treg frequency had increased (+47%) in TCZ group and decreased (-29%) in control group (p=0.02). Percentage of naïve, central memory, effector memory, TEMRA remained stable. Patients in TCZ group showed a profound decline in IFN-γ (-34%) and IL-17 (-59%) production by CD4+ T cells when compared to control group at 6 months. IFN-γ/ IL17 double producing CD4+ T cells also showed a significant decrease in TCZ group (-63% vs.+40%, p=0.009).
Conclusion: Tocilizumab treatment for 6 months significantly increased circulating Tregs and suppressed inflammatory cytokines (IFN-γ and IL-17). The favorable change in Treg to Th17 and Th1 balance suggests that tocilizumab is a promising option for controlling allograft inflammation.

W. 130. Immunoprotection of skin Allografts with Indoleamine 2,3-dioxygenase

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Background: Despite the effectiveness of skin autotransplantation, the high degree of immunogenicity of skin precludes the general 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 immunosuppression. 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 immunosuppression 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 the 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 demonstrate 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 the control group.

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 immunosuppression can also provide potential opportunities to autoimmune skin disorders such as Alopecia Areata.

W. 131. Immunosuppressant-resistant cytomegalovirus-specific T cells for advanced adoptive T-cell therapy in immunosuppressed patients

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Some patients suffer from life-threatening complications caused by normally harmless chronic viruses such as cytomegalovirus (CMV) as adverse effect of immunosuppressive therapy, *e.g*. required after transplantation. Mostly, potent anti-viral drugs can manage these complications, however many of these drugs are toxic, some patients are irresponsive or develop resistances. Thus, specific regeneration of the endogenous anti-viral immune response by adoptive anti-viral T-cell therapy (AVTT) is an attractive alternative treatment. In some cases, our conventional approach of adoptive AVTT only controls the virus temporally, probably due to malfunctions of transferred T-cells caused by immunosuppressants.

We applied electroporation to transfer nucleoprotein complexes of the nuclease CRISPR-associated protein 9 with a site-specific single guide RNA (sgRNA) to generate immunosuppressant-resistant T-cells by vector-free knockout (k.o.) of the cell-intrinsic target protein, which is required for the drug's immunosuppressive function. We proofed the concept by assessment of the function of CMV-specific T-cell products in the presence of distinct immunosuppressive drugs, upon CMV-specific stimulation.

We successfully developed a GMP-compliant protocol with a sgRNA, which efficiently knocks out the target in CMV-specific T-cell products ($T^{k.o.}$CMV-TCPs). We confirmed functionality of $T^{-/-}$CMV-TCPs in the presence of the immunosuppressant. Specificity was demonstrated by loss of function of $T^{k.o.}$CMV-TCPs in the presence of an immunosuppressant from the same class requiring a different adaptor protein. $T^{k.o.}$CMV-TCPs showed improved anti-viral cytokine production even in the presence of classical triple immunosuppression applied in organ transplantation. Currently, we are investigating off-target

effects to exclude safety concerns as prerequisite for ultimate translation of $T^{k.o.}$CMV-TCP to first in-human application.

W. 132. In Situ B Cell Activation and Selection in Human Renal Allograft Rejection

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Renal allograft rejection is often associated with graft-infiltrating lymphocytes which form tertiary lymphoid structures. Although such B cell infiltration has been shown to be associated with poor clinical outcomes, their roles in renal allograft rejection are poorly understood.

To characterize graft-infiltrating B cells, we sorted intrarenal CD19+CD38+ B cells from five patients and subjected them to single-cell RNA-seq. When compared to tonsil B cells, class switched intrarenal B cells had a characteristic gene expression profile with an upregulation of innate immune receptors and their downstream genes. In addition, intrarenal B cells upregulated inflammatory cytokines and cytokine receptors whose ligand/receptor counterparts are expressed in rejected renal tissues. These data suggest specific cross-talk pathways and circuits between B cells and other cell populations within the rejected kidney.

Finally, we cloned and expressed antibodies from the intrarenal B cells to test their reactivity. Remarkably more than half, 47 out of 87, expressed antibodies were polyreactive. Furthermore, 30 antibodies were reactive with HLA and, of these, 24 antibodies were also polyreactive. Anti-HLA antibodies were expressed even in a patient without detectable serum anti-HLA reactivity. These data suggest that in renal allograft rejection, there is strong *in situ* selection for B cells expressing polyreactive, anti-HLA antibodies. Overall, our studies define a unique population of pathogenic intrarenal B cells that likely contribute to renal allograft rejection through multiple mechanisms.

W. 133. microRNA-191 regulates T-cell clonal expansion during graft-versus-host disease

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Allogeneic hematopoietic cell transplantation is a potentially curative treatment choice for a wide variety of hematological malignancies. However, graft-versus-host disease (GVHD), which is mediated by donor alloreactive T cells, limits the success of this procedure. In this study, we investigated the role of microRNA-191 (miR-191) in GVHD using miR-191 deficient T cells (KO). Lethally irradiated (8.5 Gy) BALB/c mice were injected intravenously with 1×10^7 T cell-depleted bone marrow (TCDBM) cells along with 1×10^6 purified T cells from wild-type (WT) or KO mice in C57BL/6 background. Interestingly, all recipients in the WT group died within 35 days after transplantation, while only one out of ten animals died in the KO group in an observation period of 56 days. Body weights and clinical scores were also improved in KO T cell recipients when compared with the WT controls. Similar results were also observed in a second GVHD model (C57BL/6→C3H/HeJ). Mechanistic experiments demonstrated that miR-191 regulated alloreactive T cell clonal expansion in vitro and in vivo by supporting alloreactive T cell survival. We further demonstrated that Taf5 was a target gene of miR-191. Expression of TAF5 protein was down-regulated in activated KO T cells when compared with the WT T cells. Finally, we demonstrated that T cells deficient of miR-191 was able to preserve graft-versus-leukemia effects. Taken together, our findings reveal a critical role of miR-191 during GVHD process and demonstrate that miR-191 is a novel therapeutic target for GVHD.

W. 134. Targeting the adaptive and innate immune barriers to cell transplantation using gene editing in human pluripotent stem cells

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To enable cell therapy on a broader scale, the development of universal donor stem cell products that can be administered to multiple patients in need, has been proposed, yet a strategy controlling both adaptive and innate immune rejection has not been reported. Here we employed multiplex genome editing to specifically ablate the expression of the highly polymorphic HLA-A/-B/-C and HLA class II in human pluripotent stem cells (hPSCs). Furthermore, to prevent innate immune rejection and further suppress adaptive immune responses, we expressed the immunomodulatory factors PD-L1, HLA-G, and the macrophage 'don't-eat me' signal CD47, from the *AAVS1* safe harbor locus. Importantly, gene-edited stem cell lines could be differentiated into disease-relevant cell types, such as endothelial cells and vascular smooth muscle cells, and only showed minimal off-target effects. Utilizing *in vitro* and *in vivo* immunoassays, we found that T cell responses were blunted. Moreover, NK cell killing and macrophage engulfment of our engineered cells was minimal. Our strategy demonstrates the power of cell engineering and informs future studies aiming to generate "off-the-shelf" universal cell products that may therefore enable cell therapy on a broader scale.

W. 135. The value of a rapid test of human regulatory T cell function needs to be revised

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$CD4^+CD25^+FoxP3^+$ human regulatory T_{CELLS} (T_{REG}) are promising candidates for reshaping undesired immunity/inflammation by adoptive cell transfer, yet their application is strongly dependent on robust assays testing their functionality. Several studies, along with first clinical data, indicate T_{REG} to be auspicious to use for future cell therapies, e.g. to induce tolerance after solid organ transplantation. To this end, T_{REG} suppressive capacity has to be thoroughly evaluated prior to any therapeutic application. A 7 hour-protocol for the assessment of T_{REG} function by suppression of the early activation markers CD154 and CD69 on $CD4^+CD25^-$ responder T_{CELLS} (T_{RESP}) upon polyclonal stimulation via anti-CD3/28-coated activating microbeads has previously been published. Even though this assay has since been applied by various groups, the protocol comes with a critical pitfall. Our results demonstrate that the observed decrease in activation marker frequency on T_{RESP} is due to competition for anti-CD3/28-coated microbeads as opposed to a T_{REG}-attributable effect and therefore the protocol cannot further be used as a diagnostic test to assess suppressive T_{REG} function.

W. 136. Unique and Specific Proteobacteria Diversity in Urinary Microbiota of Tolerant Kidney Transplanted Recipients

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Purpose: Host-microbiota interactions can modulate the immune system at local and systemic levels with potential consequences for organ transplantation outcomes.However,the precise nature of these interactions and their health consequences still remain to be defined.In this study,we hypothesized that differences in urinary microbiota following kidney transplant would be associated with post-transplantation status between STAble,Minimally ImmunoSuppressed and TOLerant patients.

Material & Methods: 113 urine samples were collected 6 months to 31 years after kidney transplantation from STA,MIS,TOL and Healthy Volunteers.Urinary microbiota 16S rRNA genes were sequenced and analyzed with Simpson and Shannon indices and principal component analyses of unweighted UniFrac distances.OTUs comparison were assessed between STA,TOL,MIS and HV.

Results: TOL and STA featured a significant increase in bacterial community and biodiversity compared to HV.TOL recipients were also characterized by higher relative abundance of *Proteobacteria* at phyla

and families levels compared to STA and MIS.Furthermore, *Lactobacillales* and *Bacillales (Firmicutes)* were significantly associated with TOL and negatively correlated with CNI and mTOR inhibitors.This specific and unique microbiota profile was stable over time.

Discussion: The higher relative abundance of specific bacterial phyla and families from *Proteobacteria* or *Firmicutes* may favor stability/tolerance to the graft over time.Immunosuppressive drugs were likey to impact microbial diversity and metabolic functions with distinct profiles according to transplant status.It remains to establish whether tolerance was responsible for this microbiota profile and/or inversely.Dissecting these interactions would allow for new ways to diagnose and treat immunological disorders and promote tolerance in transplantation.

Abstract Index by Subject Area

Other – B Cells, Immunology
H.56
Other – Bioinformatics
T.53
Other – Data Analysis; Bioinformatics
T.55
Other – Developmental Immunology
T.116
Other – Eosinophilic Esophagitis Pathology
W.13
Other – Food Allergy
H.7, H.8
Other – Gene-Therapy/Immunity
H.131
Other – Immune Aberrations in Human Disease
T.118
Other – Immune Development
H.83
Other – Immune System Development in Toddlers
H.94
Other – Immunogenicity Screening
T.119
Other – Immunology
T.117
Other – Immunology and Inflammation
W.109
Other – Immunology and Metabolism
T.54
Other – Immunoregulation
H.57
Other – Immunosenescence
T.52
Other – Mathematical Modeling of Immune System Activity
H.73
Other – Micrbiota and Auto-Inflammatory Diseases
W.83
Other – Neuroimmunology
H.23
Other – Pediatric Systemic Lupus Erythematosus
H.34
Other – Skin Cancer
H.107
Other – Theoretical Model of the Immune System
H.74
Reproductive Immunology
W.119, W.120

Therapeutics/Pharmacology
H.130, H.132, H.133, H.134, H.135, T.121, T.122, T.123, T.124, T.125, T.126, T.127, T.128, T.129, T.130, T.131, T.132, T.133
Transplantation
W.121, W.122, W.123, W.124, W.125, W.126, W.127, W.129, W.130, W.131, W.132, W.133, W.134, W.135, W.136

Abstract Index by Author

X

Y

Z