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ABSTRACT SUPPLEMENT

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Allergy

Tu100. Ineffectiveness of the Dexamethasone to Protect Against Poly (I:C) Mediated Asthma Exacerbation: Potential Role of Skewed Immune Response

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Viral respiratory infections trigger severe exacerbations of asthma, worsen disease symptoms, and impair lung function. Poly (I:C), a structural mimic of double-stranded RNA, is commonly used to study immune responses linked to respiratory viral infections. The current study seeks to assess the impact of poly (I:C) on asthma exacerbations. Female BALB/c mice were sensitized and challenged with Ovalbumin (OVA), followed by intranasal administration of poly (I:C). Dexamethasone was administered intraperitoneally before poly (I:C) instillation. Mice were assessed for airway hyperresponsiveness (AHR) and lung inflammation. OVA exposure resulted in steep increase in BALF inflammatory cells, particularly eosinophils. Intriguingly, poly (I:C) at a dose of 200µg augmented lung inflammation in OVA-exposed mice. Qualitative analysis of BALF cells revealed that while the number of eosinophil was decreased but the neutrophils number were increased substantially upon poly (I:C) exposure in OVA challenged mice. Additionally, mice exposed to OVA/poly (I:C) developed AHR as reflected by an increase in sRaw. Next, dexamethosome failed to ameliorate Poly (I:C) induced asthma exacerbation. It appears that switch in inflammatory response from eosinophils to neutrophils may be responsible for failure of dexamethasone under the condition. Further, analysis of Th2/Th1 panel cytokines show that poly (I:C) modulates the immune response from Th2 towards Th1 phenotype. Overall, our data suggests major shift in immune response during poly(I:C) mediated asthma exacerbation makes the condition steroid refractory in nature.

Tu101. Male Pups Have Increased Allergen and RSV-induced Airway Inflammation After in Utero Allergen Exposure

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As children, males have a higher incidence and prevalence of asthma compared to females at a 2:1 ratio. Maternal asthma is a risk factor for childhood asthma; however, it remains unclear whether maternal asthma contributes to the sex disparity in childhood asthma. We hypothesized that, following in utero exposure to allergen, male offspring would have decreased Treg stability and function, leading to increased allergic airway inflammation. To test our hypothesis, we intranasally administered house dust mite (HDM) allergen or vehicle to pregnant Foxp3 fate mapping females (Foxp3EGFP-Cre Rosa26YFP/YFPIl13TdTomato/TdTomato) that were mated with Foxp3EGFP-CreRosa26YFP/YFP males. At 3 weeks of age, male and female pups (Foxp3EGFP-CreRosa26YFP/YFPIl13WT/TdTomato) were intranasally challenged with mock or RSV 01/2-20 clinical isolate (3x107 PFU/ml). Lungs were harvested from pups 7 days post infection. Mock challenged males exposed in utero to HDM had increased IL-13$^+$ CD4 T cells, increased ex-Tregs (Foxp3 GFP$^-$YFP$^+$), and decreased current Tregs (Foxp3 GFP$^+$YFP$^+$) compared to mock challenged, in utero HDM exposed female pups or in utero PBS exposed pups. Additionally, male pups infected with RSV had increased IL$^-$13$^+$ CD4 T cells, increased ex-Tregs, and decreased current Tregs compared to in utero HDM exposed female pups. Bronchoalveolar lavage (BAL) fluid showed increased eosinophils in male pups exposed in utero to HDM following RSV infection compared to female challenged pups. Combined, these results show sex differences in airway inflammation and Treg numbers following in utero exposure to allergen, providing a potential mechanism for the sex disparity in childhood asthma prevalence and severity.

Tu102. Molecular Endotype Identification in Two Common Skin Diseases: Atopic Dermatitis (AD) and Psoriasis (PSO)
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Sanofi

The two most prevalent chronic inflammatory skin conditions, atopic dermatitis (AD) and psoriasis (PSO), exhibit considerable patient heterogeneity. The BIOMAP consortium aims to delve into this heterogeneity of AD and PSO, to re-define taxonomy and patient stratification methods. We identify two stable molecular endotypes for each condition by analyzing skin transcriptomic data from 272 AD and 319 PSO patients. In the case of AD, one endotype showed heightened inflammation via Th1/Th2, IL17 and other cytokine pathways, while another displayed elevated PD1 and LXR/RXR activities. Similarly, in PSO, one endotype demonstrated increased inflammation with IL17 and S100 family pathways, whereas the other exhibited lower inflammation alongside heightened fibroblast activity. Key markers like CXCL1, CXCL2, CXCR3, and IL1B distinguishes the two endotypes within both AD and PSO. We are currently involved in replicating and validating our findings through collaboration with academic and industry partners in BIOMAP to further explore and characterize endotypes using the same analytical framework. Additional examination involving existing drug targets, signaling pathways, and cellular behaviors will shed light on potential avenues for innovative disease taxonomy and endotype-directed treatment approaches for patients with AD or PSO. This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 821511 (BIOMAP). The JU receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA. This publication reflects only the author's view and the JU is not responsible for any use that may be made of the information it contains.

Tu103. Piezo1 Channels Constrain ILC2 Bioenergetic and Effector Functions and Ameliorate Airway Hyperreactivity
Benjamin Hurrell
University of Southern California

Mechanosensitive ion channels sense force and pressure in immune cells to drive the inflammatory response in highly mechanical organs. Here we report that Piezo1 channels repress group 2 innate lymphoid cell (ILC2)-driven type 2 inflammation in the lungs. Piezo1 channels are induced on lung ILC2s upon activation both *ex vivo* and *in vivo*, with their expression inversely correlated with known ILC2 activation markers at the single cell transcriptomic and protein levels. Conversely, we found that genetic ablation of Piezo1 specifically in ILC2s increases their function and exacerbates the development of airway hyperreactivity (AHR). As a result, *in vivo* administration of Piezo1 agonist Yoda1 reduces ILC2-driven lung inflammation in multiple experimental models of ILC2-dependent AHR, with a notable effect on lung ILC2 effector functions. Mechanistically, Yoda1 inhibits ILC2 cytokine secretion and proliferation through a specific mechanistic process involving the modulation of transcription factor expression and activation within ILC2s. Our observations further suggest a major role for Piezo1 channels on ILC2 metabolism as Piezo1 engagement notably reduces ILC2 mitochondrial function, resulting in a reduction of oxidative metabolism and bioenergetic activity. Human circulating ILC2s express and induce Piezo1 upon activation, with Piezo1 engagement able to limit human ILC2 cytokine production, proliferation and bioenergetic activities. Conversely, *in vivo* administration of Yoda1 to humanized mice reduces human ILC2-driven AHR and lung inflammation. Our studies define Piezo1 as a critical regulator of ILC2s and we propose the potential of Piezo1 activation as a novel therapeutic approach for the treatment of ILC2-driven allergic asthma.

Tu104. The Role of BRD4/ZDHHC-1/STING Axis on Allergic Asthma Development
Terumi Midoro-Horiuti, Yoko Murakami, Sahar Fahmy, Kazuyo Kuzume, Kangling Zhang, and Randall Goldblum
University Of Texas Medical Branch, Galveston

Rationale: Early exposures to environmental estrogens (EEs) are associated with increased asthma prevalence. We have previously identified the enhanced expression of bromodomain-containing protein 4 (BRD4)/ zinc finger HHC-1 (ZDHHC1)/stimulators of IFN genes (STING) after EE exposure. We aimed to investigate the effects of EEs on the development of allergic asthma using our EE exposure model with the BRD4/ZDHHC1/STING axis. Methods: Female BALB/c mice received 10 g/ml bisphenol A (BPA), bisphenol S (BPS), or vehicle control in their drinking water during pregnancy until their pups were weaned. Half of the dams received peritoneal injections of STING inhibitor, C-176. The pups were sensitized with an intentionally "suboptimal" low dose of ovalbumin (Ova) on postnatal day 4, 1% Ova inhalation on days 18-20, and asthma phenotype was assessed on day 22. Non-sensitized female pups were saved and bred with non-exposed male mice at 8 weeks of age. The subsequent pups were sensitized, and asthma phenotype was examined up to the fourth generation. Results: F1-F4 pups from EE-exposed dams developed asthma phenotype. Pups from C-176 treated dams had decreased response to their sensitization compared to non-treated controls. Airway hyperresponsiveness and IgE anti-Ova were lower in pups with C-176 treatment. Conclusions: Early exposure to EEs, including BPA and BPS, promotes the development of experimental asthma, possibly through the BRD4/ZDHHC1/STING axis. The immune alterations may be epigenetically perpetuated, causing multigenerational effects.

W100. Cellular Iron is a Critical Regulator of ILC2 Metabolic Function and Development of Airway Hyperreactivity
Benjamin Hurrell
University of Southern California

Iron is a critical nutritional trace element. With many of the mechanisms involved in systemic iron homeostasis characterized however, attention is now increasingly turning to the role of iron in tissues and immune cells. Notably, iron has been known as a critical regulator of cellular processes, as iron deficiency impairs multiple aspects of T cell responses. Our research indicates that iron plays a pivotal role on the function of group 2 innate lymphoid cells (ILC2s), which rapidly induce a type 2 inflammation in the lungs in response to allergen exposure. Transferrin receptor 1 (TfR1) is rapidly upregulated and functional during ILC2 activation in the lungs, while blocking transferrin uptake reduces ILC2 expansion and activation. Iron deprivation reprograms ILC2 metabolism, inducing a HIF-1a-driven upregulation of glycolysis and inhibition of oxidative mitochondrial activity. Consequently, *in vivo* restriction of cellular iron availability reduces the development of airway hyperreactivity in experimental models of ILC2-driven allergic asthma. We confirmed the effects of iron on human ILC2s, as both iron uptake blockade and deprivation abrogate ILC2 effector functions in ILC2s isolated from healthy subjects. In a clinical setting, we found a negative relation between the levels of TfR1 expression on circulating ILC2s and the severity of allergic asthma in cohorts of healthy, mild, moderate or severe patients with asthma, suggesting that the expression of TfR1 and iron may directly be linked to the severity of asthma. Collectively, our studies define cellular iron as a critical regulator of ILC2 function and magnitude of type 2 airway inflammation.

W101. Local Intestinal Eosinophilic Activation Triggered by Defective Epithelial TGF-β Signalling Underlies IPO8 Deficiency

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Aberrant TGF-β signaling underlies the pathogenesis of severe congenital disorders associating developmental defects with or without immune dysregulation, including Loeys–Dietz syndrome (LDS). We have recently identified IPO8 deficiency as cause of a connective tissue disorder largely overlapping with LDS. IPO8 is a member of the β-karyopherin family, the largest group of nuclear transport receptors. We have shown that IPO8 plays a critical role during early stage of development in zebrafish by controlling phospho-SMAD nuclear translocation and TGF-β/BMP-dependent transcription. Here we report increased prevalence of treatment-resistant gastrointestinal diseases, recurrent pulmonary infections and atopic diseases including asthma, dermatitis and allergies in IPO8 deficient patients. In addition to eosinophilia, multiomics approaches combining mass cytometry, single cell transcriptomics and plasma proteomics identified altered composition of B cell and myeloid compartment with peripherical proinflammatory signature in IPO8 deficient patients. To investigate the direct intrinsic effects of IPO8 loss, we generated a full knock-out mouse model using CRISPR/Cas9 editing. Pointing to spontaneous local inflammation, we found increased frequency of activated eosinophils in the small intestine of Ipo8-/- mice. Ipo8-/- intestinal organoids showed defective SMADs translocation and increased expression of pro-inflammatory immune mediators. Overall, our data show that Ipo8-/- mice are a valid model to study how dysregulation of the TGF-β signaling dependent on IPO8 loss triggers local intestinal eosinophilic activation and immune dysregulation.

Autoimmune Diseases

Th100. A Healthy Birth After Recurrent Spontaneous Abortions Due to Antiphospholipid Syndrome: A Case Report and Literature Review

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Antiphospholipid Syndrome (APS) is an autoimmune disease characterized by persistently high titers of antiphospholipid antibodies (APLAs). Constant exposure to these antibodies result in recurrent venous thromboemboli that result in a variety of devastating consequences. APS is relatively rare at a prevalence of around 50/100,000 people. Due to the esoteric nature of this disease, however, there are presumably many more cases that are misdiagnosed initially or go completely undiagnosed. Here we present a case of a 31 year-old woman who believed herself to be infertile due to recurrent spontaneous abortions resulting in the loss of 10 fetuses. Despite her condition, she was able to give birth to a healthy baby girl with the help of obstetricians that diagnosed APS, as well as severe pre-eclampsia. Immediate emergency measures were undertaken by her care team where they performed an emergency C-section prior to her expected delivery date. Additional measures were also taken, such as rigorous blood pressure control and magnesium sulfate administration for maternal seizure prophylaxis and fetal neuroprotection. While our patient was able to have a successful birth, her condition was identified only after a staggering number of fetal losses. APS can lead to emotionally devastating recurrent spontaneous abortions, but fertility is still possible and can be maximized by rapid diagnosis and by taking appropriate measures. The goal of this paper is to outline our current knowledge of APS management and add to the current literature by identifying the successes and possible areas of improvement in the management of our patient's case.

Th101. Single Cell Transcriptomic Analysis of Graves' Disease Thyroid Glands Reveals the Broad Immunoregulatory Potential of Thyrocytes and Stromal Cells: Implications for the Role of Ectopic HLA Class II Expression in Autoimmunity

Ricardo Pujol Borrell[1], Daniel Alvarez-Sierra[2], Ana Marin-Sanchez[3], Carmela Iglesias[4], Aroa Gomez-Brey[5], Irene Bello[4], Enric Caubet[4], Oscar Gonzalez[4], Pablo Moreno[6], Nuria Ruiz[6], Carles Zafon[4] and Roger Colobran[7]
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The study of the immune response in thyroid autoimmunity has been mostly focused on the autoantibodies and lymphocytes, but there are indications that intrinsic features of thyroid tissue cells may play a role in disrupting tolerance. The overexpression of a broad spectrum of immune molecules by thyroid follicular cells (TFC) and our recent demonstration that PD-L1 is only moderately expressed by TFCs in autoimmune thyroid indicates that TFCs may actively modulate tolerance. To get a more comprehensive picture of TFC role in regulation, preparations of dispersed TFCs and stromal cells from five Graves' disease (GD) and four control thyroid glands were compared by scRNA-seq. The results confirmed the described interferon type I and type II signatures and showed unequivocally that TFC express the full array of genes that intervene in the processing and presentation of endogenous and exogeneous antigens. However, TFCs either from GD or control glands lack expression of costimulatory molecules CD80/CD86 required for priming T cells. GD fibroblasts showed widespread upregulation of cytokine genes. The results from this first single transcriptomic profiling of TFC and thyroid stromal cells provides a granular view of the events occurring in GD. The new data point at an important contribution of stromal cells and prompt a re-interpretation of the role of MHC over-expression by TFC, from deleterious to protective. This re-interpretation could also apply to other tissues, like pancreatic beta cells, where MHC overexpression has been detected in diabetic pancreas.

Th102. Single-cell Analysis of Monocyte Subpopulations in Untreated Large Vessel Vasculitis

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Giant cell arteritis (GCA) is a large vessel vasculitis of unknown cause, affecting only individuals older than 50 years of age. Autoantibodies are not detectable and specific lab markers are lacking. The disease is characterized by an acute inflammation with unspecific upregulation of acute phase reactants, which are mainly produced by innate immune cells. Affected arteries are infiltrated by monocytes and macrophages, which digest the basement membrane and, thus, allow infiltration of $CD4^+$ T cells and formation of granulomatous lesions. In essence, GCA is characterized by a strong pathophysiological role of the innate immune system. However, whether there are disease-causing defects in cells of the innate immune system or specific disease-causing cell populations is currently unknown. Here, we performed single-cell RNA sequencing of circulating monocytes from the peripheral blood of patients with active untreated GCA and healthy age-match controls. To preserve the information of potentially underrepresented monocyte populations, e.g. low frequent non-classical monocytes, we captured the individual transcriptomes from equivalent cell numbers of each monocyte subset. Using this technique, we identified a disease-specific monocyte population within the classical monocyte compartment that is not detectable in healthy individuals of the same age. This monocyte cluster disappears in GCA patients 3 months after start of therapy indicating that monocytes with this transcriptome are involved in the initiation or the very early phases of the disease, making this population a candidate for new therapies and a possible diagnostic tool.

Th103. Skewing of the Ocular BCR Repertoire Suggests a Shared Intraocular Antigen in Uveitis
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Uveitis is a heterogeneous group of ocular inflammatory diseases with a high risk for blindness. As the pathophysiology is poorly understood, current therapies are empiric and fail 30-50% of patients. To better understand patient-specific disease mechanisms, we employed single-cell RNA sequencing. We have shown that T and B lymphocytes are highly enriched and clonally expanded in the ocular fluid from a subset of patients with uveitis, suggesting an immune response triggered by intraocular antigen. Given that the eye is an immunologically sequestered tissue, we hypothesized that a common intraocular antigen might trigger inflammation in this group of patients. To study this, we analyzed the B cell receptor (BCR) sequences in our cohort. We asked whether there were shared V, D, or J segment usage amongst uveitis patients, which would suggest similarity in the specific antigens recognized by these cells. While VH segment frequencies in peripheral blood naïve B cells approximated the frequency of germline VH segments, we found enrichment amongst ocular B cells and plasmablasts of VH1 segments in 75% of patients and VH4 segments in 50% of patients. DH3 segments were also enriched in ocular B cells, whereas JH segment usage was not significantly different between the eye and peripheral blood. Skewed usage of common CDR3 regions by ocular B cells across multiple patients with uveitis suggests that a similar antigen may drive ocular inflammation in these patients. Ongoing studies aim to identify target antigens using monoclonal antibodies derived from patient BCR sequences.

Th104. Synovium $CD4^+HLADR^+$ T Effectors with Antigenic Reactivity that Sustain Inflammation and Resist Regulatory Control in Juvenile Idiopathic Arthritic Patients
Martin Wasser[1], Pavanish Kumar[2], Shi Huan Tay[2], Gerdien Mijnheer[3], Joo Guan Yeo[4], Sharifah Nur Hazirah[2], Amanda Jin Mei Lim[2], Camillus Chua[2], Phyllis Chen[2], Alessandro Consolaro[5], Marco Gattorno[5], Femke van Wijk[3], Alberto Martini[5], Thaschawee Arkachaisri[4] and Salvatore Albani[2]
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Systemic $CD4^+HLADR^+$ effectors and Tregs from arthritic patients have been shown to exhibit immunological and TCR profiles resembling synovium counterparts. Notably, circulatory $CD4^+HLADR^+$ effectors were elevated in arthritic individuals non-responsive to TNFi treatments. Why synovium $CD4^+HLADR^+$ effectors continue to sustain inflammation despite presence of Tregs and its relationship with analogous $CD4^+HLADR^+$ Tregs remains unknown. We employed CyToF to interrogate the CD4 memory landscape of JIA patients, and revealed the elevated presence of $CD4^+HLADR^+$ effectors expressing TNFa, IFNg and IL-21 within the synovium. $CD4^+HLADR^+$ effectors and Tregs positively correlated strongly across the circulatory and synovium compartments. To unravel their relationships and understand their functional properties, we sorted $CD4^+HLADR^{+/-}$ subsets and performed deep RNAsequencing. $CD4^+HLADR^+$ effectors and Tregs displayed strong transcriptomic convergence, higher TCR clonatype sharing and were enriched for 7 key pathways, including MHC-II and IFNg signalling. While synovium $CD4^+HLADR^+$ Tregs presented stable FoxP3 promoter methylation profiles and were competent suppressors, $CD4^+HLADR^+$effectors were able to resist their regulatory control. $CD4^+HLADR^+$ Tregs were unstable in the presence of Th-1 polarising and mitotic conditions, decreasing FoxP3 levels, as compared with $CD4^+HLADR^-$ Tregs. Furthermore, $CD4^+HLADR^+$ effectors responded to disease relevant antigens (HSP60) independent of APCs. In summary, this study has shown the pathogenic mechanisms through which $CD4^+HLADR^+$ effectors could contribute to persistent autoimmunity in JIA patients. Specific targeting of $CD4^+HLADR^+$ effectors in JIA may present as a potential therapeutic avenue.

Th105. T Cell-dependent Gene Programs in Keratinocytes and Dermal Fibroblasts are Associated with Inflammatory and Fibrotic Skin Disease

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The skin is an immunologically active tissue specialized to deal with a litany of stresses. Skin immune and structural cells coordinate to promote appropriate inflammatory responses and subsequent restoration of barrier integrity after insult. Single cell analyses of human skin show an array of structural cell gene programs, including disease-associated changes in keratinocytes and fibroblasts. T cell activity is implicated in many inflammatory skin diseases, but the mechanisms T cell use to promote disease-associated changes is understudied. We use 2D culture and a novel 3D organotypic skin model to demonstrate how distinct subsets of cutaneous $CD4^+$ T cells promote diverse transcriptional outcomes in keratinocytes and fibroblasts. We use these *in vitro* generated transcriptional signatures to identify T cell-dependent gene modules associated with inflammatory skin disease *in vivo*. We define a set of Th17 cell-induced genes in keratinocytes, including epigenetic modifiers, that are enriched in psoriasis patient skin and normalized in response to anti-IL-17 therapy. We also describe T cell-dependent gene programs that are enriched in scleroderma-associated fibroblasts important for fibrotic disease progression and track these populations in patient skin by spatial transcriptomic analysis. Thus, we have identified novel T cell-dependent gene programs of healthy skin that we use to explore the immune perturbations of inflammatory disease and interventional therapies.

Th106. TCR Repertoire Analysis in Human Immune System Mice Derived from Type 1 Diabetic and Healthy Control Hematopoietic Cells

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The extent to which thymic selection and formation of TCR repertoire contributes to Type 1 Diabetes (T1D) is unknown due to limited accessibility of human thymic tissue and our inability to manipulate variables *in vivo*. However, Human Immune System (HIS) mice generated with human thymus and partially HLA-matched Hematopoietic Stem Cells (HSCs) from Type 1 Diabetic (T1D) and Healthy Controls (HC) (Personalized Immune, PI mice) provide a system in which to investigate TCR repertoire development *in vivo*. Thymocyte subsets from T1D or HC PI mice were sorted, gDNA extracted and high throughput TCRβ-CDR3 sequencing carried out (Adaptive Biotech). Analysis utilizing TiRP score (Lagattuta et al., 2022) revealed increased TiRP scores in the CD4SP subset compared to DP $CD69^+$ cells in one T1D PI mouse, whereas it remained constant in the HLA-matched HC PI mouse, suggesting failed negative selection of autoreactive TCRs in the T1D system. Consistently, CDR3 hydrophobicity as a measure of autoreactivity revealed greater hydrophobicity in CD4SP cells of the T1D PI mouse as compared to the DP $CD69^+$ population. Analysis of two HC PI mice revealed increasing hydrophobicity as the cells mature from DP CD69- to DP $CD69^+$ but no change as the thymocytes mature into Tconv CD4SP. However, Treg CD4SP cells demonstrated appropriate increased hydrophobicity scores. These results support previous observations of diminished negative selection of an islet autoreactive TCR in T1D PI mice. Additional analysis of thymocytes in T1D and HC PI mice is currently in process to further substantiate these initial observations.

Th107. The Effect of Biologic Disease-modifying Anti-rheumatic Drugs on Vaccination
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Motol University Hospital

Background and objectives Vaccination provides relatively short-term protection against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), suggesting the need for booster doses. Immunocompromised individuals with immune-mediated inflammatory diseases (IMIDs) may have a markedly weakened immune response. Therefore, we investigated the persistence of the immune response, both humoral and T cell responses, after primary vaccination and the immunogenicity and safety after booster vaccination with BNT162b2. Methods This prospective observational study included a cohort of patients with axial spondyloarthritis (AxSpA) treated with Biologic disease-modifying anti-rheumatic drugs, specifically IL-17 and TNFα inhibitors. SARS-CoV-2-specific serum antibodies and virus-neutralizing antibodies, T-cell immune responses, and safety were evaluated. Result Fifteen male patients with AxSpA treated with TNFα inhibitors (73.3%) or IL-17 (26.7%) were included. After booster vaccination, the humoral response increased from 905.6 (± 186.1 SD) using Immunoblot assay and 409.1 (± 335.7) U/ml using ELISA to 989.7 (± 12.62) and 1000 U/ml, respectively. The T-cell response analyzed by specific production of IFNgamma and TNFα increased from 53.3% to 80%, with no differences between the AxSpA and healthy control cohorts. No serious AEs occurred; The AE spectrum was comparable to the general population. Conclusions Persistence of the immune response after primary vaccination and immunogenicity after booster vaccination were not affected by anti-IL17 or anti-TNFα therapy with similar AEs as in the general population.

Th108. The Integrated Cellular and Molecular Landscape of Autoimmunity
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University of Rome "Tor Vergata"

Autoimmune disorders are a heterogeneous class of diseases affecting 5 to 10% of the global population and characterized by an aberrant immune response against healthy tissues and organs. Presently, treatment options aim to control disease by inducing systemic immuno-suppression, resulting in severe side-effects. Despite progress towards understanding risk factors and pathophysiology, current therapies are limited by an incomplete picture of the mechanism of disease due to their heterogeneity, both at a disease and patient level. A comparative analysis across different patient cohorts and diseases to reveal shared and distinct associated immune pathways is currently missing. Here, we present a molecular and cellular landscape describing 11 autoimmune diseases generated by analyzing and integrating 13,054 publicly available transcriptomes of whole blood and tissue biopsies across 182 datasets. For each gene expression sample, we computationally inferred cellular composition of circulating immune cells and infiltrates (n = 20), cytokine levels (n = 275), pathway (n = 582), transcription factor (n = 727) and microRNA activity scores (n = 740). We then employed meta-analysis to integrate these biologically relevant features across all datasets separately between tissue and blood-derived samples, identifying regulatory modules that are unique and shared across diseases while minimizing the effect of cohort-specific confounders. Our ongoing work provides a foundational analysis and contributes towards the understanding of the molecular mechanisms across autoimmunity.

Th109. The Peripheral Immunological Profile Determines the Clinical Presentation and Prognosis of Patients with Autoimmune Hepatitis

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Autoimmune hepatitis (AIH) is associated with T cells dysregulation. We aimed to assess how lymphocyte phenotype (LP) at diagnosis varies with patient characteristics and if it can predict the response to immunosuppression. A prospective study from 2020-2023 included all new histologically-proven AIH diagnoses. Before of immunosuppression, an extended peripheral LP was conducted, correlating with baseline characteristics and subsequent clinical events. All patients received a uniform therapy régimen of corticosteroids (CS) (20-60mg) and azathioprine (1-2mg/Kg), excluding variant forms of AIH. Thirty-four patients were included: 70.6% women, 58 years (IQR47-73), 26(70.6%) debuted as acute hepatitis (AST/ALT>10-fold normality), 31(91.2%) presented any antibody with ANAs≥1/80 (82.4%) and xANCA (31.3%) as the most frequent. Eight (23.5%) subjects had cirrhosis. At diagnosis, cirrhotic patients showed a trend toward increased $CD8^+$TEMRA presence (51.6vs36.1,p=0.079). Acute hepatitis cases had a higher proportion of pre-switch-memory $CD27^+$BL(7.1vs3.0,p=0.003), naïve-$CD4^+$(45.8vs20.7,p=0.015), $CD3^+$(73.8vs69.3,p=0.025), $CD8^+$(23.6vs17.7,p=0.043), and lower $CD4^+$Effector$^+$Central memory phenotype (52.4vs73.6,p=0.045). After a median follow-up of 25 months, complete biochemical response (CBR) was achieved in 23(64.7%) patients being higher among those presenting as acute hepatitis compared with those with non-acute presentation (76.9%vs 25%, p=0.013), but similar regardless of cirrhosis presence (50%vs69.2%,p=0.279). CBR was associated with baseline higher values of leukocytes, platelets, AST, bilirubin, $CD4^+$ naïve(47.3vs27.0,p=0.008), $CD8^+$ naïve (26.1vs9.5,p< 0.001), and lower IgG levels (1622vs2350 IU/mL,p=0.010), $CD4^+$ effector memory (20.4vs35.5,p=0.024) and CD4 Effector$^+$Central memory (52.0vs65.0,p=0.059). Conclusion: A dominant effector $CD4^+$ and $CD8^+$ phenotype predicts incomplete biochemical response (CBR) in AIH patients. Evaluating effector T cells at diagnosis may enable early detection of challenging cases, facilitating timely intervention.

Th110. Therapeutic Potential of Imvotamab, a CD20-Targeted Bispecific IgM T Cell Engager, for the Treatment of Refractory Autoimmune Disease Patients

Racquel Domingo-Gonzalez, Isabelle Baribaud, Carrie Brodmerkel, Keely Burns, Albert Candia, Stephen Carroll, Mary Beth Harler, Kevin Hart, Genevive Hernandez, Bruce Keyt, Maya Kotturi, Rebecca Kunder, Maya Leabman, Miho Oyasu and Sivani Pandey

IGM Biosciences, Inc.

B cell depletion therapy with conventional IgGs has been used to treat autoimmune (AI) disease. However, these IgG-based therapies can fail to fully deplete tissue-resident B cells and many patients do not achieve long term disease control. Bispecific IgM antibody T cell engagers (TCEs) offer the potential to deplete tissue-resident target cells. Imvotamab (IGM-2323) is an engineered bispecific anti-CD20 IgM TCE that has been evaluated for the treatment of non-Hodgkin's lymphoma (NHL). Given the promising durable response and safety profile in NHL, we evaluated imvotamab for depletion of peripheral and tissue-resident B cells in preclinical models of AI disease. *Ex vivo* cytotoxicity assays using human peripheral blood mononuclear cells (PBMCs) or serum from healthy donors and AI patients showed imvotamab induced potent killing of B cells from both AI patients and healthy donor PBMCs, and greater complement-dependent cytotoxicity compared to rituximab. No hyper-active responses were noted for T cell activation and cytokine release following treatment of imvotamab with healthy and AI patient PBMCs. Additionally, a rituximab-resistant cell line, with significantly reduced CD20 expression levels, was killed more effectively by imvotamab than rituximab. Using a surrogate cynomolgus monkey cross-

reactive CD20xCD3 IgM TCE, IGM-2324, peripheral and tissue-resident B cell were depleted *in vivo*, including low CD20-expressing B cells. Our preclinical data show imvotamab effectively kills peripheral B cells from AI patients. Moreover, a CD20xCD3 IgM TCE can penetrate tissues to kill target cells *in vivo*. Clinical trials with imvotamab in AI patients are ongoing to evaluate the therapeutic benefit of this mechanism.

Th111. Tissue-engineered Lymph Node Fibroblastic Reticula for Localized Immunomodulation of Diabetogenic T Cells

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Current treatments for autoimmune diseases like type 1 diabetes (T1D) are limited to systemic T cell-targeting approaches, carrying significant risks. Therapies that are more localized and antigen (Ag)-specific are more desirable. Fibroblastic reticular cells (FRCs) are lymph node stromal cells that build 3D reticula and act as non-professional antigen-presenting cells with immunomodulatory effects. Upon inflammation, FRCs expand the lymph node and upregulate Ag-presenting machinery (MHC-I/II) and immune checkpoint molecules (PD-L1) while maintaining low co-stimulation (CD80/86). FRCs expressing model Ags induced Ag-specific naïve T-cell deletion *in vivo* indicating their potential for targeting Ag-specific T cells. However, their therapeutic role in Ag-specific immunomodulation against T1D has not been tested. We designed a 3D biomaterial platform for localized FRC-mediated immunomodulation of T1D. We found that biocompatible macroporous gelatin scaffolds promote murine and human FRC attachment and reticula formation during 21-day *in vitro* culture and after subcutaneous transplantation in mice (using bioluminescence imaging of luciferase-expressing FRCs). After 3-day *in vitro* co-culture with FRC reticula pulsed with selected T1D peptide Ags, we found specific $CD4^+$ and $CD8^+$ T-cell engagement via proliferation (CellTrace dilution) and CD44/CD25 upregulation. Compared to anti-CD3/CD28 stimulation, cytotoxic phenotype (Granzyme B) of engaged specific $CD8^+$ T-cell was reduced while anergic (CD73/FR4) and regulatory (CD25/FoxP3) phenotype of Ag-specific $CD4^+$ but not polyclonal $CD4^+$ T-cell was increased. Using $CD4^+$ T cells from BDC2.5 FoxP3-RFP mice, we found that Ag-presenting FRCs promoted both induction and expansion of Ag-specific T cells. Thus, our FRC platform could provide novel means for local, retrievable, Ag-specific immunomodulation for T1D.

Th112. Tolerogenic APC-targeted VaccibodyTM Vaccines Treat Disease in Mouse Models of Experimental Autoimmune Encephalomyelitis and Non-Obese Diabetes

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Nykode Therapeutics

Autoimmune diseases affect about 4% of the world population and hold great unmet medical need for the development of novel treatments. Tolerogenic vaccination strategies aim to recalibrate disease-causing effector and protective regulatory responses in an antigen-specific manner that preserve protective immunity. Nykode Therapeutics has developed a platform that targets antigens directly to antigen presenting cells (APCs) using a modular dimeric protein format known as a Vaccibody. Here, Vaccibody vaccines were designed to deliver a tolerogenic response toward disease-associated antigens via specific APC-receptor-targeting. The Vaccibody vaccines were tested for their tolerogenic potential in the Experimental Autoimmune Encephalomyelitis (EAE) model and in Non-Obese Diabetic (NOD) mice either alone or combined with co-expression of immune-modulatory proteins in a multicistronic plasmid DNA. In the EAE model, recombinant Vaccibody vaccines effectively treated EAE disease using targeting units toward two different receptors on APCs. The vaccine showed potent disease protection in preventive and early therapeutic settings associated with reduced antigen-

specific pro-inflammatory cytokine release. The response was found to be dose-dependent, antigen-specific and APC-receptor-targeting dependent. In NOD mice, vaccination with Vaccibody encoding plasmid DNA effectively delayed the development of spontaneous autoimmune diabetes; co-expression of selected immune-modulators resulted in full protection from diabetes development with a durable response also post treatment withdrawal. These data demonstrate the flexibility of novel APC-receptor-targeting Vaccibody vaccines able to deliver potent tolerogenic responses in two different mouse models of autoimmune disease.

Th113. Unveiling the Autoreactome: Proteome-wide Immunological Fingerprints Reveal the Promise of Plasma Cell Depleting Therapy

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The prevalence and burden of autoimmune and autoantibody mediated disease is increasing worldwide, yet most disease etiologies remain unclear. Despite numerous new targeted immunomodulatory therapies, comprehensive approaches to apply and evaluate the effects of these treatments longitudinally are lacking. Here, we leverage advances in programmable-phage immunoprecipitation (PhIP-Seq) methodology to explore the modulation, or lack thereof, of proteome-wide autoantibody profiles in both health and disease. We demonstrate that each individual, regardless of disease state, possesses a distinct set of autoreactivities constituting a unique immunological fingerprint, or “autoreactome”, that is remarkably stable over years. In addition to uncovering important new biology, the autoreactome can be used to better evaluate the relative effectiveness of various therapies in altering autoantibody repertoires. We find that therapies targeting B-Cell Maturation Antigen (BCMA) profoundly alter an individual’s autoreactome, while anti-CD19 and CD-20 therapies have minimal effects, strongly suggesting a rationale for BCMA or other plasma cell targeted therapies in autoantibody mediated diseases.

Th114. Using Human Colonic Explants to Recapitulate Key Areas of IBD Pathophysiology and Investigate Interventions

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AbbVie

The pathophysiology of inflammatory bowel disease (IBD) is complex. The interplay between immune cells and intestinal epithelial cells in the development of IBD is poorly understood. Human colon tissue (HCT) resections procured from patients during surgery can be used to better address mechanistic and therapeutic questions for IBD because they preserve the cellular composition and architecture of the organ. To this end, we procured HCT resections, separated the muscular wall, took 3mm biopsies from the mucosal side, and incubated them in culture. Standard incubation conditions limit our ability to study mechanisms of action (MOAs) in HCT explants due to hypoxia-induced cell death and poor tissue integrity. To prolong the tissue survival, 3mm biopsy punches of HCT were incubated in a hyperoxic environment. Histological assessment demonstrated that a high-oxygenated environment led to improved tissue survival. To investigate immune and non-immune cellular interactions, using FACS, we observed a decrease in the number of epithelial cells and an increase in the number of T cells in the lesional Crohn’s Disease explants compared to their non-lesional controls. To mimic key pathways active in IBD, we stimulated T cells within the non-IBD HCT biopsies with an anti-CD3 antibody. Similar to what is observed in IBD, we saw elevated surface expression of ICOS and CD25, markers of T cell activation, on T cells which corresponded with increased levels of IFNg and IL-17F production. A JAK inhibitor and LCK

inhibitor blocked this response. Future work includes expanding our understanding of MOAs for IBD interventions using these explants.

Th115. Utilizing High-dimensionality Mass Cytometry to Decode Complex Immunopathogenic Network for Potential Theragnostic Applications in Early Systemic Sclerosis

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Background Systemic sclerosis (SSc) is a heterogenous disease characterised by autoimmunity, vasculopathy, and fibrosis. This study aimed to characterize the peripheral immunome associated with active disease in SSc Methods Peripheral blood mononuclear cells were collected from early SSc patients (n=13 active, n=12 inactive), and healthy controls (n=10). Single-cell proteomic data was acquired using mass cytometry; data was analysed using the Extended Polydimensional Immunome Characterization platform, an unsupervised machine learning algorithm. Using the identified cell subset frequencies, we built multiple logistic regression models to classify active diseases. Correlation network between nodes was constructed by performing pairwise correlations between each node pair. Results Dysregulated peripheral immunome was observed in active SSc. CD4 T cell subsets expressing immune checkpoints were decreased, while MAIT cells expressing IL-22, TGF-b were increased in active SSc, compared to inactive SSc. NK cell subsets expressing proinflammatory cytokines and B cell subsets expressing CXCR4 were increased in active SSc, compared to inactive SSc. Using the identified immune cell subsets to evaluate active and inactive disease, receiver operating characteristic curve analyses showed an area under the curve of 0.8939. Immune cell network from inactive SSc showed more negatively connected edges and higher modularity compared to network from active SSc. Conclusions We identified dysregulated immune cell subsets and network in active SSc. Future studies to investigate their functional significance would shed light into the disease pathogenesis. The identified immune signatures could potentially be used as theragnostic tools to evaluate disease activity in SSc.

Th116. Expanding Our Understanding of PDC-E2 T Cell Responses in PBC and Their Application for Eng Treg Therapy

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Primary biliary cholangitis (PBC) is a chronic autoimmune liver disease, characterized by progressive destruction of the small intrahepatic bile ducts and portal inflammation. Treatment options are limited with heavy reliance on orthotopic liver transplant for survival in advanced cases. The adaptive immune response is implicated in disease pathogenesis both by the presence of anti-mitochondrial antibodies targeting the major autoantigen, E2 subunit of the pyruvate dehydrogenase complex (PDC-E2), in 90-95% of patients, and CD4 and CD8 T cells infiltrating the portal tracts in affected individuals. Additionally, genetic associations include a strong link to HLA Class II alleles. Here, we examined T cell responses to peptides derived from PDC-E2, with a focus on CD4 T cell responses restricted to HLA Class II DRB4*0101, an allele found in 62% of PBC patients. Using an Activation Induced Marker assay and single cell RNA sequencing, we found clonal expansion of CD4 T cells reactive to PDC-E2 epitopes in both conventional (Tconv) and regulatory T (Treg) cells. The TCR repertoires were non-overlapping and private and included TCRs specific for a novel PDC-E2 epitope restricted to DRB4*0101. CD4

Tconv cells from healthy and PBC subjects that responded to the PDC-E2 novel epitope showed phenotypic heterogeneity skewed towards the T follicular helper cell subset. Using a TCR specific for this novel PDC-E2 epitope, we created an engineered Treg that suppressed PDC-E2 specific polyclonal CD4 Tconv cells from PBC patients, indicating a potential role for engineered Treg therapy targeting PDC-E2 in PBC.

Tu105. 10X Scrnaseq Allows Characterization of Antigen Specific T Cell Populations in Peripheral Blood and Synovium of Patients with RA

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Single-cell analysis has emerged as a powerful tool for investigating transcriptomics and T-cell receptor(TCR) diversity. However, challenges remain in applying this technology to rare antigen specific T-cells found in peripheral blood and tissues of individuals with autoimmunity. To address this challenge, we conducted a pilot study using the 10x Chromium platform to obtain CITE-seq and TCR repertoire data from PBMC and synovial T-cells. The AIM assay was used to isolate T-cells from PBMC reactive to an RA-antigen-peptide-pool(RAA) or a viral-pool(CEFX), with DMSO as a control. Samples were hash-tagged, sorted, then applied to 10x where 40% of these cells resulted in high quality data. RAA and CEFX reactive T-cells demonstrated clonal expansion. Clonally expanded TCR found in activation cultures were also detected in unstimulated PBMC. Multi-modal analysis of the antigen reactive T-cells (using Unicell Deconvolve) showed eight clusters defined by merging gene expression and surface antibody markers. RAA T-cells were primarily assigned to Th1 and central memory CD4, while a substantial number of CEFX T-cells were assigned to the exhausted CD8 T-cells cluster. Synovial tissue with matched PBMC was available for 3 individuals. Synovial T-cells had an activated phenotype, and showed an increase in clonal expansion compared to peripheral T-cells. Synovial TCR were shared with TCR found in RAA or CEFX paired samples, indicating that synovial T-cells of the same specificity can be found in the periphery. In conclusion, our approach may provide valuable insights into the antigen specific T-cell responses and TCR dynamics associated with RA pathology.

Tu106. Agonistic Anti-dcir Monoclonal Antibody Inhibits Itam-mediated Inflammatory Signaling and Promotes Immune Resolution

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AbbVie

Dendritic cell inhibitory receptor (DCIR) is a C-type lectin receptor selectively expressed on myeloid cells, including monocytes, macrophage, dendritic cells, and neutrophils. Its role in immune regulation has been implicated in murine models and human genome-wide association studies (GWAS), suggesting defective DCIR function associates with increased susceptibility to autoimmune diseases such as rheumatoid arthritis, lupus and Sjogren's syndrome. However, little is known about the mechanisms underlying DCIR activation to dampen inflammation. Here, we developed anti-DCIR agonistic antibodies that promote phosphorylation on DCIR's immune receptor tyrosine-based inhibitory motifs (ITIM) and recruitment of SH2 containing protein tyrosine phosphatase-2 (SHP2) for reducing inflammation. We also explored the inflammation resolution by depleting $DCIR^{+}$ cells with antibodies. Utilizing a novel human DCIR knock-in mouse model, we validated the anti-inflammatory properties of the agonistic anti-DCIR antibody in experimental peritonitis and colitis. These findings provide critical evidence for targeting DCIR to develop transformative therapies for inflammatory diseases.

Tu107. Agonistic Effect of Alefacept on TIGIT$^+$PD1$^+$ Effector Memory CD4 T Cells in New-onset Type 1 Diabetes

Lauren Higdon[1], Laura Cooney[1], Elisavet Serti[1], Duangchan Suwannasaen[2], Kristina Harris[1], Mark Anderson[1], Cate Speake[2], Jerry Nepom[1], Srinath Sanda[1] and Alice Long[2]
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Biomarkers of treatment are an important tool in determining optimal dosage of immunomodulatory therapies, particularly in heterogenous patient populations. In the T1DAL clinical trial, C-peptide was preserved in subjects with new-onset type 1 diabetes (T1D) treated with alefacept, an LFA-3 Ig fusion protein targeting CD2-high lymphocytes for depletion. Immediate changes in T cell counts did not correlate with response, likely due to considerable heterogeneity in CD2$^+$ cell composition across subjects. Elevated levels of exhausted CD8 T cells 78 weeks after treatment and islet-specific activated CD4 T cells at baseline both correlated with response, but have limited utility as biomarkers of treatment due to timing and rarity, respectively. CD2 is expressed on all T cells, but most highly on effector memory T cells (TEM). We found that CD2-high and PD1-TIGIT- CD4 TEM were depleted as expected, but CD2-low PD1$^+$TIGIT$^+$ CD4 TEM expanded by 12 weeks after treatment and were maintained to at least 78 weeks. In comparison to PD1-TIGIT- CD4 TEM pre-treatment, expanded PD1$^+$TIGIT$^+$ CD4 TEM were proliferative (Ki67$^+$) *in vivo* with low inflammatory cytokine production; both phenotypes were enhanced 35 weeks after treatment. Prolonged expansion of PD1$^+$TIGIT$^+$ CD4 TEM appears to be specific to CD2-targeted therapy, as other T cell depleting therapies (ATG and anti-CD3) caused only transient changes in this population. Thus, anti-CD2 therapy led to expanded PD1$^+$TIGIT$^+$ CD4 TEM concurrent with reduced functionality. These findings indicate that alefacept had an agonistic effect and suggest that PD1$^+$TIGIT$^+$ CD4 TEM may represent an early biomarker of treatment with alefacept.

Tu108. An Integrated Single-cell Chromatin Landscape and Transcriptomic Map of Human Thymic Epithelium Identifies HIVEP3 as a New Player in the Control of Immune Tolerance

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Promiscuous gene expression of tissue-restricted self-antigens (TRAs) by medullary epithelial cells of the thymus (mTECs) is crucial for the control of central T cell tolerance and autoimmunity. The crosstalk between developing thymocytes and mTECs sustains their maturation into mTEChi expressing a full repertoire of TRAs whose presentation ensures the purge of autoreactive T cells. mTEC maturation relies on NF-kB signaling and results in the expression of the autoimmune regulator (AIRE) that induces the expression of a wide array of TRAs. In addition, a significant amount of TRAs is expressed in the absence of AIRE and is influenced by the developmental stage of mTEC. Thus, to uncover the missing factors implicated in mTEChi maturation and TRA expression, we carried out a sc-ATAC sequencing on isolated TECs from human and mouse thymi. Remarkably, along with NF-kB we identified a strong conservation of open chromatin at HIVEP binding sites in human and mouse mTEChi, with specific accessibility at TRA genes, therefore indicating a role for HIVEP on the control of mTEChi development and TRA expression. Importantly, sc-RNAseq analyses of human TECs revealed that HIVEP3 is the sole HIVEP family member whose expression is exclusive to mTECs. Recently, we successfully created a Nfkb2-deficient rat model. Therefore, to assess the extent of Hivep3's effect on central tolerance, individually and in combination with Nfkb2, we generated the first Hivep3-deficient rat line by CRISPR/Cas9 genome editing. Hence, we propose that HIVEP3 plays a key role in the regulation of immune tolerance through the control of mTEC maturation.

Tu109. CD2-high Auto-reactive, but Not Viral-reactive, Memory CD8 T Cell Repopulation Is Hampered Following LFA3-Ig Depletion

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Alefacept is an LFA3-Ig drug that slowed loss of beta cell function in recent onset type 1 diabetic (T1D) individuals in the T1DAL trial. Alefacept depletes CD2-high cells, but its effect on autoreactive CD8 T cells has not been described. We used mass and flow cytometry with Class I peptide-MHC tetramers on clinical and in *in vitro* depletion assays to count and characterize the CD8 population during alefacept therapy. We found that memory CD8 T cells (Tmem) in healthy and T1D individuals include both CD2-high and CD2-low subsets, all of which express higher levels than naïve CD8 T cells. CD2-high Tmem included central and effector memory, $CD57^+$, and exhausted subsets while CD2-low memory and CD57 populations express Helios. Islet-specific cells were enriched in this $Helios^+$ CD2-low Tmem population. CD2-high viral and islet specific CD8 Tmem were depleted with alefacept therapy both *in vivo* and *in vitro*. However, overall depletion of islet-specific Tmem was not significant due to their prevalence in the CD2-low population that was not depleted. Two years following treatment, CD2-high total and viral specific CD8 Tmem rebounded but islet specific cells did not. This lack of rebound among CD2-high islet-specific Tmem did not associate with clinical response, nor did it appear to be due to a natural decline over time as determined by comparison to placebo data. Together, these data suggest that islet-specific CD8 T cells are enriched for a $Helios^+$ CD2-low Tmem subset that is not depleted by alefacept but also does not preferentially expand following treatment.

Tu110. Characterizing Dysregulated B Cell Subsets in Systemic Lupus Erythematosus

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Background B cell dysregulation and production of autoantibodies against autoantigens are hallmarks of Systemic lupus erythematosus (SLE). In healthy adults, B cells with autoreactive antibodies are eliminated from the repertoire before they reach the B cell maturation stage, a mechanism that is altered in SLE. Objectives Investigating the dysregulated B cell subtypes in SLE compared to healthy control (HC) and characterize their transcriptional programs, antibody repertoires and their antigen specificity. Methods Single-cell RNA-sequencing (scRNA-seq) and BCR repertoire-sequencing of B cells from SLE (n=10) and HC (n=10). We expressed monoclonal antibodies (mAbs) encoded by dysregulated B cells in SLE (n=100) and screened their antigens specificity. Results scRNA-seq clustered memory B cells (mB) into $CD27^+$ mB (IgD-$CD27^+$CD11c-) and double negative (DN2) mB (IgD-CD27-$CD11c^+$, Fig. 1A-D). Transcriptome analyses of SLE $CD27^+$ mB cells revealed remarkable upregulation of cell activation and antigen processing and presentation (APC) pathways, as compared to HC (Fig. 1E-H). BCR analysis showed that the $CD27^+$ mB and DN2s are class-switched and have increased somatic hypermutations (SHM). Characterization of mAbs encoded by expanded and dysregulated B cells in SLE identified multiple SLE B cell mAbs that stained the HEP-2 cells and bound self-antigens on antigen arrays (Fig. 2). Conclusions Our findings revealed subsets of dysregulated $CD27^+$ mB cells encoding anti-nuclear autoantibodies in SLE. We demonstrate that clonally expanded autoimmune B cells are shared between $CD27^+$ and DN2 linages. Our results suggest that $CD27^+$ mB cells undergo cell activation, encode autoantibodies and consequently contribute to the development of pathogenic DN2 B cells.

Tu111. Circulating Pathogenic Like Lymphocytes Are Mechanistic Targets for the Induction of Immune Tolerance in Rheumatoid Arthritis

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In published work, using a high dimensionality approach, we identified a subset of pathogenic-like T cells (CPL) that (1) has a strong inflammatory function; (2) is able to recirculate through the sites of autoimmune reaction; (3) is highly enriched in synovial clonotypes; and (4) is correlating with disease activity rheumatoid arthritis (RA). The antigenic targets of CPL are still unknown. We have also previously identified a heat shock protein derived peptide (dnaJP1) as a dominant T cell antigen in RA and developed a tolerance-induction clinical trial program showing promising clinical efficacy in Phase IIa. Here, we investigated whether dnaJP1 peptide could have any effects on $CD4^{+}HLADR^{+}$ T cells. We stimulated with dnaJP1 PBMCs from a cohort of treatment naive RA patients and healthy controls, and assessed the CD4 memory landscape using our EPIC AI-powered Cytof platform, We found significantly elevated in RA patients two clusters of CPL effectors expressing TNFa with IL17A or IFNg, and proliferating in the presence of the dnaJP1. In summary, our data identifies in dnaJP1-specific CPL a mechanistic target of clinical relevant immune toleration. It also identifies CPL as an easily accessible pool of pathogenic-like cells, which may be used to advance our understanding of aberrant self-recognition, as well as to design targeted diagnostic and therapeutic tools.

Tu112. Comparative Analysis of NZWxNZB F1 and FcγR2bKO Mouse Models: Insights into Lupus Gene Signatures and Renal Pathology

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Genentech

Systemic Lupus Erythematosus (SLE) mouse models, including the NZB/W F1 model, recapitulate key aspects of human disease pathology, involving dysregulation of various immune cell types such as B cells, T cells, dendritic cells, and macrophages. This study compared the NZWxNZB F1 mouse model to the FcγR2bKO mouse model for lupus, aiming to elucidate similarities and differences in their expression profiles and renal pathology. Transcriptomic analysis revealed distinct gene signatures, with the F1 model exhibiting a strong B cell signature and the FcγR2bKO model demonstrating an intriguing neutrophil signature. Histopathological examination of kidneys from the FcγR2bKO mice revealed features resembling stage 4 human lupus nephritis with extensive, large, subendothelial immune complex deposits and small, scattered, subepithelial deposits. TLR7 transgene was introduced to accelerate disease and the resulting TLR7Tg FcγR2bKO mice have an immature neutrophil population as well as neutrophil infiltration into the glomeruli. These findings highlight the utility of TLR7Tg FcγR2bKO mouse model in recapitulating aspects of lupus pathology and offer insights into the contribution neutrophils to disease pathogenesis.

Tu113. Deciphering TNFR2-mediated Modulation of Tissue Resident Regulatory T Cells in Inflammatory and Autoimmune Disorders

Keith Mitchell
TRex Bio

Regulatory T cells (Tregs) are critical sentinels of immune homeostasis. We have found that tissue resident Tregs are in part modulated by the tissue-enriched TNF receptor superfamily member TNFR2, with functional validation demonstrating a role in expansion and activation. By integrating a TNFR2 specific gene signature with

our database of human tissue data across a range of gut and skin diseases, we evaluate TNFR2 pathway dysregulation in disease, enabling a data driven selection of indications for early clinical development. Leveraging statistics and machine learning to infer tissue specific Treg gene regulatory networks, we shed light on the intricate relationships between TNFR2, autoimmune genetics and Treg functions. Unraveling the role of TNFR2 within the Treg gene network has unveiled crucial functional and signaling pathways and highlights the target's relevance to dysregulated Treg subsets. The systems-level elucidation of these pathways enhances our understanding of Treg regulation but also provides a translational framework for therapeutic development, offering a valuable approach applicable to the exploration of future targets in the quest to develop targeted therapies for immune-related disorders.

Tu114. Decoding the Complexity of Childhood-onset Systemic Lupus Erythematosus (cSLE) Using a Multi-dimensional Approach: Enhancing Theragnosis with an Immunological-based Diagnostic Model Revealing Mechanistic Insights

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Childhood-onset systemic lupus erythematosus(cSLE) is usually more severe than adult-onset SLE. We aim to resolve the immunopathogenic complexity of SLE using high-dimensional mass cytometry(CyTOF). Machine-learning and mechanistically relevant cell clusters were used to create a diagnostic model. For discovery, peripheral blood mononuclear cells(PBMCs) from 30 cSLE patients (53 timepoints, median age:14 years) and 17 age-matched healthy controls were studied. Unsupervised analysis was done using our Extended Poly-dimensional Immunome Characterisation(EPIC) pipeline. Discovery cohort data identified predictive cell clusters using machine-learning(linear SVM, PLS-DA, random forest (RF)) with MetaboAnalyst v6.0. Cell frequencies are expressed as $CD45^+$ PBMCs percentage (median[interquartile range]) with statistical significance $p< 0.05$(Mann-Whitney U). For independent validation, 18 cSLE (median age:11.5 years) and 23 healthy individuals were used. 67 unique cell clusters formed the basis to map validation cohort data. Mapping was automated using previous unsupervised analysis and expert annotation of discovery data. A memory Treg-like population ($CD3^+CD4^+CD45RO^+CD25$-$Foxp3^+CTLA^+$) was higher in cSLE than healthy (2.75[1.90-4.36] vs. 1.28[0.83-1.78],$p < 0.0001$). Concurrently, a $CD8^+CD45RA^+BAFF^+$ T-cell population was enriched in cSLE (8.91[6.78-11.4] vs. 3.22[2.79-4.86],$p < 0.0001$). These populations and eight others were selected through collective ranking of the most important features generated by machine-learning algorithms on discovery cohort data to create a classification model. The average accuracy of discriminating cSLE and healthy based on 100 cross validations in the discovery cohort is 85.6%[85.4-85.7], with 88.9%[83.3-100] sensitivity for the validation cohort. Further studies will quantitate autoantibody titers with ELISA for comparison. In conclusion, this immunological-based classifier model can augment current clinical criteria while imparting a theragnostic perspective to cSLE.

Tu115. Deep Characterization of Autologous Living Drug, the Engineered Treg- like CD4LV-FOXP3, from Patients with IPEX Syndrome Using Multidimensional Methods

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Human $CD4^+$T cells from both healthy subjects and FOXP3 deficient patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) acquire the phenotype and *in vitro* and *in vivo* function of regulatory T cells (Treg) upon lentiviral-mediated expression of exogenous FOXP3. The resulting engineered Treg-like cells, termed CD4LV-FOXP3, are potential "living drugs" for autoimmune diseases with dysfunctional Tregs and are now tested in patients with IPEX in a Phase 1 clinical trial (NCT05241444). However, the heterogeneity of the CD4LV-FOXP3 and the cell-subsets changes of the parental $CD4^+$ T during transduction remains ill-defined. Here, we combined single-cell CITE and TCR sequencing, and other methods to investigate the $CD4^+$T cells from IPEX patients before and after manufacturing of CD4LV-FOXP3. We identified 14 conserved subpopulations with distinct transcriptome profiles from 3 $NGFR^+$ drug substances (NGFR, the co-transduced surface marker) and their NGFR- un-transduced counterparts. The composition of the subsets was comparable among different patients-derived cell products. Compared with the NGFR- subsets, $NGFR^+$ subsets had higher expression of the transgene FOXP3-lv, memory marker gene SELL, CD27 and Treg marker genes, including CCR4 and IL2RA, and lower expression of IL7R and the inflammatory cytokines IL17A, IL17F, IL13, IL22, IL5, IFNG. In addition, $NGFR^+$ clusters have constrained TCR clone expansion and cycling status. Thus, our study reveals that CD4LV-FOXP3 products preserved the heterogeneity of the parental population of cells and further confirmed, at the single-cell level, their Treg-like signatures. These findings constitute the baseline for monitoring the persistence and functional stability of the CD4LV-FOXP3 cells *in vivo*.

Tu116. Development of an Injectable Hydrogel-based Treg Immunotherapy for Uveitis: A Novel Approach Towards Disease Management

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Uveitis is a group of ocular inflammatory diseases that is mediated by undesired ocular inflammation. Studies of patients with uveitis and animal models of experimental autoimmune uveitis (EAU) indicate that regulatory T cells (Tregs) play a significant role in resolving ocular inflammation. Treg-based immunotherapy has been studied extensively. However, ensuring the viability and persistence of Tregs post-infusion remains a challenge. To enhance the therapeutic efficacy of Treg-based immunotherapy, we propose the use of hyaluronan and methylcellulose hydrogel (HAMC) to protect Tregs. In-vitro induced Tregs were encapsulated by HAMC hydrogel and were intravitreally delivered to the inflamed eye of EAU mice after disease onset. Disease development was monitored by funduscopy. HAMC-encapsulated Tregs were tracked by chemical exchange saturation transfer (CEST) using magnetic resonance imaging (MRI) non-invasively. We found that one-time intravitreal injection of HAMC-encapsulated Tregs resolved ocular inflammation and preserved visual function in EAU mice. We further demonstrated that HAMC enhances the stability and survivability of Tregs after injection into the inflamed eyes. We revealed that HAMC protects Tregs from proinflammatory environment and promotes their suppressive functions to inhibit effector T cells. After intravitreal injections, HAMC-encapsulated Tregs were able to be detected by CEST-MRI. These data supported the notion that HAMC improves the therapeutic efficacy of Tregs in EAU. Also, HAMC provides an opportunity for us to non-invasively monitor and quantify HAMC-encapsulated Tregs by chemical exchange saturation transfer (CEST) using magnetic resonance imaging (MRI), which greatly assist the optimization and evaluation of this Treg-based immunotherapy in preclinical and clinical setting.

Tu117. Development of Nanoscale Artificial Antigen-presenting Cells as Novel Treatment for Type 1 Diabetes

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Type 1 diabetes (T1D) is a T cell-mediated autoimmune disease and its pathogenesis involves autoantigen-specific $CD8^+$ T cells destroying insulin-producing beta cells in the pancreas. Here, we used a novel nanoscale Artificial Immune Modulation (AIM) platform developed by NexImmune. With this platform, T1D antigenic peptides presented on Class I MHC molecules alone or in combination with 2nd signal ligands are affixed to 60-80 nm nanoparticles (nps) composed of the polymer, polylactic acid - polyethylene glycol (PLA-PEG). The AIM nps mimic antigen-presenting cells and direct a specific T cell-mediated immune response. Our data show that murine pmel-1 $CD8^+$ T cells co-cultured with Db/gp100-PDL1-Ig nps secreted significantly lower levels of IL-2 and IFN-gamma *in vitro* compared to those treated with either uncoated nps or non-specific control nps (Db/unload-PDL1-Ig). Moreover, human MART-1 $CD8^+$ T cells incubated with HLA/MART-1-PDL1-Ig nps had significantly diminished *in vitro* killing activity of peptide loaded target cells, compared to the $CD8^+$ T cells treated with nps coated with either peptide alone or 2nd signal (PDL1-Ig) only. These results suggest that PDL1-Ig AIM nps inhibit function of $CD8^+$ T cells *in vitro* in an antigen-specific manner. We then set out to investigate the effect of PDL1-Ig AIM nps using transgenic RIP-mOVA mouse model. RIP-mOVA recipients of OT-I $CD8^+$ T cells treated by Kb/OVA-PDL1-Ig nps have been protected from autoimmune diabetes compared to the mice treated by uncoated nps ($P=0.049$). This result suggests inhibition of adoptively transferred pathogenic $CD8^+$ T cells by PDL1-Ig AIM nps *in vivo*.

Tu119. DREAMT: A Study of Abatacept in Recent Onset T1D to Determine Early Biomarkers of Response

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Abatacept, a CTLA4Ig costimulation blockade drug, has shown promise in delaying decline in newly diagnosed Type 1 diabetes (T1D). However, there is a range of responsiveness among patients necessitating a biomarker to better select patients who may benefit. This DREAMT study includes 31 subjects treated for 3 months with intense sampling for biomarker discovery including two baseline timepoints, multiple timepoints during treatment, and 12 months following treatment. Initial results showed 21/31 positive response rate, with an average greater preservation of c-peptide than published historical data, as measured by quantitative response. However, a third of subjects displayed more rapid decline in 6 months which allows for a comparison to find biomarkers of response. Analyses showed that response did not correlate with age, duration of early disease or serum drug concentrations, leading us to explore other biological factors. We did observe modest transcriptional changes from whole blood RNA-sequencing at 12 weeks, including a decrease in follicular helper T cell and CD8 T cell exhaustion gene modules, as well as CD4 regulatory T cell modules as early as 4 weeks. We will follow up on these findings using high parameter flow cytometry to look at the immune cell changes and inflammatory profiling at earlier time points. Taken together, our data suggests that immune measures as early as 4 weeks could be used to predict response to abatacept in early onset T1D allowing for better selection of patients who could benefit from abatacept therapy.

Tu120. Effect of Monocolonization with Akkermansia Muciniphila on the Diabetes Incidence and Immune Cell Subsets in Ex-germ-free NOD Mice and in Adoptive Transfers of Splenocytes to NOD-SCID Recipients

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Akkermansia muciniphila is considered as a potentially beneficial, probiotic bacterium in type 1 diabetes (T1D). In order to discriminate a direct, causative effect of Akkermansia muciniphila, from effects caused by complex changes in gut microbiota, we assessed spontaneous diabetes incidence, insulitis and carried out flow-cytometry detection of immune cell-subsets in ex-germ-free NOD mice monocolonized with Akkermansia muciniphila. Monocolonization of germ-free NOD female mice with Akkermansia muciniphila either after weaning or if followed in litters born to already monocolonized NOD females, led to slightly delayed (statistically significant at $p< 0.05$) onset of hyperglycemia in Akkermansia-colonized but not in Akkermansia-born mice compared to germ-free controls. On the other hand, although not statistically significant, the Akkermansia-born NOD females tend to keep a lower diabetes incidence compared to germ-free NOD females. No changes in insulitis scoring were documented. Adoptive transfer of splenocytes to NOD-SCID recipients led to only slightly lowered and delayed onset of diabetes in NOD-SCID mice that was not statistically significant. Among the T- and NK-cell subsets in lymphoid organs only minor differences were found; the most prominent being increased proportions of Foxp3 Tregs and Tr1 cells in SPF controls, but a reciprocal increase of IL-10 positive Foxp3 Tregs was found in the germ-free and Akkermansia-colonized NOD mice. In conclusion, in gnotobiotic, well-defined conditions, colonization of NOD mice with Akkermansia muciniphila surprisingly displayed only limited influence on the development of T1D (supported by grant no. NU21-01-00085 fom the Ministry of Health of the Czech Republic and no. 22-21356S from the Czech Science Foundation).

Tu121. Effects of Phosphodiesterase-4 Inhibitor Apremilast on Diabetogenic Immune Responses in Non-obese Diabetes Mice

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University of British Columbia

In Type 1 diabetes, islet β cells are destroyed by diabetogenic CD8 T cells. Macrophages also participate in diabetogenic CD8 T cell activation. Phosphodiesterase inhibitors Pentoxifylline and Rolipram prevent diabetes in non-obese diabetic (NOD) mice through the inhibition of inflammatory cytokine production. Our aim was to investigate effects of phosphodiesterase inhibitors (apremilast and roflumilast) on diabetogenic immune responses in NOD mice. Young pre-diabetic female NOD mice were treated with oral apremilast or vehicle for 3 weeks. Dendritic cell (DC) priming, IGRP $V7^+CD8^+$ diabetogenic T cell numbers and $Foxp3^+CD73^+$ CD4 T regular cells (Tregs) were then assessed by flow cytometry. The effects of apremilast and roflumilast on bone marrow (BM) cell differentiation and peritoneal macrophage activation were evaluated. Treatment of 5-week old NOD mice with apremilast reduced the priming of adoptively transferred 8.3 CD8 T cells. There was a decrease in IGRP $V7^+CD8^+$ T cells in the pancreatic lymph nodes (PLN) and increase in Tregs in apremilast-treated compared to control mice. The addition of apremilast or roflumilast to the culture of BM cells decreased numbers of $CD11c^+$ BMDCs. Apremilast and roflumilast also decreased CD86 expression on peritoneal macrophages stimulated by LPS. There was no demonstrable effect on peptide-induced diabetogenic T cell activation and function. Our data demonstrate that apremilast and roflumilast have inhibitory effects on BMDC differentiation, macrophage activation and diabetogenic immune responses. Treatment of NOD mice with apremilast or roflumilast at an early stage of diabetes may effectively prevent diabetes development through the inhibition of innate immune responses.

Tu122. EVO756, a Novel, Small Molecule Antagonist of MRGPRX2, Potently Inhibits Primary Human Mast Cell Activation
Sreya Bagchi
Evommune

The Mas-Related G-Protein Coupled Receptor X2 (MRGPRX2) contributes to IgE-independent mast cell degranulation and neuroinflammation upon binding various endogenous and exogenous cationic ligands. In humans, the receptor is highly expressed on cutaneous mast cells, which serve as a great tool to study MRGPRX2 biology. Thus, we examined the ability of several MRGPRX2 ligands to activate mast cells derived from human skin by tracking surface expression of degranulation markers, CD63 and CD107a, via flow cytometry. Further, we determined whether MGRPRX2 induced mast cell activation is inhibited by EVO756, a novel, potent, small molecule antagonist of the receptor. Indeed, EVO756 demonstrated potent and concentration-dependent inhibition of primary human skin mast cell degranulation in response to MRGPRX2 agonists. These findings were further confirmed by quantification of tryptase released in the culture supernatant. Therefore, EVO756, which potently inhibits MRGPRX2 mediated mast cell activation, represents a promising therapeutic for the treatment of mast cell driven diseases such as chronic spontaneous urticaria.

Tu123. Genetically-controlled Differences in Thymocyte Development in Type 1 Diabetic Immune Systems
Benjamin Vermette
CCTI - Columbia University

Type 1 diabetes (T1D) is a T cell-mediated autoimmune disease. The role of abnormal thymic selection of autoreactive T cells in driving human T1D is unknown, as thymocytes are not accessible for study in patients. We have developed a “Personalized Immune (PI)” human immune system mouse model that allows comparison of thymocyte selection in T1D patients and healthy controls (HCs). PI mice are constructed by administering bone marrow $CD34^+$ hematopoietic stem cells (HSCs) from HC or T1D donors to thymectomized NSG mice that also receive human fetal thymic tissue sharing the same T1D class II HLA risk allele with both adult human donor. Following immune reconstitution (18 to 24 weeks post-transplant), animals are sacrificed and thymocyte suspensions undergo single-cell 5’RNA sequencing (10X Genomics). A total of 3 T1D and 5 HC thymocyte suspensions from 2 T1D and 2 HC donors have been sequenced and analysis is in progress. Transcriptional profiles have revealed maturing thymocyte subsets previously identified in normal human thymus (Chopp et. al 2020). Preliminary analysis reveals statistically significant differential expression of genes driving thymocyte maturation at the immature single positive, unselected ($CD69^-$) and positively selected ($CD69^+$) double positive stages between T1D and HC thymocytes, suggesting HSC-intrinsic differences in TCR signaling. Correlations with T1D-associated single nucleotide polymorphisms in T cell signaling such as SH2B3 and Erk/MAP kinase pathway genes, which were enriched in some T1D donors and associated with defects in negative selection of a transgenic islet autoreactive TCR, will be informative.

Tu124. Hypoxia-induced Immunometabolic Reprogramming Promotes Dysfunctional Tregs
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$FOXP3^+$ regulatory T cells (Tregs) are central for maintaining peripheral tolerance and play a critical role in promoting immune homeostasis and tissue regeneration. Depending on the environment, they are able to suppress pro-inflammatory responses by different mechanisms, such as cell contact dependent mechanism or by

secretion of anti-inflammatory cytokines. Tregs have distinct metabolic needs and preferentially rely on mitochondrial metabolism to exert their suppressive function. We have recently shown that changes in the ionic microenvironment and increased Na^+ could inhibit mitochondrial respiration of Tregs through interference with the electron transport chain (ETC), leading to the acquisition of a pro-inflammatory Th1-like phenotype mimicking dysfunctional Tregs frequently observed in autoimmunity. Interestingly, mitochondrial Na^+ accumulation and up-regulation of $Na^+/Ca2^+$ exchanger activity dependent blockade of the ETC has been previously described also under isotonic hypoxic conditions. We therefore investigated by various immunological and metabolic assays the effects of hypoxia on Treg immunometabolism and functionality. Of note, temporary hypoxia induced substantial changes in human Treg immunometabolism and a phenotype closely mimicking dysfunctional pro-inflammatory Th1-like Tregs. Thus, the interference with this pathway could potentially serve as a novel target to manipulate or restore Treg function under oxygen-deprived conditions, having important implications for various diseases.

Tu125. Identification of Autoreactive Cytotoxic T Cells in Anca-associated Vasculitis

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Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a rare and severe autoimmune disease, characterized by a pauci-immune necrotizing vasculitis. ANCAs targeting proteinase-3(PR3) or myeloperoxidase(MPO) and ANCA-producing B-cells play a central role in the pathogenesis. Autoreactive T-cells are less well-studied, but recent studies suggest their importance in the AAV pathogenesis. Here, we aimed to investigate autoreactive T-cells and their cytotoxic responses. AAV or healthy control (HC) PBMCs were cultured with ANCA antigens and anti-CD28 and analyzed with flow cytometry after 24h. In addition, T-cells were analyzed with single cell RNA sequencing. Anti-$PR3^+$-AAV patients exhibited increased EMRA $CD8^+$ T-cells and $GZMB^+CD8^+$ T-cells, whereas anti-MPO^+-AAV patients had increased levels of $GZMB^+IFNy^+CD4^+$ and $GZMB^+IFNy^+CD8^+$ T-cells at baseline. MPO specifically induced expansion and proliferation of $GZMK^+IFNy^+CD4^+$ and $GZMK^+IFNy^+CD8^+$ T-cells in anti-MPO^+-AAV patients. Single cell RNA sequencing demonstrated large populations of clonally expanded $CD8^+$ T-cells in the $GZMB^+$ and $GZMK^+CD8^+$T-cell compartment in AAV patients, which was not observed in HCs. The clonally expanded $CD8^+$T-cells were characterized by expression of CD57, CD244 and CD314. Specifically, $GZMB^+CD8^+$T-cells expressed GPR56 and CX3CR1, whereas $GZMK^+CD8^+$ T-cells expressed CD56, CD161 and CD183. Moreover, both $GZMB^+$ and $GZMK^+CD8^+$ T-cells of AAV patients displayed effector cytotoxic transcriptional programs, including different granzymes, IFN-y, perforin, CCL5, KLRK1 and NKG7, which was more abundant than in HCs. This study demonstrated that clonally expanded cytotoxic $CD8^+$T-cells are present in AAV patients, and we identified an autoreactive $GZMK^+IFNy^+CD8^+$ subpopulation in anti-MPO^+-AAV patients. The identification of pathogenic autoreactive T-cells could provide novel insights into next-generation therapeutic targets for AAV.

Tu126. Insulin B Hybrid Peptides Are T Cell Targets in Type 1 Diabetes

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Recognition of the insulin B-chain has known importance in human type 1 diabetes (T1D), but its native sequence is poorly antigenic. We hypothesized that formation of hybrid insulin peptides (HIPs) through cross-linking of insulin B chain fragments to other secretory granule peptides generates potent epitopes that contribute to human disease. To identify relevant Ins B HIPs, we identified theoretical insulin B-chain hybrids that are likely to bind to high-risk HLA proteins, including HLA-DRB1*03:01 (DR0301), HLA-DRB1*04:01 (DR0401), and HLA-

DQA1*03:01-DQB1*03:02 (DQ8). Top candidate peptides for each HLA were synthesized and screened for binding using a competition assay. We then performed *in vitro* assays to assess T cell recognition of these insulin B HIPs using samples from subjects with T1D. $CD4^+$ T cells were stimulated with peptide pools, expanded for two weeks, and resulting populations of HIP-specific T cells were visualized by HLA class II tetramer staining and activation induced marker (AIM) assays. To isolate HIP responsive T cell clones and lines, tetramer-positive and AIM positive T cells were sorted, expanded, and rescreened either by tetramer staining or AIM assay. Specificity for single insulin B-chain HIPs was demonstrated through proliferation assays and the HLA restriction was confirmed by blocking with anti-DR or anti-DQ antibodies. Insulin B-chain HIPs with confirmed immunogenicity were directly enumerated responsive T cells in the peripheral blood of subjects with Type 1 Diabetes and healthy controls. In total, our results demonstrate that Ins B HIPs are relevant targets in human subjects with T1D across multiple high risk HLA types.

W102. A Novel Inhibitor of Cgas-sting-tbk1 Pathway with Broad Application in Autoimmune and Fibrotic Diseases

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Aberrant activation of cGAS-STING-TBK1 pathway via excessive production of pro-inflammatory cytokines is linked to several autoimmune diseases including monogenic diseases like AGS (Aicardi-Goutières syndrome) and SAVI (STING-associated vasculopathy with onset in infancy). Publications support importance of this pathway in lupus, SLE, CLE, and other autoimmune disorders such as Systemic Scleroderma. To overcome the existing challenges to inhibit this pathway, we have generated novel “exosite” inhibitors of TBK1 (EXO-TBKi), that disrupt recruitment by STING for downstream activation of interferon and NF-κB responses. EXO-TBKi selectively inhibit the TBK1-STING axis while sparing non-disease relevant housekeeping functions of the kinase, offering superior efficacy and improved therapeutic window. Rational and structure-based drug discovery efforts resulted in identification of a series of selective EXO-TBKi with potent binding affinities that translated into nanomolar inhibition of IFNβ in THP1 cells and primary human cell types. Orally bioavailable EXO-TBKi were evaluated in both *in vivo* and *ex vivo* models to inhibit proximal and distal pharmacodynamic markers upon stimulation with a STING agonist. We also observe this PK/PD relationship in a pathway-driven TREX-1KO model where treatment with EXO-TBKi results in dramatic reduction of pro-inflammatory cytokines including IFNβ, CXCL-10, CXCL-9 and IFIT1. Most strikingly, when assessed for activity in SLE patient derived human whole blood and PBMC, EXO-TBKi robustly suppresses pathway activation. Similarly, upon activation of pathway, EXO-TBKi reduces cytokine production in disease relevant skin inflammatory models, fibroblasts and keratinocytes. In summary, we have identified potent, selective EXO-TBK1 inhibitors, demonstrated efficacy across pre-clinical models and optimized properties towards developmental candidate nomination.

W103. BiTEing Multi-drug Resistant Rheumatoid Arthritis with CD19-T Cell Engagers

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Bi-specific T-cell engagers (BiTEs) kill B cells by engaging T cells. BITEs are highly effective in acute lymphoblastic leukemia. We treated six patients with multi-drug resistant rheumatoid arthritis (RA) with the CD19xCD3 BiTE blinatumomab. Low doses of blinatumomab led to B cell depletion and concomitant decrease of

T cells, documenting their engager function. Treatment was safe, with brief increase in body temperature and acute phase proteins during first infusion but no signs of clinically relevant cytokine-release syndrome. Blinatumomab lead to a rapid decline in RA clinical disease activity, improved synovitis in ultrasound and FAPI-PET-CT and reduced autoantibodies. High dimensional analysis of B cells documented an immune reset with depletion of activated memory B cells, which were replaced by non-class switched, IgD positive naïve B cells. Together, these data demonstrate the principle feasibility to effectively treat B cell mediated autoimmune disease with BiTEs.

W104. Inferring Disease Mechanisms in Lupus Nephritis: Lessons from the Accelerating Medicines Partnership (AMP) in SLE Consortium

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Gaining insights into the immune mechanisms underlying lupus nephritis (LN) remains a major challenge towards the development of more effective and specific therapies for this potentially fatal disease. We analyzed the single-cell RNA-sequencing data generated as part of the AMP SLE consortium. Kidney biopsies from 156 LN patients and 30 healthy controls were profiled, yielding 73,440 immune cells and 474,873 tissue cells. Our analysis showed that pre-fibrotic disease is dominated by myeloid cells, with an expansion of disease-specific proinflammatory and phagocytic subsets of macrophages, in a manner correlated with disease activity. The emergence of irreversible tissue damage is associated with an increase in the relative frequencies of dendritic cells, B cells, $GZMK^{+}CD8^{+}$ T cells and memory $CD4^{+}$ T cells, and a decrease in the interferon response. *In vitro* experiments, in which cells were stimulated with several different cytokines and lupus-related antigens, and the resulting transcriptional changes were compared to those observed *in vivo*, suggested that the differentiation of proinflammatory macrophages is driven by TLR7 ligands, while that of phagocytic macrophages is promoted by apoptotic debris. Differential expression analysis of parietal epithelial cells (PECs) identified the upregulation in disease of several receptors whose ligands are expressed by immune cells. Additional analysis suggested that the production of Galectin-9, TWEAK and plasminogen activator, urokinase receptor (PLAUR) by phagocytic macrophages, and of IFNγ by $CD8^{+}$ T cells, may promote PEC activation, proliferation and migration, leading to glomerulosclerosis. We provide further evidence supporting these predictions by analyzing a spatial transcriptomics dataset of LN samples.

W105. Insulin-reactive T Cells are Not Deleted in Participants with Early Stage T1D Treated with Oral Insulin

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Oral insulin (OI) given for 6 months daily (67.5 mg, n=49), but not biweekly (500 mg, n=36, p=0.0066), reduced islet autoantibody levels at 12 months in early stage T1D, suggesting immune modulation. Since T cells regulate autoantibody production, we evaluated insulin-reactive T cells before and during treatment. Insulin-specific CD4 and CD8 T cell frequencies were assessed using Class II tetramer and Class I multimers in HLA-DR*0401(n=31) and HLA-A2 (n=30) participants, respectively, at enrollment, baseline and 3 months. Insulin-specific T cells were reproducibly identified in >80% of samples. No significant changes in T cell frequency were detected following 3 months of treatment. Yet, increased CD4 insulin-specific T cell frequency from baseline to 3 month correlated with reduced insulin autoantibody levels at 6 months in daily OI treated participants (n=15, r=0.51), suggesting linkage between insulin-specific T cells and antibody levels. No change in insulin-specific CD8 T cell frequency was confirmed across participants (n=42) with a range of HLA using tetramer in a CyTOF panel, and a $CXCR3^{+}tbet^{+}$ memory phenotype, which was enriched in islet-specific cells, also showed no change. By comparison, insulin peptide-reactive CD8 T cell responses detected by IFN-γ ELISPOT were similar at baseline between the groups but lower at 6 and 12 months in daily OI participants (n=18) compared to biweekly treatment (n=16, $p< 0.01$ at both timepoints). Thus, insulin-reactive CD4 and CD8 T cells can be detected in early stage T1D and are not depleted, suggesting that daily OI may functionally alter T cell responses.

W106. Integrated Soluble Proteomics: Predicting Renal Involvement in Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE), a clinically diverse autoimmune disease, challenges in predicting its varied manifestations. While cytokine profiles have been used, their predictive accuracy is still limited. This study aimed to evaluate soluble proteomics and autoreactomics in predicting SLE renal involvement. We included 88 plasmas from 16 controls and 72 SLE patients categorized into: Group2 - without renal disease nor anti-nuclear antibodies, Group3 - with anti-dsDNA but no renal disease, Group4 - both anti-dsDNA and renal disease, and Group5 - anti-RNA autoantibodies with some renal disease. Here, we utilized a high-throughput and sensitive human proteomic assay (Olink ExplorerNGS) consisting of 1472 analytes. Univariate analysis showed that there are 186 dysregulated proteins in Group2, 398 in Group3, 444 in Group4, and 375 in Group5 compared to controls. Pathway analysis revealed upregulation in toll-like receptor, interleukin-10, and cytokine/chemokine signaling in Group3, Group4, and Group5. Notably, interferon-gamma production/signaling was prominent only in Group4 with renal disease. Unsupervised UMAP identified four clusters, with cluster4 comprising patients with renal disease. Cluster4 showed significantly higher levels of HAVCR2, BST2, IL10, CD300, and IFNG than other clusters. Random Forest model based on HAVCR2, BST2, TNFRSF1B, CXCL9, CD300, and IFNGR was able to predict patients with renal involvement with ~91.0% accuracy. Of interest, HAVCR2 and BST2 are both expressed in the kidney, suggesting the tissue-specific damaging signatures with cytokines/chemokines may provide a superior RF model. The study underscores the importance of a comprehensive approach, combining molecular signatures and inflammatory mediators, for enhanced prediction and monitoring of severe manifestations.

W107. Interferon-induced Mitochondrial Dysfunction in Myositis: from Mice to Men

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Myositis are autoimmune and inflammatory disorders leading to skeletal muscle weakness and disability. Human studies suggest a role for type I and II interferons in the pathogenesis of the disease. We have shown that ICOS pathway invalidation in NOD mice results in a switch of autoimmunity from diabetes towards myositis, providing a valuable murine model. Indeed Icos-/- NOD mice exhibit reduced muscle strength and muscle histopathological features including macrophage and IFNg-secreting CD4 T cell infiltration. Spatial transcriptome analysis revealed a strong downregulation of genes encoding proteins involved in key mitochondrial metabolic processes, mitochondrial dynamics and structure stability in myofibers from diseased mice close to inflammatory infiltrates. IFNg-blocking antibodies dramatically reduced mitochondrial abnormalities and haltered myositis clinical progression, indicating a pathogenic role for IFNg as a mediator of mitochondrial dysfunction in myofibers. Bulk transcriptome analysis of muscle biopsies from myositis patients revealed a negative correlation between IFNg and mitochondrial gene expression signatures. In addition, exposure of human myoblasts to IFNg *in vitro* significantly diminished mitochondrial gene expression. All these data support a link between IFNg and mitochondrial dysfunction and suggest a self-maintenance loop between mitochondrial dysfunction and inflammation, opening perspectives for mitochondria therapy in myositis.

W108. Investigating Immunological Profiles of Thymus in Myasthenia Gravis by scRNA-seq

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OBJECTIVE: To characterize the immunological signatures of thymus in patients with AChR-MG. METHODS: We collected matched thymus samples and peripheral blood monocytes (PBMCs) from two patients diagnosed with early-onset AchR-MG. To identify and characterize immune cell subsets within the thymus and PBMCs, we applied high-resolution single-cell RNA sequencing (scRNA-seq) technology. After removing the dead cells and following sequencing, we performed bioinformatic analysis. Public available dataset of age and sex-matched healthy controls (HC) were also included. RESULTS: A total of 39,943 single cells derived from thymocytes and PBMCs passed QC. Twenty-four immune cell clusters were identified. Comparing thymocytes, we observed a significant decrease in double positive T cells, along with an increase in CD4 T cells, B cells, NK and DCs in MG patients than HC. In PBMCs, we observed a significant increase in double positive T cells and CD4 T cells, while NK cells, DCs and monocytes were decreased in MG patients. Overall, our findings indicate a shift of double positive T cells from thymus to periphery, and an increase of B cells, NK cells and DCs in the thymus of MG patients. SUMMARY/CONCLUSION: Our study highlights the presence of an immune cell imbalance between the thymus and peripheral circulation in MG patients. Further investigations are necessary to confirm the underlying mechanisms and elucidate the role of these imbalanced immune cells in the pathogenesis of MG.

W109. JAK2 Overexpression in Mesenchymal Cells: Role in the Immunopathogenesis of UC

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Cells of mesenchymal origin, known as fibroblasts and their progenitors, are present in colonic mucosa, serve as immunosuppressors under homeostasis, and are suggested to be among key contributors to the immunopathogenesis of ulcerative colitis (UC). An increase in pathological fibroblasts known as Inflammatory Fibroblasts (UC-iFibs) was observed in UC and these cells expressed high levels of JAK2. While targeting JAK pathways has shown therapeutic promise, the mechanistic relevance of JAK2 upregulation in UC-iFibs remains unknown. We hypothesize that increased JAK2 signaling within UC-iFibs governs their pathological activity and promotes inflammation in UC. Human UC-derived mesenchymal cells were used in this study. Mesenchymal lineage- and fibroblast-specific conditional JAK2 overexpressing mice (Grem1CreJAK2VF$^{+/-}$ B6 and Col1αCreJAK2VF^{+} WASPKO respectively) were used in two models of UC-relevant colitis (TNBS and WASP KO). JAK2high UC-iFib primary human cultures treated with JAK2-specific inhibitor show significantly reduced expression of UC-relevant pathologic responses (IL-6, IL-15, IL-33, PD-L1). *In vivo* induction of JAK2 overexpression within fibroblasts at the initiation of chronic inflammation in Col1αCreJAK2VF^{+} WASP KO mice aggravated the clinical and histological features of colitis, and increased UC-relevant type 2 inflammatory responses. Importantly, JAK2 overexpression within mesenchymal lineage cells in Grem1CreJAK2VF$^{+/-}$ B6 mice was sufficient to induce colitis with UC-relevant histological features and increased pathologic type 2/17 responses. Overexpression of JAK2 in mesenchymal cells also aggravated TNBS colitis. Our data suggests that overexpression of JAK2 within mesenchymal cells is critical to the pathological activity of UC-iFib and is among the key events contributing to the development of UC.

W110. Local Delivery of IL-35 mRNA Therapeutic Using Lipid Based Nanoparticles Vector Demonstrates High Efficacy to Suppress Autoimmune Hepatitis

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IL-35 is a pleiotropic immunosuppressive cytokine suppressing T and B lymphocytes proliferation and functions, inducing regulatory T and B cells and activating Myeloid-suppressor-cells limiting inflammatory responses. The clinical use of cytokine is limited by their short half-live (less than 1 hour) and peripheral large immunosuppressive action caused by a non-specific biodistribution. We have evaluated the therapeutic potential of IL-35-encoded mRNA vectorized with Lipid-based nanoparticles (LNP) for a local delivery and expression of IL-35 into the inflammatory microenvironment. Efficacy of mRNA IL-35/LNP therapy was assessed in acute autoimmune hepatitis mouse model induced by Concavalin A characterized by T and B cell activation, increased of pro-inflammatory cytokines (IL-2/IL-6/IFNγ/IL-12p40) and hepatotoxicity (ASAT/ALAT). IL35 mRNA/LNP treatment reverses inflammation and liver-induced damage associated with a significant decrease of pro-inflammatory cytokines and transaminase levels even at low dose of mRNA injected (dose 0,5µg to 4ug) (*p< 0,05, n=3 experiments, n=10-13 mouse) as compared to untreated or empty LNP control groups. Further phenotypic analysis showed that IL-35 mRNA therapy decreases T cell activation (CD25) and favors expression of the inhibitory receptor PD-1 on B and T cells *in vivo*. Altogether, our results illustrate that localized IL-35 expression, through the use of mRNA/LNP therapy, has the potential to reverse acute autoimmune hepatitis inflammation and open potential new perspectives to use this novel RNA Therapeutic strategy for treatment of multiple auto-immune or inflammatory diseases.

W111. Loss of Thymic Tolerance to Perilipin-1 is Associated with Adipose Autoimmunity in Aire-deficient Mice

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Acquired generalized lipodystrophy (AGL) is a clinical syndrome characterized by immune-mediated loss of body fat, hepatic steatosis and metabolic dysregulation. Recently, we reported autoantibodies to a lipid droplet protein, perilipin-1 (PLIN1) in patients with AGL as well as in humans and mice with defects in autoimmune regulator protein (AIRE), a master regulator of thymic tolerance to tissue antigens. Significantly, adipose tissue of Aire-/- mice showed profound mononuclear infiltrates, reminiscent of panniculitis observed in AGL. We thus sought to define the immunologic determinants of adipose autoimmunity in Aire-/- mice. We found that Plin1 was expressed by medullary thymic epithelial cells in wild type but not Aire-/- mice, suggesting that it is an Aire-dependent antigen. Aire-/- mice had dramatically reduced body fat stores and their livers showed evidence of microsteatosis, suggesting disrupted lipid metabolism, which are features of AGL. By flow cytometry, infiltrate in the Aire-/- fat pads was dominated by activated CD4 and CD8 T cells. To better understand immune populations involved in the attack on adipocytes, we performed single-cell RNA sequencing of the adipose $CD45^+$ stromal vascular fraction. We observed a significant expansion of Th1-like and memory CD4 T cells and exhausted and memory CD8 T cells in the Aire-/- mice, consistent with an ongoing immune response. Furthermore, we have identified over 20 expanded T-cell clones, with current work ongoing to characterize their TCR specificities. Taken together, our data establish Aire-/- mice as a model of AGL and provide the first characterization of cellular heterogeneity in adipose autoimmunity.

W112. Non-canonical Translation of Long Non-coding RNAs are a Source of Neo-antigens in Pancreatic Beta Cells

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Genome Wide Association Studies (GWAS) have revealed the presence of type 1 diabetes (T1D) risk variants in non-coding regions of the human genome, including long non-coding RNAs (lncRNAs). lncRNAs are defined as RNA molecules of more than 200 nucleotides that lack coding potential, but there is increasing evidence that short peptides can be translated from small open reading (smORFs) frames in lncRNAs. These micropeptides could affect β cell homeostasis and constitute an unexplored source of neo-antigens, possibly contributing to the perpetuation of the β cell destruction by T cells. In this study, we aimed to identify lncRNA-derived peptides that act as autoantigens in pancreatic islets. We combined RNA sequencing of ribosome complex-bound RNA from basal and polyinosinic-polycytidylic acid (PIC)-treated human β cells (EndoC-βH1) and mass spectrometry (MS) analysis of the nascent peptides of ribosome complexes to identify candidate lncRNA-derived micropeptides. We then utilized a published algorithm to identify micropeptides likely to be bound and presented by HLA-DRB1*03:01 (a prevalent high-risk HLA-DR allele in our patient population). After confirming binding to HLA-DRB1*03:01 using an *in vitro* competition assay, we evaluated T cell recognition of candidate micropeptides using activation induced marker assays and HLA class II tetramer staining. Our results validate an approach for the rapid identification of lncRNA capable of generating micropeptide translation products and suggest that such micropeptides can function as neo-epitopes that drive T cell activation. Our future work will further characterize the translation of micropeptides and the relevance of micropeptide specific T cells in individuals with T1D.

W113. Non-Citrullinated α-Enolase and Vimentin Peptides Activate Seropositive DRB1*0401 Rheumatoid Arthritis CD4 T Cells Uncovering a Novel Antigen-Specific CXCR5$^+$CXCR3-CCR6- Population

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While the CD4$^+$ T cell response to citrullinated antigens has known importance in seropositive Rheumatoid Arthritis (RA), recognition of unmodified autoantigens and the phenotype of responding cells is not well characterized. By stimulating PBMC from DRB1*0401 RA patients with unmodified α-Enolase and Vimentin peptide libraries, we established a panel of DR restricted T cell lines with specificity toward six α-Enolase and two Vimentin peptides. These unmodified peptides were pooled and T cell reactivity to them was compared to a pool of previously published citrullinated epitopes in samples from patients with seropositive RA and age and sex matched controls through an *ex vivo* activation induced marker assay. RA individuals had both a 3 – fold greater antigen specific T cell response (mean 40.9 vs 13.5 specific T cells per 106 CD4$^+$, respectively, p = 0.019), and a 3 – fold greater memory T cell response to the unmodified pool, vs controls (mean 11.0 vs 3.48 specific T cells per 106 CD4$^+$, respectively, p = 0.0056). The magnitude of responses was similar to those elicited by the citrullinated peptide pool. Notably, in RA patients, the response to the unmodified peptides included a population of CXCR5$^+$CXCR3-CCR6- T cells that was largely absent in controls (mean 1.60 vs 0.375 specific T cells per 106 CD4$^+$, respectively, p =0.014). Our findings demonstrate that antigen specific T cells in seropositive RA patients also recognize non-citrullinated autoantigens, and these cells exhibit a peripheral TFH-like phenotype with known disease relevance.

W114. Nonclinical Toxicology and Safety Studies of MTX-101, an Inhibitory KIR2DL X CD8 Targeting Bispecific CD8 Treg Modulator, Enabling Clinical Development as Therapeutic for the Treatment of Autoimmune Disease

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In healthy individuals, CD8 Treg selectively kill self-reactive CD4 T cells. In autoimmune disease, CD8 Treg appear dysfunctional and insufficient to eliminate self-reactive CD4 T cells, in part due to expression of the autoimmune checkpoint KIR2DL. MTX-101 is designed to target and selectively activate only CD8 Treg to restore immune balance, reduce inflammation, pro-inflammatory cytokines and disease pathology. MTX-101, at repeat doses up to 100mg/kg, was evaluated in a GLP toxicology study using a humanized CD34$^+$-engrafted NSG(IL-15Tg) mouse model. In-life safety, during dosing and recovery, and terminal evaluations demonstrated that MTX-101 was well-tolerated with no drug-related findings observed. While high levels of target expressing cell binding was seen, no changes of immune cell frequency or activation status were noted. No cytokine release was detected. High exposure over course of study was observed with PK consistent to PK in normal Balb/c mice and cynomolgus monkeys. MTX-101 was evaluated for cytokine release in human whole blood or PBMC (multiple assay formats), with no elevation of cytokine detected. *In vitro* immunogenicity evaluation suggests low risk of ADA, consistent with a lack of impact to PK of MTX-101 *in vivo*. MTX-101 was assessed for off-target binding via the Retrogenix protein library microarray platform. No strong binding was observed other than expected interactions to its KIR2DL and CD8 targets. In conclusion, nonclinical safety studies support clinical entry into a Ph1a/b healthy adult and patient study. MTX-101 is a promising therapeutic approach to address an underlying cause of autoimmune disease via enhancement of CD8 Treg function.

W115. Non-enzymatic Asn Deamidation of GAD65 Generates HLA-DRB1*03:01- Restricted Neoepitopes in Type 1 Diabetes

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In type 1 diabetes (T1D), GAD65 antibodies are most common in individuals with HLA-DR3 genotypes. Furthermore, administration of GAD65 in a clinical trial improved glycemic control in subjects with T1D who were HLA-DR3 positive. It can be expected that HLA-DR3 presents important epitopes from GAD65, but only two HLA-DRB1*03:01 restricted epitopes have been described. Notably, binding pocket 4 of HLA-DRB1*03:01 has a strong preference for Asp. Consequently, non-enzymatic Asn to Asp deamidation, a post-translational modification recently documented in inflamed islets, could generate neo-epitopes that are presented by HLA-DRB1*03:01. We used a systematic approach to define wild type and Asn deamidated GAD65 peptides that are bound and presented by DRB1*03:01. Ten wild type and six Asn deamidated GAD65 peptides had measurable binding to HLA-DRB1*03:01. Unmodified peptides corresponding to the Asn deamidated peptides had weaker or undetectable binding. One unmodified GAD65 peptide elicited detectable T cell responses in tetramer-based assays, while two Asn deamidated GAD65 peptides elicited tetramer-positive responses in subjects with T1D. Two additional Asn deamidated peptides elicited positive responses in an activation-induced marker assay. Clones recognizing the newly identified Asn deamidated GAD65 epitopes were isolated, confirming the validity of the observed T cell responses. In total, our data suggests that Asn deamidated GAD65 is more immunogenic than unmodified GAD65 in DR3 positive subjects. These wild type and deamidated epitopes can be applied to characterize GAD65 specific T cell responses in HLA-DR3 positive individuals during diabetes progression and in the setting of clinical trials.

W116. Novel B Cell Subsets as Potential Biomarkers in Idiopathic Inflammatory Myopathies: Insights into Disease Pathogenesis and Disease Activity

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Idiopathic inflammatory myopathies are a heterogeneous group of rare autoimmune disorders characterized by progressive muscle weakness and the histopathologic findings of inflammatory infiltrates in muscle tissue. The diagnosis of IIM is based on clinical manifestations and is complicated due to the lack of reliable clinical biomarkers. Although their pathogenesis remains indefinite, the detection of specific autoantibodies and the evidence of the high effectiveness of depleting therapies suggest that B cells could be implicated. However, little is known about changes in peripheral blood B cell subsets in IIM patients and how these changes are related to disease activity. Therefore, we isolated PBMCs from 27 patients and 24 healthy controls and explored the landscape of peripheral B cells in this disease by multiparametric flow cytometry. We found significant numerical decreases in memory and double negative subsets, as well as an expansion of the naïve compartment relative to healthy controls, that contribute to defining disease-associated B cell subset signatures and correlating with different clinical features of patients such as the Visual Analog Scale (VAS) for disease activity and the Manual Muscle Testing (MMT8) muscle strength scale. Additionally, we determined the potential value of these subsets as diagnostic biomarkers, thus positioning B cells as neglected key elements possibly participating in the onset or development of idiopathic inflammatory myopathies.

W117. Novel Method of Dermal Fibroblast Detection in Primary Skin Tissue Biopsies
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AbbVie

Introduction: Skin fibrosis is a prevalent characteristic observed in autoimmune skin diseases such as psoriasis and systemic sclerosis. Despite progress in using skin biopsies to advance our understanding of these diseases, effectively profiling immune cells and pathological fibroblasts to precisely characterize the dysregulations driven by these pathologic cells poses a persistent challenge. Methods: We have devised a comprehensive 15-color flow cytometry panel designed to immunophenotype rare tissue-resident fibroblast populations and common tissue-resident immune cells in healthy human and cynomolgus monkey dermal sections. Human dermal sections obtained from GenoSkin were shipped overnight at room temperature and cynomolgus skin samples were shipped overnight cryopreserved with CryoStor CS10 freezing medium. Both tissues were mechanically dissected and further treated with Collagenase IV enzymatic digestion or Miltenyi's MACS Whole Skin Dissociation Kit for tissue disruption. Results: Digestion of human punch biopsies processed with Collagenase IV produced qualitatively superior results in comparison to Miltenyi's MACS Whole Skin Dissociation Kit. Substantial levels of antibody saturation on target receptors were retained after skin tissue disruption followed by staining with fluorescently labeled antibodies for both Collagenase IV and Miltenyi's MACS Whole Skin Dissociation methods, however, Collagenase IV resulted in higher yield for our cells of interest. Conclusion: We observed similar immune profiling in human and cynomolgus dermal sections and sustained levels of antibody-mediated target saturation when using both tissue disruption methodologies evaluated in this study.

W118. PanTreg: A Cell-therapy, System-biology - Based Approach for Therapy of Autoimmunity
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Current efforts for CarTreg are mostly focused on engineering patient's Treg specific for a single antigen, deemed relevant for the etiopathogenesis of the disease, These approaches have inherent imitations. We have engineered a first in class lentivirus (PanTreg herein) which: a) binds specifically pro-inflammatory cytokines such as TNF and IFN; b) upon binding, induces expression of FoxP3, TGFb and IL-10 in transfected human polyclonal T cells. We have developed and validated this construct *in vitro* and found that PanTreg are superior to Treg in regulating human T effector function We have tested PanTreg in *in vivo* in a humanised model of GVHD and found that: i) home to microenvironments where inflammatory cytokines are present, and ii) are induced by such inflammatory cytokines in producing locally tolerogenic cytokines, thus inducing immune tolerance, with an effective clinical control based on the induction of active immune tolerance. Indeed, we tested two different does of PanTreg and compared their efficacy against autologous polyclonal, untransduced Treg. No animals died in the experimental arm treated with teh highest dose of PanTreg ($p< 0.001$ vs control) In conclusion, we describe here our initial experience with a first in class type of CarTreg, where the scFv is specific for an inflammatory cytokine. This approach has the potential to provide with a single construct an innovative approach, and perhaps a permanent solution, to many diseases, in which such cytokines are relevant, by inducing active tolerance rather than suppressing, or "sponging out" the relevant cytokine

W119. Peripheral Blood Mononuclear Cells from Patients with Systemic Lupus Erythematosus Induce Salt Sensitivity of Blood Pressure in Humanized Mice

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Systemic lupus erythematosus (SLE) is also a risk factor for cardiovascular disease (CVD) and hypertension. SLE and CVD are on the rise, and it is not known if this is attributable to the increased dietary salt in the modern diet and SSBP. We hypothesized that high salt intake increases both blood pressure and the proinflammatory milieu in mice humanized with the immune cells of patients with SLE. We humanized female NSG/MHCI/MHCII-/- immunodeficient mice with peripheral blood mononuclear cells (PBMCs) isolated from patients with SLE (n=5) and controls without autoimmune disease (n=4). The blood pressure was measured with radiotelemetry, immune phenotyping was performed with flow cytometry, and vascular reactivity was assessed in mesenteric arteries using wire myography after two weeks of a 4% high salt diet. High salt treatment significantly increased systolic blood pressure in lupus mice compared to the controls (135.5 vs. 123.8 mmHg, p=0.014). Antigen presenting cell infiltration (macrophages, monocytes, dendritic cells), their activation (CD86) and proinflammatory markers (IL-23 p19, TNF-alpha), and oxidative stress (Isolevuglandins, Nox2) were significantly higher in the kidneys of lupus mice. These mice exhibited vascular dysfunction ($p< 0.05$). Also, the lupus mice tended to excrete more albumin than the controls (17.24 vs. 25.89 µg/ml p=0.142), suggesting greater kidney injury. In conclusion, our findings suggest that immune cells from patients with SLE, in response to high salt intake, increase blood pressure and the proinflammatory milieu in the kidney, aorta, and spleen; impair vascular function; and worsen albuminuria in immunodeficient mice.

W120. Pharmacodynamic Demonstration of Immunological Tolerance Induced by KAN-101, a Novel Liver-targeted Therapy for Celiac Disease

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Anokion

Celiac disease (CeD) is a chronic inflammatory disorder triggered and maintained by ingestion of gluten and which has no approved pharmacologic treatment. KAN-101 is an investigational therapy composed of a liver-targeting glycosylation signature conjugated to a gliadin-derived peptide designed to induce tolerance as a therapeutic approach to the treatment of CeD. The Assessment of KAN-101 in CeD (ACeD) study evaluated safety and tolerability of KAN-101 and immune biomarker responses following a 3-day oral 9 gram gluten challenge (GC) in CeD patients who received multiple doses of KAN-101 at 0.15, 0.3, or 0.6 mg/kg or placebo (NCT04248855). No serious adverse events or dose-limiting toxicities were observed in the study. Adverse events observed during the study were mild to moderate in severity, resolved within hours of onset, and were consistent with symptoms experienced by CeD patients upon ingestion of gluten. Blood biomarker data demonstrated antigen-specific tolerance to gliadin, induction of gliadin-specific T cell anergy and control of the broader immune response to gluten, indicating bystander suppression activity. KAN-101 also modulated a key disease biomarker, GC IL-2, which has been reported to be associated with symptomatic responses in CeD patients. Cytokine responses following KAN-101 administration demonstrate a shift from activating (IL-2) to regulatory (IL-10) upon repeat exposure of liver-targeted antigen. In conclusion, the ACeD Ph1 clinical trial demonstrated that KAN-101 was safe and tolerated in CeD patients, induced immunological tolerance to gliadin and modulated a biomarker of clinical efficacy. KAN-101 is currently being tested in Ph2 studies in CeD patients.

W121. Preclinical Pharmacology of S-1117, a Novel Engineered Fc-fused IgG Cleaving Enzyme, for Chronic Treatment of Autoantibody-mediated Diseases

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Seismic Therapeutic

Pathogenic autoantibodies are key effectors of inflammation, promoting complement activation and immune cell responses that cause tissue damage in autoantibody-mediated diseases such as myasthenia gravis. IgG-proteases represent a new therapeutic opportunity. Here we present S-1117, a novel Fc-fused pan-IgG protease, engineered using a proprietary machine learning enabled platform to reduce immunogenicity and augment manufacturability and stability while maintaining enzyme activity. S-1117 cleaves soluble IgG and the BCR on memory B cells with comparable potency and significantly reduces antibody-dependent cytotoxicity (ADCC) and immune complex-mediated PBMC activation *in vitro*. When tested *in vivo*, a deep reduction of human IgG (>90%) occurred within 30 minutes after dosing. A PK/PD model developed for human projections predicts rapid, deep and sustained reduction of IgG levels. In addition, in an anti-glomerular basement membrane (GBM) model, a prototype Fc-fused pan-IgG protease reduced complement activation, IC deposition, which correlated with reduced proteinuria, blood urea nitrogen and renal pathology. In summary, S-1117 is a novel engineered pan-IgG protease that demonstrates rapid and sustained reduction of IgG levels and reduces multiple pathological manifestations in a murine nephritis model. Given its ability to address multiple pathogenic mechanisms as a single drug, it has the potential to achieve improved clinical outcomes in autoantibody-mediated diseases.

W122. Profiling of Immune Infiltrates in RR-EAE Reveals Dynamic Changes in Myeloid Cells

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Relapsing-remitting experimental autoimmune encephalomyelitis (RR-EAE) is characterized by a pattern of relapses and spontaneous recovery occurring over a period of weeks to months. This pattern is very similar to the clinical signs of disease observed in multiple sclerosis (MS) patients over many years. The immune response in MS is primarily driven by autoreactive T lymphocytes that recognize myelin peptides. However, the mechanisms involved in the induction of the disease and clinical relapses are not well understood. To this end, we performed deep profiling of spinal cord and infiltrates at the different phases of the disease (onset, peak, remitting, and relapsing) using single-cell RNA sequencing and multi-parameter flow cytometry in SJL mice immunized with PLP139- 151. This line of experimentation revealed drastic changes in immune cell composition over time with unique cellular compositions revealed during the different disease phases. Further analysis uncovered marked changes in the number of myeloid cells such as macrophages, dendritic cells, and monocytes and respective subsets during each disease phase. In addition, the infiltrating myeloid cells had distinct transcriptional signatures which might define their role throughout the phases of the disease. Our comprehensive analysis of immune infiltrates identifies functional heterogeneity of key myeloid cell subsets that in concert with other cells could be contributing to disease pathogenesis.

W123. Single Cell Analysis of CD8 T-cells Reveals Autoantigen Specific Signatures of Teplizumab Treatment in at Risk Subjects from the Trialnet-10 Study

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In the Trialnet TN10 Anti-CD3 Prevention study, at risk subjects with multiple AABs treated with Teplizumab (a humanized FcR non-binding anti-CD3) exhibited a reduced incidence of progression to type 1 diabetes, manifested as an increased median time to clinical diagnosis of two years as compared to placebo treated controls. A comprehensive analysis of immune cells in peripheral blood revealed that a partially exhausted population of $TIGIT^+KLRG1^+$ $CD8^+$ T cells was strongly associated with clinical response. Prior work did not investigate changes in antigen specific T cells following treatment. Therefore, we characterized the number, phenotype, and transcriptional profiles of beta cell and EBV specific $CD8^+$ T-cells in baseline and longitudinal 3-30 month post-treatment samples from TN10 participants. Using HLA class I tetramers corresponding to established islet epitopes, we quantified beta cell and EBV specific $CD8^+$ T-cells in peripheral blood and sorted a portion of these cells for single cell RNA-Seq analysis using a previously published approach. Clustering analysis of the resulting transcript data revealed distinct cell states of interest, including antigen naïve, effector-like, and exhausted T cells. Combinatorial tetramer staining combined with indexed assembly of antigen specific T cell receptors allowed an assessment of T cell receptor diversity and clonal expansion. Beta cell specific T cells had more limited TCR sharing and were largely non-exhausted at baseline but exhibited increased exhaustion after treatment corresponding to delay of disease onset. Together, this analysis uncovered attributes of antigen specific T cell function that correlate with response to anti-CD3 treatment.

W124. STAT1 Gain-of-function Leads to Dysfunctional T Regulatory Cells

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Understanding the pathogenesis of Type 1 diabetes (T1D) is critical in developing effective treatments. T1D results from immune dysfunction leading to the destruction of insulin-producing β-cells, influenced by genetic predispositions and environmental factors. However, the exact mechanisms remain elusive. This study explores the role of signal transducer and activator of transcription 1 (STAT1) gain-of-function (GOF) mutations in T1D, known for causing immune dysregulation, including T1D and thyroiditis. We developed a novel NOD.STAT1 GOF knock-in mouse model, incorporating the R247W mutation associated with human STAT1 hyperactivity and T1D. This model replicates key aspects of the human STAT1 GOF phenotype, with the mice exhibiting autoimmune diseases, including colitis and skin inflammation. Notably, T cells from these mice secreted elevated levels of IFN-γ, mirroring human pathology. Furthermore, crossing these mice with T cell-restricted BDC2.5 NOD mice resulted in accelerated diabetes onset. Our single-cell analysis revealed decreased CD25 transcript levels in T regulatory cells, confirmed by flow cytometry. These findings highlight a critical function of STAT1 in modulating T regulatory cells, suggesting potential therapeutic interventions using JAK inhibitors.

W125. Unsupervised and Supervised Machine Learning Promotes Translational Research of Autoimmune Diseases

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The immune system erroneously attacks and damages healthy tissues in autoimmune diseases like Systemic Lupus Erythematosus (SLE). High-dimensional mass cytometry (MC) measures over 50 proteins in single cells and is a powerful technology to profile perturbations of the immune system and their impact on patients' health. To discover patterns in MC data that enable biomarker discovery and lay the foundation for diagnostic and prognostic applications, the Extended Polydimensional Immunome Characterization (EPIC) data mining platform was introduced. Here, we present two extensions. First, a module dubbed group similarity analysis (GSA) facilitates pattern discovery in clustering outputs. Upon segmenting aggregated cytometry data, the proportions of cell clusters give rise to immune signatures that are visualised by Uniform Manifold Approximation and Projection (UMAP). Subsequent silhouette analysis of the two-dimensional UMAP projections enables quantitative comparison of batch effects and biological stratification. Second, we apply supervised learning for biological sample classification. We developed a leave-one-batch-out training strategy to estimate the classification accuracy where new data are first mapped to a trained self-organising map (SOM) to assign them to annotated cell populations. Subsequently, their frequencies predict the health status of the corresponding sample. To illustrate the analytics pipeline, we used a dataset of 133 samples from SLE patients and healthy controls acquired in 10 independent experimental batches. Unsupervised GSA and supervised learning demonstrate that immune signatures can stratify blood samples into four distinct groups. This work paves the way for future applications where cytometry data mining provides decision support for the medical practitioner.

Autoinflammatory Diseases

Th117. Skeletal Muscle DUPD1 Links Gut Inflammation with Obesity-associated Metabolic Diseases

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Inflammatory bowel disease (IBD), comprising of Crohn's Disease and Ulcerative Colitis, is a debilitating disease with no known cause. Although extensive work has linked numerous genetic loci to IBD, most of these associations remain poorly understood. Some studies have suggested that IBD and metabolic diseases are associated. Whether these two diseases are indeed linked and what the molecular and pathophysiological mechanisms behind this association is unknown. Here, we show that DUPD1, a phosphatase of unknown function mechanistically links both IBD and metabolic disease. Specifically, Dupd1-/- mice were protected from high fat diet (HFD)-induced obesity, fatty liver, and glucose intolerance. Dupd1-/- mice were also protected from dextran sodium sulfate (DSS) and Helicobacter hepaticus induced colitis, as well as DSS/Azoxymethane (AOM) induced colitis-associated colon cancer (CAC). The expression of DUPD1 is largely restricted to the skeletal muscle. Indeed, using skeletal muscle specific conditional DUPD1 knockout mice (Dupd1fl/fl Myf6Cre), we show that DUPD1 exerts its colitogenic effects from the skeletal muscle by promoting systemic inflammation and through the modulation of autophagy within the skeletal muscle. Our study suggests that IBD has a metabolic component and that the skeletal muscle acts as an immunological organ capable of regulating inflammation at distant sites. This study further implicates DUPD1 as a potential novel therapeutic target in IBD and obesity-associated metabolic diseases.

Tu127. A Novel Translational Assay Improves Diagnostic Evaluation of A20 Haploinsufficiency
Urekha Karri and Daniella Schwartz
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A20 (TNFAIP3) is a ubiquitin editing protein that negatively regulates multiple pro-inflammatory pathways. Heterozygous TNFAIP3 mutations cause the pleomorphic immune dysregulatory disease, haploinsufficiency of A20 (HA20). These include frameshift, null, truncating, or missense mutations, all of which reduce protein stability and expression. The current gold standard assay for pathogenicity uses A20 overexpression (transient transfection), followed by expression of NF-kB luciferase activity downstream of TNFα. We hypothesized that this assay might not accurately capture the effects of missense mutations of homeostatic protein stability leading to potential underdiagnosis of HA20 in these patients. To test this hypothesis, we used the luciferase assay to test 4 frameshift/truncating variants and 4 missense variants from our cohort of 60 HA20 patients (52 are frameshift/truncating/null and 8 missense). While A20 mediated NF-kB suppression was absent in all frameshift/truncating variants, results were not affected by missense variants. We next measured A20 protein expression in peripheral blood mononuclear cells (PBMCs) from 25 healthy subjects and 25 patients with frameshift/truncating and missense variants using western blot and ELISA. A20 is sensitively detected by western and ELISA, and expression was reduced in PBMC in all patients with TNFAIP3 variants. Together these results suggest that a better preclinical diagnostic assay is needed to determine pathogenicity of TNFAIP3 mutations, especially missense variants. Future assay (western blot and ELISA) will include the measurement of WT vs mutant A20 stability in transfection models and primary cells, as well as the assessment of TNF and LPS induced NF-kB activation in primary cells.

Tu128. Biologic Therapies Imprint Epigenetic Modifications in the Skin-homing T Cells of Atopic Dermatitis Patients
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UMC Utrecht

Biologic therapies, including the IL-4 receptor blocker dupilumab and JAK inhibitors like upadacitinib, have revolutionized the management of atopic dermatitis (AD). However, prolonged treatment is necessary to sustain disease remission. While both therapies are clinically effective, only dupilumab has demonstrated successful dose reduction, suggesting potential disease-modifying effects. This study aimed to investigate the long-term effects of dupilumab or upadacitinib treatment on the epigenetic profile of pathogenic skin-homing T cells in AD. Using flow cytometry, $CD4^{+}CLA^{+}$ memory T cells were isolated from AD patients at baseline and after 52 weeks of dupilumab or upadacitinib treatment. DNA methylome and RNA transcriptome analyses were conducted using the EPIC array and RNA sequencing, respectively. Non-atopic healthy controls were included. Our analysis identified 408 differentially methylated regions (DMRs) between non-atopic individuals and AD patients, involving pathways like cytokine-cytokine receptor interactions, NF-kB, and T cell receptor signaling. Some of these DMRs were associated with a respective change in the transcriptome. Following treatments, dupilumab modulated 6 AD-related DMRs, with 4 rectifying in the direction of healthy controls. On the other hand, upadacitinib modulated 40 AD-related DMRs, the majority of which shifted further towards the AD profile. Interestingly, we identified 240 DMRs and 12 DMRs that were modulated in response to upadacitinib and dupilumab, respectively, independent of AD-related DMRs. In conclusion, our analysis highlights distinctive effects of dupilumab and upadacitinib treatment on the epigenetic level. The observed discrepancy in the direction of modification between treatments may suggest disease-modifying properties with clinical consequences upon therapy cessation.

Tu129. Deep Phenotyping Identifies Inflammatory Clonal B Cell Expansions in IBD

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B cells play an important role in gut homeostasis. Dysregulated B cell populations have been reported in patients with inflammatory bowel disease (IBD), but how these B cell perturbations contribute to disease remains largely unknown. We studied the B cell immunophenotype, serological profile, transcriptome, and B cell receptor (BCR) repertoire from patients with active IBD. This multi-omic approach identified several alterations in IBD suggesting host-microbiome influences and a pathogenic role of B cells. Increased antibodies against Klebsiella Pneumonia, Lactobacillus species, and Enterococcus faecalis indicated microbiome dysregulation. An inflamed phenotype was evident with up-regulation of interferon and NOD-like receptors signalling pathways. The BCR repertoire in blood and mesenteric lymph nodes draining inflamed regions of bowel showed a global reduction in somatic hypermutation compared to healthy controls, despite the presence of well formed germinal centres. Shared BCR clones were uniquely present between patients with Crohn's disease (CD), but not in healthy controls nor in patients with Ulcerative colitis. These shared clones were present in both lymph nodes and circulating plasmablasts, suggesting a specific CD-associated antigen pattern driving inflammatory B cell responses. These results further elucidate the dysregulation of B cells in IBD. The identification of shared B cell clones across patients with CD sheds further light on the pathogenesis CD and the interaction with the microbiome. These findings warrant further work to potentially help in the diagnosis of disease endotypes and for possible novel treatment strategies.

Tu130. Generation of Gut-specific-engineered Treg-like Cells (GI-CD4LVFOXP3) for Pediatric Crohn's Disease Treatment

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Stanford University

Crohn's disease (CD) is a predominant form of inflammatory bowel disease characterized by chronic inflammation in the gut that affects millions of people worldwide. The CD is more prevalent in the pediatric population and less likely to respond to conventional therapies. Further, gut damage induced by uncontrolled inflammation often requires surgery and hospitalization and is a source of poor quality of life and suffering for many children as well as their families. To provide alternative treatments, we are developing gut-specific-engineered Treg-like cells (GI-CD4LVFOXP3), a patient-specific "living drug" that could control excess inflammation in the gut and support gut repair. Immunophenotyping of peripheral blood from CD patients showed the signature of inflammation and an increase in effector-T cells expressing gut-homing receptor $\alpha4\beta7^+$ integrins. We are generating GI-CD4LVFOXP3 Tregs from $CD4^+$-T cells expressing gut-homing receptor $\alpha4\beta7^+$ integrins isolated from peripheral blood to promote gut homing and transient expression of ST2 to enhance the responsiveness to danger signal IL-33. GI-CD4LVFOXP3 showed expression of Tregs markers and gut repair molecule amphiregulin and IL-22. Enteroids derived from CD patients but not from HD controls showed alteration in polarity, differentiation capacity, and transepithelial resistance. We aim to obtain data supporting the therapeutic efficacy of GI-CD4LVFOXP3 using autologous gut organoids, as preliminary evidence that GI-CD4LVFOXP3 will be a potent therapeutics for CD and co-culture of GI-CD4LVFOXP3 and enteroids will demonstrate the efficacy of these cells in reversing epithelial gut damage.

Tu131. Identification and Characterization of PD-1 Agonists for the Treatment of Autoimmune and Inflammatory Diseases

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Seismic Therapeutic

The development of autoimmune diseases is the result of a dysregulated immune response against self-antigens, resulting in cell dysregulation and tissue damage. Upon chronic exposure to antigens, inhibitory receptors (IR) are upregulated as a feedback mechanism to limit T-cell effector activity. IR deficiency is associated with autoimmunity in mice and humans. Similarly, blockade of IR is efficacious for the treatment of cancer but is often associated with new onset of immune-related adverse events. Given that loss or blockade of IR pathways can result in autoimmunity, we hypothesized that agonism of these pathways should restore normal immune homeostasis. However, the generation of agonistic antibodies presents some challenges. For instance, to elicit strong receptor agonism, super-clustering mediated by the binding of the constant region (Fc) of an antibody to FcgRs, must occur. While FcgRs are widely expressed on antigen-presenting cells (APCs), they can be functionally classified as activating or inhibitory. Of note, FcgRIIb is the only inhibitory Fc receptor, and APCs derived from mice deficient in this receptor exhibit exaggerated cytokine release. Most PD-1 agonist antibodies in clinical development contain a wild-type Fc and can trigger unwanted production of pro-inflammatory cytokines by engaging activating FcgRs. To mitigate such liability, we designed novel PD-1 agonistic antibodies that selectively bind FcgRIIb, prevent APC activation and limit immune responses *in vivo*.

W126. Human OTULIN Mutations Can Cause Distinct Inflammatory Disease Phenotypes Depending on the Protein Domain That Is Mutated

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Pyoderma gangrenosum (PG) is an extremely rare and severe inflammatory and necrotic skin disorder. For the first time, we define a monogenic basis for PG resulting from bi-allelic mutations in the human gene OTULIN. We report patients with recurrent PG since childhood, each with a homozygous OTULIN R57C mutation. OTULIN encodes a deubiquitinase that cleaves linear ubiquitin chains added to proteins by the linear ubiquitin chain assembly complex (LUBAC). This balance between removing and adding linear ubiquitin is crucial to regulating immune signaling. Other bi-allelic mutations in OTULIN result in a fatal systemic autoinflammatory disorder called OTULIN-related autoinflammatory syndrome (ORAS), while mono-allelic OTULIN mutations leads to defective skin immunity to Staphylococcus aureus infections. All previously reported mutations in OTULIN affect the catalytic domain and compromise deubiquitinase function. In contrast, the R57C mutation affects a domain responsible for protein-protein interactions. We demonstrate that R57C reduces OTULIN's ability to bind LUBAC but preserves its deubiquitinase activity. Patients with the R57C OTULIN mutation show perturbations in circulating immune cell frequencies and gene expression patterns distinct from patients with catalytically dead OTULIN or healthy controls. Further, the patients show dysregulated cytokine production in peripheral blood. These discoveries add to the emerging spectrum of inborn errors of immunity caused by defects in linear ubiquitin regulation and suggests a role for OTULIN in maintaining skin immune homeostasis.

W127. *In Vitro* and *In Vivo* Efficacy of Selective Small-molecule PKC-theta Inhibitors
Robert Whitener
Evommune, Inc.

T cell mediated inflammatory diseases (TMDs) constitute a diverse group of illnesses in which an uncontrolled immune response damages self-tissue. Patients diagnosed with TMDs such as atopic dermatitis, psoriasis, and inflammatory bowel disease generally respond well to broad immunosuppressants. However, these therapeutics often come with significant side effects, highlighting the need for more targeted approaches. The Protein Kinase C – theta isoform (PKCt), which is expressed nearly exclusively in T cells, represents an attractive T cell restricted target for pharmaceutical intervention. Here we report results from both *in vitro* and *in vivo* studies of novel small molecule inhibitors (SMIs) which are highly specific for PKCt. Using primary human PBMCs in *in vitro* models of T cell activation, our SMIs potently inhibited T cell activation, without affecting cellular viability. Similarly, in *in vitro* models of whole primary human skin biopsies, tissue resident T cells also failed to activate after PKCt inhibition. Furthermore, *in vivo* studies in mice demonstrated dose-dependent inhibition of inflammation after oral administration of SMIs highly specific for PKCt, including an oxazolone-induced model of atopic dermatitis and a SCID mouse T-cell transfer colitis model. These data suggest that a highly specific SMI for PKCt could have utility in the treatment of T-cell mediated inflammatory diseases.

W128. Inhibition of Vascular Occlusive Crisis in Sickle Cell Disease
Kumarpal Shah
Endocrine Technology, LLC.

Problem: The sickling of red blood cells contribute to hyper activation of alternate complement system. This contributes to major morbidity and mortality of vascular occlusive crisis.(Lombardi Et Al. in “Factor H interferes with adhesion of Sickle red cells to vascular endothelium: a novel disease-modulating molecule” Haematologica 2019; Volume 104 (5) :919-929). Proposed Solution: Since 80% or more complement activation occur at C3 amplification loop, inhibition of this loop will lead to complement inhibition and reduce severity of vascular crisis and its related costs. Our Work: We have developed an ultra small molecule of sulfonic polymer that act as inhibitor of Factor H, Factor B and Factor D. This will contribute to downstream inhibition of vascular crisis. Appropriate formulation strategy will provide alternate cost-effective solution to reduce morbidity and mortality related to Sickle cell related vascular occlusive crisis and its exuberant cost (Patent pending Kumar shah "IMMUNOTHERAPY OF SICKLE CELL DISEASE(SCD) WITH VASSO OCCLUSIVE (VOCs) CRISIS" 12/26/2023). Available for review if requested.

W129. Necrotising Fasciitis as a Presentation of NFKB1 Haploinsufficiency-related CVID: A Case Report and Review of the Literature
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Trauma-induced sterile necrotising fasciitis (NF) is an under-reported presentation of NFKB1 haploinsufficiency, compared to CVID-related manifestations. We report a case with intramuscular injections-induced ulcers, abscesses and scarring from age four years. At 26 years age, she underwent caesarean section, complicated by presumptive wound infection requiring ICU admission, debridement surgeries and skin graft. Wound healing was delayed with significant hernia and scar. At 26 years she was found to have sever pan-hypogammaglobinaemia, diagnosed as CVID , and started on immunoglobulin replacement, which continued subcutaneously. At age 32 years, she underwent hernia repair with abdominoplasty, which was complicated by NF, and neutrophilia (30),

leading to significant loss of the anterior abdominal wall. Her CVID-phenotype was of sinopulmonary infections, psoriasis, and recently liver derangement. Her variant was paternally inherited heterozygous copy-number-loss leading to pathogenic deletion of all NFKB1 coding exons2-24, which might explain the severe early onset. NF was reported only in NFKB1 truncating variants. Her father is well, illustrating reported incomplete penetrance of NFKB1-NF that will inform genetic-counselling. She had normal neutrophil-oxidative-burst (DHR) on PMA stimulation, which could be NFKB1-indepednet. Further neutrophil function assessment is being arranged. Delayed wound healing with peripheral neutrophilia mimics leukocyte-adhesion-defects not corrected by immunoglobulins-replacement. Nevertheless, deep-tissue trauma triggers NF in NFKB1-haploinsufficiency without infection. Steroids and anti-IL1 blocking are reported to accelerate wound healing, in keeping with reported high IL1B/IL8. Further studies are required to elucidate the mechanism of necrotising-fasciitis in NFKB1 haploinsufficiency and inform management.

W130. NK-like CD8 T Cells Are Essential to Immune-related Adverse Events Pathogenesis of Cancer Immunotherapy

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Columbia University

Immune checkpoint inhibitors (ICI) targeting PD-1 and CTLA-4 are major game changers in cancer therapy. Nevertheless, their use is limited by the occurrence of immune-related adverse events (irAEs), which can target different organs and significantly impact the quality of life of the patients. To unravel the cellular mechanisms involved in irAEs at the protein level, we developed a T cell-specific high-dimensional panel of 42 markers for spectral flow cytometry to investigate peripheral blood T cell landscape before and during ICI treatment in patients with and without irAEs. Unsupervised cluster analysis before ICI treatment revealed that patients developing irAE have a more prominent subpopulation of CD27- CD28- PD-1- $CD8^+$ TEMRA T cells than those without irAEs. This population is characterized by the expression of the NK markers CD56 (NCAM), NKp80 (KLRF1) and CD57. We observed a higher proportion of CD27- CD28- $CD8^+$ T cells expressing NK cell markers in irAE patients by gating analysis. Interestingly, these cells have been previously associated with different autoimmune diseases (e.g., rheumatoid arthritis) and effector cell phenotypes (cytotoxicity and IFNg secretion), supporting the concept that irAEs are associated with losing tolerance to self-antigens. Current investigations aim to determine the origin of these cells and whether the lack of the canonical co-activator CD28 is compensated by the expression stress-sensing activating NK receptors, including CD56 and NKp80, and by high-self antigen reactivity. We propose a new mechanism mediating irAEs through bystander NK-like CD8 T cells, which amplify ICI-mediated tissue inflammation.

W131. Piroxicam Induces Colitis in NSGS Mice Humanized with IL10-deficient HSPCs

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Stanford University

Very early onset inflammatory bowel disease (VEO-IBD) is a life-threatening form of pediatric IBD that affects children younger than 6-years-old. In these genetically susceptible patients, an uncontrollable immune response to antigens of unknown origin results in excessive and chronic intestinal inflammation for which current treatments are often insufficient. The most severe form of VEO-IBD is caused by loss-of-function (LOF) mutations of the IL10, IL10RA, and IL10RB genes which are crucial for immune tolerance through IL-10 signaling. Although Il10-deficient mice provided critical insights in IBD, there is a need for humanized mouse

models that allows for testing novel cell and gene therapies as a definitive cure. To meet this clinical need, we created a novel humanized mouse model of IL10 deficiency by engrafting NSGS mice with human CD34$^+$ hematopoietic stem and precursor cells (HSPCs) in which the IL10 gene has been knocked out by a multi-guide CRISPR/Cas9 strategy with high efficiency. Upon successful reconstitution with human immune cells across disease-relevant tissues, we provided the mice with 200 ppm of piroxicam in their diet to trigger intestinal inflammation. After 7-14 days of piroxicam treatment, mice that were reconstituted using IL10-deficient human cells showed an increase in fecal Lipocalin-2 (fLcn-2) compared to mice that were engrafted with HSPCs that were either wildtype or in which a functional copy of IL10 cDNA was inserted. There results illustrate that we were able to induce colitis in IL10-deficient humanized NSGS mice which paves the way for testing novel cell and gene therapies to treat IL10-deficient VEO-IBD.

Basic Science of Immunology - Adaptive Immunity

Th118. ANB032, a BTLA (B and T cell Lymphocyte Attenuator) Checkpoint Receptor Agonist, Modulates Dendritic Cell (DC) Maturation and Function

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AnaptysBio

BTLA is a co-inhibitory checkpoint receptor that regulates T cell, B cell, and DC function. The interaction of BTLA with its ligand, HVEM, induces inhibitory signals that regulate immune cell function. ANB032, an investigational non-depleting BTLA agonist antibody, does not compete with the binding of BTLA to HVEM. Previous *in vitro* studies demonstrated that upon binding to BTLA and simultaneous Fc receptor engagement to an opposing cell, ANB032 induced inhibitory signaling, reduced T cell proliferation, and reduced inflammatory cytokine secretion (Th1, Th2, Th17, Th22). Although the expression of BTLA on subsets of DCs has been reported, BTLA's role in modulating DC maturation and function has not been thoroughly investigated. To address the functional role of BTLA on DCs, an LPS-mediated maturation assay with monocyte-derived DCs was performed to confirm that mature DCs highly express BTLA. ANB032 reduced HLA-DR expression, co-stimulatory molecule expression, and inhibited inflammatory cytokine production from DCs challenged with LPS. When co-cultured with allogenic naïve T cells, ANB032-treated DCs increased the generation of Foxp3$^+$ Tregs and decreased production of Th1 and Th2 cytokines. Therefore, BTLA agonism by ANB032 inhibits a broad range of immune cells and modulates DC function, while inducing Tregs, and potentially restoring immune balance, which may provide therapeutic value in the treatment of autoimmune and inflammatory disease. ANB032 is currently being studied in a Phase 2 clinical trial in atopic dermatitis (NCT05935085).

Th119. Helios Regulates CD8 T cell Activation in Response to TCR Affinity

Gerardo Suárez-Rojas[1], Rosa Rubio[2], Adrian Albarrán-Godínez[1], Erika Magallón-Gayón[3], Eduardo Magallón[2], Rosa Aguilar-Fuentes[3], Diego Esperante[1], Rocio Chavez-Santoscoy[3], Iris Madera-Salcedo[2], Florencia Rosetti[2] and José Crispín[2]
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Helios (Ikzf2) belongs to a family of transcription factors that regulate lymphoid differentiation and function. In thymic-derived FoxP3 regulatory T cells, Helios promotes suppressive function. Previously, we observed that Helios is induced in CD8 T cells rendered anergic. The aim of this work was to analyze Helios induction and function in CD8 T cells. We quantified Helios expression in OT-I cells exposed to ovalbumin (OVA) presented in different contexts: (a) ubiquitous self-antigen; (b) tissue-restricted self-antigen; (c) bacterial antigen; (d) tumor-

associated antigen. Helios was produced only by exposure to self- and tumor-associated antigens. We mounted an *in vitro* assay to identify factors that modulate Helios expression. Productive CD8 activation, achieved by high affinity ligands and pre-activation of antigen presenting cells, inhibited Helios expression. In contrast, activation with low-affinity ligands led to robust Helios induction. Pharmacological dissection of signaling pathways indicated that activation of the IL-2-STAT5 pathway inhibited Helios induction. Accordingly, IL-2 blockade or STAT5 inhibition released Helios expression in CD8 T cells activated with high affinity ligands. *In vivo*, Ikzf2-deficient OT-I cells exhibited altered proliferation when exposed to OVA as a self-antigen or associated with Listeria. scRNA sequencing revealed that Helios controls genes involved in cell cycle, cytokine response, and metabolic adaptation. Helios deficiency caused an abnormal expansion of effector cells. In summary, Helios fine-tunes proliferation and effector differentiation during CD8 T cell activation. IL-2-STAT5 activation inhibits Helios expression during strong CD8 stimulation. This mechanism could differentially regulate the activation of lower affinity clones during immune responses.

Th121. Rosnilimab, a PD-1 Agonist Antibody that Binds to a Membrane Proximal Epitope Leading to Optimized PD-1 Agonistic Signaling

Stephen Parmley, Stephen Parmley, Benjamin Szlyk, Richard Frank, Matthew Hsu, Polina Brodsky, Yangsu Ren, Cailin Sibley, Paul Lizzul, and Martin Dahl
AnaptysBio

Checkpoint agonism represents a promising class of therapies for the treatment of autoimmune and inflammatory diseases, including rheumatoid arthritis (RA) and ulcerative colitis (UC), where unmet needs persist despite available therapies. Rosnilimab is an investigational PD-1 agonist IgG1 antibody designed to optimize inhibitory signaling through the PD-1 receptor. Rosnilimab depletes PD-1[high] pathogenic T cells and agonizes remaining PD-1[int] T cells. Binding to membrane proximal regions of checkpoint receptors and simultaneous Fc interactions with Fc receptors on opposing cells contributes to immune synapse formation between the immune cell and the antigen presenting cell, leading to clustering and agonistic activity. Similar binding properties also optimize the potential for depletion of high PD-1 expressing T cells. The binding epitope of rosnilimab was mapped to a membrane proximal region of the PD-1 receptor, while the epitope of a reference antibody (ref1) was mapped to a membrane distal region. Rosnilimab demonstrated greater reduction of T cell proliferation, inflammatory cytokines and genes related to T cell activation, and depletion of PD-1^{+} T cells compared to ref1 *in vitro*, consistent with the hypothesis that membrane proximal binding improves agonistic activity and target cell depletion. These mechanistic data, translational *in vivo* and *in vitro* data, robust Phase 1 healthy volunteer data, and unmet needs provide rationale for ongoing global Phase 2 studies of rosnilimab in RA (NCT06041269) and UC (NCT06127043).

Th122. Sequential Phases of Follicular Helper T Cell Development and Their Impact on Humoral Immune Responses to Vaccination

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Follicular helper T (Tfh) cells play a crucial role in the generation of high-affinity antibodies after vaccination. While the signals triggering Tfh differentiation from naïve T cells have been investigated, those governing subsequent developmental stages leading to optimal effector function remain poorly understood. We employed

fate mapping and reporting strategies for the cytokine IL-21 to elucidate the sequential developmental stages of Tfh differentiation. These stages include a progenitor-like stage (Tfh-Prog), a fully developed effector stage (Tfh-Full), and a post-effector Tfh stage characterized by retention of transcriptional and epigenetic features despite the absence of IL-21 production (Tfh-Ex). We also confirmed sharing of transcriptional features identified in different Tfh stages with human Tfh subsets after vaccination. Notably, Tfh stages are not strictly dictated by anatomical location and have distinct abilities to persist *in vivo*. Our findings reveal that the progression through Tfh stages is intrinsically regulated by the transcription factor FoxP1, while extrinsic control is exerted by follicular regulatory T cells. By selectively deleting later Tfh stages (Tfh-Full and Tfh-Ex), we demonstrate that these cells not only mediate germinal center (GC) B cell differentiation and control epitope dominance during early GC responses, but also maintain vaccine-specific B cells in the GC during its contraction, favoring the formation of protective antibodies. Collectively, these studies elucidate the sequential phases of Tfh development and clarify their role in promoting humoral immunity. Interventions aimed at fine-tuning the progression through Tfh developmental stages could improve humoral responses to vaccination.

Th123. Tissue-specific Sex Differences in Pediatric and Adult Immune Cell Composition and Function
Mahina Tabassum Mitul
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Sex-based differences in immune cell composition and function can contribute to distinct adaptive immune responses. Prior work has quantified these differences in peripheral blood, but little is known about sex differences within human lymphoid tissues. Here, we characterized the composition and phenotypes of adaptive immune cells from male and female *ex vivo* tonsils and evaluated their responses to influenza antigens using an immune organoid approach. In a pediatric cohort, female tonsils had more memory B cells compared to male tonsils direct *ex vivo* and after stimulation with live-attenuated but not inactivated vaccine, produced higher influenza-specific antibody responses. Sex biases were also observed in adult tonsils but were different from those measured in children. Analysis of peripheral blood immune cells from *in vivo* vaccinated adults also showed higher frequencies of tissue homing CD4 T cells in female participants. Together, our data demonstrate that distinct memory B and T cell profiles are present in male vs. female lymphoid tissues and peripheral blood respectively and suggest that these differences may in part explain sex biases in vaccine and virus responses.

Th124. Unraveling the Mechanistic Regulation of IL-2 Signaling in T Cells and Diseases
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IL-2 signaling is critical in maintaining immune homeostasis that requires precise regulation. To identify potential regulators of IL-2 signaling, we performed genome-wide CRISPR knockout screening in IL-2-dependent cells derived from a patient with Adult T cell Leukemia (ATL). ATL cells are $CD4^+CD25^+$ with regulatory T cells (Treg)-like phenotype and constitutively active JAK-STAT signaling. Our CRISPR knockout screening showed enrichment of sgRNAs targeting PRDM1 that encodes Blimp-1 which inhibits IL-2 production in T cells; however, its role in IL-2 signaling remains unknown. Conditional knockout (CKO) of Prdm1 in mouse $CD4^+$ T cells and Tregs resulted in increased expression of IL-2R subunits and pSTAT5 which was validated during an influenza infection where IL-2 signaling was enhanced in T follicular helper (TFH) and T follicular regulatory (TFR) cells in the absence of Blimp-1. Further, adoptive transfer of Prdm1 CKO Tregs into Rag2-/- mice resulted in augmented

IL-2 signaling with attenuated ability to suppress inflammation in T cell induced colitis. Consistently, CRISPR/Cas9-mediated deletion of PRDM1 in human CD4+ T cells and natural Tregs led to an enhanced IL-2 signaling. Blimp-1 ChIP-Seq in human Tregs demonstrated its direct binding to the key gene regulatory elements of IL-2 signaling. Finally, single-cell RNA-Seq analysis of ATL cells from acute patients showed a lower percentage of PRDM1+ populations leading to aberrant IL-2 signaling in these patients due to impaired regulation by Blimp-1. Thus, Blimp-1 is a pivotal regulatory node in IL-2 signaling that could be modulated for synchronizing T cell responses in human diseases such as ATL.

Tu132. Anti-inflammatory NKT Cell Activation Induces Expansion of Regulatory B Cells That Ameliorates Inflammation

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Invariant Natural Killer T (iNKT) cells are characterized by its capacity to secrete diverse cytokines after its activation. Such cytokines can modulate the function of multiple immune cells, including its differentiation towards a regulatory profile, which enables these cells to modulate the immune response and restore the immunological tolerance. Here, we incorporated iNKT cells-activating glycolipids and ovalbumin (OVA) into liposomes, in order to both activate iNKT cells and modulate the immune response in an antigen-specific manner on an OVA-specific inflammatory model. Mice were treated intranasally with liposomal formulations, in order to evaluate the recruitment and expansion of regulatory immune cells given the activation of iNKT cells. Physicochemical and functional characterization of the formulations were assessed previous to the administration. Characterization of the immune cell population on pulmonary tissue was assessed through flow cytometry after treatment. Histopathologic characteristics of pulmonary tissue was also evaluated. *In vivo*, we found that the immunization of glycolipid-containing liposomes led to the expansion, activation and production of cytokines from iNKT cells. Activation of iNKT cells led to the expansion of both regulatory T and B cells. Additionally, the production of IL-10 was enhanced in the aforementioned cells. The administration of the liposomal formulation alleviated the pulmonary inflammatory response, reducing the production of mucus, goblet cell hyperplasia and leukocyte infiltration. Herein, we present a novel strategy to alleviate the inflammatory response through the expansion regulatory cells given by the activation of iNKT cells, becoming the first step to generate a cutting-edge strategy to reduce inflammatory disorders.

Tu133. Cell-intrinsic Glucocorticoid Biosynthesis and Sensing Restrains Th17 Pathogenicity

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CD4+ IL-17A-producing T (Th17) cells are heterogenous; homeostatic Th17 (Th17Hom) maintain homeostatic tissue functions and inflammatory Th17 (Th17Inf) drive pathogenic autoimmune tissue inflammation. While IL-23 has a recognized role in driving Th17Inf, the pathways that preserve Th17 cells in a homeostatic state have remained enigmatic. Our study reveals that endogenous glucocorticoid (GC) production downstream of Cyp11a1, a key enzyme for steroid hormone production, distinguishes Th17Hom from Th17Inf. TCR signaling initiates Cyp11a1 expression, which is amplified and maintained co-operatively by TGFβ1 and IL-6, cytokines pivotal for Th17Hom differentiation. Additionally, Th17Hom exhibit heightened GC sensitivity compared to Th17Inf, providing additional context for the reported steroid resistance of Th17 cells. Both Cyp11a1 and the GR

(glucocorticoid receptor, encoded by Nr3c1) are crucial in maintaining homeostatic Th17 phenotype and limiting pathogenicity during autoimmune central nervous system (CNS) inflammation. Lastly, TGFβ1 rescues impaired GC sensing in Th17Inf, suggesting therapeutic potential for treatment of steroid-resistance. This study advances our understanding of the pathways underlying Th17 cell state regulation *in vivo* and highlights the clinical implications of cell-intrinsic GC biosynthesis and sensing in shaping the pathogenic potential of Th17 cells.

Tu134. Comprehensive Open-source Workflow for Multiplex Imaging Datasets Improves Resolution of Single Cells and Lymphatic Tissue Architecture

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Automated high-parameter multiplex immunofluorescence approaches are marketed as all-in-one solutions, yet do not account for technical and biological variability in target marker expression impacting data quality. To address this, we created a workflow consisting of customized open-source packages, original code, and ImageJ macros employable without significant computing resources or coding knowledge. A novel deconvolution method greatly improved single cell and structural detail. Autofluorescence mitigation allowed for isolation and recovery of low-intensity markers. With GPU acceleration and parallel processing, we achieved ~800% improvement in processing times (4-6 hrs on a standard PC for a 13-cycle 350GB 3D dataset). We applied the workflow to human spleen (n=5) and lymph node (n=7) sample images acquired with the CODEX system. Our workflow allowed for significant adjustments needed to isolate and clarify those markers (CD11c, CD5, CD1c, CD68, CD163) lost during automated processing, improving resolution of cell subtypes and stromal architecture. Segmented cells were analyzed according to marker expression, spatial coordinates, and signed distance from the nearest segmented vessel. Leiden clustering and expert cell annotation indicated distinct expression patterns typical of follicular, stromal, and endothelial cellular subtypes. Neighborhood analysis found significant interactions indicative of niche lymphatic functions (Spleen: Tregs with Tregs, Proliferating cells, $CD8^+$ T cells; Lymph Node: Tregs with Macrophages; $p< 0.05$). The workflow is thus a viable tool to address challenging features of multiplex image datasets, and for improving resolution over existing methods to enhance quantitative spatial analysis.

Tu135. Deliniation of Secondary Lymphoid Organ Immune Landscaping with Multiplex Imaging Analysis (mIF)

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Background: what regulates the follicular/germinal center (GC) immune reactivity in human is not well understood. Imaging methodologies can greatly improve our understanding for the development of B cell responses. Methods: FFPE tissues from four tonsils and two reactive LNs were used for analysis by mIF assays, i)(CD20/CD3/CD4/CD8/PD1/TIGIT/GITR/ICOS/CXCR3/CD57/Ki67/Bcl6/GATA3/Tcf1/Blimp1/GrzB/SYTO(, ii) (CD20/CD11c/CD163/CD68/FDC/CD123/MPO/CD31/CXCL13/IL21/IL10/SYTO) and iii) suppressor CD4 T cells (CD20/CD4/FOXP3/CD25/MCT1/MCT4/Helios/Neuropilin/IL10/SYTO). Imaging data were processed with HistoCytometry (FlowJo10) and computational modules for topological analysis. Results: a high phenotypic heterogeneity of the follicular helper CD4 T cell (Tfh) was found among the tonsillar tissues as well as the LNs.

FloxJo FlowSOM clustering analysis showed specific Tfh cell phenotypes with distinct localization across the follicular areas. The majority of FOXP3hiCD25hi/lo CD4 T cells (Tregs) were extrafollicular with a diverse -metabolism- profile. A lower frequency of monocytic subsets was found in LNs compared to tonsils in follicular and extrafollicular areas. Neighboring analysis also revealed differences between tonsils and LNs with respect to GC B and Tfh cell positioning. Conclusion: we present an experimental pipeline allowing for a high dimension analysis of GC relevant immune cells in health. Application of such pipeline could provide clues for the interplay between cell types and possible association with B cell responses as well as to lead in novel diagnostic tools especially for certain lymphomas.

Tu136. Differential Expression of Activation and Effector Molecules in Neonatal CD8 T Cells

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Viral infection is one of the top causes of neonatal morbidity and mortality. Cytotoxic CD8 T cells are necessary for the anti-viral immune response and have unique features in early-life. These include enhanced proliferation, but early cell death. To ascertain the functional properties of stimulated neonatal CD8 T cells, we quantified activation and cytotoxic molecule production compared to healthy young adult counterparts. Naive CD8 T cells ($CD8^+CCR7^+CD45RA^+$DAPI-) were sorted using Fluorescence-activated Cell Sorting (FACS) from whole blood of healthy young adults (< 30 years) and full-term neonates at birth (cord blood). Cells were then stimulated with anti-human CD3 and CD28 in complete RPMI medium with IL-2. On days two and three after stimulation, cells were re-stimulated using Phorbol 12-myristate 13-acetate (PMA) and ionomycin for four hours. Cytokine secretion was prevented by adding Brefeldin A/Monensin. Cell surface molecules and intracellular cytokines were quantified using flow cytometry. Activated neonatal CD8 T cells produced significantly increased TNFα and IL-2 after restimulation compared to their adult counterparts. Conversely, IFNγ production and expression of activation marker, CD25, and Fas ligand were not significantly different. Preliminary experiments show enhanced viability and decreased cytokine production in the presence of Rapamycin, an mTOR complex 1 inhibitor, in both neonatal and adult cells. Increased TNFα and IL-2 production in neonatal CD8 T cells after 2-3 days of stimulation may indicate unique roles for these cytokines in early-life. Further work is needed to decipher molecular programs driving this phenotype and the roles of TNFα in early-life immunity.

Tu137. Efficient Generation of Human Immune System Rats Using Human $CD34^+$ Cells

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Human immune system (HIS) mice generated using human $CD34^+$ stem cells, serve as a pivotal experimental model for the *in vivo* evaluation of immunotherapies. Yet, these mice have posse certain limitations including small size. In contrast, rats, due to their size and comprehensive immune system, hold promise for experiments that require assessment of a complete immune response. While immunodeficient rats have been produced, humanizing them with $CD34^+$ hematopoietic stem cells even when expressing human SIRPalpha remains inefficient and suboptimal. In this study, we introduce an efficacious method for long-term immune humanization, achieved through intrahepatic injection of human $CD34^+$ cells in irradiated newborn immunodeficient (Rag1 and Il2rg deficient) rats expressing human SIRP. By 12 weeks post-transplantation, significant immune reconstitution was evident in the blood, further increasing over 18 and 24 weeks. Importantly, and in contrast to HIS mice, the proportion of human immune mononuclear cell subsets mirrored that of human blood: predominantly T cells (52-98%), followed by B (5-25%), NK (~3%) and monocytes (~2%). These included diverse T cells subsets (naïve, memory, Treg), as well as a normal range of B cell subsets. At sacrifice, immune humanization was also high in

spleen, thymus, bone marrow and lymph nodes. In conclusion, we describe for the first time a method to efficiently generate HIS rats. Furthermore, HIS rats show the development of a human immune system that more closely resembles humans compared to HIS mice. HIS rats have the potential to be a useful model for translational immunology.

Tu138. High-throughput Serology of Identical Twin Ageing Demonstrates Stability and Genetic Independence of Antibody Memory

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CDI Labs

Introduction: Recent advances in high-throughput synthetic biology tools enable massively multiplexed serologic profiling of antigen-specific antibody repertoires by proteome microarray and phage-display immunoprecipitation sequencing (PhIP-Seq). However, little remains known about the impact of individual genetics on these global profiles or repertoire stability across time. Methods: In order to determine the impact of genetics and time on anti-viral and anti-self antibody repertoires, we obtained serum samples from twenty pairs of monozygotic twins (TwinsUK) at an initial timepoint and again after at least a decade of ageing. We ran all eighty samples on three platforms: human proteome microarrays (HuProt, >21,000 full-length proteins, CDI Labs), human proteome PhIP-Seq (HuScan, >700,000 49-mer peptides, CDI Labs Canada), and pan-viral PhIP-Seq (VirScan, >480,000 62-mer peptides, CDI Labs Canada). IgG hits across each platform were tallied in relation to negative controls. Results: Strikingly, all patients on all platforms had highly-correlated-hits across their paired samples before and after 10^{+} years of ageing (median Jaccard similarity 0.49, 0.41, 0.33 for VirScan IgG, HuScan IgG, and HuProt IgG, respectively). Patient profiles poorly correlated with their identical twin on all platforms (0.14, 0.05, 0.08, respectively) although this was significantly higher than between unrelated individuals (0.10, 0.02, 0.07, respectively; Mann-Whitney $p < 0.0001$ for all comparisons). Conclusion: Immunologic memory has long been known to remain stable for years against certain individual antigens; herein we demonstrate that this applies across the majority of known viral and self-antigens and manifests uniquely even among genetically identical individuals.

Tu139. Human Naïve CD4^{+} T Cell Survival is Maintained by a Novel CD46-KLF/SP Axis

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Signals mediated by autocrine activation of the human-specific complement receptor CD46 during T cell receptor (TCR) stimulation are vital to Th1 induction in human CD4^{+} T cells. However, how exactly CD46 mediates this role at a molecular level is currently undefined. CD46 is expressed in different isoforms that can bear either one of two distinct cytoplasmic tails: CYT-1 or CYT-2. Nuclear translocation of CYT-1 is a critical requirement for the expression of genes coding for nutrient-influx-channels and mTORC1 activity that mediate metabolic adaptations needed for Th1 responses. The lack of a DNA binding domain in CD46-CYT-1 precludes it from acting directly as a transcription factor (TF) and we hence hypothesized that CYT-1 regulates gene expression via direct interaction with specific TF activator and/or repressor complexes. Indeed, CUT&RUN experiments in conjunction with ELISA and microscale thermophoresis experiments identified key members of the KLF/SP TFs family as central interacting partners of CD46-CYT-1 in human CD4^{+} T cells, with CYT-1 largely fostering KLF/SP TFs binding to the target DNA motifs. Unexpectedly, CYT-1-assisted KLF/SP binding shapes dominantly the transcriptional output of naïve CD4^{+} T cells and governs their survival via negative regulation, specifically, 'death

caspase machinery' (caspases 2, 3, 8, and 9). Aligning with these findings, naïve CD4+ T from patients with a germline frameshift mutation in the CYT-1-binding region of KLF/SP TF displayed survival defects *ex vivo*. These data define a novel and critical human-specific pathway of gene regulation and further underpin the vital role of intracellular/autocrine complement in regulating normal cellular activity.

W132. Investigating the Role of Slc7a5 Amino Acid Transporter Using a Mouse Model of Multiple Sclerosis

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Multiple sclerosis (MS) is an autoimmune disease mediated by the infiltration of autoreactive T cells into the central nervous system (CNS) leading to chronic demyelination. Excessive demyelination leads to axonal damage and neurodegeneration. Upon demyelinating injury, myelin producing oligodendrocyte precursor cells (OPCs) are recruited to the site of damage, where they differentiate into mature oligodendrocytes and restore myelin in an endogenous process known as remyelination. Unfortunately, with disease progression, remyelination fails to occur for reasons not fully understood. One possibility for remyelination failure is unresolved inflammation in the CNS caused by the proliferation and differentiation of effector T cells in response to damage. Amino acids are essential in providing T cells with the fuel to proliferate and differentiate during an immune response. Hence, I will examine the role of the large neutral amino acid transporter, Slc7a5, in effector T cells in mice under immune mediated demyelination. Experimental autoimmune encephalomyelitis (EAE) was induced in mice lacking Slc7a5 in CD4+ T cells (Slc7a5 f/f CD4-Cre). We found that the Slc7a5 f/f CD4-Cre mice displayed a significant decrease in EAE severity scores compared to control mice. Moreover, flow cytometry analysis of T cell population in the spinal cord of these Slc7a5f/f CD4-Cre EAE mice revealed a significant decrease in proinflammatory T cells. Our results suggest that T cells utilize the Slc7a5 transporter and amino acids to initiate an inflammatory response in mouse models of MS, and that both amino acids and Slc7a5 may be potential novel immunomodulatory targets for preventing disease progression in MS.

W133. Lifespan Changes in CD4 T Cells and Their Implications to Aging and Neurodegenerative Diseases

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Using single-cell RNA sequencing techniques, we recently revealed lifespan changes within the CD4 T-cell compartment of mice, including a marked accumulation of cytotoxic CD4 T cells (CD4 CTLs) in late stages of aging. Here we aim to characterize the CD4 T cell landscape across the human lifespan, especially focusing on cytotoxic subtypes and their activation properties. Furthermore, we aim to determine whether individuals with mild cognitive impairment (MCI) exhibit a more advanced stage of immunological aging. Peripheral blood mononuclear cells (PBMCs) were isolated from human healthy male and female individuals and from elderly individuals diagnosed with MCI and were subjected to flow cytometry analysis using broad markers of CD4 T cell subsets including cytotoxic, exhaustion and regulatory T cells (Tregs). We revealed significant age-related changes in the CD4 T cell landscape with a marked increase in CD4 CTL frequencies. We also observed correlations between age and frequencies of exhausted and Treg subsets. Intriguingly, elderly individuals with MCI exhibited a distinct tendency for heightened age-related alterations in the CD4 T cell landscape. Moreover, principal component analysis of flow cytometry data revealed that the features of age-related changes in the CD4 T cell compartment roughly predict chronological age and MCI with top features related to cytotoxic functions of CD4 T cells. Our findings reveal substantial alterations in the CD4 T cell landscape during human lifespan which

were more abundant in individuals with MCI. We suggest that these changes gradually reduce the capacity of tissue repair and may thus linked to the biology of aging and age-related disease susceptibility.

W134. Natural Killer T Cells Promote Class-switch Recombination Towards IgG Subtypes Against T-Independent Antigens

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T-independent (TI) antigens such as bacterial capsular polysaccharides, among others, are known for activating "innate" B cells without help from T cells. This immune response is frequently restricted to the production of IgM isotype antibodies and does not induce a memory response. Activation of Natural Killer T (NKT) cells trough the reference ligand α-Galactosylceramide (αGC), induce the secretion of factors and cytokines, like IFN-γ and IL-4, that not only promote their differentiation into plasma cells but also induce class-switch recombination towards the production of IgG antibodies. Structural analogues of α-GalCer such as AH10-7 and OCH induce polarized secretion either towards IFN-γ or IL-4, respectively. Here we aim to evaluate the effect of AH10-7 and OCH in the process of class-switch recombination against TI antigens. We used two different TI antigens, Phosphorylcholine or NP-Ficoll, admixed either with free or liposome-contained ligands of NKT cells and administered intraperitoneally in mice. 7 days later, serum and splenocytes samples were obtained to evaluate the presence of antigen-specific antibodies by ELISA, and to evaluate the expression of IgG subtypes in B cells subsets and plasma cells trough flow cytometry. AH10-7 induced the production of IgG1 specific against the antigens, which correlated with the presence B cell and plasma cells expressing high levels of IgG1 and also IgG3. Recently we also detected that AH10-7 promoted the induction of Follicular Helper NKT cells, probably interacting with antigen-specific B cells. This work supports the use of glycolipid ligands as adjuvants against T-independent antigens such as those from pathogens.

W135. Next-generation B Cell ImmunoSpot® Assays Permit In-depth Assessment of the B Cell Response Elicited by SARS-CoV-2 Infection And/or COVID-19 mRNA Vaccination

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Cellular Technology Limited

The affinity distribution of the antigen-specific memory B cell (Bmem) repertoire in the body is a critical variable that defines an individual's ability to rapidly generate protective antibody specificities. Defining the Ig class and IgG subclass usage of such antigen-specific Bmem is also critical since it forecasts the anamnestic, recall response to be engaged upon antigen re-encounter. Here, we leveraged multiplexed B cell ImmunoSpot® assays to comprehensively define the magnitude and Ig class/IgG subclass usage of the SARS-CoV-2 Spike-specific Bmem repertoire present in convalescent subjects following PCR-verified infection, and/or after COVID-19 mRNA vaccination. Beyond evidencing past SARS-CoV-2 infection more reliably than assessment of plasma IgG reactivity against the intracellular nucleocapsid (NCAP) protein, direct assessment of the Bmem repertoire also provided invaluable insights into the affinity distribution and cross-reactivity profile of the Spike-specific Bmem repertoire. To do so, the frequency of receptor-binding domain (RBD)-specific antibody-secreting cells (ASC) in the test sample was first determined through quantification of individual spot-forming units (SFU) in wells seeded with decreasing cell inputs. Using additional aliquots of cryopreserved PBMC, and seeding the test sample in many replicate wells at the so-called "Goldilocks number" to generate ~50 SFU based on the previously calculated frequency, the affinity distribution of the RBD-specific ASC repertoire was evaluated against the prototype (Wuhan/1) and additional variant strains of SARS-CoV-2. Collectively, B cell ImmunoSpot® assays

offer tremendous value for future B cell immune monitoring efforts owing to the ease of implementation, economical utilization of PBMC, high-throughput capacity, and suitability for regulated testing.

W136. Overcoming Obstacles to Porcine Thymic Transplantation for Xenograft Tolerance of Human T Cells

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Xenotransplantation could alleviate the inadequate supply of organs for transplantation. However, toxic immunosuppression is required to prevent xenograft rejection. Instead, we aim to induce xenograft tolerance by transplanting porcine thymic tissue. In human immune system (HIS) mice, human T cells develop in porcine thymus grafts and demonstrate specific tolerance of the donor pig. However, these mice also demonstrated reduced HLA-restricted immune responses and T cell homeostasis. We compared selection of human thymocytes in NSG mice receiving human $CD34^+$ Hematopoietic Stem Cells (HSCs) with either autologous human fetal thymus (HU/HU mice) or SLA-inbred (SLAhh) miniature swine thymus (SW/HU mice). The proportion of CD4/CD8 double positive (DP) thymocytes undergoing positive selection, as measured by the expression of CD69, was significantly reduced in SW/HU thymus compared to HU/HU thymus. TCR diversity was reduced in $CD69^+$ DP and SP thymocyte populations of SW/HU vs HU/HU mice. TCRβ CDR3 hydrophobicity reflects affinity for self-peptide/MHC and positive selection (CD69- to $CD69^+$ DP) was associated with increased hydrophobicity in SW/HU and HU/HU thymocytes. A decrease or no change in hydrophobicity occurred in the $CD69^+$ DP to CD4SP transition in HU/HU mice but hydrophobicity increased during this progression in SW/HU mice, suggesting altered negative selection. CDR3 hydrophobicity among thymic Tregs in HU/HU mice was increased compared to non-Treg CD4 SP cells, indicating appropriate diversion of strongly self-reactive thymocytes, whereas SW/HU thymocytes showed no hydrophobicity change during this transition. We currently aim to normalize thymic selection in the swine thymus by introducing stem cell-derived human thymic epithelial cells.

W137. Reversal of Thymic Involution Delays Age-Associated Mortality of Toxoplasma gondii Challenged Mice.

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Effective adaptive immunity is dependent on T cell development in the thymus, which undergoes age-related involution, leading to reduced output of naïve T cells. This is suggested to compromise T cell immunity and increase risk of disease severity in the elderly. However, it is unknown whether enhancing thymic function would ameliorate the immune defects observed in the elderly, due to a limiting number of models allowing efficient reversal of thymic atrophy. We developed a novel mouse model where thymic involution can be reversed by inducible enforcement of Myc expression in thymic epithelial cells. Toxoplasma gondii is an age-sensitive parasitic infection that induces death in wild-type (WT) mice over 9 months (M) (Gardner et al, 1977. Johnson et al, 1995). Antibody depletion of T cells in aging hosts challenged with Toxoplasma gondii rescued this phenotype, prolonging their survival. This suggests T cells mediate the death of aged mice. 18M mice that underwent reversal of the involution process had enhanced thymic function and partial restoration of the age-associated reduction in naïve T cell numbers. When challenged with Toxoplasma gondii they displayed delayed death compared to 18M WT controls, suggesting reversal of involution confers improved immunological protection against infection to the aging host. These findings identify a role for thymic involution in the age-associated mortality of Toxoplasma gondii infection. We are using this model to investigate the mechanisms

governing death in aging hosts and enhanced outcome in thymic rejuvenation. Overall, these findings reveal the potential clinical significance of thymic regrowth in improving the age-related defects in adaptive immunity.

Basic Science of Immunology - Innate Immunity

Th125. BCL6 Constrains Expression of HLA Class II and Antigen Presentation Machinery in Endothelial Cells, in a Selective Manner

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We hypothesized that the transcriptional repressor BCL6 could block expression of STAT1-target genes, in particular IFNγ-stimulated HLA II expression in endothelial cells (EC). Human EC (n=4) were transduced with GFP alone, BCL6 overexpression (OE) vector, GAPDH shRNA or BCL6 shRNA (BCL6 KD). In parallel, EC were treated with BCL6-BTB domain inhibitors (BTBi) that enhance BCL6 binding to target genes. After IFNγ treatment, HLA expression was measured by RNA-Seq and flow cytometry. BCL6-bound target genes were identified in silico using public datasets of ChIP-Seq (ENCODE) and motif prediction (Transfac, JASPAR). BCL6 OE reduced, while BCL6 KD augmented, IFNγ-induced gene expression of HLA II. Concordantly, BCL6 OE reduced and KD enhanced HLA-DR but not -ABC surface expression. Treatment with BTBi strongly blocked IFNγ induced HLA-DPA1, DPB1, DRA, DRB1, DQA1 and DQB1 transcripts by more than 90%. Class II transactivators CIITA and NLRC5 were reduced by BTBi, and augmented by BCL6 depletion. BTBi abolished IFNγ-induced HLA-DR and –DP, but not HLA-ABC, protein expression. There was no effect of BCL6 using any of these approaches on IFNγ inducible HLA class I gene expression. Many BCL6 binding sites are distributed across the HLA class II genomic region, particularly between HLA-DRB1 and -DQA1 loci, and within the -DPB1 locus. In contrast, there was a stark absence of BCL6 motifs near HLA-A/B and HLA-C. BCL6 is a novel and selective modifier of HLA gene expression in endothelium. BCL6 may be an untapped target to prevent pathogenic expression of HLA II in organ transplants.

Th126. Crosstalk Between Antiviral and Wound Healing Responses of Human Respiratory Epithelial Cells

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The respiratory epithelium coordinates an antiviral defense during acute viral infection and repairs tissue damage post-infection, but it is unclear how the balance between these functions is regulated. Here we studied the effects of Type III interferons (IFN-λ), cytokines secreted by epithelial cells during the innate antiviral response, on wound healing of primary human bronchial epithelial cells (HBEC) using a scratch assay. Monolayers of low-passage HBEC pre-treated with IFNλ1 or IFNλ2 for 16 hours were wounded with a standardized scratch. Wound repair kinetics were quantified using ImageJ's MRI Wound Healing Tool and video microscopy. The standardized wound healed in 24 hours, whereas IFNλ1-treated monolayers healed more slowly, covering 50% of the initial wound in 48 hours ($p < 0.0001$). IFNλ2 had no significant effect. Targeting the IFN-λ receptor with siRNA KD rescued wound healing post-IFNλ1 treatment, but KD of MX1, an interferon-stimulated gene hypothesized to participate in cell motility, did not. Prior studies showed that influenza A-induced IFN-λ disrupted murine lung repair by p53-dependent inhibition of cell proliferation. We found that IFNλ1-treated HBEC showed a trend, though not significant, toward decreased proliferation over the scratch assay time course (48 hours). Trajectory analysis showed that IFNλ1 diminished cell migration distance, with IFNλ1-treated cells traveling 75% as far as control cells over 24 hours ($p < 0.0001$). With the literature, our data indicate at least two mechanisms of crosstalk

between antiviral defense and wound healing in the respiratory epithelium, which may serve to delay tissue repair until after the resolution of acute viral infection.

Th128. Immune Prophylaxis and Therapy of Vascular Complications of Diabetes
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The Problem: Vascular complications of diabetes contribute to major economic social burden of $ 412.9 Billions in USA (Parker et al “Economic Costs of Diabetes in the U. S. in 2022” in Diabetes Care, ADA Statement (doi.org/10.2337/dci23-0085) Immune pathogenesis: The Recent advances in Genomics and Fundamentals of Immunology pertains to the Factor H and how it interacts with glycation abnormalities in the host to cause immune system dysfunction and vascular complications of diabetes (de Boer ECW, van Mourik AG and Jongerius I (2020) Therapeutic Lessons to be Learned From the Role of Complement Regulators as Double-Edged Sword in Health and Disease. Front. Immunol. 11:578069.doi: 10.3389/fimmu.2020.578069 and Reily C. et al, Glycosylation in health and disease, *Nature Reviews - Nephrology*, Vol. 15, June 2019, 346-366). Our Work: 80 % of complement dysfunction is attributed to "C3 Amplification Loop" that cause explosive vascular damage in diabetes. A method to inhibit C3 amplification loop is detailed with sulfonic polymer that may contribute to prevention of vascular complications and its therapy in future.

Th129. Induction of Tolerogenic Dendritic Cell Phenotype Through Natural Killer Cells
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Abbvie

Dendritic Cells (DCs) play a crucial role in maintaining the balance between autoimmunity and tolerance. Harnessing the ability to induce a tolerogenic phenotype in DCs, known as TolDCs, holds great promise for restoring immune tolerance and managing autoimmune diseases. In this study, we generate human TolDCs through co-culture with autologous Natural Killer (NK) cells. We co-cultured monocyte derived DCs with autologous NK cells overnight at a 5:1 ratio. Following this, the cells were treated with either LPS or a media control for four hours before undergoing FACS sorting. DCs cultured alone were also sorted as controls. We characterized these DCs using a combination of functional assays, epigenomic profiling using ATAC-Seq, and transcriptomic profiling using RNA sequencing. The DC-NK co-culture induced a consistent tolerogenic phenotype in DCs compared to DCs cultured alone, including lower expression of costimulatory molecules (CD80, CD86, CD40 and MHC-II), as well as a consistent change in cytokine expression, with decreased IL1 and IL6 levels and increased IL10 levels in addition to increased activity of TCF and KLF transcription factors, with known roles in tolerance. Additionally, these NK co-cultured DCs compared to DCs cultured alone were anergic to LPS stimulation and induced blunted T cell responses. Results indicate that this reprogramming is induced at epigenomic level. Our findings provide compelling evidence supporting the regulatory role of NK cells through TolDC induction, holding significant promise for restoring immune tolerance and managing autoimmune diseases.

Th130. Interleukin-1beta Enables Endothelial Surface Expression and Trans-presentation of Interleukin-15 by Relieving let-7c-3p-mediated Translational Suppression

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Expression of interleukin-15 (IL-15) on the surface of human graft endothelial cells (ECs) bound to the IL-15 receptor alpha (IL-15Ralpha) subunit can increase the activation of cytotoxic T lymphocytes (CTLs), potentiating allograft rejection. Surface expression of this complex can be induced by alloantibody-mediated complement activation and, ultimately, autocrine/paracrine IL-1-mediated activation of NF-kappaB. We identify 8 constitutively expressed alternatively spliced IL-15 transcripts in human ECs. IL-1β does not alter the expression level of any IL-15 transcript but induces surface expression independently of RNA Polymerase II-mediated transcription while requiring new protein translation. By small RNA sequencing, we found that IL-1βcauses an NF-kappaB-mediated reduction in the level of microRNA Let-7c-3p, thereby relieving a translational block of IL-15 surface protein. Let7c-3p antagonist can induce EC surface expression of IL-15/IL-15Ralpha in the absence of complement activation or of IL-1, enabling IL-15 trans-presentation to boost CD8 T cell activation. The complexity of IL-15 regulation requires caution in interpreting increased total IL-15 mRNA or protein levels as a surrogate for trans-presentation.

Th131. Regulation and Dysregulation of Immune Responses by Human Skin Dendritic Cell Subset

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Dendritic cells (DCs) are key antigen-presenting cells that control both immunity and tolerance. Understanding the principles by which DC control these responses have provided a rich basis for studying and improving clinical outcome in treating human diseases. Part of the complexity is due to the existence of distinct DC subsets, each bearing different microbial receptors, surface molecules and cytokine expression. The biological raison d'être for separate DC subsets has been the focus of many studies, including our own. Our research focuses on skin-migrated DC2 lineage cells, specifically on two subtypes differentiated by CD5 expression. The $CD5^+$ DCs are highly efficient at priming cytotoxic $CD8^+$ T cells and induce inflammatory T helper cell responses compared to their CD5- counterparts. Their elevated levels in inflamed psoriatic skin plaques, where high effector T cell responses are seen, compared to distal cutaneous tissue, suggest they are critical players in the pathogenesis. On the other hand, $CD5^+$ DC numbers are reduced in malignant tissues and correlate with cancer patient survival. Consistent with this notion, we demonstrate, using human patient cells and mouse models, that CD5 functions to trigger an inflammatory pathway of DC maturation and effector T cell activation. Deletion of CD5 on DCs educes tumor rejection and immunotherapy response. During successful immunotherapy, $CD5^+$ DCs increased, which was critical for their interaction with CD5hi T cells. Thus, understanding the function and development of CD5-expressing DCs in immune regulation provides insight into how ICB immunotherapies work and identifies CD5 on DCs as a potential therapeutic target.

Immuno-engineering and Cellular Therapies

Th132. A Tolerogenic Lipid Nanoparticle Delivery System for Antigen-specific Immune Tolerance
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The immunogenicity of mRNA lipid nanoparticles (LNPs) is advantageous for vaccines but detrimental for antigen-specific immune tolerance (ASIT). To unlock the potential of mRNA-mediated antigen delivery in tolerance, we developed a tolerogenic LNP (tLNP) system to co-deliver mRNA and immunomodulators to antigen-presenting cells *in vivo*. Relative to mRNA-only LNPs, tLNPs exhibit comparable biodistribution and mRNA delivery while reducing the associated inflammatory response in mice. In a prime-boost mouse model of ovalbumin immunization, tLNPs reduced antigen-specific IFNγ and IgG1 production ($P < 0.0001$ vs mRNA-only LNPs). We identified a lead tLNP composition that inhibited antigen-specific CD4 proliferation and Th1/Th2 cytokine secretion, increased antigen-specific Tregs *ex vivo*, and reduced autoimmune infiltration of pancreatic islets in pre-diabetic NOD mice after 2 injections ($P=0.02$ vs controls). Subsequently, we tested whether tLNPs could induce ASIT and prevent diabetes in NOD mice. Female NOD mice injected biweekly from 5-15 weeks with our lead tLNP (islet autoantigen mRNA and immunomodulator) had dramatically reduced diabetes incidence relative to buffer-injected controls (13.3% vs 66.7% diabetes free at 30 weeks, $P=0.0002$) and delayed diabetes onset. LNPs carrying mRNA only or irrelevant antigen with immunomodulator were ineffective (66.7% and 53.3% diabetes free at 30 weeks), revealing both autoantigen and immunomodulatory components are essential for ASIT. Furthermore, tLNPs inhibited mouse and human dendritic cell maturation *ex vivo* and increased the frequency of splenic Tregs in NOD mice ($P=0.006$) in an antigen-specific manner. In conclusion, this novel tLNP system induces ASIT in NOD mice and could enable mRNA therapeutics for tolerance across multiple indications.

Th133. CAR T Cell Therapy Targeting CD84 Alone or in Combination with CD19 for the Treatment of B Cell Malignancies
Ane Altuna Mongelos
Fundació de Recerca Clínic Barcelona-Institut d'Investigacions Biomèdiques August Pi i Sunyer

Most B cell acute lymphoblastic leukaemia (B-ALL) patients (>80%) achieve complete remission with CD19-directed CART cell therapy, but negative or low-antigen expressing leukemic cells may lead to relapse. In contrast, chronic lymphocytic leukaemia (CLL) patients have significantly lower response rates with this type of treatment. CD84 (SLAMF5) is an immunoreceptor overexpressed in B cell malignancies that emerges as a novel target for their treatment. A CD84-directed second-generation CART cell (CART84) was engineered (anti-CD84scFv-CD8αTM-4-1BB-CD3) and proved to be cytotoxic towards aggressive B cell lymphoma cell lines (Ramos) and B-ALL (NALM-6) *in vitro*. Moreover, CART84 controlled Ramos tumour progression and increased survival of CART84-treated NSG mice compared to the control group. We designed a CD19 and CD84 dual CAR T cell (CD19/CD84-DUAL1) using an "AND" strategy. C164S and C181S mutations were introduced to prevent dimerization of the hinge domains (CD19/CD84-DUAL1m). Subsequently, two different CD19 and CD84 dual CART cells were engineered employing an "IF BETTER" strategy (CD19/CD84-DUAL2m: anti-CD19scFv-4-1BB-CD3/anti-CD84scFv-4-1BB; CD19/CD84-DUAL3m: anti-CD19scFv-4-1BB-CD3-anti-CD84scFv-CD28) in an attempt of improving the efficacy of CD19-directed CART cells for CD19low B-ALL relapses. CD19/CD84-DUAL1m, CD19/CD84-DUAL2m and CD19/CD84-DUAL3m specifically killed $CD19^+/CD84^+$ NALM-6, but not $CD19^-/CD84^+$ MOLM-13 (acute myeloid leukaemia) cells *in vitro*. In conclusion, CART84 cells are highly cytotoxic towards aggressive B cell lymphoma both *in vitro* and *in vivo*, thus supporting the t use of CD84-

targeted CAR T cells to treat B cell malignancies. Furthermore, CD19/CD84-directed dual CAR T cells could be used to rescue CD19low B-ALL relapses after CD19-directed CART cell therapy.

Th134. Gene-edited Allogeneic Regulatory T Cells as 'Off-the-shelf' Immunomodulatory Therapy for Transplantation

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Regulatory T cells (Tregs) are a pivotal component in maintaining immune homeostasis and have therapeutic potential in autoimmune diseases and transplant rejection. However, the clinical application of Tregs is hindered by several challenges, including donor variability, manufacturing complexities, and the time-intensive nature of Treg expansion. These limitations are particularly pronounced in acute disease phases or scenarios where autologous Tregs are sparse or dysfunctional. Here, we explored the feasibility and potency of unmatched, allogeneic Tregs as an 'off-the-shelf' immunomodulatory cell therapy. In a humanized skin transplant mouse model, allogeneic "third-party" Tregs displayed significantly reduced ability to suppress graft rejection. Gene editing of B2M and CIITA in human Tregs allowed efficient silencing of MHC class I and II, respectively. Targeted insertion of HLA-E cDNA provided partial protection of B2M-edited Tregs against allogeneic NK cell lysis *in vitro*. Silencing of MHC class II was necessary to shield allogeneic Tregs from killing by expanded allo-specific T cell lines *in vitro*. The combination of HLA-E-B2M knock-in and CIITA knockout enhanced the suppressive activity of allogeneic, unmatched Tregs in the humanized skin transplant model. With respect to long-term graft survival, allogeneic MHCI/II-edited Tregs had comparable suppressive activity to autologous Tregs. Peripheral blood analysis of humanized mice indicated the presence of circulating MHC-silenced Tregs at day 20 after cell infusion. Further *in vivo* experiments are ongoing to validate these results. Our results suggest that MHC gene editing can enable more efficacious allogeneic 'off-the-shelf' Tregs.

Th135. IL-10 Producing Tolerogenic Dendritic Cells Modulate B Cell Responses *in vitro* and *in vivo*

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Tolerogenic dendritic (tolDC) cell-based therapies offer the possibility of restoring immune regulation by targeting only the detrimental immune responses against disease-associated antigen (Ag)s. IL-10-modulated DC generated from monocytes by IL-10 gene transfer (DCIL-10) have been proven to modulate T cell responses, to promote the differentiation of regulatory T cells, and to prevent autoimmune diabetes in pre-clinical models. Since IL-10 regulates B cell responses by modulating antibody (Ab) production and excessive B cell activation, and DC are involved in providing second signals to B cells for their full activation, we dissected the impact of DCIL-10 on B cell responses *in vitro* and *in vivo*. We demonstrate that DCIL-10 promoted allogeneic human naïve and memory B cell activation and proliferation and induced isotype switching by promoting IgG4 secretion in memory B cells. DCIL-10-mediated modulation of naïve B cells is cell-to-cell contact dependent, while of memory B cells is IL-10 dependent. In a newly developed in humanized mouse model treatment with allogeneic DCIL-10 prevented IgG Ab secretion. Finally, treatment with Ag-pulsed DCIL-10 directly promoted B cell maturation and induction of Ag-specific IgG release, and indirectly prevented the expansion of Ag-specific B cells, overall reducing the secretion of Ag-specific IgG *in vivo*. Collectively these data demonstrate for the first time that IL-10-producing DC directly and indirectly modulate Ag-specific B cell responses. The ability of IL-10-engineered DC to

regulate, in addition to T, B cell responses, opens new perspectives for defining an innovative cell-based approach to promote Ag-specific tolerance in the context of autoimmune diseases.

Th136. Patient-derived Lung Cancer Organoids as 3D-testing Platform for Advanced Personalized Therapies

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Lung cancer stands as the leading cause of global cancer-related deaths, prompting a critical need for new treatment options. While chemotherapy and radiotherapy are standard treatments for certain lung cancers, their efficacy is not sustained. Targeted antibodies and immune-checkpoint inhibitors (ICI), effective in a small subset of patients, encounter challenges due to high tumor heterogeneity. Adoptive cell therapy which involves the infusion of natural tumor-reactive T cells or genetically modified T cells to express chimeric antigen receptor (CAR), represents a promising alternative. To address tumor resistance mechanisms and personalize new therapies, models reflecting individual tumors are crucial. We successfully established patient-derived lung tumor organoids and matched healthy organoids from surgically resected patient tissue. These organoids closely resemble the original tissue, validated through histology, DNA sequencing, and single-cell RNA sequencing. Acting as patient avatars, we systematically tested each patient's treatment regimen with our organoid platform *ex vivo*. Furthermore, proteomics analysis showed potential to identify therapy resistance mechanisms. To develop alternative therapy options, we screened each patient organoid line for lung tumor-associated antigens (TAAs). As a proof of concept, we identified suitable TAAs for specific T cell targeting, employing and generating corresponding CAR-T cells. These CAR-T cells demonstrated specific tumor organoid killing compared to healthy controls, indicating their potential as a therapeutic alternative. Our study establishes patient-derived organoids as avatars, utilizing a diverse set of immunotherapeutic approaches to inform and personalize therapy for lung cancer patients.

Th137. Precision Therapy for Lupus: Targeting Follicular Helper T Cells Using Chimeric Antigen Receptor Natural Killer Cells

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Aberrant follicular helper T (Tfh) cell responses are a core feature of autoimmune disease. Notably, Tfh cells promote the development and expansion of pathogenic autoantibodies in systemic lupus erythematosus (SLE), a chronic autoimmune condition that disproportionately impacts young Black, Asian, and Hispanic women. Patients with SLE exhibit elevated frequencies and tissue infiltration of Tfh cells, particularly during disease flares, and mouse models suggest that Tfh cells are necessary and sufficient for the development of SLE-like disease. Hence, targeted depletion of Tfh cells is a compelling yet largely unexplored therapeutic strategy to achieve SLE

remission. Our lab developed a novel programmed death-ligand 1 (PD-L1) chimeric antigen receptor (CAR) that selectively targets Tfh cells based on their unique phenotypic feature – high surface expression of programmed cell death protein 1 (PD-1). At the 2023 FOCIS meeting, I presented functional experiments showing that human PD-L1 CAR natural killer (NK) cells efficiently kill Tfh cells and spare off-target leukocytes *in vitro*. This year, I will share findings from our latest *in vivo* studies evaluating the therapeutic efficacy of PD-L1 CAR-expressing lymphocytes in mouse models of SLE. We rigorously tested the capacity of our innovative Tfh-targeted cellular therapy to alleviate SLE-like disease in spontaneous genetic, inducible, and humanized mouse models. PD-L1 CAR-engineered cells are highly selective for PD-1-high Tfh *in vivo* and demonstrate no overt toxicity. In addition to their therapeutic potential in autoimmune disease, PD-L1 CAR NK cells offer versatile applications for targeting Tfh in contexts such as transplant rejection, lymphoma, and chronic infection.

Th138. Rapamycin Maintains FOXP3 Expression and Modulates Metabolic Function in Expanded Regulatory T Cells

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Introduction: Cell-based therapies employing regulatory T cells (Tregs) offer promise for reinstating immune tolerance in transplantation and autoimmune disorders. *In vitro* expansion of Tregs, however, is challenging due to the loss of the Treg master transcription factor FOXP3 during long-term culture. This study investigates the mTOR inhibitor rapamycin's potential for preserving FOXP3 expression during Treg expansion, alongside its downstream metabolic effects. Methods: Tregs from an 83-year-old donor underwent 3-week *in vitro* expansion with or without rapamycin. Multi-color flow cytometry tracked cellular phenotype, while Seahorse assays assessed metabolism, and Treg suppression assays measured functionality. Additionally, *in vivo* experiments were performed to test rapamycin-treated Tregs' capacity to mitigate disease onset in a GVHD mouse model. Results: Rapamycin-treated Tregs exhibited significantly higher FOXP3 expression beyond 2 weeks of expansion ($p < 0.0001$), indicating enhanced Treg stability compared to untreated cells. Moreover, these cells displayed altered mitochondrial function, demonstrated by elevated oxygen consumption rate ($p=0.042$), reduced proton leak ($p=0.0063$), and improved respiratory coupling efficiency ($p=0.0097$). Differential expression of activation and senescence markers further suggested rapamycin's potential for modulating crucial factors influencing Treg stability *in vitro*. Discussion: These findings shed light on phenotypic attributes of rapamycin-treated expanded Tregs, indicating that it may play a role in sustaining Treg stability during prolonged culture. Further, the observed correlations between rapamycin treatment and other cellular functions offer valuable insights into its utility for generating cells for clinical cell-based therapies.

Th139. Regeneration of a Sustained Antiviral Response in Transplant Recipients by Tacrolimus Resistant Antiviral T Cell Products

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Viral complications are major risk factors for immunocompromised patients after solid organ transplantation (SOT). SOT patients depend on lifelong immunosuppression, dampening endogenous T cell response against viral infections. In particular, reactivation of cytomegalovirus (CMV), Epstein-Barr virus (EBV) and Influenza A virus (IAV) cause severe threads to SOT patients. EBV for one elicits posttransplant lymphoproliferative disease (PTLD), leading to mortality rates of up to 25% in SOT patients. Furthermore, respiratory viruses as influenza-A virus (IAV) can cause severe destruction of the respiratory tract, while vaccination responses in those patients remain insufficient. To regenerate a broad, protective antiviral T-cell response in immunocompromised patients and prevent viral complications, we have established a GMP-compliant manufacturing for tacrolimus-resistant CMV-, EBV- and IAV-specific T cell products (TCPs) ready-to-use for adoptive T cell therapy. After specific peptide-driven enrichment of effector T cells, tacrolimus-resistance is induced through a vector-free CRISPR-Cas9-based knockout of FKBP12, the adaptor protein for tacrolimus. With that, maintenance of effector cytokine production and target specific killing capacity of TCPs is secured, while preventing anti-allogenic rejection of the graft by immunosuppression. The safety of tacrolimus-resistant TCPs is verified using in-depth CITE-sequencing and mass spectrometry. Additionally, efficacy of e.g. IAV-specific TCPs is assessed using a human-based native infection model. Currently, patient data from immunocompromised individuals is acquired. Developed single-virus-specific T cells further lay the basis for FKBP12KO multivirus-specific TCPs addressing spectrum combinatorial treatment with an equally constituted antigen-specific T cell pool. These next generation ATMPs have potential long-term regeneration of pathogenic responses in immunocompromised patients and help in the fight against viral threads.

Th140. SH2D2A is an Indicator of Favourable Prognosis in Bladder Cancer and is Enriched in Activated Treg Cells

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The significance of RNA sequencing (RNA-seq) in modern medical research and diagnostics cannot be understated, and its importance has brought about an expansion in the quality and accessibility of the method and its data output. The conventional approach that has defined much of science begins with measurable phenomena, natural or otherwise, and proceeds, by identification of the mutations or aberrations that produce it, to a gene's function. While this approach has produced a great body of scientific progress, its Achille's heel is its reliance on measurable phenomena. With the expanding availability of RNA-seq and other such high dimensional data it is now possible to consider a complementary approach working in the opposite direction i.e., from transcriptomics up towards protein function. This approach may prove fruitful in producing information on the function of cytosolic-bound proteins such as adapter proteins. To test the validity of this method, we made use of several public datasets to interrogate the cancer-specific role of the adapter protein SH2D2A: a protein enriched in T and NK cells, and a known interactor of the kinase LCK, whose function remains uncertain. We found that SH2D2A is a favourable marker for prognosis in urothelial bladder cancer (BLCA). Digging further, we identified a population of $SH2D2A^{+}$ $FOXP3^{+}$ IL2RAhi activated Tregs as the main expressors of SH2D2A in BLCA. This suggests that the expression of SH2D2A in these Tregs contributes to a beneficial prognostic effect. Further comprehension of SH2D2A's function in these cells holds the potential for advancing treatment in BLCA.

Th141. Single Cell Isolation of CD4+ T Cells Specific for NY-ESO-1 Presented on HLA-DRB3*02:02

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Natural transgenic T cell receptors (tTCR) represent an emergent type of cell therapy against cancer, recognizing peptides expressed both on the intracellular and extracellular level. Recognizing intracellular antigens constitutes a significant advantage in comparison to CAR therapy that can only recognize extracellular surface proteins, which are difficult to define as Tumor Associated Antigens (TAA), especially on solid tumors. However, it must be taken into consideration that TCR therapies against TAAs could potentially generate off-tumor on-target toxicity. In order to prevent this risk, this work is focused on NY-ESO-1, from the cancer/testis antigen family, expressed on several types of tumors but not relevantly on healthy tissues besides testicles. Our work is focused on class II-presented peptides, namely NY-ESO-1119-143 on HLA-DRB3*02:02 (DR52b), expressed approximately by half of the Caucasian population. Specific CD4+ CD25- T cells against NY-ESO-1119-143 presented on HLA-DRB3*02:02 were obtained after 30 days of cell culture and two rounds of cell sorting at days 15 and 30. T cell purity and specificity were assessed using CD4 and CD154 markers, respectively. Purity was maintained at 97%, and specificity increased significantly in the second cell sorting in comparison with the first one. Single cell sequencing by Rhapsody® system was performed to obtain TCR sequences as well as RNA and protein expression profiling.

Th142. T Cell Receptor Precision Editing of Regulatory T Cells for Celiac Disease

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Background: In celiac disease, HLA-DQ2.5 facilitates the presentation of deamidated gluten-derived peptides to antigen-specific CD4+ T cells, triggering immune activation and enteropathy. The adoptive transfer of engineered (e) gluten-specific regulatory T cells (Tregs) may suppress the effector function of pathogenic T cells. Methods: Five TCRs recognizing the immunodominant DQ2.5-glia-a1a(clones S2, LS2.8) and glia-a2 epitopes (clones D2, JR5.1, S16) were tested in a TCR-deficient NFAT-luciferase cell line. We next replaced the endogenous TCR of human primary T cells and Tregs through homology-directed repair targeting the TCR alpha and beta constant loci using AAV and CRISPR-Cas9 technologies. The same strategy was applied to murine T cells and Tregs, and evaluated in HLA-DQ2.5+ C57BL/6 transgenic mice exposed to gliadin via oral gavage. Results: Jurkat cells transfected with the mRNA of any of the five gluten-specific TCRs exhibited peptide-specific NFAT activity. Human primary eCD4+ T cells upregulated CD25 and CD71 with a similar mean functional avidity (EC50) for either specific peptides but not for glia-a1a/a2 overlapping epitopes. Human eTregs demonstrated superior suppressive activity compared to polyclonally expanded Tregs in an EC50-dependent manner. In the HLA-DQ2.5 mouse model, eCD4+ T cells migrated and strongly proliferated in the small intestine (duodenum/jejunum/ileum) and to a lesser extent in the draining (liver/celiac/duodenal/mesenteric) lymph nodes compared to the spleen. This proliferation was suppressed only in the presence of gluten-specific eTregs. Conclusion: Redirecting Tregs to a single immunodominant gliadin-derived peptide could be sufficient for selective trafficking into the gut and draining lymph nodes. These cells hold therapeutic potential for restoring gluten tolerance in celiac patients.

Th143. TCR Discovery and Detection of Antigen-presentation on Cells Using the Dextramer® Technology
Leiying Lyn Zhu, Thomas Blicher, and Liselotte Brix
Immudex

The antigen-specific interaction between the T Cell Receptor (TCR) and cognate peptide MHC (pMHC) complex is at the heart of the successful development of T cell-based therapies. The TCR:pMHC interactions are generally weak, which could be a limitation and hinder the discovery and study of TCRs. The increased avidity of MHC Dextramer reagents has already been shown to enable detection of low affinity antigen-specific CD8, CD4 T cells as well as unconventional T cells. Here, we demonstrate a smooth workflow for discovery of pMHC-specific TCR sequences using single-cell RNA-seq and dCODE Dextramer® reagents, and how TCR functionality can be verified using custom-made TCR monomers and TCR Dextramer® reagents. To date, we have developed TCR Dextramer® reagents recognizing a range of different pMHC specificities, including HLA-E*01:03/peptide, MR1/5-OP-RU and Mamu-A*01/peptide. Finally, we show that TCR Dextramer reagents have been successfully used to specifically detect antigen-presentation on target cells – a useful tool for evaluation of antigen-presentation on variant target cells. This study shows a complete workflow from TCR discovery through TCR validation and final evaluation of target expression using TCR Dextramer®. A set of analytical tools useful in development of TCR immunotherapeutics and cell therapies.

Th144. The Hospital Exemption is the Best European Pathway Towards the Final Authorization of an Academic Proposal in CAR-T Therapy
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By now, authorization for the use of cell and gene immunotherapies is just possible through pharma companies and conventional commercialization after full market authorization by general drug agencies (FDA in US and EMA in Europe). In this presentation we want to share our experience in a hospital of Barcelona, centred in the obtention of authorization based in the "hospital exemption" rule as an exemple of a middle step for arrive to authorize academic products. At least in Europe and for autologous procedures like the present for CAR-T immunotherapies, we want to present why this proposal can be considered the best solution for Public European Health Systems towards the final authorization of an academic proposal, providing global and future access to patients. By now Varnimcabtagene autoleucel (ARI-0001) and cesnicabtagene autoleucel (ARI-0002h) are the 2 first examples that allowed us to treat more than 250 patients. ARI-0001 is also under evaluation of EMA thanks to prime designation of this product. Access, sustainability and development of new options (mainly done by academic centers) should be the main aspects to consider and improve when we want consider new advanced cell-gene therapies.

Th145. Tolerogenic IL-2 Containing Nanoparticles Induce CD4 and CD8 Regulatory T Cells and TGF-β Producing Regulatory NK Cells That Provide Essential Support for the Tregs Which Suppress Immune-Mediated Disorders
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We have previously reported that a brief course of tolerogenic nanoparticles (NPs) decorated with anti-CD2 and containing IL-2 and TGF-β induced CD4 and CD8 iTregs and NK cells which had long-lasting protective effects

from lupus-like disease in mice via a TGF-β-dependent mechanism. Using human peripheral blood mononuclear cells (PBMCs) to induce fatal graft-versus-host disease (GVHD) in NSG mice, we document here that TGF-β-producing NK cells induced by the tolerogenic NPs are essential for the development and maintenance of the Tregs and that the tolerogenic effects of IL-2 delivered by the NPs to the Tregs are TGF-β-dependent. Blockade of TGF-β signaling converted the tolerogenic response into an immunogenic response that markedly decreased the mice survival. Remarkably, the depletion of NK cells from the PBMCs had identical deleterious effects. *In vitro* stimulation of PBMC with either anti-CD2 or anti-CD3 antibody-decorated NPs induced most NK cells to express intracellular TGF-β. Importantly, the safety of the NPs was improved by eliminating the encapsulation of TGF-β since the NPs could locally stimulate target cells to produce the TGF-β for Treg induction. These results indicate for the first time, to the best of our knowledge, that NK cells can be induced to become essential tolerogenic cells and that the TGF-β produced by these cells associates with the long-lasting duration of the Treg therapeutic effects. This suggests that therapeutic agents which can concomitantly induce tolerogenic adaptive and innate responses have an increased ability to prevent and treat chronic immune-mediated inflammatory disease.

Th146. Type-2 Functionality in Sustaining CAR T Cell Longevity Linked to 8-year Leukemia Remission

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Despite a high response rate in chimeric antigen receptor (CAR) T therapy for acute lymphocytic leukemia (ALL), ~50% of patients relapse within the first year, representing an urgent question to address in the next stage of cellular immunotherapy. To investigate the molecular determinants of ultra-long CAR T persistence, we obtained single-cell multi-omics sequencing data from >1 million pre-infusion CAR T cells from 82 pediatric ALL patients enrolled in the first two CAR T ALL clinical trials and 6 healthy donors. Our analysis revealed a notable role of type-1 function, which was highly represented but showed no discernible correlation with CAR T persistence. Surprisingly, we identified that elevated type-2 functionality in CAR T infusion products was significantly associated with patients maintaining a median B cell aplasia duration of 8.4 years. Ligand-receptor interaction analysis uncovered that type-2 cytokines regulated terminal effector cells showing dysfunctional signatures. *In vitro* functional studies showed that adding IL-4 during CAR-specific activation alleviates CAR T cell dysfunction and enhances functional fitness. Serial proteomic profiling of post-infusion sera from patients revealed a higher level of circulating type-2 cytokines in 5-year or 8-year relapse-free responders. In a leukemic mouse model, type-2 high CAR T cell products demonstrated superior expansion and antitumor activity particularly upon leukemia rechallenge. Additionally, the restoration of antitumor efficacy in type-2 low CAR T cells was attainable by augmenting their type-2 functionality. Our findings underscores an unexpected role of type-2 functionality in maintaining a balanced homeostatic state within the entire CAR T population associated with ultra-long-term complete remission.

Tu141. Alloantigen-specific CAR Tregs Protect Islets from Autoimmune Attack

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Type 1 Diabetes (T1D) is driven by T cells that kill beta cells in pancreatic islets. Islet transplantation is a therapeutic option; however, transplants are subject to attack by both alloimmune cells and pre-existing

diabetogenic cells. We have previously shown that regulatory T cells (Tregs) engineered to express a chimeric antigen receptor that detects the alloantigen HLA-A2 (A2 CAR), suppress anti-HLA-A2 immunity in various pre-clinical models. However, the ability of A2 CAR Tregs to mediate bystander suppression of immunity targeting antigens distinct from HLA-A2 was unknown. We hypothesized that in the context of islet transplantation, A2 CAR Tregs may suppress killing of HLA-A2$^+$ islets by islet autoantigen-specific T cells. To test this possibility, NSG mice were transplanted with islets from HLA-A2$^+$ NOD mice and injected with diabetogenic (BDC2.5) CD4$^+$ T cells (sorted Foxp3-negative) alone or with A2 CAR Tregs. Whereas BDC2.5 T cell-treated mice rapidly developed hyperglycemia, the majority of A2 CAR Treg-treated mice were protected. A2 CAR Tregs strongly engrafted in the mice and significantly reduced BDC2.5 T cell engraftment and proliferation in the blood and endogenous islets. In protected A2 CAR-Treg treated mice, the proportion of Foxp3-expressing BDC2.5 T cells progressively increased over time, suggestive of infectious tolerance. Remarkably, both the HLA-A2$^+$ islet graft and HLA-A2-negative endogenous pancreas were protected in A2 CAR Treg-treated mice. These data show that A2 CAR Tregs mediate bystander suppression of autoreactive T cells *in vivo* and support the development of this approach to control allo- and autoimmunity in islet transplantation.

Tu142. CAR-Tregs Synergize with Anti-thymocyte Globulin and Rapamycin to Delay Skin Allograft Rejection

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Regulatory T cell (Treg) therapy is as an alternative to traditional post-transplant immunosuppression. Modified Tregs expressing a donor-specific chimeric antigen receptor (A2-CAR) show promise in delaying HLA-A2$^+$ skin and heart graft rejection. However, in the absence of adjunct immunosuppression their persistence and suppressive effect is transient. To enhance A2-CAR-Treg efficacy, we investigated the impact of anti-thymocyte globulin (ATG, rabbit anti-mouse), rapamycin, and A2-CAR-Tregs as a combination therapy in a haplo-mismatched 2W-Ova-BALB/c x B6.HLA-A2 F1 skin to B6 recipient transplant mouse model. ATG recipients received two intraperitoneal injections (500ug/dose, day -1 and 3) and rapamycin (1mg/kg) was given for 10 consecutive days from day 15. The median graft survival for untreated or A2-CAR-Treg alone was 14 days. Treatment with ATG alone, ATG and A2-CAR-Tregs, or ATG and rapamycin moderately extended median graft survival to 20-26 days. However, the combination of ATG, rapamycin and A2-CAR-Tregs resulted in significant prolongation, extending median graft survival to 41 days. Mechanistically, ATG and rapamycin increased A2-CAR-Treg engraftment and proliferation, as measured by increased ki67 expression. Ongoing studies are determining effects on donor-specific antibodies as well as donor T cells specific for the 2W or Ova transgenes. In summary, these data show that A2-CAR-Tregs can be successfully used in combination with ATG and rapamycin and have important implications for the application of A2-CAR Tregs in humans.

Tu143. Enhancing Regulatory T Cell Therapy with Orthogonal IL2-IL2R Systems to Restore Immune Tolerance in Type 1 Diabetes

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Regulatory T cell (Treg) therapy aims to restore immune tolerance, but only a small fraction of infused Tregs persist long term. We investigated whether an orthogonal IL2-IL2R system could enhance the persistence and function of Tregs in the NOD mouse model of autoimmune diabetes. A low-affinity orthogonal IL2 ligand, 3A10, induced STAT5 phosphorylation and proliferation selectively in ortho-IL2R$^+$ Tregs at high concentrations *in vitro*, but failed to aid ortho-IL2R$^+$ islet antigen-specific Tregs in preventing diabetes. While a high-affinity orthogonal IL2 ligand, 1G12, weakly stimulated wt-IL2R at high concentrations. Thus, the selectivity of 1G12 for ortho-IL2R was dose dependent. At low doses, 1G12 prevented diabetes with or without infusion of ortho-IL2R$^+$ islet antigen-specific Tregs. These results show that the use of the ortho-IL2-IL2R system *in vivo* depended on ortho-IL2's relative intrinsic affinity for wt-IL2R and avidity for ortho-IL2R. To improve the potency and selectivity of the ortho-IL2-IL2R system, we tethered the orthogonal IL2 ligands to the ortho-IL2R via a peptide linker. 3A10 tether selectively promoted greater pSTAT5, CD25 and Bcl2 expression, proliferation, and survival of engineered Tregs *in vitro* and *in vivo* when compared to empty vector-expressing Tregs without IL2 and 1G12 tether. However, empty vector-expressing Tregs provided IL2 up-regulated these markers greater than 3A10 tether. Nonetheless, both 3A10 tether and 1G12 tether enabled complete diabetes prevention using as few as 2,000 engineered islet antigen-specific Tregs. Collectively, our study demonstrates that an orthogonal IL2 autocrine system can be an effective cell engineering strategy for augmenting Treg cell therapy for autoimmune diseases.

Tu144. Fate Mapping of Human T Cell Subsets During Therapeutic T Cell Expansion

Avery Lam
Stanford University

Adoptive T cell therapies represent a major advance for patients with otherwise-refractory blood cancers, but patient responses can vary widely, due in part to the inherent heterogeneity of the manufactured T cell product. Decades of work have identified multiple specific T cell subsets/phenotypes in the infusion product that can predict clinical efficacy. It is not clear how and where these desirable cells arise from within a mixed population during *in vitro* expansion. We investigated the contribution and fate of multiple T cell subsets towards the final therapeutic T cell product, by performing lineage tracing on naive, central memory, and effector memory cells expanded either alone or together within a pool. We used lentivirus-based proteomic barcodes along with single-cell mass cytometry to follow each T cell subset over time. Central memory and effector memory T cells expanded within a pool upregulated several costimulatory (OX40) and coinhibitory receptors (PD-1, LAG-3), but otherwise exhibited similar activation kinetics to their counterparts expanded alone. On the other hand, naive T cells expanded within a pool exhibited augmented activation, upregulating CD25, HLA-DR, and the proliferation marker Ki-67. This coincided with a restoration of cell cycle progression in the pooled cells, compared to naive T cells expanded alone which progressively returned to G0/G1 over time. Overall, pooled expansion has divergent effects on different T cell subsets with important implications for therapeutic T cell manufacturing strategies.

Tu145. Super Consultancy Robotic Model for Diabetes
Kumarpal Shah
Endocrine Technology, LLC.

Problem: Diabetes and its treatment is complex, Extremely costly and outcome is heterogenous. There are many unanswered questions in the treatment and its cure potential. Unanswered questions are how to protect Beta cells?, prevent and treat target organ damage? at cellular/sub cellular levels that are beyond evidenced based medicine and how to optimize drug therapy?, clinical responses and preventive therapies.? Potential Solutions: During recent GTC meeting in 2024 NVIDIA announces new robotics products that are not only automated but can be trained with artificial intelligence. Humanoid Robot --GR00T stands for "Generalist Robot 00 Technology," and with the race for humanoid robotics heating up, this new technology is intended to help accelerate development. GR00T is a large multimodal model (LMM) providing robotics developers with a generative AI platform to begin the implementation of large language models (LLMs). training with vast amount of data input/(https://www.therobotreport.com/nvidia-announces-new-robotics-products-at-gtc-2024/) Solution: Based on our patented technology for the cure of Type 1 and Type 2 diabetes - we plan to develop Super consultancy model for diabetes treatment and its cure. The target group are various end group users such as hospitals, physicians, endocrinologists, researchers and biotechnology companies. It is expected when successfully developed --it will reduce cost and allow rapid improvement in the diabetes and its complications.

W138. A Bispecific CD8-dependent Immunoconjugate to Crosslink and Eliminate Antigen-specific B Cells
David Scott
Uniformed Services University of Health Sciences

We previously used regulatory or cytotoxic T cells expressing an antigenic epitope to modulate or kill B cells producing adverse/inhibitory antibodies. We call these "BAR" T cells in analogy to chimeric antigen receptor (CAR) T cells. Herein, we describe an immunoconjugate that can target B cells by crosslinking a CD8 effector cells in a model of hemophilia A (HA). HA is an X-linked recessive bleeding disorder in which patients with mutations in the F8 gene lack pro-coagulant protein FVIII. Typically, HA patients are treated with intravenous infusion of Factor VIII prophylactically or on demand. Due to lack of tolerance to FVIII, approximately 30% of HA patients develop an adverse immune response leading to antibodies that neutralize FVIII, which are a major impediment to therapy. Inhibitors primarily bind to epitopes on the immunodominant C2 and A2 domains, both critical for FVIII activity. We have designed and produced a bispecific antibody that expresses the C2 domain of FVIII, as well as an anti-human CD8 arm, as an approach to eliminate inhibitors. The construct brings cytotoxic T cells in proximity to FVIII-specific B cells to initiate killing. Using FVIII-specific hybridomas as targets, we have demonstrated via ELISpot that this cytotoxic CD8 T cell conjugate is capable of killing FVIII-specific B cell populations in the presence of human $CD8^+$ T cells. These data support the use of bispecific CD8-dependent immunoconjugate to module adverse immune responses. (Supported by NIH R21 HL152318.)

W139. CAR-Ts Are Getting Viral! a New Method for Allogenic CAR-T Cell Therapy Development
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Autologous Chimeric Antigen Receptor (CAR)-T cell therapies are effective against B cell lymphomas, yet are hindered by challenges such as suboptimal T cell quality, treatment delays, and financial constraints. Allogeneic CAR-T therapy, if successful in preventing Graft versus Host disease (GvHD) and rejection, could overcome

these limitations. Prior strategies involving genome editing to eliminate T Cell receptor (TCR) and Major Histocompatibility Complex class I (MHC-I) expression have encountered issues of genotoxicity, off-target editing, and procedural complexity. This study introduces an innovative approach that sidesteps genome editing by employing a chimeric protein. The chimeric protein, comprising a single chain variable fragment targeting CD3ε fused to US6 protein from Human Cytomegalovirus, serves as a retention motif. This construct hijacks CD3 within the endoplasmic reticulum (ER) while inhibiting the Transporter Associated with Antigen Processing (TAP), thereby abolishing class I presentation. Expression of the CD3-US6 chimeric protein in T cells successfully downregulated TCR and HLA-I expression. Furthermore, CD3-US6 T cells demonstrated reduced reactivity against allogeneic stimuli *in vitro* and *in vivo* compared to control T cells. The rejection response elicited on CD3-US6 T cells was also attenuated. Evaluation of CAR-T cell activity in both control and CD3-US6 T cells revealed comparable efficacy *in vitro* and *in vivo*. In summary, we show that the expression of CD3-US6 and CAR in T cells achieves effective TCR and MHC-I downregulation while preserving CAR-T cell functionality. This innovative strategy avoids the pitfalls associated with genome editing, presenting a promising avenue for the development of allogeneic CAR-T cell therapies.

W140. Engineering Stable and Lymphodepletion-resistant T Regulatory Cells for Inducing Immune Tolerance During Transplantation
Sameena Nikhat
Mayo Clinic

Organ transplant recipients require a life-long regimen of expensive immunosuppressive drugs that cause long-term side effects including predisposition to diseases and cancers. Regardless, 40-70% experience transplant rejection within 10 years, suggesting an urgent need to develop more efficient strategies for inducing immune tolerance. Regulatory-T cells (Tregs) mediate targeted suppression of immune responses to maintain immune homeostasis. However, current Treg immunotherapies show two significant challenges: First, Tregs lose stability and convert into T-effector cells (Teff) while maintaining their target specificity, which is detrimental for the graft. Second, lymphodepleting conditioning regimens administered to transplant recipients can deplete Tregs besides Teff, resulting in poor tolerance to the graft. To address this we aimed to develop resilient Tregs that are resistant to conversion or conditioning regimens for use in allo-/xeno-transplantation. By knocking-out surface receptor CD2, we generated Tregs that are resistant to siplizumab (α-CD2)-containing conditioning regimens. Compared to $CD2^+$ Tregs, CD2-KO Tregs exhibited greater stability, maintaining consistently higher levels of Treg-related proteins like FOXP3 and CTLA4, throughout several days in culture. This difference was particularly notable during later phase of expansion. They were also more efficient in suppressing allogeneic immune responses *in vitro*. In multiple *in vivo* experiments that involved injecting autologous Treg-depleted PBMCs into a mouse model of graft-vs-host disease, CD2-KO Tregs demonstrated either comparable or enhanced efficacy in mitigating disease and improving survival rates than $CD2^+$ Tregs. The mechanisms underlying enhanced immunosuppressive capabilities of CD2-KO Tregs are currently under investigation, and these findings hold immense potential for development of improved Treg-based therapies.

W141. Fixation of Cynomolgus Monkey and Human Whole Blood for Assessment of Pharmacodynamic Effects Using Flow Cytometry
Ariel Calderon, Denny Nguyen, and James Sheridan
Abbvie

Identification of robust intracellular pharmacodynamic effects in global clinical trials remains a challenge in drug development. Rapid cell preservation reagents have reduced the costs and complexity of performing FACS-

based immune studies in the global trial setting. However, a complication can be chemical alteration of antigen-antibody epitopes that antagonize their association, limiting the identification of immune subsets and cell surface proteins linked to function. Additionally, shipment of samples may take more than 24 hours, at which time, sensitive signaling molecules like pSTAT5 are no longer viable to use as biomarkers. In order to circumvent these logistical challenges, we compared three whole blood fixation reagents to determine their viability in the global trial setting. To test these reagents, healthy cynomolgus and human whole blood was stimulated with either IL-2 or IL-15 and preserved using either Proteomic Stabilizer (SmartTube inc.), Whole Blood cell stabilizer (Cytodelics Inc.), or Cyto-Chex (Streck Inc.) to determine the stability of phosphorylated signaling molecules across various immune cell populations. Impact of sample fixation on checkpoint and functional related proteins was evaluated. We observe the preservation of cell surface antigens utilizing these reagents to identify major immune cell subsets while preserving measurements of transient pharmacodynamic effects. These studies provide an attractive solution to determine test article effects on transiently expressed biomarker activities. Whole blood fixation protocols enable the ability to monitor discrete changes in checkpoint and functional markers via flow cytometry in the global clinical trial setting.

W142. Impact of the Gastrointestinal Microbiota on the Immune Population Obtained During the Apheresis of Patients Undergoing CAR-T Cell Therapy for Multiple Myeloma

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CAR-T cell therapy shows promising results in hematologic malignancies but is curative for only a fraction of patients, emphasizing the need for strategies to improve outcomes in non-responsive cases. This study aims to understand if the composition of the gastrointestinal microbiota impacts the immune population obtained during the apheresis of patients undergoing BCMA-directed CART (Clinical trial NCT04309981) therapy for multiple myeloma and the characteristics of the final CAR T cell product infused. Twenty-seven patients were included, and microbiota composition was analyzed by amplifying and sequencing the V3-V4 region of the 16S rRNA; sequence reads were processed by QIIME2. A panel of 35 stool metabolites was assessed using nuclear magnetic resonance, and comprehensive clinical and CAR-T production data were collected. We investigated correlations between $CD4^+$ immune subsets at the apheresis stage and their gut microbiota composition, as well as corresponding metagenomic and metabolomic profiles. Memory repertoire analysis uncovered correlations between $CD4^+$ naïve, $CD4^+$ central memory, and effector memory cells with bacterial families and metabolites. Notably, the Actinomycetaceae family and succinate emerged as independent predictive markers for $CD4^+$ central memory and effector memory levels in the CAR T cell infusion product, influencing the persistence of CAR T cells post-infusion. Phenotypic, metabolite, and functional characterization of *in vitro* expanded CAR T

cells supplemented with succinate validated clinical results. This study establishes a correlation between the gastrointestinal microbiota composition and the immune population characteristics during apheresis and the final infusion product in BCMA-directed CAR-T therapy for multiple myeloma patients.

W143. Lactic Acid Enhances Human Regulatory T Cell Phenotype and Function

Karoliina Tuomela[1], Majid Mojibian[2], Vivian Fung[2], Manjurul Haque[2], Dominic Boardman[2], Ramon Klein Geltink[2] and Megan Levings[2]
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Cell therapy using regulatory T cells (Tregs) represents an approach for targeted suppression of pathological immune responses in autoimmunity and transplantation. Clinical translation of Treg therapy faces numerous challenges, one of which is expansion of homogenous and optimally suppressive cell populations. Given the association between lactic acid (LA) and enhanced Treg function in tumours, we hypothesised that supplementation of LA during expansion may be favourable for Treg expansion. To test this possibility, naïve human Tregs were stimulated with anti-CD3/CD28 beads with or without 15 mM LA from day 3 onwards. Without LA, Treg viability declined after day 9, which was fully prevented by LA supplementation. Culture in LA also significantly enhanced FOXP3 expression and suppressive function *in vitro*, accompanied by increased glycolysis. LA supplementation was also tested during the manufacture of Tregs expressing chimeric antigen receptors (CARs) targeting HLA-A2. Not only did LA enhance viability and purity, but it also significantly reduced exhaustion marker (PD-1, TIM-3, and LAG-3) expression, likely due to diminished CAR tonic signalling. *In vivo* experiments, in which human HLA-A2-CAR Tregs were injected into HLA-A2-transgenic immunodeficient mice, revealed that LA-cultured CAR Tregs maintained higher phenotypic stability and cell numbers over 4 weeks, with lower exhaustion marker expression in blood and tissues. Thus, LA has multimodal effects on human Treg manufacturing resulting in enhanced purity, viability, and function as well as reduced exhaustion. These data demonstrate the benefit of a straightforward culture media modification that leads to substantial improvement in expansion of optimal Treg products for cell therapy.

W144. Manufacturing of CD19/CD20 Bispecific CAR-T Cell Therapy (IMPT-514) for the Treatment of Multiple Sclerosis, ANCA Vasculitis, Inflammatory Myositis, and Systemic Sclerosis

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ImmPACT Bio

Many autoimmune disorders are known to be B cell driven, characterized by pathogenic autoantibody producing B cells that contribute to the pathology of disease through damage of tissues and organs. Examples of B cell driven autoimmune diseases include Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis effecting small blood vessels, inflammatory myositis (IM) effecting muscles, systemic sclerosis (SSc or scleroderma) effecting muscles and connective-tissues, and multiple sclerosis (MS) effecting the central nervous system. Standard of care therapies for these autoimmune diseases include immunosuppressive medications such as methotrexate, prednisone, azathioprine, etc., but these treatments are hindered by severe side effects and have demonstrated variable effectiveness with a high risk of disease recurrence. Recent case studies used CD19 CAR-T cells to treat systemic lupus erythematosus/lupus nephritis, systemic sclerosis, and anti-synthetase syndrome and have demonstrated remarkable efficacy and potential for longevity of effect in treating different B cell mediated autoimmune diseases. We report the successful manufacturing of IMPT-514, a CD19/CD20 bispecific CAR-T cell product manufactured using $CD62L^+$ cells isolated from ANCA-associated vasculitis, IM, SSc, or MS patients and demonstrate elimination of autologous B cells in-vitro. All IMPT-514 products

manufactured from patient samples showed robust CAR expression and VCN and retained a predominantly naïve and central memory phenotype at harvest. In addition, IMPT-514 products secreted interferon-gamma (IFN-γ) when cocultured with donor-matched B cells and effectively eliminated autologous B cells. In summary, IMPT-514 demonstrates good potency against the intended B cell target representing a key potential advancement in the evolution of CAR-T therapy in the autoimmune realm.

W145. Microfluidics-based Bioprocessing Platform for Treg Cell Therapy Manufacturing Applications

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King's College London

In cell and gene therapy (CGT) manufacturing, large vessels known as bioreactors are used to support cell culture operations by providing a controlled microenvironment that mimics the physiological niche which the cells require to survive. The large fluidic volume of these vessels, which can often exceed 5 litres, poses issues which affect both the cost of the manufacturing process and the quality of the end product. An example of this is in chimeric antigen receptor (CAR) T cell therapy manufacturing, whereby the multi-step, labour-intensive manufacturing process drives the high price-point of £280,000 per patient. GMP-grade reagents that are used in the manufacturing process, such as the lentivirus used for T cell transduction, can account for as much as 50% of the materials costs. The large volume nature of bioreactors used in the process is often associated with low process efficiency and batch variability, resulting in a high-cost, heterogeneous product. While CAR T cells are currently licensed for the treatment of haematological cancers, there is potential for the use of regulatory T cells (Tregs) in the manufacturing process, to produce a CAR Treg therapy that can be used for the treatment of auto-inflammatory diseases. Here we present a closed-system, perfusion microfluidic bioreactor for the production of functional CAR Tregs from primary, human Tregs. The integration of a porous membrane allows continuous perfusion and transmembrane flow, which can be used to significantly increase Treg expansion and transduction efficiency, relative to standard plasticware.

W146. Optimized Platform for the Personalized Production of Transgenic TCR T Cells (TCR-READY)

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Chronic Lymphocytic Leukemia (CLL) is the most common hematologic malignancy in adults in Western countries, with a significant increase in incidence with age. While hematologic cancer treatments have improved, especially thanks to the success of Chimeric Antigen Receptor T cell therapy (CAR-T), its application in CLL has been limited, highlighting the need for alternative therapies. CARs offer a therapeutic strategy designed to recognize tumor-associated antigens (TAAs) on cell surfaces. However, the dual presence of TAAs in both tumor and healthy cells poses a risk of inadvertent recognition and severe side effects. In contrast, Tumor-Specific Antigens (TSAs), specifically neoantigens arising from genomic mutations in cancer cells, offer a more specific target with lower risks. The main challenge of this strategy is the time required to identify, design, and develop neoantigen-directed cell therapies. Our proposal aims to address this challenge by optimizing the identification of neoantigen-specific T cell receptors (TCRs) and subsequently inducing them as transgenic TCRs (tTCRs) in T lymphocytes. The therapy will target neoantigens that can be recognized by both $CD4^+$ and $CD8^+$ T cells, with the former typically underutilized in cancer immunotherapies, adding additional innovative value. Sequenced TCRs specific to candidate neoantigens will serve as recognition modules, transduced into T cells using lentiviral

vectors. In summary, this project will establish an optimized platform for tTCR production, aiming to overcome the time limitation and enable the effective application of personalized TCR-based therapy to CLL patients.

Immunogenetics

Tu146. A Polymorphism in Heterozygous IFIH1-Δ14 Leads to Severe Systemic Inflammatory Responses Like Hemophagocytic Lymphohistiocytosis

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Even common viral infections that are usually mild and self-limiting can lead to hospitalization and severe clinical outcome in specific situations such as immunosuppression, preterm birth, and chronic diseases. Systematic inflammatory reactions fulfilling criteria for hemophagocytic lymphohistiocytosis (HLH) can be the initial manifestation of primary immunodeficiency. A twelve-month female infant who was previously healthy, demonstrated fever for two weeks and subsequently generalized skin rashes, suggesting a viral infection. However, her manifestation did not resolve but aggravated with sustained fever, pancytopenia, splenomegaly, increased soluble IL-2R, and decreased fibrinogen fulfilling HLH criteria. We identified a heterozygous splicing variant (NM_022168.3:c.2807+1G>A) (rs35732034) in IFIH1 gene associated with innate immunity using targeted panel sequencing and subsequent Sanger confirmation. We also revealed the alternative splicing resulting in skipping of exon 14 (Δ14) with RNA transcript sequencing. This splicing variant inducing loss-of-function mutation has an allele frequency of 0.0044~0.0065 in Korean population. Therefore, we suggest that IFIH1-Δ14 polymorphism should be considered in the case of exceptionally severe clinical courses, especially revealing systemic inflammatory reactions in common viral infections.

Tu147. CTLA4 Somatic Mosaicism Reveals Deleted Cells Show Little Difference for Big Effect

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CTLA4 controls a pivotal immune tolerance checkpoint, and its haploinsufficiency leads to a diverse autoimmune and inflammatory syndrome with ~67% penetrance. We report a unique case of gonosomal mosaicism involving a 250KB deletion encompassing the CTLA4 locus in an individual with adolescent-onset autoimmune cytopenia's and inflammatory bowel disease responding to CTLA4-Ig therapy. His daughter possesses the same variant in the germline state and has severe neonatal-onset disease. Quantitative PCR of the proband indicated the deletion at 6% in whole blood, with notable enrichment in memory B and T, Treg, and TFH cells, reaching nearly 50% of cells in some T-cell subsets. This cell-autonomous preferential expansion of CTLA4-deficient T-cells and development of autoimmune disease contrasts with studies of chimeric mice, where the presence of CTLA4-wildtype T-cells completely suppressed autoimmune disease. Single-cell RNAseq demonstrated that deletion-bearing cells were polyclonal, exhibiting a trend towards a higher immunoglobulin somatic mutation rate and an increased proportion of IgA switched cells in memory B cells. No significant transcriptional differences were observed compared to CTLA4 wild-type memory B cells. Similar polyclonal patterns were observed in Treg and TFH subsets, with deleted cells showing no transcriptional distinctions from their wild-type counterparts. This case represents the first instance of a mosaic deletion of CTLA4 associated with immune dysregulation. Acknowledging the limitation of deriving broad conclusions from one study, the selective accumulation of CTLA4-haploinsufficient memory lymphocytes, means low-level mosaicism for haploinsufficient cells may have delayed

clinical implications, either for somatic deletions, or for residual recipient chimerism post-HSCT for CTLA4 haploinsufficiency.

Tu148. Enhancing Drug Indication Expansion in Immunology with Sanofi Genetic Data Lake

Clement Chatelain, Samuel Lessard, Jennifer Sloane, Katherine Klinger, Emanuele de Rinaldis, and Shameer Khader
Sanofi

The Sanofi Genetic Data Lake (SGDL) is a comprehensive platform integrating various genome-centricdata, including genome-wide association studies, single cell eQTL, and epigenetic data, to uncover relationships between immune diseases, cell types, and therapeutic targets. This integration aims to strategically position therapeutic targets in immune diseases, enhancing probability of technical and regualory success of clinical trials. Specifically, the platform identifies targets with broad associations, facilitating multi-indication drug development. We applied SGDL to explore new indications for IL6-targeted therapies, focusing on IL6, IL6ST, and IL6R genes. Through Mendelian randomization and colocalization analysis, combining disease GWAS and eQTL data from multiple tissues and immune cell types, we discovered causal links between IL6 expression in CD16 monocytes and various cardiovascular diseases, contrasting with decreased allergic asthma risk. Similarly, IL6R and IL6ST expressions were associated with distinct risks across cardiovascular, respiratory, and autoimmune diseases. Notably, IL6ST expression in T cells and whole blood was strongly linked to both rheumatoid arthritis and polymyalgia rheumatica. Our findings not only reaffirmed known associations of the IL-6R pathway with cardiovascular and immune diseases but also revealed a new link between IL6ST and polymyalgia rheumatica. This discovery is supported by the recent US approval of Sarilumab, an IL-6R inhibitor, for treating polymyalgia rheumatica. Overall, SGDL, utilizing multiomics data, machine intelligence, and large language models-based technologies, proves to be useful to deepen understanding of immune target biology.

Tu149. Single-cell CRISPRi Screen in Human Macrophages Identifies Immune Dysregulation in Crohn's Disease

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Icahn School of Medicine at Mount Sinai

Common and rare genetic variant Crohn's alleles are enriched in loci containing genes expressed in myeloid cells, with many high effect genes being loss-of-function, notably NOD2. We employed CRISPRi screens and single-cell transcriptomics (Perturb-seq) to assess 41 IBD candidate genes, within 500kb of a Crohn's- or IBD-associated SNP and 11 genes with known macrophage differentiation (such as CSF1R, SPI1 and PPARA). Following CRISPRi transduction, cells were cultured under hypoxia for 7 days with MDP, the NOD2-specific peptidoglycan agonist. Single cell sequencing demonstrated a median knockdown efficiency of 73% (median of 148 cells) per target. Among most differentially expressed genes of the integrated library, we observed two core, correlated perturbation clusters. The larger cluster contained numerous genes involved in MDP response, notably, NOD2, SNX20 (adjacent to NOD2), and RIPK2; the smaller cluster included PAF1, CSF1R, PPARA, CEBPB and ATG16L1. Complementary analysis using non-negative matrix factorization to dissect cell type from state confirmed the two core perturbation patterns. PPARA-targeted cells demonstrated downregulation of CSF1R transcript and the PI3K/Akt pathway. Some targets markedly altered mitochondrial expression and validation of three genes, STK11, CEBPB, and NRBP1, that upregulated the mitochondrial genes expression in our screen was performed by CRISPR knockout. The STK11 and NRBP1 knockouts both decreased mitochondrial ROS and favored glycolysis; CEBPB also decreased mitochondrial ROS, and both CEBPB and

NRBP1 decreased mitochondrial mass. Given the central role of macrophage differentiation and cell state in gut hypoxia and with chronic microbial stimulation, these Perturb-Seq studies highlight mitochondrial function in Crohn's pathogenesis.

Tu150. The Type 1 Diabetes-associated Risk Variant of BACH2 Alters T Cell Receptor Clonal Diversity in CD4$^+$ Regulatory T Cells and Drives a CD8$^+$ Effector T Cell Phenotype in Pancreatic Lymph Nodes
Leeana Peters, Richard Musca, Travis Hill, Melanie Shapiro, Maigan Brusko, and Todd Brusko
University of Florida

The transcription factor BACH2 contains a variant conferring risk for Type 1 Diabetes (T1D; rs729298038G>A; OR=1.18; MAF=0.18) and previously linked to reduced expression in naïve human T cells. We sought to examine the impact of BACH2 genotype on tissue phenotypes and T cell receptor (TCR) repertoire from organ donors derived from the Network for Pancreatic Organ donors with Diabetes (nPOD) program. We employed mass cytometry (n=12 control (mean age 16.9; 33% female), n=10 T1D, mean age 19.3, 30% female) and flow cytometry as a validation method (n=18 control, n=6 T1D) to examine immune phenotypes from pancreatic lymph nodes (pLN). We utilized a custom (985,971 loci) Affymetrix array for genotyping samples. Lastly, we assessed TCR (TRB) repertoire clonality by comparing Shannon entropy. We observed that heterozygous (AG) donors possessed more CD8$^+$ T cells co-expressing effector T cell associated chemokine receptors CXCR3 and CXCR5 as compared to homozygous protective (GG) donors (p=0.0165). Moreover, pseudotime analysis of mass cytometry data indicated AG subjects possessed higher mean CD8$^+$ T cell pseudotime, indicative of more terminally differentiated effector T cells (p=0.0362). Accordingly, TRB repertoire analysis revealed increased clonality in sorted pLN CD8$^+$ T cells (p=0.0340) and Tregs (p< 0.0001) from AG donors. These data support a role for the T1D-associated risk allele of BACH2 in controlling CD8$^+$ T cell and Treg immunophenotypes. These findings provide novel insights on how genetic risk variants alter immune regulation in the context of T1D and may offer novel opportunities for therapeutic targeting of this pathway to restore immune tolerance.

Tu151. Towards Complete Haplotype Resolution: A Novel Approach for Characterizing the Immunoglobulin Heavy Chain Locus
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Immunoglobulins play a pivotal role in adaptive immune responses, and understanding their biology is crucial for unraveling mechanisms underlying infectious diseases, vaccine development, and autoimmune disorders. Despite this, the immunoglobulin heavy chain (IGH) locus has only been fully resolved in a limited number of individuals. Here, we present a novel approach utilizing Oxford Nanopore Technology (ONT) sequencing with adaptive sampling (AS) for accomplishing haplotype-resolved IGH loci. To overcome the many complexities of the locus, we employ ultra-long sequencing to span problematic regions and large structural variants. High molecular weight gDNA from blood mononuclear cells is sequenced and enriched for the IGH locus with AS on the ONT PromethION platform, generating reads with an N50 > 60 kilobases. This enables us to bioinformatically separate the parental origin of the reads into two separate haplotypes, typically generating a single contig per haplotype with 1-3 phase blocks. We validate this method by comparing to a well-established framework for IGH characterization, using SMRT sequencing and IGenotyper. We see comparable accuracy and enhanced contiguity using our approach on the same donors. Preliminary analysis uncovers genetic variation throughout the IGH locus, previously unreported or incompletely described. This includes a notable 150 kb segmental duplication encompassing five constant genes. Coupled with antibody repertoire sequencing, haplotype-resolved

germline data enables us to investigate recombination sites, intergenic regions, and promoters of IGH and how they might affect the adaptive immune response. This novel approach has the potential to significantly advance our understanding of the genetic underpinnings of immune response variability.

W147. Estimated Prevalence and Clinical Manifestations of TNFAIP3 Variants Associated with Haploinsufficiency of A20 (HA20) in the United States

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TNFAIP3 encodes the regulatory protein A20, which is a potent inhibitor of multiple inflammatory signaling pathways. High-penetrance heterozygous germline mutations in TNFAIP3 cause early-onset immune dysregulation, recently described as the rare monogenic disease Haploinsufficiency of A20 (HA20). The prevalence of HA20 in the United States is likely underreported and limited by ascertainment biases. To determine the prevalence of pathogenic TNFAIP3 variants and associated clinical phenotypes, we used data from the All of Us Research Program, a multidisciplinary consortium that integrates electronic health records (EHR) and whole genome sequencing (WGS). Of 244,845 WGS participants (mean age, 58.6 years; 51.3% White; 59.3% female), 475 individuals harbored 101 TNFAIP3 variants with definitive pathogenicity (n=11; frameshift, nonsense, or splice site) or predicted pathogenicity (n=90; missense; CADD-Phred ≥ 25; SIFT=deleterious; PolyPhen=probably damaging; MAF < 0.01%). 87 participants (mean age, 58.1 years; 62.1% White; 52.9% female) with 31 high probability pathogenic variants (CADD-Phred ≥ 30; SIFT=deleterious; PolyPhen=probably damaging; MAF < 0.01%) were identified. In this group, inflammatory phenotypes (94.9%) were present in individuals with linked EHR data (n=59), including rash (44.1%), gastrointestinal inflammation (62.7%), arthritis/arthralgia (71.2%), neurologic complications (57.6%), vascular complications (52.5%), cytopenia (39.0%), infection (57.6%), genitourinary complications (44.1%), autoimmunity (37.3%), and psychiatric diagnosis (49.2%). The mean prevalence of high probability pathogenic variants was 1:2782 (95% CI, 1:2258-1:3427, Wilson/Brown). The mean prevalence of definitively pathogenic variants was 1:14403 (95% CI, 1:8993-1:23067, Wilson/Brown). This suggests that 14,400-148,000 Americans suffer from A20-insufficient disease. Studies are ongoing to functionally validate the high probability pathogenic TNFAIP3 missense variants.

W148. Genome-wide Autoimmune Genetic Variant Perturbations Connect Genetic Risk to Primary T Cell Expression and Function

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~24 million people in the US have an autoimmune disease, but we still lack knowledge about how disease is caused and need more effective treatments. Genome-wide association studies have identified thousands of genetic associations with autoimmune diseases, but the genetic variants that cause each disease are mostly unknown due to tight linkage disequilibrium between causal and non-causal variants and because most associations fall within enigmatic non-coding regions. To better determine variants that cause disease, we employed three approaches in primary T cells. We tested ~18,000 autoimmune variants for allele-specific effects on cis-regulatory element (CRE) activity with massively parallel reporter assays (MPRAs), identifying ~500 with allele-specific activities. These variants are enriched ~50-fold for causal variants. We next tested ~1000 variants in putative CREs for their effects T cell function using CRISPR-interference screens. We found 42 variant CREs that modulate proliferation, including several that interact with the MYC promoter, and 37 variant CREs that alter IFNg production. To directly link variants to genes they regulate, we used a single cell CRISPRi screen. 15/23

targeted variant CREs had effects on nearby gene expression including a type 1 diabetes-associated variant CRE that promotes IL2RA expression but also repressed 4 other nearby genes including IL15RA. We also identified an inflammatory bowel disease variant CRE that controls PPP5C expression and effector and metabolic expression programs. Thus, using MPRA and CRISPRi screens in primary T cells, we prioritized likely causal variants and defined their effects, which will help define mechanisms and potentially targetable pathways for autoimmune diseases.

W149. Mechanistic Investigation of Human DPP9 Deficiency
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Inflammasomes serve as sensors of infection and cellular stress. Although studies of murine inflammasomes have shed key insights on their human counterpart, the regulation and components differ between mouse and human. For instance, the mouse genome contains homologs of human NLRP1 but not CARD8. However, studying human inflammasomes *in vivo* remains technically prohibitive. DPP9 is a negative regulator of human NLRP1 and CARD8, and patients with DPP9 deficiency exhibit pancytopenia and other immune-associated defects. However, Dpp9 deletion in conventional laboratory mouse strains do not recapitulate the loss of hematopoietic cells seen in patients, suggesting specie-specific functions. We study human DPP9 deficiency using a humanized mouse model called MISTRG6, which enables the development of human immune system after engraftment with primary human hematopoietic stem and progenitor cells (HSPCs). Using novel CRISPR editing approaches, we efficiently knock out Dpp9 in human HSPCs and found that DPP9 deficiency led to pancytopenia due to the loss of human stem cells *in vivo*. We then sought to identify the inflammasome involved and found that CARD8 activation induced loss of human HSPCs, since simultaneous Card8 and Dpp9 deletion rescued pancytopenia and HSPC loss. In contrast, NLRP1 was dispensable for Dpp9-induced pancytopenia. Unexpectedly, pharmacological and genetic inactivation of caspase-1 failed to rescue Dpp9-associated cytopenia, suggesting alternative regulatory mechanisms of CARD8 activation *in vivo*. Taken together, we identify a novel regulatory pathway of human hematopoiesis by a human-specific inflammasome, and find potential therapeutic targets for patients with DPP9 deficiency.

Immunology Across the Ages

W150. Age-associated Increase in Cytotoxic T Lymphocyte-associated Protein 4 (CTLA-4) Expression on Effector T Cells: Potential Implication in Autoimmunity and Immunosenescence
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Cytotoxic T lymphocyte-associated protein 4 (CTLA-4) is a crucial immune checkpoint inhibitor expressed on $CD4^+Foxp3^+CD25^+$ regulatory T cells (Tregs), essential for maintaining immune homeostasis. Interestingly, CTLA-4 is also expressed on $CD4^+$Foxp3-CD25- effector T cells (Teffs). We observed a significant reduction in CTLA-4 expressing Teffs in adult lupus (n=41), compared to age-matched healthy controls, suggesting a putative role of CTLA-4 on Teffs in maintaining immune homeostasis. We, therefore, hypothesise there is a natural age-related increase of CTLA-4 expression in Teffs to counteract the increased exposure to exogenous immune

stimuli. To achieve this, we re-mined the mass cytometry data from PBMCs from 193 healthy individuals (0 to 95 years old) from the Extended Polydimensional Immunome Characterization (EPIC) database.1 Batch effect correction, clustering, and annotation of cell subsets based on marker expression were done. Expectedly, the CTLA-4$^+$ naive Tregs correlated negatively with age (Spearman correlation, r = -0.6497, p< 0.0001) while memory Tregs correlated positively with age (r = 0.6609, p< 0.0001). Interestingly, both naive and memory CTLA-4$^+$Foxp3-CD25- Teffs increased with age (r = 0.3837, p< 0.0001; r = 0.4533, p< 0.0001 respectively). CTLA-4$^+$ Teffs significantly differed between the 0-6 and 6-12 versus 40-60 and 60-95 year-old age groups (Kruskal-Wallis test; Dunn's multiple comparison tests, p< 0.0001). Our results suggest that CTLA-4 expression on Teffs may be important for immune homeostasis by increasing the cellular activation threshold to stimuli. Conversely, its increase may contribute to immunosenescence, resulting in increased susceptibility to infection and reduced vaccination responses in the elderly. 1. Yeo JG et al. Nat Biotechnol. 2020 Jun;38(6):679-684.

W151. Analysis of T Cell Response Induced by a Fifth Dose of Inactivated SARS-CoV-2 Vaccines Based on the Omicron Variant or on a Trivalent Vaccine Containing the Ancestral, Delta and Omicron Variants by Multiparametric Flow Cytometry

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The COVID-19 pandemic pushed the rapid development of vaccines to protect the population from severe disease and death caused by SARS-CoV-2. We previously showed that four doses of CoronaVac®, a SARS-CoV-2 inactivated vaccine, induce the activation of Spike (S) specific CD4$^+$CD137$^+$OX40$^+$ T cells. Here, we studied the effect in CD4$^+$ and CD8$^+$ T cell activation in subjects vaccinated with a fifth dose of an inactivated vaccine based on the Omicron BA.1 variant or a trivalent vaccine based on the Wuhan, Delta and Omicron BA.1 variants. Peripheral blood mononuclear cells were collected from 6 subjects before and after immunization (28 days), and stimulated with a megapool (MPs) of peptides derived from the S protein of the ancestral virus (WT), the Delta and the Omicron (BA.1 and BA.2) variants. Activated CD3$^+$ T cells were visualized by Barnes-hut distributed Stochastic Neighbor Embedding (bh-SNE) and clustered through the EMGM algorithm. CD3$^+$ T cells were grouped in 24 clusters, where 3 clusters showed changes in their frequency after vaccination. C17 (activated fully differentiated CD4$^+$ memory T cells) increased its frequency following stimulation with WT-MP-S. C21 (CD8$^+$ central memory T cells) reduced its frequency when stimulated with WT-MP-S and BA.2-MP-S. Finally, C24 (effector CD8$^+$ memory T cells), decreased following stimulation with WT-MP-S. The cellular response induced by a fifth dose of SARS-CoV-2 vaccine is complex and not restricted to the induction of CD4$^+$CD137$^+$OX40$^+$ T cells. We speculate that additional functional markers will improve our ability to evaluate the effects of SARS-CoV-2 boosters in T cell function.

W152. Immune Alterations Typically Associated with Inflammaging Characterize Severe Infertile Men

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Infertile men are at high risk of premature development of non-malignant and malignant non-communicable diseases (NCDs). The association among male health status, immune asset, and infertility is still undefined. By multiparameter phenotyping and scRNAseq we delineated the immune status of relatively young (median age of 37 years) severe infertile men with either oligo-astheno-teratozoospermia (OAT) or idiopathic non-obstructive azoospermia (iNOA). Overall, infertile men are characterized by a pro-inflammatory environment in the semen and the peripheral blood defined by enrichment in neutrophils, inflammatory dendritic cells and soluble mediators, and reduction of lymphoid cells. These features are typically observed in healthy elderly men and are comprehensively defined as inflammaging, an unresolved systemic inflammation observed in aged individuals and associated with the emergence of several NCDs. Mechanisms leading to inflammaging include cellular senescence, oxidative stress, and immune cell dysregulation. Notably, scRNAseq of T cells from peripheral blood of OAT and iNOA men revealed an overall high rate of differentiated/effector cells with T cells from OAT patients displaying a signature of exhaustion whereas T cells from iNOA patients having a signature of senescence. Both features further sustain the hypothesis of an early aged immune system in infertile men and revealed a different inflammatory nature between OAT and iNOA men. This study provides first demonstration that, despite being overall young and clinically healthy individuals, infertile men present an immune profile typically associated with inflammaging, and that OAT and iNOA constitute two different diseases with the potential to develop different secondary infertility-associated NCDs.

W153. Lymphocyte Subpopulations in Korean Children

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Introduction: We aimed to determine the relative and absolute numbers of lymphocyte subpopulations in Korean children according to gender and age. Methods: We recruited patients with no evidence of any infectious, immunologic, or hematologic disorders from 3 hospitals, grouped into two groups according to age: group 1 (5 – 9 years) and group 2 (10 – 17 years). The lymphocyte subpopulations were analyzed by flow cytometry with the EuroFlow Primary ImmunoDeficiency Orientation Tube. Lymphocytes were divided into three major groups: T cells, B cells, and NK cells. T cells were divided into $CD4^+$ T cells, $CD8^+$ T cells, and CD4-CD8- T cells and further subdivided according to the maturation stage into naïve, CM/TM, EM, and TD T cells. B cells were divided into GC cells, unswitched and immunoglobulin-switched memory B cells, and plasma cells. Results: Total lymphocyte count and B cells, especially pre-GC B cells, in group 1 were higher than those in group 2. On the other hand, the memory $CD4^+$ T cells (CM/TM and EM) increased in group 2. The higher B cells and $CD8^+CD27^-$ TD T cells in males were observed as those in females. Inter-laboratory differences were found in most subpopulations of T cells and B cells. Conclusion: We provided the relative and absolute numbers of lymphocyte subpopulations in Korean children, which could be used as reference ranges to monitor and predict the immune status in the pediatric population. Age, gender, and laboratory-related factors might influence the distribution of lymphocyte subpopulations.

W154. Transcriptional Dynamics in Peripheral Immune Cells Across Healthy Aging

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Immune aging is a dynamic process driven by complex internal and external interactions. Here, we sought to untangle the complexity of the healthy human immune system across age using deep molecular profiling. We first built a peripheral immune cell reference characterizing more than 1.8 million peripheral blood mononuclear cells (PBMCs) across 108 healthy people (age range:11-65 years) using scRNAseq, identifying a total of 71 distinct cell subsets. We then applied this reference to study the transcriptional dynamics of immune cells in our longitudinal aging cohort of healthy young (n=47, 25-35yrs) and older (n=45, 55-65yrs) adults followed over the course of 2-3 years (final dataset ~20 million PBMCs). We observed that T cells exhibit substantially more age-related changes in transcription than other immune cell subsets, suggesting that they may reveal clues to understanding the molecular basis of immune age. Age-related transcriptional changes in T cell subsets were closely associated with T cell differentiation state (i.e., naïve T cells> central memory>>>effector memory) but were not uniformly associated with age-related compositional shifts, were distinct from CMV-related expression changes and were stably maintained over the course of more than one year. Moreover, an age-related accumulation of GZMK$^+$ effector memory CD8 T cells was linked with increased GATA3 expression in the naïve compartment of older adults and with upstream STAT6 signaling – suggesting that gradual, age-related alterations of transcriptional networks in T cells may skew their differentiation trajectories and in turn, lead to shifts in the compositional landscape of immune cells as we age.

Immunometabolism

Th147. The CD43 Sialomucin Promotes the Expression of GLUT-1 on the Membrane of T Cells

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CD43 is a type I transmembrane sialomucin abundantly expressed in T cells. It has been implicated in thymocyte selection and maturation and the migration, adhesion, and activation of mature T cells. We hypothesized that its long and rigid structure positions CD43 as one of the initial molecules conveying environmental cues essential for complete T-cell activation. Specifically, we asked whether CD43-mediated signals participate in the metabolic adaptations that T cells undergo in response to activation signals, where aerobic glycolysis is essential. To this end, we evaluated the expression levels of the glucose transporter Glut-1 by flow cytometry, immunofluorescence, and qPCR in normal human peripheral blood CD4$^+$ T cells, and Jurkat cells following activation with TCR/CD43, TCR/CD28, CD43, or CD28. In CD4$^+$ T cells, GLUT-1 membrane expression increased ~10-fold in response to TCR/CD43 stimulation, surpassing that of TCR/CD28, while the single stimulation with CD43 or CD28 failed to increase the membrane GLUT-1 levels over basal values. Conversely, Jurkat cells exhibited an increase in both total and membrane GLUT-1 expression with the CD43 or CD28 single stimuli at 48h but not with the TCR/CD43 or TCR/CD28 stimuli. These results prompted us to explore whether CD43 expression correlates with Glut-1 expression in cancer. The GEPIA2 platform revealed 8 different types of cancer with CD43 overexpression and a positive correlation between CD43 and Glut-1 in acute myeloid

leukemia. Our data uncovers a role for CD43-dependent signaling in promoting Glut-1 expression in both normal T lymphocytes and lymphoid tumor cells, expanding the functions of this molecule.

Th148. The Impact of Cognitive Behavioral and Mindfulness Intervention on Gut-immune-brain Interactions in Crohn's Disease

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Crohn's disease (CD) involves chronic stress and inflammation along with microbiome and metabolic changes, reflecting the potential role of the gut-immune-brain axis in the disease process. Our research showed that a 3-month trial (NCT05085925) of cognitive behavioral and mindfulness daily exercise (COBMINDEX) intervention improves the well-being of CD patients, associated with changes in systemic inflammation and microbiome profiles. We hypothesize that COBMINDEX rebalances the microbial composition and immune regulation. CD patients were analyzed for distress, well-being, microbiome, and immune profiles at recruitment and 3 months post-intervention. Samples were categorized into Top Responders (TR) and Poor Responders (PR) based on Harvey Bradshaw Index (HBI) and psychological surveys, compared to healthy controls (HC). PBMCs were analyzed by flow cytometry for CD4 T-cell subsets. Serum samples underwent metabolomics analysis using Liquid Chromatography Mass Spectrometry. We demonstrate that frequencies of exhausted, effector memory, and regulatory CD4 T cells were significantly increased in the PR as compared to the TR group, along with a reduction in the frequency of naïve CD4 T cells. Metabolite profiling of serum samples showed distinct patterns between TR and PR individuals such as the PR group had higher levels of the secondary bile acids Tauroursodeoxycholic Acid and Fructoselysine. Overall, both the landscape of CD4 T-cells and the metabolite profile in the TR group exhibited a greater similarity to the HC group. These observations highlight the potential of COBMINDEX to alleviate the pathology of CD via microbiome-derived metabolites that can impact the inflammatory properties of T cells.

Tu152. Associations of Senescence and Mitochondrial Function in T Cells: Flow Cytometry Panel Design and Outcomes

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Introduction: Cellular senescence, an irreversible growth arrest, plays a crucial role in aging and related diseases. In T cells, senescence leads to functional decline, contributing to immune senescence. This study aims to explore the links between senescence and mitochondrial function in T cells to better understand immune aging. We designed a flow cytometry panel to investigate these associations. Methods: Senescence was characterized in peripheral blood from >65-year-old individuals (n=9) using flow cytometry. Our panel assessed senescence, activation, cellular stress and DNA damage markers. Additionally, we evaluated Senescence-Associated β-Galactosidase (SA-β-gal), mitochondrial mass, membrane potential, and Reactive Oxygen Species (DCFDA) to assess immune cell functionality and metabolism. Results: A reverse correlation was found between $DCFDA^+$ and $SA\text{-}\beta\text{-}Gal^+$ populations in Non-Treg cells ($r = -0.78$, $p = 0.01$), indicating an interplay between oxidative stress and senescence. In $CD4^+$ populations, $CD28^+$ cells inversely correlated with Mitotracker ($r = -0.85$, $p = 0.003$), suggesting a link between CD28 expression, mitochondrial mass, and T cell function. Furthermore, H2AX, a marker for mitochondrial damage, inversely associated with TMRE in $CD4^+$ cells, indicating a potential link between DNA damage and mitochondrial function. Discussion: These findings shed light on regulatory mechanisms governing senescence and mitochondrial dynamics in T cell subsets. Understanding these associations may lead to targeted interventions to mitigate immune aging and enhance

immune function, particularly in elderly patients. This flow cytometry panel offers potential for assessing senescence in various disorders.

Tu153. ATP FLOW: A Rapid Method for the Analysis of Cell Metabolism in Live Cells Using Flow Cytometry

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T cell activation and differentiation depends on dynamic changes in essential metabolic pathways such as glycolysis, oxidative phosphorylation and fatty acid metabolism. A deeper understanding of the metabolic regulation of immune cell function is needed for the improvement of immunotherapy approaches. Methods are needed to interrogate metabolic pathways at single-cell resolution within a heterogeneous population of rare cell sub-types. Puromycin incorporation into nascent polypeptides has been used for metabolic profiling by flow cytometry (Scenith method [1]). However, this method is time consuming, requiring cell fixation and permeabilization. Additionally, this method does not provide a direct measurement of available cellular ATP. Puromycin can stimulate polypeptide synthesis [2] and prevent insulin-stimulated increase of glycolysis [3]. Puromycin labeling is not reliable across cell types and does not accurately measure translation rates in energetically challenged cells [4]. We have developed a direct and simplified approach to measure ATP levels in live cells by flow cytometry, allowing for the discrimination of rare sub-populations while interrogating important cellular metabolic pathways, such as glycolysis and oxidative phosphorylation. This method is rapid – results can be obtained in < 1hour (compared to > 2 hours with puromycin) and yields an accurate measurement of cellular ATP production and its dependency on metabolic pathways of interest. Using primary human and murine leukocytes, we show that this method accurately depicts the metabolic changes occurring during T cell activation, differentiation and memory formation. This method will facilitate research of cellular metabolic pathways in many cell types across species and fields of study.

Tu154. Evidence for a Role of Pro-regenerative Macrophages in Enhancing Beta Cell Survival, Proliferation, and Function

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Type 1 diabetes (T1D) is a chronic condition arising from a loss of immune tolerance to insulin-producing beta cells, leading to immune cell-mediated beta cell destruction. Our previous research revealed that in diabetic mice islet-resident macrophages shift towards a tissue-regenerating phenotype, characterized by reduced expression of inflammatory cytokines compared to healthy mice. This observation led us to hypothesize that macrophages with a tissue-regenerating phenotype could play a role in promoting beta cell survival and regeneration. We polarized bone marrow-derived macrophages (BMDMs) from mice into a pro-regenerative (M2-like) phenotype through stimulation with IL-4 and IL-13. Co-culture of 100 Bl/6 mouse islets (intact or dispersed) with 100,000 M2-like macrophages for 6 days through either direct or indirect co-culture systems led to a significant (25%, $p< 0.05$) increase in beta cell survival, as measured by CytoCalcien fluorescence intensity. Additionally, direct co-culture of dispersed or intact mouse islets with M2-like macrophages for 72-hours resulted in elevated beta cell proliferation (range 0.08 to 2.83%), assessed through flow cytometry measuring EdU^+ cells within the insulin+ cell gate. Beta cell function, assessed by glucose-stimulated insulin secretion, also improved (10.7 pM vs 44.8 pM, $p< 0.05$) following both direct and indirect co-culture of intact mouse islets with M2-like macrophages for 72-hours. These findings suggest that pro-regenerative (M2-like) macrophages enhance beta cell survival,

proliferation and function through secreted factors. Future studies aim to better understand the mechanistic basis for these effects and explore macrophage-mediated islet regeneration using *in vivo* transplantation models.

W155. Immune Alterations in Patients with Congenital Urea Cycle Disorders

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Urea Cycle Disorders (UCD) comprise a group of metabolopathies sharing similar clinical phenotypes, in which acute hyperammonemia (AH) crises occur often. Interestingly, intercurrent infections are the most frequent and dangerous precipitant of AH and metabolic decompensation. Although concurrent, it is currently unknown whether infections are associated with immunological alterations in these patients. Thus, we herein analyzed different phenotypic and functional immune function parameters in UCD patients and healthy controls. *In vitro* lymphoproliferation against different polyclonal and memory recall cell antigens, T helper cell subsets, cytokine secretion in culture supernatants, total immunoglobulin serum levels, and the glycosylation status of serum immunoglobulins, were analyzed. Similar lymphoproliferative responses to either polyclonal or memory recall cell antigens were observed in patients with respect to controls. However, significantly reduced counts of Th17 and Th1 cells and decreased IL17 and IFNɣ secretion levels were observed in patients with respect to controls. Moreover, patients revealed significantly higher proportions of terminally differentiated effector memory (TEMRA) CD4 and CD8 T cells. Besides, a state of marked hypogammaglobulinemia associated with significantly reduced frequencies of plasmablasts and memory B cells was detected in UCD patients. Moreover, serum immunoglobulins displayed a strikingly altered glycosylation pattern. These data show that UCD patients present alterations in different biomarkers of immune function, suggesting a state of immunocompromise that would renders patients more susceptible to infections. The latter would further aggravate the HA status increasing the morbidity/mortality risk.

W156. Over Expression of GATA2 in Atherogenic Macrophage Induce Development of Atherosclerosis

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The loss of efferocytic activity – the phagocytic clearance of apoptotic cells – in cardiac macrophages is required for the accumulation of necrotic cells that causes atherosclerotic plaque. Our lab demonstrated that defects in macrophage efferocytosis occur by over-expression of hematopoietic transcription factor GATA2. In this study, we investigated the pathways that regulate GATA2 expression in macrophages and identified the GATA2-regulated genes that promote atherogenesis. We found higher GATA2 expression in the circulating monocytes of ~50% of atherosclerosis patients. However, there was no correlation between monocytic GATA2 expression, plasma cytokines, monocyte intravascular recruitment, or monocyte proliferation. However, PDGF-AB/BB was elevated in some patients, and *in vitro*, this induced GATA2 expression in macrophages. There was no correlation between monocyte GATA2 expression and plaque macrophage GATA2 expression, with GATA2-expressing macrophages identified in the plaques of all patients. In the plaque, GATA2 drives the proliferation of macrophages and reduces their sensitivity to apoptosis. Although GATA2 can be expressed from two promoters, we determined that only the internal (IG) promoter is used by macrophages. We also determined that atherogenic stimuli induces GATA2 expression from the IG promoter via NF-κB, Ahr, and STAT1, while AP-1 negatively regulates GATA2 expression. ChIP-seq identified the GATA2 transcriptome, which include MIR12136, a microRNA that negatively regulates a plethora of efferocytic receptors, apoptosis-regulatory proteins, and proteins required for the degradation of engulfed apoptotic cells. This study provides further insights into the

mechanism by which GATA2 impairs efferocytosis during atherosclerosis and may identify future therapeutic targets for the treatment of atherosclerosis.

W157. Oxidative Stress is a Shared Characteristic of ME/CFS and Long COVID

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More than 65 million individuals worldwide are estimated to have Long COVID (LC), a complex multisystemic condition, wherein patients of all ages report fatigue, post-exertional malaise, and other symptoms resembling myalgic encephalomyelitis / chronic fatigue syndrome (ME/CFS). With no current treatments or reliable diagnostic markers, there is an urgent need to define the molecular underpinnings of these conditions. By studying bioenergetic characteristics of peripheral blood lymphocytes in over 16 healthy controls, 15 ME/CFS, and 15 LC donors, we find both ME/CFS and LC donors exhibit signs of elevated oxidative stress, relative to healthy controls, especially in the memory subset. Using a combination of flow cytometry, bulk RNA-seq analysis, mass spectrometry, and systems chemistry analysis, we observed gender-specific aberrations in ROS clearance and damage pathways. While ME/CFS females exhibit higher total ROS and mitochondrial calcium levels, ME/CFS males have normal ROS levels, but larger changes in lipid oxidative damage. The higher ROS levels correlate with hyperproliferation of T cells and can be attenuated by metformin, suggesting this FDA-approved drug may be an effective treatment. Thus, we report that both ME/CFS and LC are mechanistically related and can be diagnosed with quantitative blood cell measurements. We also suggest that effective, patient tailored drugs might be discovered using standard lymphocyte stimulation assays.

Immuno-oncology

Th149. Aire-expressing Tumor-associated Macrophages Promote Cancer Immune Evasion

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Peripheral immune populations expressing the Autoimmune Regulator (Aire) gene have been suggested to play critical roles in immune self-education, maternal-fetal tolerance, and commensal tolerance in the gut, but their roles have not been defined in the tumor microenvironment (TME). TMEs are populated by highly heterogenous myeloid populations with either tolerogenic or anti-tumor properties. Understanding the immunosuppressive programs that promote tumor tolerance in myeloid populations could lead to new therapeutic targeting strategies. Here we demonstrate the presence of Aire-expressing macrophages (aTAMs) across multiple mouse tumor models (MC38 and B16F10) and characterize them as an immunosuppressive, tumor-promoting macrophage subtype. These aTAMs display a unique transcriptional and metabolic profile characterized by increased immunosuppressive and proliferative features. The Aire gene is central to the formation of these aTAMs, as Aire-deficient mice exhibit a decrease in the aTAM population and in the gene signature that characterizes this cell population. Ablation of aTAMs (Aire-DTR) improves tumor control across multiple immunotherapy-resistant tumor models in a CD8 T cell-dependent manner resulting in increased CD8 T cell activation and inflammatory remodeling of the myeloid compartment. Notably, peripheral loss of Aire (Vav1-Cre x Airefl/fl) or aTAM ablation (Aire-DTR) synergizes with anti-PD-1 to improve anti-tumor immunity demonstrating a previously undiscovered role of extrathymic Aire. Furthermore, aTAMs can be found in human scRNA-seq tumor datasets across multiple

tumor types and are associated with poor prognosis. Our data identify a novel macrophage population and demonstrate that extrathymic Aire in this phenotypically and functionally distinct population plays a critical role in inhibiting anti-tumor immunity.

Th150. Exclusion of PD-1 from the Immune Synapse: A Novel Strategy to Modulate T Cell Function

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Targeting immune checkpoint receptors on T cells is a common cancer treatment strategy. Frequently, this is accomplished through antibodies targeting the ligand of inhibitory co-receptors. Blocking the immune checkpoint PD-1 binding to its ligands PD-L1 and PD-L2 prevents downstream signaling and enhances anti-tumor T cell responses. This approach improved cancer patients' outcome. However, only one-third of the patients respond to these treatments. To better understand the mechanism of anti-PD-1 antibodies, we explored the location of PD-1 within the immune synapse. Surprisingly, we discovered that anti-PD-1 antibodies, besides blocking the interaction between PD-1 and its ligands, also removed PD-1 from the synapse. We demonstrated a correlation between removing PD-1 from the synapse by anti-PD-1 antibodies and the extent of T cell activation. Interestingly, a short version of the anti-PD-1 antibody, F(ab')2, failed to remove PD-1 from the synapse and activate T cells. Using syngeneic tumor model, we showed a superior anti-tumor effect to anti-PD-1 antibody over the shorter version of the antibody. Our data indicates that anti-PD-1 antibodies activate T cells by removing PD-1 away from the synapse and changing the location of PD-1 or other immune receptors within immune synapse could serve as an alternative, efficient approach to treat cancer.

Th151. Helios Limits CD8 T Cell Antitumor Capacity

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Helios (Ikzf2) is a transcription factor associated with suppressive function in thymic-derived CD4 regulatory T cells. Previously, we documented Helios expression in CD8 cells exposed to antigen presented as self or associated with malignant cells. Here, we analyzed whether Helios is induced in tumor infiltrating CD8 T cells and determined the effects of its absence. Helios expression was documented in CD8 T cells infiltrating tumors from patients with colorectal cancer. It showed a statistically significant correlation with tumor stage. In mice with B16 melanoma, Helios upregulation was observed in tumor infiltrating CD8 T cells. Cells from tumor draining lymph nodes remained Helios negative. Helios was induced by antigen activation, as it was not observed in OT-I cells from tumors devoid of ovalbumin. Helios deficiency in T cells (Cd4-Cre) and in CD8 T cells (E8III-Cre) decreased melanoma and colon adenocarcinoma growth in mice. This was associated with decreased abundance of terminally exhausted CD8 T cells and increased frequency of TCF-1$^+$ cells. We generated Pdcd1 and Ikzf2 double KO mice (dKO). dKO mice, controlled tumor growth better than mice with isolated deficiencies. Tumors did not grow in a large fraction of dKO mice. Tumor infiltrating CD8 T cells from dKO mice produced higher levels of IFN-g and granzyme B. These results indicate that Helios is induced in CD8 T cells by the tumor microenvironment and that its presence curbs the anti-tumor capacity of CD8 T cells.

Th152. HIV-associated Lung Cancer Exhibits an Immunoregulatory Tumor Microenvironment

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Non-small cell lung carcinoma (NSCLC) is a leading cause of mortality among people with HIV (PWH). We have previously found that circulating markers of T cell dysfunction persist among PWH subsequently diagnosed with cancer, but the role of immune dysfunction within the tumor microenvironment (TME) has not been explored. Using a matched cohort of NSCLC tissue from PWH (n=18) and people without HIV (PWOH) (n=19), a tissue microarray was prepared and imaging mass cytometry was used to quantify expression of cellular markers while maintaining spatial integrity. Linear mixed effects model and AI-based pageRank mathematical algorithm based on spectral graph theory were used to quantitate differences in findings. $CD8^+$ T cells from the TME of PWH demonstrated distinctly different immune profiles compared to PWOH. Notably, $CD8^+$ T cells from PWH showed enriched representation of subsets with elevated expression of PD-1 and Lag-3, as well as activation and proliferation markers (57% of all $CD8^+$ T cells in PWH compared to 27% in PWOH, $P< 0.001$). Among tumors from PWH, tumor-associated macrophages revealed increased expression of immunoregulatory molecules (PD-L1, PD-L2, B7-H3, B7-H4, IDO1 and VISTA; 59% of TAMs in PWH compared to 18% among PWOH, $p< 0.001$). Using spectral graph theory to confirm imaging mass cytometry data, HIV^+ vs HIV^- tumors could be discriminated with 84.6% accuracy. In conclusion, we show that that the TME from PWH demonstrate an altered and unique immune landscape, with evidence of T cells and macrophages with immunoregulatory phenotypes and evidence of exhaustion, which may portend altered outcomes to immunomodulatory therapies.

Th153. Peripheral Blood as a Source to Identify Candidate Neoantigens and Reactive TCRs

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Adoptive transfer of neoantigen-specific tumor infiltrating lymphocytes has demonstrated antitumor activity in selected patients. So far, the development of T-cell therapies relies on the obtention of a tumor biopsy to isolate neoantigen-reactive T cells and to extract tumor DNA and RNA to identify neoantigens. Nevertheless, the obtention of a tumor biopsy is often not feasible and it may underrepresent the tumor clonal diversity of the tumor. Last year, we showed that circulating neoantigen-specific CD8 and CD4 T cells could recognize neoantigens derived from non-synonymous mutations detected in circulating tumoral cell free DNA (Garcia-Garijo A et al., FOCIS 23). Moreover, they were preferentially enriched in the $PD\text{-}1^{hi}$ T and the PD1hi cells co-expressing CD39. To further characterize neoantigen-reactive $CD8^+$ with the above phenotypes, we sequenced the TCRs of the corresponding neoantigen reactive lymphocyte from one metastatic breast cancer patient. The most frequent alpha-beta TCR pair were shared by both populations; this TCR and some additional alpha-beta pairs were cloned and transduced into PBMCs. All these evaluated TCRs recognized neoantigen KIAA1524p.S57I but not the wild type peptide. The same procedure was applied to the $CD4^+$ T-cell populations identifying two additional different neoantigens. These results confirmed that the different enriched clonotypes were neoantigen-specific and highlight the polyclonal nature of both $PD\text{-}1^{hi}$ and $PD\text{-}1^{hi}CD39^+$ KIAA1524p.S57I-reactive populations from PBL. In conclusion, by sorting different T cell subsets from peripheral blood, we were able to detect eight different TCRs targeting three neoantigens that were originally identified using plasma-derived cell free DNA.

Th154. Pre-existing Skin-resident CD8 and γδ T Cell Circuits Mediate Immune Response in Merkel Cell Carcinoma and Predict Immunotherapy Efficacy

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Merkel cell carcinoma (MCC) is an uncommon, aggressive neuroendocrine skin carcinoma with two etiological subtypes. Polyomavirus-driven MCCs harbor public viral antigens, but few, if any, private somatic mutations, whereas UV-induced virus-negative MCCs harbor a nearly uniformly high tumor mutation burden. Both subtypes are immunogenic, with response rates equivalent to those of immune checkpoint blockade (ICB); thus, MCC is a powerful and unique human model for tumor immunotherapy. To identify biomarkers that predict response, we integrated bulk (n=65 patients) and single-cell RNA-seq (302,597 cells from 59 patients) and spatial transcriptomics (n=20 patients) from a cohort of 110 patients. Our dataset highlights the importance of pre-existing immune cells and cytokines in the MCC tumor microenvironment (TME). In non-responders, MCCs show evidence of increased tumor proliferation, which correlates with lower immune infiltration and increased IL-1. In responders, tumors have increased levels of 1) type I/II interferons, 2) tissue resident (Trm) CD8+ T cells, or Vδ1 γδ T cells in MHC class I low tumors. Multi-modal scRNA-seq analyses showed that $CD8^+$ Trm and Vδ1 γδ cells functionally converge to generate antitumor immunity by acquiring overlapping antigen-specific transcriptional programs and clonal expansion of public TCRs that putatively recognize tumor antigens. Spatial transcriptomics demonstrated significant co-localization of T cells with B and dendritic cells, which supply chemokines and co-stimulation. Finally, in a subset of patients, Trm cells are clonally expanded after ICB, highlighting their therapeutic importance. Collectively, our unique dataset identified potentially clinically actionable cytokine circuits in human cancers.

Th155. Role of Cell Adhesion Receptors in Recruitment and Functional Plasticity of Tumor Associated Macrophages

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Tumor-associated macrophages (TAMs) drive the protumorigenic responses and facilitate tumor progression via matrix remodeling, angiogenesis, and immunosuppression. Leukocyte migration and their interaction with extracellular matrices via cell surface integrins are essential in the immunological responses. However, little is known about the expression dynamics of integrin cell adhesion receptors and their correlation with functional plasticity of macrophages in tumor microenvironments. Our present data show that in a 4T1 mouse mammary tumor model, at day 10, when palpable tumors are detected, $CD11b^+Ly6C^{hi}$ monocytes in both bone marrow and blood showed higher expression of integrin α5β1 along with CCR2 and CXCR4. At day 15, while higher expression of integrin α5β1 was found on tumor-infiltrating monocytes ($Ly6C^{hi}MHCII_{low}$) and M1-TAMs ($F4/80^+Ly6ClowMHCII^{hi}$), higher levels of integrin αvβ3 were observed on the M2-TAMs ($F4/80^+Ly6C_{low}MHCII_{low}$). Additionally, upregulated expression of integrin α5β1 and αvβ3 was shown in $CD206^{hi}$ and $MERTK^{hi}$ cells in different TAM subsets (M1 and M2) in tumors and lungs at day 30 post induction. Further analysis suggested that $CD11b^+Ly6G^-F4/80^+α5^+$ TAMs displayed increased levels of inflammatory cytokines and $CD11b^+Ly6G^-F4/80^+αv^+$ TAMs displayed a pro-tumorigenic phenotype with higher il-10, arg1, and tgf-β transcripts. Blocking of integrin α5 and αv during macrophage differentiation reduced expression of M2 marker (CD206 and CD163) and VEGF and

arg1transcripts. Bioinformatics analysis revealed the involvement of ptk2 (FAK) and SRC protein kinases in the functional polarization of TAMs in breast tumors. In conclusion, our data shows that signaling via integrin play an essential role in the recruitment, polarization, and tumor environment mediated reprograming of TAMs.

Th156. Secondary Inhibitory Signals on Human Kidney Microvascular Endothelium Confer Peripheral T Cell Tolerance: Insight from Checkpoint Inhibitor Toxicity

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The microvascular endothelium of normal human kidney expresses high levels of HLA-DR without inflammation. This surprised us given what is known of HLA class II antigen processing. Would it not be dangerous to have HLA-DR exposed to circulating protein debris that could be endocytosed and processed to antigens recognized by T cells? Using tissue cytometry and T-cell assays to investigate the role of HLA-DR and secondary inhibitory signals on human kidney microvascular endothelial cells (KMEC) we found: 1) Sensitized T cells are activated when peptide is presented by KMEC of the appropriate HLA-DR specificity. Blockade of CD58 or HLA-DR reduces T cell activation while blockade of CD274 (PD-L1) enhances activation. 2) T cell co-stimulatory and inhibitory molecules on native and transplanted kidneys are identified by flow cytometry. CD274 (PD-L1) expression is high on all KMECs and T cells within the kidney express CD279 (PD-1). Inhibitory molecules B7H3 (CD276) and B7H4 are also expressed on KMEC. 3) Biopsies of kidneys injured with PD-1/PDL-1 checkpoint inhibitors show an intense perivascular T-lymphocytic infiltrate. 4) Human fetal kidneys express endothelial HLA-DR and CD274 at a similar time in development. Taken together, we propose an endothelium-based mechanism of peripheral tolerance whereby constitutive high expression of CD274 (PDL-1) limits activation of sensitized T cells, even if HLA-DR bound peptide is recognized. This could inhibit autoreactive T cells that escape thymic deletion throughout the lifespan of humans. Findings in the kidney are likely applicable to other human organs which express HLA-DR on their microvascular endothelium.

Th157. Study of CD4$^+$ Th1, Th2, Th17 and Treg Cell Subpopulations in Myelotoxicity Related to Chemotherapy in Pediatric Patients with Acute Lymphoblastic Leukemia

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INTRODUCTION Acute lymphoblastic leukemia represents 25% of pediatric cancer. Myelotoxicities are frequently produced in low-middle-income countries due to the high doses of antineoplastics in chemotherapy, which may increase the risk of mortality. CD4+ cells coordinate response against cancer cells; evidence suggests that Th1, Th2, Th17, Treg, and their cytokines are essential in progressing and responding to treatment. For this reason, monitoring these cell populations could allow the evaluation of the presence and severity of myelotoxicities and survival in ALL. OBJECTIVES To determine the changes related to myelotoxicity in CD4$^+$ Th1, Th2, Th17 and Treg cells during the induction to remission phase in pediatric patients with ALL. METHODS We included ten pediatric patients with a recent diagnosis of ALL at Juan I. Menchaca Hospital, Civil of Guadalajara. Blood samples were collected at diagnostic consultation, during, and at the end of the RI. PBMC were separated, and staining was performed to identify the CD4$^+$ subpopulations, which were determined by flow cytometry. Complete blood count and the Common Terminology Criteria documented the evaluation of myelotoxicities. The Research and Ethics Committees approved the study protocol. RESULTS Patients who completed the IR phase of chemotherapy with mild and/or moderate myelotoxicities present an increase in the frequency of Th1, Th2, and Th17 cells, with a decrease in Treg. In contrast, patients with severe myelotoxicities

show an increase in the percentage of Treg and a reduction in Th1, Th2, and Th17 cells. CONCLUSION Treg cell population is related to severe myelotoxicities and could represent a therapeutic target.

Th158. The Novel Trem1 Ligand Regulate Trem1-mediated Tumor Immune Surveillance by Enhancing Antigen Presentation

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Tumor-associated macrophages (TAMs) can modify T cell activity through antigen presentation and cytokine secretion within the tumor microenvironment. M1-polarized macrophages can activate cytotoxic T cells and initiate tumor immune surveillance. Triggering receptor expressed on myeloid cells 1 (Trem1) is explicitly expressed on TAMs, and its signaling pathway enhances inflammatory responses and promotes macrophage M1 polarization. However, it is unclear how Trem1-mediated responses activate T cell responses and regulate tumor growth. Our recent research demonstrated that Trem1 plays a role in presenting tumor antigens to T cells, which helps eliminate tumors. We conducted investigations using Trem1 knockout (KO) mice and found that Trem1 has a significant anti-tumor function in the tumor microenvironment. Using the BioID assay, we discovered a new Trem1 ligand from necrotic tumor cells that can activate Trem1-mediated responses as an agonist. According to surface plasmon resonance, the binding affinity between the ligand and Trem1 is approximately KD=5.99E-8, indicating a high binding affinity. The mechanisms of the findings suggest that the novel Trem1 ligands promote Trem1-Dap12-mediated endocytosis, leading to the phosphorylation of Tyrosine-protein kinase (SYK) and enhancing tumor-antigen presentation, which in turn activates tumor-specific T cells. In addition, we generated a recombinant soluble form of the Trem1 ligand and observed its capacity to induce tumor elimination in a Trem1-dependent manner. The study shows the potential of using soluble Trem1 ligands for tumor therapy through a Trem1-mediated anti-tumor mechanism.

Th159. TMEM176B is a Druggable Intrinsic Driver of Protumoral Th17 Lymphocytes

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A potential role for Th17 lymphocytes in tumor immunity remains controversial. Intratumoral Th17 cells have been associated with good but also with bad prognosis in cancer and particularly in anti-PD-1 therapy. The dual role that Th17 cells seem to play in cancer can be explained by the great heterogeneity and/or plasticity of these cells. Developing a pharmacological strategy to modulate Th17 cells polarity is therefore highly relevant for cancer immunotherapy. Here, we unveil the immunoregulatory cation channel TMEM176B as a suppressor of anti-tumoral Th17 responses. Accordingly, inhibition of TMEM176B using BayK8644 triggers Th17 effector responses and reprograms subsets of exhausted $CD8^+$ T cells under PD-1/PD-L1 blockers in two different murine cancer models. Adoptive cell transfer of Th17 cells modulates subsets of exhausted $CD8^+$ T cells and improves the anti-tumor efficacy of PD-1 blockers. Mechanistically, intrinsic expression of TMEM176B drives a regulatory program within Th17 lymphocytes. Pharmacological inhibition of TMEM176B as well as its genetic deletion were associated with an effector phenotype of Th17 cells, increased phosphorylation of AKT, improved metabolic fitness and impaired immune-regulatory capacities *in vitro*. WT but not $Tmem176b^{-/-}$ Th17 cells were able to undermine terminal $CD8^+$ T cell exhaustion and mitochondrial fitness. In melanoma patients, Th17-related genes were positively correlated with stem-like exhausted $CD8^+$ T cells. At the molecular level, in silico docking studies suggest that BayK8644 blocks critical amino acids required for ion transport by TMEM176B. These

findings highlight the potential of TMEM176B inhibitors to modulate Th17 lymphocytes and thus enhance responses to PD-1/PD-L1 blockers.

Tu155. A Model for Acute Myeloid Leukemia Sensitivity and Resistance to T Cell Killing
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Acute myeloid leukemia (AML) is a malignant disease of the bone marrow characterized by abnormal proliferation and differentiation of myeloid progenitor cells. Although survival rates have increased with improved care and treatment, overall survival remains suboptimal. Lack of markers specific to AML but not normal myeloid cells limit development of targeted immunotherapies, and allo-hematopoietic stem cell transplantation has significant risks of morbidity and mortality. Our lab has developed an engineered polyclonal type 1 regulatory T (Tr1)-like cells, called CD4IL10, which kill most AML cells *in vitro* via perforin and granzyme B degranulation. However, some AML samples were resistant to CD4IL10 killing. Herein, we developed an *in vivo* model to study genes involved in AML sensitivity to CD4IL10 killing using gene edited U937 cells, a promyelocytic cell line used as a model for AML. To establish the model, NSG mice were engrafted with U937-WT-Luc+ cells, and on day 5, we injected CD4IL10 cells and monitored tumor progression. On day 8, we observed a reduction in tumor burden in CD4IL10-treated mice vs untreated mice. To investigate an effect of a specific gene on AML sensitivity to killing, we used CRISPR/Cas9 to knockout ICAM1 from U937-WT-Luc+ cells. ICAM1 is a critical component of the immune synapse required for T cell killing of tumor cells. In our *in vivo* model, tumor burden was significantly reduced in CD4IL-10-treated U937^{-}WT mice in comparison to U937^{-}ICAM1^{-}KO mice. The following studies validate our *in vivo* model to test mechanisms of AML sensitivity and resistance to T cell killing.

Tu156. Biolegend Kappa and Lambda Monoclonal Antibodies for Light Chain Analysis on B Cells: A Performance Evaluation
David PILLON, Derrick Lee and Micahel Li
BioLegend

The analysis of kappa and lambda surface light chains is a critical test for assessing the presence of a single, abnormal clone of cells (known as clonality) in blood cancer research. When designing an antibody panel for flow cytometry targeting kappa and lambda light chains, it is important to consider the specificity of the antibodies and the level of antigen expression in the populations of interest. Polyclonal antibodies have traditionally been used in designing antibody panels for the detection of light chains in B cell lymphoproliferative disorders research. These polyclonal reagents have become the gold standard in kappa/lambda detection to characterize B cell disorders, however, several monoclonal antibodies have also been developed in recent years. This could be an advantage for researchers in the field as monoclonal kappa and lambda antibodies offer higher manufacturing reproducibility while preserving high specificity and sensitivity. In this study, we compared the performance of BioLegend monoclonal kappa and lambda light chains conjugated antibodies with gold standard polyclonal kappa and lambda antibodies. Our findings indicate that BioLegend monoclonal antibodies exhibited significantly higher median fluorescence intensities and stain indices than the polyclonal antibodies. These results demonstrate a consistent and comparable performance of our monoclonal antibodies. Additionally, researchers could benefit from our wide conjugate offering and our capabilities to conjugate antibodies to a variety of suitable fluorophores for expanded flow cytometry panel selection to better understand the biology of blood cancers.

Tu157. Characterization of the Epichaperome in T Cells from Healthy Donors and Heme-malignancies Patients

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The epichaperome was defined as an integrated network of proteins in cancer cells which supports tumor-associated microenvironment and cancer cell survival. Previously, we reported the biochemical tools used to measure epichaperome abundance along with the correlation between epichaperome abundance and sensitivity to epichaperome inhibitor, PU-H71. PU-H71 inhibitor showed promise in single-patient trial (Sugita et al. 2021, NPJ Precis Oncol). The patient progressed to acute myeloid leukemia (AML) after failing allo-transplant, the patient harbored a novel PML-SYK fusion. After PU-H71 treatment, the patient achieved and sustained complete remission for over 5 years. We found changes in immune cells. This led to the hypothesis of epichaperome having a role in modulating immune response, also suggesting a function in healthy immune cells. Thus, we evaluated the epichaperome levels in T cells in patients with leukemia (AML or CLL) and healthy donors. At the baseline healthy T cells exhibit epichaperome levels with an average value of 1.7 (epichaperome probe over control). Epichaperome levels were consistent across CD4+ and CD8+ subpopulations of healthy T cells. In contrast, the epichaperome levels of T cells in a malignant state appeared to be aberrant. Our results showed significantly higher levels of epichaperome in T cells in patients with leukemia when compared with healthy individuals.

Tu158. Detecting and Dissecting Tissue Resident Memory T Cells as an Early Melanoma Biomarker

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Tissue-resident memory T cells (TRM) are enriched in nonlymphoid barrier organs where they typically reside long-term without recirculating. These cells are functionally highly specialized for their local environment and have been demonstrated to provide protective tissue immunosurveillance against infection and cancer. TRM exhibit a robust cytotoxic phenotype and have been found to play a vital role in promoting anti-tumor immunity across several cancer types. Tumor infiltrating TRM are also associated with favorable clinical outcomes. In contrast to the concept of strict tissue compartmentalization and retention, some recent evidence from both human and murine studies suggests TRM can re-enter the circulatory system as “ex-TRM.” Considering that ex-TRM appear to retain the phenotypic, functional and transcriptional signature (including TCR repertoire) of their parental population, we hypothesized that they can be leveraged as an early melanoma biomarker. Such a biomarker would be useful for early cancer detection as well as an analysis of the immunotherapeutic sensitivities of a given tumor. Using single cell RNA and TCR sequencing, we have profiled the immune landscape of stage 0-2 cutaneous melanomas and matching blood samples. Our data demonstrate the presence of TRM in these early tumors and points to the biomarker potential of these cells. In addition, our comprehensive transcriptomic analysis provides new insight into the evolution of the overall immune compartment in early primary melanomas.

Tu159. Engineering the Prostate Cancer Microenvironment: A 3D Bioprinted Model Using Dynamic Gelatin-based Hydrogel with Laponite® for Therapeutic Studies

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An *ex vivo* model for the prostate cancer tumor microenvironment (PCa-TME) remains elusive due to the disease's complexity and clinical heterogeneity. Recent advancements in *in vitro* models, such as organoid technology and 3D bioprinting, have shown promise, but there is a need for ideal bioinks that can support cellular interactions and accurately predict drug responses. This study aimed to develop a 3D bioprinted model of PCa-TME using engineered gelatin methacrylate (GelMA)-based hydrogels enhanced with the rheology modifier Laponite®. We hypothesized that tunable viscoelastic hydrogels mimic the dynamic properties of the extracellular matrix in PCa-TME to support paracrine signaling among normal, cancer, and immune cells. The viability, proliferation, and migration of cells using dynamic GelMA-based hydrogels were investigated using standard prostate cell lines such as WPMY-1, LNCaP, DU145, and PC3, along with two cancer-induced cell lines with chronic exposure to cadmium, i.e., WPMY-Cd and BPH-Cd. Results revealed that the dynamic GelMA-based hydrogels supported the desired cellular properties in 3D bioprinted mono- and co-cultures of normal and cancer cells. Bioprinted structures with dynamic GelMA bioinks had improved printability, shape fidelity retention, and interconnected porosity. The live/dead assay showed a significantly higher number of dead cells than live cells (p-value < 0.001) in the 3D bioprinted PCa-TME model after 5 days of treatment with a drug candidate, 1,4-dihydroxy-2-naphthoic acid (DHNA). The novel 3D-bioprinted PCa-TME model is currently being evaluated for macrophage polarization and transcriptome analysis for primary and secondary metastasis.

Tu160. Enhancing Tumor Apoptosis with the Combined Use of Pentoxifylline and Chemotherapeutic Agents in Hodgkin Lymphoma

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Hodgkin Lymphoma (HL) is a B-cell neoplasm predominantly treated with chemotherapy, notably doxorubicin (DOX) and bleomycin (BLM), which are associated with severe adverse effects. Moreover, the cure rate decreases to 75% in advanced-stage patients. Therefore, novel strategies are required to optimize treatment efficacy, minimize adverse effects, and enhance clinical outcomes. Pentoxifylline (PTX) functions as an inhibitor of the NF-κB pathway and enhances chemotherapeutic-induced apoptosis in various cancer cell lines by increasing the expression of proapoptotic genes. Consequently, we propose utilizing PTX as an adjuvant therapy to augment the antitumor effect of chemotherapeutics agents in the HL cell line Hs-445. To this end, we assessed apoptosis, cell cycle, senescence, mitochondrial membrane potential ($\Delta\Psi m$), and caspase activities through flow cytometry. We found that PTX induces a considerable cell death compared to DOX and BLM, particularly when administered in conjunction with BLM and triple therapy. This cell death primarily occurs via apoptosis, as evidenced by the upregulation of caspases-3, -8, and -9 activity, with a synergistic effect observed when combined with DOX, and BLM. Additionally, PTX induces a loss of $\Delta\Psi m$, with the most significant impact observed in combination with BLM or triple therapy. Furthermore, PTX abolishes DOX-induced cell arrest in G2, leading to an increase in the percentage of cells in G1. Notably, PTX does not induce senescence and reverses senescence induced by DOX, and BLM. In conclusion, PTX is a potent inducer of apoptosis in HL, augmenting the cytotoxic effects of DOX and BLM while reversing senescence induced by these agents.

Tu161. Flow Cytometry–based Functional Assay to Study Rituximab, Obinutuzumab and Blinatumomab Mechanisms of Action and Capture Donor Heterogeneity

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Many B-cell targeting biologics already available today have proven efficacious treatment modalities, and more are in clinical development. The result is a wide range of therapeutics that differ in structure, specificity, and mechanism of action (MoA). As patients further constitute a very heterogeneous population, patient stratification and treatment personalization are a major challenge to achieve the highest treatment efficacy and the lowest magnitude of adverse events in an individual patient. In this study, we used a whole blood–based functional assay strategy to capture subject heterogeneity in terms of engagement of effector cells and activation of bystander cells, and to characterize drug MoA. Using three flow cytometry panels, we examined the activation phenotype of whole blood immune cells after overnight stimulation with Rituximab, Obinutuzumab or Blinatumomab. As expected, Rituximab and Obinutuzumab were associated with target cell depletion and NK activation, with a strong antibody-dependent cellular cytotoxicity (ADCC) in Obinutuzumab. Blinatumomab was associated with a strong T cell response and a high target cell activation. In bystander cells, membrane markers and cytokine production were strongly induced by Obinutuzumab and Blinatumomab. Interestingly, in patient samples, the effector response was higher than in healthy donors, whereas bystander activation was weaker. In both healthy donors and patients, the range of responses was variable, demonstrating the ability to capture subject heterogeneity. The proposed approach allowed to capture donor heterogeneity and drug MoA. We believe it might represent a valuable option to guide treatment personalization and needs to be further evaluated in translational research.

W159. Immunological Correlates of Successful Treatment of Stage IV Pulmonary Lymphoepithelioma-like Carcinoma (LELC) with Adoptive EBV-specific T Cell Immunotherapy

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Pulmonary lymphoepithelioma-like carcinoma (pLELC) is associated with Epstein-Barr virus (EBV) infection. A stage IV pLELC patient was treated with EBV-CTLs as part of a compassionate drug access programme. Adoptive T cell therapy was carried out following chemotherapy. The patient went into full remission but eventually relapsed. We aimed to assess systemic immunologic changes in this patient associated with treatment response and subsequent relapse. Peripheral blood from 7 treatment intervals were profiled using mass cytometry and analysed using the Extended Polydimensional Immunome Characterisation (EPIC) discovery tool. Treatment resulted in dramatic decline in plasma EBV DNA titre, a known indicator of clinical outcome, and near complete metabolic response. Upon relapse, EBV DNA titres rebounded. Decreasing EBV titre was correlated with decreased circulating Ki-67$^+$ CD27$^+$ memory B cells (R2=0.76, p=0.01), which may reflect concomitant targeting of endogenously infected B cells by EBV-CTL. By contrast, the frequency of CD27$^-$ CXCR5$^-$ naïve B cells (R2=0.88, p=0.002) was inversely correlated with EBV titre, possibly replacing the depleted memory B cell population. Clinical outcome in response to EBV-CTL treatment is thus reflected both in plasma EBV DNA titres and the circulating immunome. Our case study demonstrates for the first time the clinical benefit of chemotherapy + EBV-CTLs in pLELC and the utility of monitoring plasma EBV DNA titres in this treatment. Further studies in larger cohorts will determine the functional contribution of these immune subsets to disease control and specific interactions of EBV-CTL with the immune system.

W160. Immunopeptidome Overlap Between HLA-DR Alleles in Heterozygous Dendritic Cells Pulsed with the Same Antigen

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The anti-tumor immune response is crucial for the elimination of tumor cells. Typically, the presence of Tumor-Infiltrating Lymphocytes (TILs) often correlates with favorable patient prognoses. But, among anti-tumor T cells, $CD4^+$ T lymphocytes play a pivotal role in the initial phases, acting as orchestrators of the immune response in two possible ways: as effector or regulatory cells. Previously, we compared the CDR3-TCR motifs expressed by *in vitro* expanded and then sorted CD4-TILs and CD8-TILs. The results suggested a more homogeneity among breast cancer samples in the CD4-TILs compared to CD8-TILs, suggesting this could be attributed to a less diverse immunopeptidome presented by MHC-II. Therefore, immunopeptidomic data from dendritic cells (DCs) pulsed with MCF-7 cell line extract were obtained from HLA heterozygous donors. Our objective was to analyze peptide overlap, comparing the proteins presented by different MHC class II molecules (HLA-DR alleles). Key findings include: (i) significant peptide and protein overlap among different HLA-DR alleles in both pulsed and non-pulsed samples; (ii) identification of only 4.5% of MCF-7 cell line proteome in pulsed DC samples; (iii) influence of certain alleles, such as DRB1*13:02, by the donor-paired allele and increased exclusive proteins in others, like DRB1*03:01 or DRB1*04:04. This study suggests that the presentation of cancer-related proteins may be influenced by the behavior of HLA-DR alleles. Consequently, understanding differences in peptide-loaded HLA-DR alleles is crucial. Such insights could inform the design of therapies utilizing DCs pulsed with peptides, enhancing the intervention of $CD4^+$ TILs across diverse patient populations.

W161. Loss of Heb Alters the Pathogenesis of β-Catenin-induced T cell Acute Lymphoblastic Leukemia

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Acute Lymphoblastic Leukemia (ALL) is the most common cancer among children in the US. Approximately 5% of T-cell ALL (T-ALL) cases involve acquired focal chromosomal duplications encompassing a region downstream of the Myc oncogene, characterized as a Notch1-dependent Myc enhancer (N-Me). The lack of mouse models with such N-Me duplications has limited our ability to study the molecular mechanisms of the disease. We found that mouse leukemias resulting from conditional aberrant activation of β-catenin with simultaneous ablation of the transcription factor HEB in CD4+CD8+ double-positive thymocytes bear N-Me duplications. The ablation of HEB fundamentally changes the mechanism of leukemogenesis induced by the simple activation of β-catenin, which is characterized by TCRα-Myc/Pvt1 reciprocal translocations. Based on the finding that HEB binds N-Me at a site that overlaps Notch-1 binding and the conserved motifs of other regulators, we propose that HEB has a central regulatory role in the oncogenic process. We hypothesize that HEB binding regulates the access of transcription regulators to N-Me, rationing Myc expression. Loss of HEB in the context of activated β-catenin enables access of these regulators to N-Me, and triggers super-enhancer properties and changes in chromatin conformation, leading to excessive Myc expression and leukemogenesis. Ultimately, the proposed study aspires to determine the role of HEB in the initiation, progression, and treatment of human T-ALL

W162. Macrophage-Drug Conjugate (MDC): A Promising Paradigm for Cell-based Immunotherapy for Solid Tumors

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Solid tumors remain challenging for therapeutic treatments, requiring the search for innovative treatment strategies. Macrophages known to infiltrate tumors due to signals from cancer cells, play a pivotal role in tumor dynamics. This study presents a method for loading macrophages with ferritin-drug complexes introducing the concept of a Macrophage-Drug Conjugate (MDC). Ferritin's protein-cage structure makes it an efficient drug carrier, while macrophages demonstrate a marked ability to internalize substantial amounts of ferritin. We have discovered a novel process where drug-loaded macrophages transfer ferritin to adjacent cancer cells that we called the "TRAnsfer of Iron-binding protein" (TRAIN). Crucially the TRAIN process requires direct cell-cell contact and the formation of an immune synapse-like structure. Macrophages loaded with ferritin conjugated to the cytotoxic drug exhibited highly potent anti-cancer activity in various orthotopic solid tumor models (glioblastoma, ovarian cancer, pancretic cancer leading to complete tumor elimination) and in patient tumor samples. Next, macrophages phagocyte cancer cells and activate immune system and immune memory leading to anti-tumor resistance. Our work lays the foundation for translating this potent adoptive cell therapy into clinical trials for solid tumor treatment.

W163. NK-mediated Protection Against Tumor Recurrence in CMV$^+$ Bladder Cancer Patients Upon BCG Treatment

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The intersection of viral infections and solid tumors presents a complex landscape for immunotherapy strategies, where treatments such as intravesical instillation of BCG are standard first-line therapy in non-muscle-invasive bladder cancer (NMIBC), yet its efficacy remains limited with a 32-42% recurrence rate. We found that cytomegalovirus (HCMV) reactivates in response to BCG-therapy and spreads to NMIBC tumors, including infection of monocytes, fibroblasts, and even tumor cells, suggesting a viral influence that extends beyond traditional perspectives on cancer treatment. Approximately 40-50% of HCMV-seropositive individuals harbor a subset of "adaptive" NK cells, which exhibit enhanced capacity for antibody-mediated immune responses. Adaptive NK cells expand in response to HCMV-reactivation and may hold a key to overcoming treatment resistance. Using a cohort of NMIBC patients treated at Mount Sinai, we found that HCMV-seropositive NMIBC patients respond better to BCG-therapy than their HCMV-seronegative counterparts and HCMV-seropositive patients with adaptive NK cells had a perfect response. Further, we profiled urine-derived CD45+ cells and bladder biopsies of patients during BCG-therapy and observed that adaptive NK cells infiltrate and establish residency in bladder tissues and colocalize with HCMV-infected cells within the bladder. Additionally, we observed significant elevation in plasma IgG1/IgG3 after exposure to BCG and unique to HCMV-seropositive NMIBC patients, suggesting a role for antibody-dependent cellular cytotoxicity (ADCC) mediated by adaptive NK cells. HCMV's role in cancers has been understudied, especially its impact on the immune landscape of solid tumors. Our results provide critical evidence for targeting HCMV and adaptive NK cells for improving immunotherapies for solid tumors.

W164. PD-L1 is Fascinating but IDO Needs Attention in Non-HCV and Non-HBV-associated Hepatocellular Carcinoma Patients

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Background/Aim: Hepatocellular carcinoma is one of the most common forms of liver cancer that is modulated by the immune system. Programmed cell death ligand-1 (PD-L1) has emerged as a novel therapeutic target in cancers. Indoleamine 2,3-dioxygenase (IDO) is an immunosuppressive enzyme that is associated with poor prognoses in cancers. Our aim was to investigate the PD-L1 expression, and clinicopathological features of non-HCV and non-HBV-associated HCC patients, including IDO expression. Methods: Immunohistochemical analysis was performed to analyze the expression of PD-L1 and IDO. FFPE tumor tissues (n=50) were obtained from the pathology department, at Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, Pakistan between 2005 and 2022. Furthermore, it was a rare group of patients with no previous history of any viral hepatitis. For statistical analysis, chi-square or Fisher exact test and Mann–Whitney U-test was performed. Results: Of 50 tissue specimens, PD-L1^{+} was observed in 21 [high: 12 (24%), low: 9 (18%)] and PD-L1- was observed in 29 HCC patients. IDO+ was observed in all 50 specimens [high: 42 (84%), low: 8 (16%)]. Additionally, both PD-L1 and IDO had high expression in 11 (22%) patients. While both PD-L1 and IDO had low expression in 2 (4%) patients. Furthermore, in IDO^{+}/PD-L1- group, 20 (69%) out of 29 patients died while in the IDO+/PD-L1^{+} group, 9 (43%) out of 21 patients died. Conclusion: Evaluation of IDO and PD-L1 expression may add therapeutic advantage in non-HCV and non-HBV-associated HCC patients that overexpress IDO. Further validation in a larger cohort is warranted.

Infectious Diseases

Th160. Modeling COVID-19 in a Humanized Mouse with Functional Human Neutrophils

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The murine and human immune systems exhibit notable differences in proportions, activities, and mechanisms, particularly in the case of neutrophils—a crucial cell type influencing immunity and pathogenesis. Neutrophils play a vital role in defending against injury and infection by engaging in phagocytosis, releasing reactive oxygen species (ROS), antimicrobial peptides, proteases, and forming neutrophil extracellular traps (NETs). While these functions are conserved, significant species-specific differences can be observed in the maturation, migration, and function of neutrophils. Humanized mice bearing a functional human immune system generated by transplantation of human hematopoietic stem and progenitor cells into genetically modified mice serve as an invaluable tool to study the development and function of the human immune system *in vivo*. However, a major limitation of all current humanized mouse models has been absence of mature human neutrophils in circulation and tissues. To overcome this, we generated a new version of humanized mouse model named MICTRG6 (acronym for genes modified) in the C57Bl/6N strain that improves human myelopoiesis and enables development of functional human neutrophils. To establish a comprehensive humanized mouse COVID-19 model, the MICTRG6 model was further adapted using adeno-associated virus (AAV)-driven gene therapy to deliver human ACE2 to the lungs. This model faithfully replicates the human neutrophil response to SARS-CoV-2 infection. Human and mouse neutrophils utilize different effector mechanisms and dynamics in response to SARS-Cov-2 infection. These inter-species distinctions underscore the importance of tractable models supporting

human immune cells and have significant implications for understanding pathologies, including hyper-inflammatory responses in chronic viral infections.

Th161. Neutrophil Transcriptomic Profile During Streptococcus Pneumonia Infection in Mice
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Streptococcus pneumoniae (*S. pneumoniae*) is a Gram-positive bacterium and the main cause of bacterial pneumonia worldwide. When *S. pneumoniae* colonizes the lower respiratory tract, it is recognized by cells from the host triggering the immune response. In the early stage of lungs infection, neutrophils have a crucial role in bacterial clearance through the release of proinflammatory cytokines, phagocytosis, and neutrophil extracellular traps (NETs) release. However, studies have reported that neutrophils can exert immunomodulatory activity and modulate the inflammation in the lungs. Previous reports of FSC_{low} and FSC^{high} neutrophil subpopulations present in infected mice showed that the ratio between these 2 subsets change with course of infection but is unknown if this change is related to the presence of a key unidentified component. Therefore, the study aim was to evaluate the transcriptomic profile of purified neutrophils during *S. pneumoniae* infection in mice to determine a key factor that could modulate the ratio of the described neutrophil subpopulations. For this, C57BL/6 mice were intratraqueally inoculated with 3x10^7 colony forming units, then, 12 and 24 hours post infection, neutrophils were purified from broncho alveolar fluid and RNA-seq was performed. Results showed 4000 differentially expressed genes and gene set enrichment showed cellular pathways related to hypoxia response, damage response, neutrophil response, response cancer, neuroinflammation response, among others. In conclusion, these results shows that neutrophils participate are multifaced cells that help in microbe elimination but also in other unexpected pathways. Funding: FONDECYT 1231905 & 1211060 and Millennium Institute on Immunology and Immunotherapy ICN09_016 (former P09/016-F).

Th162. Pulmonary Hypertension in a Cystic Fibrosis Child Following COVID 19: Case Report
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Background: COVID 19 pandemic was a disaster for the Globe. Cystic fibrosis (CF) also has been identified as a possible risk factor for poor outcome and long term consequences. Besides, overlaps between CF exacerbations and COVID 19 were associated with diagnostic dilemmas. Case description: we report a case of a 12 year old CF girl (CFTRdele2/I175V), chronically infected with *pseudomonas aeruginosa*, who developed pulmonary hypertension following COVID 19 infection. Patient was admitted to the University ''Muratsan'' hospital in December 2020 with shortness of breath, 3 day fever, increased cough with greenish phlegm, low oxygen saturation, decreased appetite and myalgia. PCR for SARS COVID 19 was positive, labs showed significantly high CRP, ESR and leucocytosis. Meropenem and amikacin, steroid pulse therapy were initiated. Besides disease specific changes ground glass opacities are found on CT. She was discharged at 16 day with clinical, laboratory and radiological improvement on home oxygen therapy, inhaled antibiotic and protocol based treatment. Patient was readmitted 2 weeks later with severe respiratory distress, low oxygen saturation, sleep disturbances. She was tested negative for respiratory viruses, laboratory tests and X-ray didn't found remarkable cause for deterioration: CRP was 18 mg/l, she cultured negative for Pseudomonas aeruginosa, atypical mycobacteria. Echocardiography detected significant pulmonary hypertension. Bosentan and sildenafil were added. Conclusion: Pulmonary arterial hypertension should be suspected in a CF patient presenting after COVID 19 with worsened respiratory status, unexplained desaturation, night sleep disturbances. Discussion: The prevention of COVID 19 in CF cohort should be prioritized.

Th163. Rapid Diagnostic Test for Assessment of Cellular Immune Response in a Viral Infection as a Model

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During the recent SARS-CoV-2 outbreak, there has been a recognized need of evaluating the adaptative immunological status, not only in infected individuals, but also in the vaccinated population. While the humoral immune response has been extensively assessed, the cellular immune response has received less attention due to the challenges of transferring it into a rapid diagnostic test. Consequently, there is a demand for the development of a rapid and user-friendly test for detecting activated T cells. This study is based on measuring interferon-gamma production as an indirect assessment of specific T cells. It uses the early expression of transcripts, amplifying them through PCR to targeted it in a paper-based rapid test. SARS-CoV-2 infection serves as a model for evaluating IFNγ transcripts in COVID-19 patients using a lateral flow assay. Whole blood of infected and non-infected individuals (both vaccinated) is incubated with Spike and Nucleocapsid peptide mixture followed by an immunomagnetic separation of the $CD3^+$ T cells and IFNγ double-tagging RT-PCR. In parallel, non-stimulated blood sample are included as IFNγ basal expression. IFNγ increment is assessed by comparing the intensity of LFA signal from stimulated versus basal samples with a smartphone. Moreover, a time course is conducted to track the signal over time in the individuals with a specific T cell response. In summary, IFNγ-LFA is a one-day test, capable of assessing the cellular immune response up to two months and suitable for point-of-care applications as it requires minimal equipment.

Th164. SARS-CoV-2 Vaccination Provides Boosted Mucosal Protection in Previously Infected Young Children

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Understanding the basis for generation of protective humoral responses to SARS-CoV-2 vaccination in young children is critical for refining immunization strategies and minimizing the severity of COVID-19. It has been suggested that generation of mucosal IgA could improve the efficacy of vaccines directed at viral respiratory pathogens, however evidence regarding the production of mucosal IgA in children who received current SARS-CoV-2 mRNA vaccines is quite limited. To delineate the generation of mucosal IgA responses following vaccination and/or natural infection in young children, we assessed mucosal IgA responses in saliva (n=116) and nasal swabs (n=103) of children under 5 years of age stratified by vaccination status and history of prior SARS-CoV-2 infection. Analysis of serial matched serum and saliva specimens following SARS-CoV-2 mRNA vaccination revealed that despite robust IgG responses in the serum, post-vaccination IgA responses in saliva were significantly higher in children with a history of COVID-19 infection. Similarly, analysis of nasal swabs obtained from a convenience sample of young children demonstrated that SARS-CoV-2 specific IgA levels were significantly higher in children with a history of COVID-19, or both a history of COVID-19 and SARS-CoV-2 vaccination, than in children with SARS-CoV-2 vaccination without prior COVID-19 disease. These results indicate that the ability of current SARS-CoV-2 mRNA vaccines to generate mucosal IgA responses is quite limited in young children who have not previously had COVID-19. This study highlights the importance of considering both mucosal and circulating responses when evaluating vaccine-induced humoral immunity in pediatric populations.

Th165. Single-cell Analysis of Thymic Dysregulation and Viral Tropism After Neonatal Roseolovirus Infection

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Herpesviruses, including the roseoloviruses, have been linked to autoimmune disease. The ubiquitous, chronic nature of herpesvirus infections in which acute infection occurs years before onset of autoimmune disease onset makes establishing a causal association challenging. We showed that murine roseolovirus (MRV), which is highly related to human roseoloviruses, induced disruption of central tolerance after neonatal infection, resulting in development of autoimmunity in adult mice, long after resolution of acute infection. This suggested that MRV induces durable immune dysregulation. In these studies, we utilized single-cell RNA sequencing to identify the tropism of MRV in the thymus and determine cellular processes that were disrupted by neonatal MRV infection. We found that MRV infection resulted in major shifts in inflammatory, differentiation and cell cycle pathways in infected thymocytes. We identified the cellular tropism of MRV in the thymus to include double negative, double positive, and CD4 single positive thymocytes, as well as medullary thymic epithelial cells. We also establish the inflammatory response to thymic infection, which was characterized by a type I IFN as well as an IL-36 response. We identified transcriptional changes that suggested disruption of interactions between antigen presenting cells and thymocytes that could alter survival and selection. Our results provide the first complete picture of roseolovirus tropism and roseolovirus-induced transcriptomic disruption in the thymus after neonatal infection. These studies define how MRV alters the microenvironment of the thymus after neonatal MRV infection, providing insight into how roseoloviruses disrupt central tolerance and promote development of autoimmune disease.

Th166. The Experience of the Artificial Intelligence Tools' Use in Prediction of Long- COVID-19 in Armenia

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Long COVID-19 is a condition that lasts for weeks, months and years following the initial SARS-CoV-2 infection with a broad symptomological spectrum. Persisting neuropsychiatric manifestations, including fatigue, dyspnoea, cognitive impairments, and disturbances in concentration, anxiety, and sleep, may endure beyond 6–12 months post-acute infection. The post-COVID condition is hypothesised to emanate from immune dysregulation and the presence of circulating "microclots" comprising amyloid, fibrin, and fibrinogen, with plasmapheresis demonstrating efficacy in their removal. The advent of computational tools for the prognostication of deteriorative risk employs a specific scoring algorithm (0-4) predicted on clinical variables. Prioritisation of patients for administration of Therapeutic Plasma Exchange (TPE), this model leverages patient-specific data including the latest laboratory test results, age, body mass index (BMI), risk factors, underlying medical conditions, and clinical symptomatology. The algorithm integrates 30 laboratory parameters, encompassing albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, blood cells and a comprehensive inflammatory marker profile. In the annum 2023, an investigation involving 198 subjects highlighted the model's utility across diverse symptomatologies including respiratory/allergic, neurological, cardiovascular, and musculoskeletal domains, accounting for 20-40% of presentations. For severe manifestations, a therapeutic regimen comprising up to seven TPE sessions over a six-month trajectory was administered. The utilisation of AI-driven prognostic tools in long-COVID-19 management has evidenced favour able outcomes in both clinical efficacy and patient quality of life, as well as had a pivotal role in physician decision-making process.

Th167. The Lung Innate and Adaptive Response in Pediatric Acute Respiratory Distress Syndrome

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Children are susceptible to viral lung infections due to immune immaturity. Occasionally, viral lung infections progress to acute respiratory distress syndrome (ARDS) with poor clinical outcomes. Here, the pulmonary innate and adaptive immune pathways that contribute to pediatric ARDS pathogenesis is studied with a theragnostic translational goal of identifying prognostic immune signatures associated with greater disease severity and druggable targets. 19 children with pediatric ARDS and 12 children with no lung injury (controls) were studied. Deep tracheal lavage samples were obtained and $CD3^+$ T cells and $CD14^+$ myeloid cells isolated using fluorescence-activated cell sorting (FACS). Transcriptomics analysis using the NanoString nCounter system was performed to characterize lymphocyte and myeloid gene expression. Gene set enrichment analysis was performed on DAVID Bioinformatics Resources 6.8 and upregulated pathways identified. 115 lymphocyte genes were significantly up-regulated. Pathways associated with ongoing inflammation, specifically T cell receptor signaling, immune activation (type I and II interferon, TNF and downstream NFKB pathways) and cell adhesion/migration genes were up-regulated in pediatric ARDS compared to control. Severity of pediatric ARDS was specifically associated with upregulation of T cell cytotoxic (GZMA, GZMB, GNLY), exhaustion/inhibition (CTLA4, PDCD1, LAG3, TIGIT) and apoptosis (ABL1, ATG5, BAX, BCL2) genes. Myeloid genes (169) were predominantly downregulated with decreased antigen presenting function (MHC class II HLA genes), pattern recognition (TLR2, TLR4) and anti-inflammatory potential (CD163, IL10). These results aid in understanding how the over-exuberant inflammatory responses are skewed and may provide theragnostic opportunities associated with disease severity and druggable targets.

Th168. Therapeutic Effectiveness of Interferon-α2b Against COVID-19 with Community-acquired Pneumonia

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Background. The announced effectiveness of IFN-α in the treatment of patients with severe COVID-19 has been ambiguous: some work indicated a lack of positive effects, while at the same time, the effectiveness of such a treatment was confirmed in cohort studies as well as clinical trials. Our work aimed to assess the clinical effectiveness of IFN-α in hospitalized patients with COVID-19. Methods. A prospective cohort study included 130 adult patients with COVID-19 pneumonia who were randomized to the main (81 patients) and control (49 patients) groups. The main group received, in addition to standard therapy, received IFN-α2b administered in the form of a nasal spray, 2 spray doses 4 times a day, which was a total of 80,000 IU per day for 10 days. Results: Adding IFN-α2b to the standard therapy for patients with severe COVID-19 reduces the length of the hospital stay by 3 days ($p < 0.001$). In the group of patients receiving IFN-α2b, the SpO2 index before and after treatment increased from 94 (92–96, Q1–Q3) to 96 (96–98, Q1–Q3) ($p < 0.001$), while the percentage of patients with normal saturation increased (from 33.9% to 74.6, $p < 0.05$), but the level of SpO2 decreased in the low (from 52.5% to 16.9%) and very low (from 13.6% to 8.5%) categories. Conclusions: Considering the contradictory results obtained regarding the strength of the response to type I IFNs in patients with severe COVID-19, more accurate information is required for the appropriate therapeutic use IFNs.

Tu162. A New Fast Detection Approach for Microbial and Infectious Disease Assays in Digital PCR
James Qin
Qiagen Inc.

Healthcare-associated (nosocomial) infections (HAIs) represent the most common adverse event among hospitalized patients worldwide and is a major public health concern in hospitals. There are 6 most common types that attributed to approximately 722,000 infections and over 90,000 deaths according to CDC; among them, healthcare-associated urinary tract infection (HA-UTI) and surgical site infection (SSI) contribute to prolonged stay, disability, mortality, and multidrug-resistant. Regularly surveillant on pathogens from clinical and wastewater specimens is helpful in keeping check on antimicrobial usage and drug-resistance and are important for infectious disease management and prevention for nosocomial infections and multi drug resistance (MDR) monitoring. Here we introduce QIAcuity dPCR system and nanoplate technology that is fast, sensitive, more consistent at lower concentrations, and show easy adaptability for 4/5-plexes microbial assays and eliminated the need for standards making it a viable, low cost, fast, sensitive option for microbial detection for infectious diseases. It automates and streamlines the digital PCR workflow of partitioning, thermocycling and imaging, and generates absolute quantitation data in approximately 2 hours. It's ideal for use with QIAGEN GeneGlobe microbial assays, custom lab-developed tests (LDTs) in clinical research or commercial qPCR assays for microbial detection. In this study, we randomly selected five probe-based assays involved in UTI and wastewater from predesigned, web-lab validated microbial assay portfolio, and compared the assay in 1-plex and 5-plexes. Data show comparable performance, high precision and sensitivity when run with pool of synthetic long oligos and genomic positive reference DNAs in 4-logs of dilutions. Data is comparable with quantitative-PCR.

Tu163. Alterations in γδ T Cells and the Effect of IVIG in Multisystem Inflammatory Syndrome After SARS CoV-2 Infection in Children
Andrea Reitsma, Ayantika Sen, Jing Guo, Anika Shah, Mahil Rao, Yueh-hsui Chien, Sheri Krams, and Olivia Martinez
Stanford University

Multisystem Inflammatory Syndrome in Children (MIS-C) is a late onset severe hyperinflammatory condition associated with SARS CoV-2. As part of the Pediatric Research Immune Network on SARS-CoV-2 and MIS-C (PRISM) trial, we collected whole blood from children at baseline prior to treatment, and at 28 days, 6 months, and 12 months post-diagnosis (n=33 MIS-C and n=25 COVID). Analysis of 37 cell immune subsets by mass cytometry demonstrated significant increases in B cells (p=0.0002), $CD4^+$ T cells (p=0.0193) and non-classical monocytes (NCMo) ($CD11c^+HLA\text{-}DR^+CD14^-CD38^-$) (p=0.0026) in MIS-C. The proportions of T cells (p=0.0145), dendritic cells (DC) (p=0.0001), and classical monocytes (p=0.0162) were significantly decreased in MIS-C patients. By 28 days post-diagnosis immune cell subsets in MIS-C patients had returned to the levels in COVID patients. Although the proportion of γδ T cells (γδT) in the MIS-C and COVID groups were similar at diagnosis, γδT were significantly elevated in MIS-C at 28 days and 180 days (p=0.0068, p=0.0058). Further analysis of γδT identified four subclusters of effector memory phenotypes dysregulated in MIS-C patients. Immune cell subsets before or after treatment with IVIG were analyzed. DC (p=0.0445), γδT (p=.0003) and NCMo (p=0.0010) were all significantly decreased after treatment. There were no changes in other T cell or B cell populations and all populations returned to baseline levels by 28 days post-treatment. Thus, IVIG, an effective treatment for MIS-C, rapidly decreases DC, NCMo and γδT in the circulation. Further, our study suggests an unappreciated role for γδ T cells in MIS-C.

Tu164. An mRNA Vaccine for Lymphatic Filariasis (LF)
Varsha Hariharan, Tanmayee Vagesna, Camar Ameril, Justin Richner and Ramaswamy Kalyanasundaram
University of Illinois

Lymphatic filariasis (LF), caused by thread-like parasites transmitted through mosquitoes, affects over 120 million people in 72 countries, leading to severe disability and lymphatic system impairments. Despite significant attempts to control the infection by preventative chemotherapy, over 856 million individuals remain at risk. There is currently no vaccine available to protect the vulnerable population. In our laboratory, a tetravalent recombinant fusion protein (BmHAXT) vaccine was developed for human lymphatic filariasis, which is currently moving towards clinical trials. However, the cost of manufacturing a protein vaccine is high, and in addition needs an adjuvant and a cold chain for storage as well as transportation, which adds to the cost. Addressing these challenges and given the recent success in mRNA SARS- Cov19 vaccine, in this study, we evaluated the potential of a BmHAXT mRNA vaccine in a mouse model. Our results show that following three doses of immunization with the mRNA vaccine, mouse developed significant antigen-specific IgG antibody titer and showed significant protection against a challenge infection, levels comparable to those of the recombinant fusion protein vaccine. Analysis of cellular immune responses showed that the mRNA vaccination also generated antigen-specific central memory T cells.

Tu165. Asymptomatic Brain Infection with Herpes Simplex Virus Induces the Expression of Senescence-related Biomarkers in the Central Nervous System
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Previously, we have described that asymptomatic brain infection with herpes simplex virus type 1 (HSV-1) induces an earlier onset, and more severe form of experimental autoimmune encephalomyelitis (EAE) in mice, which is an autoimmune disease related to the central nervous system (CNS) that has biological similarities with multiple sclerosis (MS) in humans. Noteworthy, asymptomatic brain infection with HSV-1 in humans is common, and likely leads to persistent inflammation in this tissue. However, the effects of this type of infection in the brain and CNS are not fully understood. Here, we explored the expression of cellular senescence biomarkers in the CNS after asymptomatic brain infection with HSV-1, with or without later EAE induction. Importantly, we observe a significant increase in senescence markers in brain and spinal cord after infection and/or EAE, which varies depending on the treatment. Overall, these findings suggest that asymptomatic brain infection with HSV-1 induces senescence in the CNS, which in time could contribute to increased onset or severity of neurodegenerative diseases. Additional studies related to these findings could provide a better understanding of the long-term effects of persistent CNS infection with neurotropic pathogens as HSV-1 and minimize their effects over these tissues.

Tu166. Comparative Analysis of Multiplexed Immunoassay Platforms for Inflammatory Markers in a COVID-19 Cohort
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In this study, we evaluated three distinct immunoassay platforms designed for the simultaneous detection of multiple inflammatory markers. Our assessment focused on three performance metrics - detectability, correlation,

and differential expression - using serum samples from the NIH IMPACC study. The scrutinized platforms included the fluorescent bead-based Luminex assay, the proximity extension-based Olink assay, and the novel proximity ligation assay platform, Alamar NULISAseq. Our findings provide valuable insights, revealing that the Alamar platform exhibited the highest overall detectability, followed by Olink and Luminex. Notably, protein measurement correlations between Alamar and Olink were generally stronger than those observed between either of these platforms and Luminex. Furthermore, we conducted a freeze-thaw experiment using the Alamar assay, demonstrating minimal differences between 1 and 2 freeze-thaw cycles for serum samples. This additional experiment adds a layer of robustness to our findings. Through our study, we offer insights into the comparative performance of these assays, contributing to a refined understanding of their strengths and limitations. This is particularly pertinent when applied to complex biological samples, as exemplified by the sera from the COVID-19 cohort. Our research contributes to the selection of protein assays and facilitates improved data interpretation for future studies involving intricate disease cohorts.

Tu167. Detection of Immune Repertoire and Transcriptome Changes in a Murine Tuberculosis Model with Microsampling Technology

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This study introduces a novel approach in tuberculosis (TB) research using murine models, focusing on non-terminal blood sampling for immunological profiling during disease progression. Current approaches rely primarily on the evaluation of lung lesions after euthanasia, with a large number of animals needed for time-course and dose-response studies. Measuring blood biomarkers in small volumes obtained serially could provide useful insights and reduce the number of animals needed. Hence, we evaluated the utility of small-volume blood samples for profiling T- and B-cell receptor repertoires and transcriptomes in mice (n=16) at different stages of TB progression (early, middle, and advanced—defined by lung histopathology and mycobacterial loads). We isolated RNA from 100 uL of whole blood for TCR/BCR profiling using the Cellecta DriverMap™ adaptive immune receptor (AIR) assay as well as transcriptome quantitation of ~4700 key mouse genes using a targeted RNA-sequencing panel. The results showed substantial changes in clonotype usage and diversity over the course of TB disease, most prominent in the BCR IgH (e.g, top 10 clonotype abundance count in control, early, advanced disease, respectively: 62 + 47, 1792 + 589, 3559 + 286, $p < .01$, mean + SD, n=3-4). Blood transcriptome profiling showed abundant up-regulation of immune and inflammatory genes (e.g, up-regulated genes vs control: 182 (early), 382 (advanced), q.05 FC1.5). The results support direct testing of non-terminal serial blood samples to characterize disease progression and therapeutic or vaccine interventions.

Tu168. Evaluation and Establishment of Serum Pepsinogen Levels in Health Checkup Patients Using the MAGLUMI® X3 Analyzer

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Gachon University Gil Medical Center

Serum pepsinogen(PG) I and PG I/II ratio are associated with *Helicobacter pylori* infection, serving as valuable indicators for screening precancerous lesions like atrophic gastritis. Although commonly accepted cutoff values for atrophic gastritis are 70 ng/mL for PG I and 3.0 for PG I/II ratio, these values still need standardization across different methods and vendors. PG I and PG II, and *H. pylori* IgG were measured in 154 healthy adults using MAGLUMI® X3 analyzer(SNIBE Co., Ltd, China) based on the CLIA. *H. pylori* IgG testing revealed 58 positive

and 96 negative cases. In the *H. pylori* positive group, both PG I(114.24±80.22 vs. 80.48±39.70 ng/mL) and PG II(15.38±10.65 vs. 7.78±3.99 ng/mL) significantly increased, while the PG I/II ratio decreased(8.11±3.31 vs. 10.36±1.8)(P< 0.001). In endoscopic findings, 46 patients showed atrophic gastritis, while 108 displayed other findings such as erosive or superficial gastritis. Only PG II(14.08±7.69 vs. 9.18±7.87 ng/mL) and PG I/II ratio(8.22±2.89 vs. 10.07±2.43) significantly increased in patients with atrophic gastritis(P< 0.001). Serum PG effectively reflects gastric corpus atrophy but may not always consistent with atrophic gastritis found by endoscopy. Relying solely on traditional reference values should be challenging in populations with a high *H. pylori* prevalence or healthy individuals. PG level cannot replace endoscopic examination entirely. Thus, a comprehensive assessment, including serologic and endoscopic examinations alongside *H. pylori* testing, is beneficial for a more comprehensive understanding of health status. Also, optimal cutoff values for MAGLUMI Pepsinogen II and Pepsinogen I/II ratio were established as 12.38 ng/mL and 7.56 for atrophic gastritis through this study.

Tu169. Evaluation of an mRNA Vaccine for Dirofilariasis in Dogs
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University of Illinois, Rockford

Dirofilariasis is a disease of canines caused mainly by the filarial nematode *Dirofilaria immitis*. Our laboratory developed a recombinant protein vaccine for lymphatic filariasis disease, which is also caused by a filarial parasite *B. malayi*. The genomes of *D. immitis* and *B. malayi* was compared and it showed significant similarity of over 88%. The three major vaccine candidates of *B. malayi*, the heat shock protein 12.6 (HSP), thioredoxin peroxidase-2 (TPX) and abundant larval transcript (ALT-2) showed 97%, 96% and 93% sequence similarity respectively. In this study, we evaluated the potential of rBmHAXT as a prophylactic vaccine for *D. immitis*. Recent advances in the mRNA vaccine field, especially during the Covid-19 pandemic provided an exciting opportunity for developing an mRNA vaccine for other infectious diseases including parasitic infections. Thus, in this project, we constructed two different mRNA formulation, one the tetravalent (HAXT) mRNA and the second a mixture of all four antigens (H+A+X+T) as an mRNA vaccine for *D. immitis*. Initial ELISA and confocal microscopy studies confirmed that the dog cell line, Madin-Darby Canine Kidney (MDCK) cells can express and secrete HAXT protein following transfection with HAXT DNA. Subsequently, mice were immunized three times with the mRNA vaccine, and challenged with *D. immitis* L3 larvae. Our results showed that the mRNA vaccine induced significantly high titer of IgG antibodies compared to controls. Challenge study results are awaited. Preliminary studies showed that the mRNA immunization induces significant protective immunity. The mRNA vaccination also generated significant antigen-specific central memory T cells in mice.

Tu170. High Burden of Viruses and Bacterial Pathobionts Drives Heightened Nasal Innate Immunity in Children
Timothy Watkins
Yale University

Studies during the COVID-19 pandemic showed that children had heightened nasal innate immune responses compared to adults. To evaluate the role of nasal viruses and bacteria in driving these responses, we performed cytokine profiling and comprehensive symptom-agnostic testing for respiratory viruses and bacterial pathobionts in nasopharyngeal swab samples from children tested for SARS-CoV-2 in 2021-22 (n=467). Samples were collected from a period of low SARS-CoV-2 circulation (June-July 2021) and a period of high SARS-CoV-2 circulation (January 2022) to account for seasonal differences in virus circulation and effects on the nasal innate immune response. Respiratory viruses and/or bacterial pathobionts were highly prevalent in both cohorts,

especially in young children, and were detected in both symptomatic and asymptomatic children (82% of symptomatic and 30% asymptomatic children; 90% and 49% for children < 5 years). Virus detection and load correlated with the nasal interferon response biomarker CXCL10, and the previously reported discrepancy between SARS-CoV-2 viral load and nasal interferon response was explained by viral co-infections. Bacterial pathobionts correlated with a distinct pro-inflammatory response with elevated levels of IL-1β, TNF, and myeloid cell recruitment based on RNA-seq analysis. Furthermore, paired samples from healthy 1-year-olds collected 1-2 weeks apart revealed frequent respiratory virus acquisition or clearance with the mucosal immunophenotype changing in parallel. These findings reveal that frequent, dynamic host-pathogen interactions drive nasal innate immune activation in children.

W165. A Multi-modal Longitudinal Model of Sepsis Reveals Immune Dynamics Associated with Mild, Severe and Fatal Disease

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Sepsis remains a significant global health concern, posing complex challenges due to its diverse origins and limited treatment options. Immune responses during sepsis are heterogeneous and may include a mixture of immune paralysis, hypo-responsiveness, or hyper-inflammation. Previous work primarily examined the role of the immune system in sepsis through cross-sectional data or by focusing solely on a singular aspect or data modality, thereby overlooking the complexity of the system. Instead, here, we take a systems-level approach and hypothesize that the timing, sequence, and interaction between multiple players in the immune system are crucial in determining the course and outcome of sepsis. Specifically, we built a multi-modal, longitudinal disease model of sepsis by profiling the peripheral blood of 26 sepsis patients, with varying clinical progressions, across 4-9 time points using mass cytometry (CyTOF), mass-spec proteomics, cytokine arrays, whole-transcriptome sequencing and detailed clinical measurements. Collectively, this rich dataset enabled us to align patients in pseudo-time and construct a shared axis that describes sepsis and recovery. By overlaying features from multiple modalities, we describe the molecular units and biological pathways that participate in sepsis and reveal their dynamics throughout the disease. Finally, by using a clinically heterogeneous cohort, we found specific events, or molecular “misbehaviors” that are associated with worse clinical courses or outcomes. Overall, our work helps to decipher - in detail - the immune system’s failure(s) during sepsis and across different outcomes. This work paves the way towards reliable, actionable, immune monitoring tools and immune-directed interventions for sepsis.

W166. Abnormal STAT3 Signaling in Fibroblasts Promotes *Staphylococcal* Growth and Abnormal Tissue Response to Infection

Miao Chen
Columbia University

Autosomal-dominant Hyper IgE syndrome due to dominant-negative mutations in STAT3 (STAT3DN) leads to infections, atopy, and connective tissue abnormalities. In addition to recurrent pyogenic pneumonia, especially due to *Staphylococcus aureus*, AD-HIES patients demonstrate evidence of abnormal wound healing, which includes development of pneumatoceles following infection, unexplained spontaneous bowel perforations, and non-inflammatory vascular aneurysms. Here, we developed a mouse model of infection-induced abscess and pneumatocele generation via intratracheal infection of transgenic Stat3DN Mut mice and littermate controls with *S. aureus* (USA300). We found that one to two weeks following infection, the Stat3DN Mut mice develop a higher rate of lung parenchymal abscesses compared to WT mice and these abscesses are more severe and larger

(7/20 vs. 1/16). 4-8 weeks after I infection, large cavitary lung lesions were observed via CT only in STAT3DN mice (2/6 vs. 0/8). Moreover, *S. aureus* growth was significantly higher when cultured with STAT3DN fibroblasts, implying increased bacterial adhesion to cells/extracellular matrix. RNA-seq of STAT3DN dermal fibroblasts following *Staphylococcal* co-culture showed alterations in the expression of disintegrins and metalloproteinases involved in proteoglycan remodeling and structure of basement membranes (decreased expression of NTN4). Mice with STAT3 loss of function mutations provide a novel model for infection-induced pneumatocele formation, and STAT3 loss of function in fibroblasts creates a permissive environment for *S. aureus* growth, tissue adhesion, and impaired extracellular matrix and basement membrane remodeling. These findings may help shed light on the consequences and tissue targets of genetic and pharmaceutical alterations of the STAT3 pathway.

W167. High Prevalence of Inborn Errors of Immunity in a Pediatric Population Attending a Pulmonology Service in Pretoria, South Africa

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University of Pretoria

Introduction: South Africa has a high burden of infectious diseases, especially respiratory infections. Primary immunodeficiency disease/inborn errors of immunity (IEIs) have been overshadowed by secondary causes, such as HIV. In contrast to HIV, there is no coordinated service to diagnose and manage IEIs in the country. Methods: A new pediatric pulmonology referral service for children with problematic airway infection was established in 2023 in Pretoria, South Africa. A retrospective audit was performed of all the files opened between January and December 2023. Results: A total of 145 patients were seen. The median age was 7 (IQR 5 – 12) years and 65% were male. The primary referral diagnosis was recurrent sinopulmonary (68%), upper respiratory tract (16%), and multisystem (8%) infections. Secondary complaints were mostly allergic (35%), ranging from asthma, allergic rhinitis, and eczema. No patients had HIV or cystic fibrosis. IEI was diagnosed in 58% of cases. Of the IEIs, combined variable immunodeficiency (33%) and specific antibody deficiency (31%) were the most common, followed by transient hypogammaglobulinemia of infancy (14%) and chronic granulomatous disease (6%). Secondary immunodeficiency (8%) was mostly caused by repeated use of oral cortisone (80%). A genetic diagnosis was obtained in 23% of patients. Conclusion: We found a high prevalence of IEI in a pulmonary service in South Africa where IEI is known to be underreported. These data raise concerns about the lack of awareness, diagnostic capacity and access to genetic testing in the country.

W168. Human Immune Cells Promote SARS-CoV-2 Dissemination and T Cells Promote Lung Pathology

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Columbia University

We have developed a novel human immune system (HIS) mouse model in NOD/LtSz-scid IL2R gamma null (NSG) mice engineered to express hACE2 under physiologic control. Using CRISPR, the hACE2 gene was knocked into the endogenous murine ACE2 locus in NSG zygotes. hACE2-specific qPCR revealed hACE2 expression in 14 different tissues of these mice, mirroring hACE2 expression in humans. Upon short-term (1- 2 weeks) intranasal infection with SARS-CoV-2, the virus disseminated to at least eight different tissues, including the brain, heart, and intestine. This spread of infection was not dependent on the presence of T cells but was markedly enhanced by the presence of human immune cells. Histology of the native mouse lung after infection revealed thickened interstitium and inflammation around the airways, reminiscent of human COVID-19 patient histology, and the presence of SARS-CoV-2 N protein. Nanostring analysis also revealed an active immune response to virus in multiple tissues, including brain and intestine. Human innate immune activation was greater

in mice lacking T cells compared to mice with T cell reconstitution. This mouse model is also amenable to the study of post-acute sequelae of SARS-CoV-2 infection (PASC), as long-term (8-week) infection resulted in marked lung pathology with more inflammation present in mice with T cells, the persistence of viral proteins, and intrapulmonary T cell responses to a broad range of SARS-CoV-2 peptides. This model will permit long-term SARS-CoV-2 studies and further investigation of the role of T cells and other human immune components in the pathogenesis of acute COVID-19 and PASC.

W169. Humanized Monoclonal Antibody Targeting the Nucleoprotein of Human Respiratory Syncytial Virus Elicits Protective Cellular Immunity in a Murine Infection Model

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The human Respiratory Syncytial Virus (hRSV) is the main respiratory virus that causes infant hospitalization, leading to acute lower respiratory tract infections. The absence of approved vaccines for infants against hRSV highlights the need to explore innovative therapeutic formulations. This becomes particularly relevant in enhancing the efficacy of licensed prophylactic agents such as the monoclonal antibody (mAb) Palivizumab. Like Palivizumab, the recently approved mAb, Nirsevimab, exhibits neutralizing capabilities against the fusion protein of hRSV. Our laboratory has identified the nucleoprotein (N-hRSV), expressed on the surface of hRSV-infected cells, as an interesting target. Our team has successfully developed a humanized mAb anti-N-hRSV, and its impact in protecting against hRSV infection has undergone a comprehensive study. Using a BALB/c murine infection model, we observed that treating humanized mAb anti-N-hRSV significantly reduces the pathology associated with hRSV infection. The reduction in viral load and neutrophil infiltration in lung tissue evidenced this. Additionally, we performed co-culture assays of dendritic cells with purified T cells from the spleens of immunized mice. By flow cytometry we detected the activation of $CD4^+$ T cells which has been evidenced by an increase in CD25 and CD69 markers. Moreover, ELISA assays showed an increase in IL-2 secretion in the mice immunized with anti-N-hRSV and infected, compared with the mice not treated with anti-N-hRSV antibody. Notably, we observed the promotion of effector and central T cell immunological memory. Accordingly, our results suggest a promising preclinical profile for the humanized mAb anti-N-hRSV as a potent candidate against this pathogen.

W170. Identification of an Immunoreactive Protein Candidate for Developing a Diagnostic and Controlling Measure for Pythium Insidiosum Infection Using Proteome-based Mass Spectrometric Analysis and Cell-free Protein Synthesis

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Pythiosis is a devastating infectious disease caused by a fungus-like pathogen, *Pythium insidiosum*. The disease processes high morbidity and mortality in affected humans and animals worldwide. Effective diagnostic and therapeutic modalities still need to be improved. Besides, a basic understanding of the disease and the causative agent is trivial. In this study, we explored and characterized immunoreactive proteins of *P. insidiosum*, which are excellent candidates for being a diagnostic marker and drug or vaccine target. Crude extract of the pathogen was subjected to protein separation using 2-dimensional gel electrophoresis for Western analyses against three representative sera from patients with active pythiosis. A total of 55 protein spots were recognized by all tested sera, but not a control serum, and thus subjected to isolation and liquid chromatographic-mass spectrometric (LC-MS/MS) analysis against our in-house *P. insidiosum* genome-derived proteomic library. As a result, eight spots generated a peptide profile that can match 15 proteins in the library. By employing the cell-free protein

synthesis (CFPS) workflow, we can simultaneously express these proteins *in vitro* to validate their immunoreactive properties using Western blot analysis against anti-HisTag antibody and three pythiosis sera. Only the CFPS-based recombinant protein S01, annotated as a chaperone DnaK, demonstrated immunoreactivity. S01-coated ELISA showed a significant signal of an array of pythiosis sera (n = 23) compared with control sera (n = 24). In conclusion, we successfully identified and produced an immunoreactive chaperone DnaK protein of *P. insidiosum*, a potential target for developing a diagnostic and control measure for pythiosis.

W171. Immune Response to SARS-CoV-2, a Case of Original Antigenic Sin and Interference by IFNa-alpha Autoantibodies? A Second-third Wave COVID-19 Study of Unvaccinated Patients in Barcelona

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After four years, the features of SARS-CoV-2 infection are well known but the extent of tissue damage that is due to the immune response rather than to direct viral action is not clear at the patient level. In a cohort of 235 unvaccinated individuals including controls and asymptomatic, 128 were followed for and average of 25 days from symptoms onset. In these patients loess regression analysis of the isotype Ig responses to M, and N structural proteins revealed that only in five the response followed a primary pattern (IgM preceding IgG and IgA). By contrast, the response to RBD followed in most cases a primary pattern. There was a clear correlation between antibody titer, including anti-RBD and disease severity AS assessed by the WHO eight point score. Spectral flowcytometry of fresh PBMNCs showed that isotype switched B cells and Tfh lymphocytes appeared in circulation earlier and at higher level in mild than in moderate/severe cases, this including the $CD4^{+}CXCR5^{+}CXCR3^{+}$ subset while in severe patients were the plasmablasts and plasma cells which appeared earlier. IFN-alpha levels showed a consistent negative correlation with the antibody responses; interestingly, in 16 patients positive for IFN-alpha antibodies the antibody responses were stronger than in anti-IFN-alpha negative patients. Overall theses finding will support the proposed view that the response to SARS-CoV-2 structural proteins are modulated in part by prior infections to circulating coronavirus (original antigenic sin). Immune response to SARS-CoV-2 is influenced by the effect of anti-IFN-alpha antibodies this hindering the interpretation of the responses.

W172. Immune Responses During COVID-19 Breakthrough Cases in Vaccinated Children and Adolescents

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Vaccine effectiveness against SARS-CoV-2 infection has been somewhat limited due to the widespread dissemination of the Omicron variant and its subvariants , and the immune response dynamics of the naturally

infected with the virus. Methods: Twelve subjects between 3-17 yo, vaccinated with two doses of CoronaVac®, were followed and diagnosed as breakthrough cases starting 14 days after receiving the second dose. Total IgGs against different SARS-CoV-2 proteins and neutralizing capacity of these antibodies were measured in plasma. The activation of $CD4^+$ and $CD8^+$ T cells was evaluated in peripheral blood mononuclear cells stimulated with peptides derived from the proteins from the wild-type (WT) virus and Omicron subvariants by flow cytometry, as well as different cytokines secretion by Luminex. Results: 2 to 8 weeks post-infection, as compared to 4 weeks after 2nd dose of vaccine, there was a 146-fold increase in neutralizing antibody titers against Omicron and a 39-fold increase against WT SARS-CoV-2. Subjects showed an increase in total IgG levels against the S1, N, M, and NSP8 proteins of the WT virus. Activation of $CD4^+$ T cells showed a significant in response to BA.2 subvariant ($p< 0.001$). Finally, the secretion of IL-2 and IFN-ɣ cytokines showed an increase trend after infection. Conclusion: SARS-CoV-2 infection in pediatric population vaccinated with an inactivated SARS-CoV-2 increased neutralizing antibodies against Omicron and increased specific IgG antibodies for different SARS-CoV-2 proteins. Also, $CD4^+$ T cell activation was increased, suggesting a maintained conserved cellular response against the Omicron subvariants, whereas Th1-type cytokine secretion tended to be decreased.

W173. Integrated Transcriptomic and Immune Repertoire Analysis Reveals Innate and Adaptive Immune Response to Vaccination

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We present an examination of immunological responses elicited by Td (Tetanus-diphtheria), PPSV23 (pneumococcal), and Shingrix (herpes zoster) vaccines. Utilizing techniques for genome-wide expression and adaptive immune receptor (AIR) profiling, along with single-cell transcriptome analysis of isolated T and B cells, we present the temporal landscape of the immune response, post-vaccination. Blood samples were collected from a single donor, pre- and post-vaccination, employing Mitra microsampling technology and Tempus tubes for sample collection. RNA and DNA were extracted, and the DriverMap™ targeted RNA-Seq expression profiling assay was used for genome-wide profiling (19K panel), and DriverMap™ AIR immune repertoire profiling assay run to profile T and B cell receptors from RNA and DNA. The NGS results were analyzed using the MiXCR (MiLaboratories) pipeline (AIR panel) and Salmon analysis (targeted RNA-Seq 19K panel). Pathway enrichment analysis of the whole transcriptome shows that innate immune response, with significant activation of interferon and cytokine signaling, is prominent on Day 2 after immunization, followed by activation of adaptive immunity on Days 7-10, which gradually declines to baseline levels by Days 15-22. Adaptive immune responses to vaccinations involved both branches of TCR and BCR signaling with significant activation of immunoglobulin clonotypes specific for vaccine antigens. A detailed characterization of specific cell types responsible for adaptive immune responses was obtained through T/B cell sorting experiments and 10x Genomics analysis. This study highlights the importance of integrated transcriptome, immune repertoire, and single-cell analysis profiling to monitor the response to vaccination in understanding the complex interplay of innate and adaptive immunity.

W174. Investigating Airway Immune Responses to Acute Respiratory Viral Infections in Individuals with Chronic Disease

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Benaroya Research Institute

Acute respiratory viral infections (ARVI) are the most frequently occurring global illness producing significant morbidity and mortality, especially in populations with chronic disease. In our Human Immunology Project Consortium - Respiratory Virus (HIPC-RV) study, we seek to understand the molecular and cellular immune signatures of the vulnerable host response to ARVI to identify novel therapies and individuals at risk for clinical complications through systems immunology approaches. In this preliminary study, we sought to understand changes in upper airway mucosal responses before, during, and after ARVI in a cohort of adults with rheumatoid arthritis (RA) as well as age, sex-matched healthy controls. We evaluated induced sputum samples obtained at enrollment and one month after ARVI using high-dimensional flow cytometry (n=8 with additional collections ongoing), and single cell transcriptomics using the 10X flex protocol (n=2). We found that the proportions of B cells, CD4 T cells, and CD8 T cells were elevated at one-month post-ARVI compared to enrollment in both RA participants and healthy controls. Additional studies into the single cell profiles of these samples will help identify changes in transcriptional profiles over the same time period. Together, these studies provide novel insights into the dynamic changes in upper airway mucosal immune responses to ARVI.

W175. Investigating the Role of Natural Killer Cells in the Control of Latent Epstein-Barr Virus Infection

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Stanford University

Epstein–Barr virus (EBV) is a ubiquitous gamma-herpesvirus that persists as a chronic infection in over 90% of the adult human population. EBV infections are mainly asymptomatic and subside with the virus transitioning to latency in a subset of memory B cells. Failure to control latent EBV infection can result in a variety of malignancies, including lymphoproliferative diseases in immunocompromised people. Studies in both experimental models and humans suggest that NK cells are critical in the host defense against EBV. In previous work, we demonstrated NK cells expressing the inhibitory receptor NKG2A were specifically able to recognize and kill latently infected autologous B cell lines. We also showed that HLA-E-presented peptides derived from EBV latent cycle proteins can impair NKG2A recognition and its downstream inhibitory signaling, potentially leading to NK cell activation. To better characterize the phenotype of NK cells that respond to EBV-infected B cells, we generated a panel of EBV-lymphoblastoid cell lines (EBV-LCL) and performed co-culture experiments with primary NK cells and autologous EBV-LCL. We developed a novel mass cytometry panel of NK functional and phenotypic markers and determined that in addition to NKG2A, EBV-LCL-responsive NK cells express increased NKp30 and CD32 and downregulate CD16 which may contribute to NK cell activation and killing of EBV-LCL. Further, we generated HLA-E knockout EBV-LCL using CRISPR and determined that autologous NK cell cytotoxicity increased, suggesting that disruption of the NKG2A:HLA-E immune checkpoint axis enhances killing of EBV-infected B cells and would be an attractive therapeutic option for EBV-associated malignancies.

W176. Joint Sequence and Structure Embeddings of Spike Proteins for Predicting Viral Dynamics
Dhananjay Bhaskar, Lila Schweinfurth, Akiko Iwasaki, and Smita Krishnaswamy
Yale University

Predicting which viral strains will become predominant in human populations is challenging due to the complex relationship between the structure and composition of viruses and factors such as binding affinity and immunogenicity. However, a comprehensive understanding and anticipation of viral evolution is crucial for effective vaccine design and therapeutic interventions. Traditional methods in virology have been limited by considering sequence or structural data in isolation. Addressing this limitation, our study introduces ViSENet (Viral Sequence Evolution Network), a model that learns joint representations from protein structure and sequence data, along with key properties like binding affinity and immune escape, to create a more holistic model of viral evolution. ViSENet employs a deep transformer-based autoencoder to learn viral spike protein sequence embeddings that are chronologically organized, and from which viral sequences can be reconstructed. This is complemented by a geometric scattering autoencoder that captures the tertiary information of pertinent domains in the spike protein from AlphaFold-predicted structures. By marrying these two data representations in a joint latent space, our model surpasses the predictive performance of previous unimodal methods. Furthermore, by utilizing the neural ODE framework to navigate through the joint latent space, our model can project viral evolution dynamics several weeks into the future. Such forecasting capabilities are demonstrated through accurate predictions of COVID-19 and influenza evolutionary patterns, showcasing the potential of our approach in guiding future vaccine and therapeutic development.

W177. Repeated Exposure to SARS-CoV-2 Antigens Induces Exceptionally Broad and Potent Neutralizing Immunity to Major *Sarbecoviruses* in Humans Including SARS-CoV-1
Linqi Zhang and Peng Chen
Tsinghua University

Human antibody response to SARS-CoV-2 has become increasingly complex due to emerging variants and spread across the world, resulting in multiple waves of breakthrough infections with distinct antibody potency and breadth. Here, we identified two individuals stood out in their exceptional plasma neutralization to a diverse panel of *sarbecoviruses* including major SARS-CoV-2 variants, SARS-CoV-1, and ACE-2-using bat and pangolin coronaviruses. Looking into their immunization and infection history has revealed rather unusual episodes of antigen exposure, involving multiple intramuscular vaccination with inactivated virus, adenovirus-based, mRNA as well as intranasal vaccination with adenovirus-based vaccine, and intranasal infection/breakthrough infection with wildtype and/or Omicron BF.7. To study the molecular basis of their antibody potency and breadth, we have isolated a total of 963 monoclonal antibodies from the two individuals. Of which, 254 antibodies exhibit neutralization activity to the infecting wildtype and BF.7 subvariant. Among these, many demonstrated exceptionally broad and potent neutralizing activity against a diverse panel of coronaviruses including SARS-CoV-1, all SARS-CoV-2 variants tested including the most recent Omicron subvariant EG.5.1, JD.1.1 and JN.1, as well as ACE-2-using bat and pangolin coronaviruses, resembling that of corresponding plasma samples. Crystal and cryo-EM structural analysis revealed some unique features of antibody epitope and recognition. Taken together, we have identified two individuals with remarkable antibody breadth and potency against wide range of human and animal coronaviruses. The discovery of these broad and potent monoclonal antibodies serves as the first and important step to inform the development of next-generation vaccine against diverse human coronaviruses and beyond.

Mucosal Immunology

Tu171. A Pathogenic Role of Activated Mucosal-associated Invariant T Cells in an Animal Model of Ulcerative Colitis

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Background & Aim: We have previously reported that the activation status of the circulating mucosal-associated invariant T (MAIT) cells in patients with ulcerative colitis (UC) is associated with the severity, and these cells infiltrate the inflamed lesion. These observations imply that MAIT cells are involved in the pathogenesis of inflammatory bowel disease (IBD). However, the role of MAIT cells in the setting of IBD has not been unveiled. Therefore, we investigated the role of MAIT cells in an animal model of UC. Methods & Results: We utilized major histocompatibility complex-related molecule 1 deficient mice (MR1-/-), which lack MAIT cells, and isobutyl 6-formyl pterin (i6-FP), which is a synthetic antagonistic MR1 ligand. Wild-type (WT) C57BL/6 mice administered an oral i6-FP or MR1-/- were sensitized with oxazolone to induce colitis. MR1 deficiency or i6-FP treatment resulted in reduced severity of colitis. Splenocytes and colonic lamina propria lymphocytes were isolated from mice receiving i6-FP. Treatment with i6-FP resulted in reduced MAIT cell production of pro-inflammatory cytokines such as IFN-g and TNF. Reduced cytokine production by MAIT cells were also observed in peripheral blood mononuclear cells from the patients with UC when incubated with i6-FP. MR1-/- and i6-FP-treated WT were orally administered FITC-dextran. Although MR1 deficiency resulted in increased intestinal permeability, i6-FP administration did not affect gut integrity in mice. Conclusion: These results indicate that MAIT cells have a pathogenic role in colitis and suppressing activation of these cells may reduce the severity. Thus, MAIT cells are potential therapeutic targets for IBD.

Tu172. Immune Therapy of CHAPLE Syndrome (Complement Hyper Activation, Angiopathic Thrombosis and Protein Losing Enteropathy Syndrome)

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Problem: CHAPLE Syndrome (Complement Hyper activation, Angiopathic Thrombosis and Protein Losing Enteropathy) is an extremely rare life threatening genetic pediatric disorder. This is due to the deficiency of CD 55 receptor protein that is also known as Decay accelerator Factor, DAF or Cromer Blood Group located at gene chromosome 1 at Q32.2). The disease has an over all frequency of (Less than 1 in Million or < 1 / 1 000 000) cases. The total number of cases in USA are less than 10 and globally less than 100 cases are reported. FDA has recently approved the drug Pozelimab (Veopoz) under fast tract rare disease program and has a cost of $ 34,615.38 for single dose vial The Need: A cost effective solution is urgently needed for this life threatening disease to reduce the burgeoning problem of rare diseases and its medical management cost structure. Our Work: Based on "the recent advances in Genomics and Fundamentals of Immunology" we have identified a small molecular drug for factor D inhibitor that will reduce cost structure by 10 folds in next 5 years and further 10 folds long term (Kumar Shah, MD CHAPLE SYNDROME, Patent Pending USA) and will require extreme competitive medical management and clinical trials for the positive outcome

Tu173. MyD88 in Stromal Cells Regulates Antimicrobial Defenses in Intestinal Macrophages in Early Stage of IBD

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The role of fibroblasts in the regulation of macrophage antimicrobial defense in the gut remains unknown. MyD88 is suggested to shape fibroblast responses in the intestinal microenvironment. We examined the role of fibroblast-intrinsic MyD88 signaling in regulation of antimicrobial activity in macrophages. Mice lacking MyD88 within fibroblasts showed a decrease in the activity of antimicrobial defense pathways in the colon, accumulation of immature Arginase 1^{+} macrophages with low production of antimicrobial peptide cathelicidin, development of dysbiosis, and aggravation of DSS-induced colitis. Priming of bone marrow-derived monocytes with colonic fibroblasts lacking MyD88 resulted in generation of macrophages with low production of antimicrobial molecules and decreased bactericidal function. The production of IL-6 and CCL2 downstream of MyD88 was critically involved in fibroblast-mediated regulation of macrophage antimicrobial function. Thus, fibroblast-intrinsic MyD88 signaling contributes to colonic homeostasis by regulating macrophage antimicrobial defense, and its disruption may predispose animals for the development of intestinal inflammation.

Tu174. Peripheral Regulatory T-Cells Specific for Commensal Bacterial Flagellins Control Th17-Mediated Inflammation and Polyposis

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RORgt^{+} regulatory T-cells (Tregs) express high levels of IL10 and control microbial-instigated T-helper 17 (Th17) inflammation. In inflammatory bowel diseases and colorectal cancer (CRC), loss of the anti-inflammatory properties of Tregs contributes to deregulated inflammation and escalation of pathogenic Th17 responses. We have established that increased numbers of specific pathobionts (such as Parvimonias Micra) lead to increased polyp loads in the small bowel and colon of CRC-prone mice, as well as changes in the epigenome and gene expression in host cells, and long-term changes in microbiome composition. Conversely, RORgt^{+} Tregs generated in a microbial specific manner by protective bacterial strains (such as those from the Lachnospiraceae family) may be highly resilient in controlling inflammation and have therapeutic potential. The Lachnospiraceae flagellin CBir has been established in mouse models as an important antigen in the immune response for inflammatory bowel diseases (such as Crohn's), where it generates specific, protective RORgt^{+} Tregs. Our exploration is focused on the impact of CBir-specific Tregs, their distribution, stability, and influence on polyposis in colorectal cancer. Mice colonized with Lachnospiraceae A4 were injected with naïve CD4^{+} CBir TCR transgenic T-cells. These cells were allowed to proliferate for 4 days in the mice. Flow cytometry of extracted mesenteric lymph nodes, small bowel, colon, and spleen aim to detect proliferation of donor CD4^{+} T-cells and conversion into Tregs. Ongoing analyses determine the impact of polyposis on activation of CBir TCR specific Tregs, CBir TCR Treg suppression of inflammation and polyposis, and Treg stability in the course of disease.

Tu175. Probiotic Delivery of Galectin-1: A New Therapeutic Strategy for Inflammatory Bowel Diseases

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Inflammatory bowel diseases (IBD) are chronic and progressive inflammatory conditions that severely affect quality of life. Although introduction of TNF-α-neutralizing monoclonal antibodies has revolutionized IBD therapy,

a third of patients do not respond to this treatment. Thus, the search for new therapeutics to improve disease outcomes and quality of life remains a major area of interest. Recently, we reported dysregulation of the β-galactoside-binding protein galectin-1 (Gal1) and its glycosylated ligands in patients with IBD and validated its therapeutic potential in experimental colitis. This work encouraged us to find a selective delivery method of Gal1 through oral administration. We genetically engineered Lactococcus lactis (L. lactis) to deliver Gal1 as a new therapeutic approach for intestinal inflammation. We generated a novel construct for Gal1 expression in L. lactis, yielding higher amounts of bioactive human Gal1 as evidenced by Western-blot, ELISA and glycan-binding assay. Supernatants of Gal1-secreting L. lactis induced more rapid wound closure in human colon cells ($p< 0.05$), compared to L. lactis or Gal1. Using the 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced mouse model of colitis in Gal1-deficient (Lgals1-/-) mice, we demonstrated active delivery of Gal1 to the gut with daily intragastric administration of 1x109 CFU of Gal1-secreting L. lactis. Treatment with Gal1-secreting L. lactis significantly decreased weight-loss and macroscopic inflammation in these animals ($p< 0.05$). Additionally, WT mice show a significant decrease in local IFN-γ expression ($p< 0.05$). Thus, oral administration of genetically-modified L. lactis offers an effective targeted delivery system of human Gal1, as a novel potential therapeutic approach for IBD patients.

W178. Oral Pathobionts Promote CRC Development by Alteration of the Host Epigenome and Immunomic Microenvironment

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Gut microbiota play a major role in the immunological and clinical outcomes of colorectal cancer (CRC). Previously, using metagenomic studies, we and other researchers detected the enrichment of Parvimonas micra (P. micra), an oral pathobiont in human feces and colon mucosa of CRC patients. These findings lead us to test the hypothesis that P. Micra enrichment causes tumor-associated phenotype in host cells. We focused on tumor cells and the immunomic microenvironment. CRC-prone mice; TS4CreAPClox468 were antibiotic treated and then given a healthy human microbiome transplant (FMT), either with or without P. micra. Analysis of fecal pellet 16S rRNA sequencing revealed establishment of the human microbes in both groups. Addition of P. micra changed the composition of the bacterial community, enriched the population of other CRC-associated bacteria, and increased tumor load. DNA methylation sequencing of crypt epithelial cells in CRC prone mice using the RRBS (Reduced Representation Bisulfite Sequencing) method revealed differential methylation in tumor development pathways, including regulation of Wnt signaling and epithelial-mesenchymal transition pathways. By analyzing the methylation data and the RNA sequencing results in tandem, we also found deregulation of circadian genes in the P. micra group, which is another CRC related pathway. Methylation changes were also detected in the DNA of naïve CD4 T-cells isolated from the mesenteric lymph nodes. The experiment was repeated with tamoxifen-inducible CRC mice. Ongoing analyses of the DNA methylation and gene expression data (combined with immunohistology) aim to determine biological processes that are affected by and contribute to the carcinogenesis process.

Neuroimmunology

Th169. Greater Composite Confirmed Disability Progression in Non-hispanic Black and Hispanic or Latino Individuals with Relapsing Multiple Sclerosis: Analysis of Self-reported Identity and Genetic Ancestry in the OPERA I and II Trials

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Background: Individuals of underrepresented racial and ethnic backgrounds with multiple sclerosis (MS) may experience a more severe disease course. Ocrelizumab is a B-cell–depleting therapy approved for the treatment of MS. Objectives: To assess whether persons with relapsing MS (pwRMS) self-identifying as non-Hispanic Black (NHB) or Hispanic/Latino (HL) exhibit differences in disability progression vs those identifying as non-Hispanic White (NHW) and whether such differences are confirmed by genetic-ancestry analysis. Methods: We examined longitudinal outcomes in pwRMS who self-reported as NHB (n=65), HL (n=213) or NHW (n=1350) in the pivotal ocrelizumab RMS trials (NCT01247324, NCT01412333). Genetic-ancestry for consenting participants (n=1138) was determined using ADMIXTURE, with 1000 Genomes Project data used for reference-populations. Self-reported identity or genetic-ancestry and 24-week composite-confirmed-disability-progression (cCDP) risk were evaluated using time-to-event analysis. Differences in $CD19^+$ B-cell levels between subgroups were assessed using the Mann-Whitney U-test. Results: In the comparator interferon treatment group, pwRMS self-identifying as NHB or HL showed greater risk for 24-week cCDP than NHW individuals (hazard ratio [HR] [95% CI] NHB: 3.0 [1.4–6.5]; P=0.006; HL: 2.3 [1.6–3.4]; P< 0.001). Genetic analyses confirmed the association between proportion of African (HR, 4.4 [1.7–11.2]; P< 0.001) or admixed-American (HR, 5.8 [2.7–12.4]; P=0.002) ancestry and greater risk for cCDP. Baseline $CD19^+$ B-cell levels were higher in NHB or HL pwRMS and were reduced with ocrelizumab treatment in all subgroups (all, P< 0.001). Conclusions: NHB and HL pwRMS showed greater risk for cCDP while receiving interferon treatment. Genetic analysis confirmed the higher progression risk in pwRMS with African or admixed-American ancestry.

Th170. Longitudinal Analysis of Immune Cell Changes in FUS-ALS Patients Treated with a FUS Antisense Oligonucleotide

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Columbia University

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease with significant genetic, pathological, and clinical heterogeneity. Individuals with a mutation in the fused in sarcoma (FUS) gene experience one of the most aggressive and early-onset forms of ALS (FUS-ALS), as well as mislocalization and aggregation of FUS protein. To test the therapeutic potential of FUS knockdown, our previous work generated a knock-in FUS-ALS mouse model exhibiting denervation of neuromuscular junctions and glial activation followed by motor neuron death, features which were reduced with a single injection of FUS antisense oligonucleotide (ASO) at birth. Further, in a first-in-human trial, we showed that treatment with FUS ASO slowed the rate of functional decline as measured by the ALS Functional Rating Scale-Revised. Now, the efficacy of FUS ASO (i.e., jacifusen) is being tested further in an expanded access program in which paired blood and CSF samples are collected. Considering the role of the immune system in neuron function and survival, as well as the alterations to gliosis seen in our mouse model, this study sought to characterize immune cell changes in ASO-treated FUS-ALS participant blood and CSF. Analysis of single-cell RNA sequencing and TCR sequencing data revealed significant changes to immune cell frequencies and phenotypes, as well as altered clonal expansion and migration of clones between the blood and CSF, across treatment. These findings were validated further with methods such as flow cytometry and

cytokine profiling, and provide important insight into immune-related functional consequences of FUS knockdown and their potential relationship with clinical outcomes.

Th171. Mitochondrial Derived ATP and Its Metabolites Modulate the Balance of B-cell Cytokine Responses: Implications for MS Pathogenesis and Therapeutics

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Among key antibody-independent B-cell functions are their capacity for context-dependent secretion of distinct cytokine profiles that can shape local immune responses in both health and disease. Abnormal B-cell cytokine responses have now been implicated in the pathophysiology of multiple human immune-mediated conditions, including multiple sclerosis (MS). Surprisingly little is known, however, about the mechanisms involved in the regulation of B-cell cytokine expression. Our goal was to investigate fundamental mechanisms underlying regulation of the balance between B-cell pro- and anti-inflammatory cytokine responses and their impact on CNS inflammation. We find that B-cell cytokine production is metabolically regulated both *in vitro* and *in vivo*, with particular involvement of mitochondrial respiration (OXPHOS), and that distinct modes of B-cell activation shift their metabolic state, regulate the extent of ATP release by the B cells, and trigger the coordinated and modular regulation of ATP-pathway molecular machinery that enables ATP and its metabolites to function as a '4th signal', that reciprocally modulates the balance between pro- and anti- inflammatory cytokine expression. We further find that inhibition of either B-cell OXPHOS or ATP signaling could attenuate CNS inflammation *in vivo*, as well as restore the balance of B-cell cytokine responses in MS. Furthermore, we demonstrate that BTK inhibition, an emerging B-cell-targeting autoimmune disease therapeutic approach, could modulate B-cell cytokine responses through decreasing OXPHOS. Together, our study reveals a fundamental mechanism involving metabolic regulation of B-cell pro- and anti-inflammatory cytokine responses and points to non-depleting therapeutic strategies that restore the B-cell cytokine balance in MS by targeting metabolism.

Th172. Vagus Nerve Stimulation Alters Circulating Ly6clow Monocyte Populations

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The Feinstein Institutes for Medical Research

Neuronal pathways, including the vagus nerve-based inflammatory reflex, are physiological regulators of immune function and inflammation. Electrical vagus nerve stimulation (VNS) regulates tumor necrosis factor production in the spleen by a mechanism requiring acetylcholine signaling through the α7 nicotinic acetylcholine receptor expressed on cytokine-producing macrophages. While it is understood that VNS attenuates the release of proinflammatory cytokines from splenic macrophages, its effects on the immune cell populations are not yet clear. Here, we subjected mice to electrical VNS for four minutes at a continuous current of 250 uA and 16.5 ms pulse width at 30 Hz. Two hours following stimulation, whole blood mononuclear cells were isolated and processed for single-cell RNA sequencing (scRNASeq) as per 10X Genomics standard single-cell protocol. Samples were sequenced using NextSeq 2000 and analyzed utilizing the Seurat platform. Analysis of the scRNASeq data from sham (n = 12,030 cells) and VNS treated (n=10,057) samples identified 12 unique clusters based on annotated canonical markers and the Immgen database. Further investigation of the monocyte cluster using differential genes identified significant (log fold change > 2, and an adjusted p-value < 0.05) downregulation of the following genes: Wfdc21, Adgrl3, and S100a8. Within monocytes, we observed an increase in anti-inflammatory Ly6clow monocytes when comparing sham (n=7,538 cells) and VNS-treated (n=2,971 cells) cells. Overall, this study highlights the importance of investigating the complex interplay between the nervous and the immune systems and underscores the systemic changes that occur during vagus nerve stimulation.

W179. A Comprehensive Analysis of T Cell Dynamics and Clonality in the Blood and CNS Across Sporadic and Familial ALS

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Columbia University

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder characterized by the rapid loss of motor neurons. While 90% of ALS cases are sporadic, the remaining 10% have a family history usually characterized by mutations in several ALS-associated genes including aggressive Fused in Sarcoma (FUS) mutations. Although reactive microglia in the central nervous system (CNS) and regulatory T-cell dysfunction have been observed across ALS, we lack a comprehensive understanding of how the immune response is mounted and whether it differs between sporadic and familial ALS. Here we built a groundbreaking pipeline leveraging multi-tissue single-cell RNA and TCR-sequencing to perform phenotypic analysis and lineage tracing to identify the spatiotemporal trajectory of clonal T-cells in ALS and their phenotypes across tissues. Our cohort included >80 paired CSF and PBMC samples from pre-symptomatic/symptomatic FUS-ALS, sporadic ALS, and postmortem motor cortex and brain border tissues from ALS patients, alongside paired PBMC/CSF samples from multiple sclerosis patients and healthy control subjects. We identified shared expanded T-cell clones across FUS-ALS patients and peripheral and central compartments, exhibiting high levels of granzymes and other cytotoxic genes. Microglia-like cells and gamma-delta T-cells are enriched in FUS-ALS CSF, and high levels of MAIT cells showed elevated LIGHT signaling, which has been implicated in motor neuron killing. Enhanced CCL5-CCR4 signaling, generated by natural killer and CD8 effector memory T-cells, received by regulatory T-cells, was observed exclusively in FUS-ALS CSF. These findings highlight a unique FUS-ALS immune environment in the CNS offering potential for immunotherapy strategies to modulate neuroinflammation.

W180. Antigen-specific Interactions Between Endothelial Cells of the Blood-brain Barrier and Naive CD8 T Cells

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It was shown that antigen-specific interactions between endothelial cells of the blood-brain barrier (BBB-EC) and activated CD8 T cells are key drivers of some pathologies affecting the central nervous system (CNS), including cerebral malaria or Susac syndrome. However, little is known about antigen-specific interactions between BBB-EC and naïve CD8 T cells, in non-inflammatory conditions. We used a mouse model, in which BBB-EC conditionally and inducibly express the influenza virus hemagglutinin (HA) protein as well as a TCR transgenic mouse line in which ~95% of CD8 T cells recognize the HA512-520:Kd complex, to assess antigen-specific interactions between BBB-EC and naïve CD8 T cells, in the absence of inflammation. *In vitro* co-culture experiments showed that BBB-EC are able to directly activate HA-specific naïve CD8 T cells. After adoptive transfer of naïve HA-specific CD8 T cells in mice expressing or not HA in BBB-EC, we observed specific induction of activation and proliferation of the transferred cells in HA-expressing recipients. This antigen-specific interaction leads to an accumulation of the HA-specific CD8 T cells in the CNS without patent clinical manifestations. The CNS-infiltrating CD8 T cells persist up to 90 days after injection and based on their expression of CD69 and CD103, appear to acquire a tissue-resident memory phenotype. We assessed the consequence of this CNS-infiltrating CD8 population on a subsequent neuro-inflammatory challenge. Mice expressing HA on BBB-EC and injected with HA-specific CD8 T cells (but not CD8 of unrelated specificity) are surprisingly protected from EAE. The mechanisms underlying this phenotype are currently under investigation.

W181. CLEC16A-driven Mitophagy Limits Astrocyte Proinflammatory Activities

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Astrocytes are abundant glial cells in the central nervous system, with important roles in the pathology of multiple sclerosis (MS). In a genome-wide CRISPR-based forward genetic screen we identified CLEC16A, a gene linked to MS susceptibility, as a suppressor of astrocyte pro-inflammatory activities. Gene and small molecule perturbation studies in mouse primary and human embryonic stem cell-derived astrocytes in combination with metabolic and bulk and single-cell transcriptional analyses established that CLEC16A promotes mitophagy, limiting the accumulation of mitochondrial products that activate NF-κB, the NLRP3 inflammasome and gasdermin D. Astrocyte-specific Clec16a inactivation increased NF-κB, NLRP3 and gasdermin D activation *in vivo*, worsening experimental autoimmune encephalomyelitis, a pre-clinical model of MS. Moreover, we detected disrupted mitophagic capacity and gasdermin D activation in astrocytes in MS. These findings identify CLEC16A-driven mitophagy as a suppressor of astrocyte pathologic responses and a candidate therapeutic target in MS and other neurological diseases.

W182. Data-driven Positioning of Immune and Inflammation Related Drugs to Target Neuroinflammation Diseases

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Sanofi

Immune pathways drive diverse biological functions and disease phenotypes. Data-driven drug repurposing of existing portfolio of immune-disease related drugs to new indications can accelerate drug development cycle times to clinical trial by leveraging demonstrated product safety profile, efficacy in a related disease indication or mechanism of action. In this study we developed an end-to-end drug repurposing strategy leveraging compound signatures and multi-omics evidence from the Sanofi Disease Data Ecosystem to match existing immunology drugs and potentially new indications in neuroinflammatory diseases. To enable this, we build an Sanofi Therapeutic Signature database, with 5,900 compounds and >3,800 target perturbation profiles (RNAi/CRISPR, ligand, ovExp, Perturb-Seq) in >200 diseases & 190 cell model systems and integrated with immunology-related clinical trial data from Citeline's Pharmaprojects. For a subset of targets with compound signature available in immune cells, we used an artificial intelligence-based transcriptomic fingerprinting scoring system (AITxFP) to compute similarity of compound transcriptomic profile to prioritize compounds reversing disease signatures. We further integrated the signatures with multi-omics evidence including transcriptomics enrichment, genetic evidence, knowledge graph, protein-protein networks, and literature-based associations from PubMed. Further, an indication priority score was computed for all immunology targets in each selected neuroinflammatory disease (for example: Alzheimer's disease). We validated two compounds from the prioritized ranked list in a disease relevant iPSC-derived neuro tri-culture model. In conclusion, our data-driven end-to-end drug indication positioning approach can expand the drug value and accelerate patient impact by reducing the cycle time of drug discovery, development, and positioning.

W183. DNA Methylation and RNA Expression on Immune Cells in the Cerebrospinal Fluid of Patients with Neurosyphilis

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Neurosyphilis (NS) is a complication of syphilis that manifests with a range of neurological conditions, including stroke and neuropsychiatric disease. Although the prevalence of both syphilis and neurosyphilis is rising, the pathogenesis of NS and its sequelae after treatment are not well understood. We longitudinally analyzed DNA methylation and RNA expression profiles from cerebrospinal fluid (CSF) cells from diagnosis to one year post-treatment from 12 adults with laboratory confirmed NS (CSF venereal disease research laboratory reactive and >5 cells/uL) and 11 controls who had syphilis without neurosyphilis (non-NS), matched for age, sex, HIV status, and serum rapid plasma reagin titer. We utilized the Illumina MethylationEPIC platform to assay DNA methylation at single nucleotide resolution from ~850K CpG sites. DNA methylation profiles from the CSF of participants with NS were significantly different from those of participants with non-NS. Among the pre-treatment CSF samples, status of NS vs non-NS was the primary driver of methylation differences among the samples, with 1660 differentially methylated CpG sites (false discovery rate < 0.01 and Effect size > 10%), which were enriched in genomic regions regulated by the GATA3 transcription factor. More than 80% of these DNA methylation changes persisted after treatment. Cell population imputation found B cell elevation within the CSF of patients with neurosyphilis, which resolved post-treatment. This pattern corresponded to concomitant RNA expression changes among B-cell genes in the same samples. Despite treatment for NS, these results suggest persistent CNS immune dysregulation. Future studies must examine whether these sequelae are clinically meaningful.

W184. Unraveling TIM-3 as a Myeloid Immune Checkpoint in Autoinflammatory Diseases

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In the central nervous system, TIM-3 functions as an immune checkpoint inhibitor modulating pro-inflammatory responses. While TIM-3 has been extensively studied in terminally differentiated IFNγ-secreting $CD4^+$ T helper cells and dysfunctional $CD8^+$ T cytotoxic cells, the immunoregulatory role of TIM-3 in myeloid cell biology remains unexplored. Recent discoveries link germline loss-of-function mutations of TIM-3, specifically Y82C, 197M, and T101I variants, as potentially causal in subcutaneous panniculitis-like T cell lymphoma (SPTCL), with nearly 20% of SPTCL patients also developing systemic lupus erythematosus (SLE). Individuals harboring heterozygous TIM-3 variants exhibited reduced TIM-3 surface expression and higher pro-inflammatory cytokine production by immune cells. In multiple sclerosis (MS) patients, we observed surface TIM-3 downregulation on myeloid cells *ex vivo*, which was restored after B cell depletion therapy. These findings in human myeloid cells from autoinflammatory diseases strongly suggest TIM-3's regulatory role in myeloid-mediated inflammation. *In vitro* gene perturbation by shRNA knockdown and CRISPR-Cas9 knockout on human primary myeloid cells revealed low TIM-3 expression upregulated CD80 and CD86, prompting a pro-inflammatory macrophage phenotype, whereas loss-of-TIM-3 increased susceptibility to inflammasome activation with upregulation of IL-1β secretion and caspase-1 cleavage upon LPS priming and nigericin stimulation. Employing proteome profiler human phospho-kinase array and antibody array assays, downstream intracellular mediator candidates and activated signaling pathways, such as LYN, WNK, HSP60, NFκB, and MAP Kinase demonstrated TIM-3 signaling in human myeloid cells. Targeting downstream candidates via TIM-3 perturbation offers compelling therapeutic potential. TIM-3 surface and signaling blockade together could address immune dysregulation in autoinflammatory diseases like SPTCL and MS.

Reproductive Immunology

Tu176. A Case of Postpartum Hemorrhage Due to Possible Ig-E Mediated Dysregulation
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Postpartum hemorrhage (PPH) is a common postpartum complication that affects almost 6% of deliveries worldwide. The most common cause is uterine atony of various etiologies (near 90% of cases). Here we present a patient with atonic uterus associated hemorrhage possibly secondary to an IgE-mediated autoimmune dysregulation of connective tissue. A 20 year old female presented with PPH after vaginal delivery which was unresponsive to conservative hemostatic measures. An emergency hysterectomy was performed, and during the procedure, an extremely distended uterus was discovered. Postoperatively we interviewed the patient to find a possible etiology of her complication. The patient revealed prior history of scoliosis, atopic dermatitis, and PEP(polymorphic eruption of pregnancy). Her history was also notable for an autoimmune condition that was in the process of being officially diagnosed prior to her current pregnancy. Her mentioned manifestations are all IgE-mediated processes and are thus reminiscent of an incomplete Hyper-IgE immunoglobulinemia syndrome (HEIS). Current literature linking gestation and immune signaling support this suspicion because most of pregnancy is characterized by Th2-dominant signaling. This signaling is mediated by cytokines IL-4 and IL-13, both of which are also signals for IgE-class switching. IgE has been linked to destruction of certain types of collagen that is also prevalent in the uterus. The relationship between autoimmunity and pregnancy outcomes is a fledgling field of research. This complex relationship between immune response and postpartum complications deserves further study and may indicate more extensive screening for immune dysregulation in pregnant patients to possibly decrease intra and postpartum complications.

Tu177. Chronic Inflammation of the Male Genital Tract Affects Fertility by Decreasing Sperm Quality and Inducing Inflammation in the Female Genital Tract After Insemination
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Urogenital inflammation has been proposed as a cause of male infertility, suggested to account for at least 15% of male infertility cases. However, supporting evidence from animal models is scarce. Thus, we herein aimed to assess whether chronic prostatitis may cause infertility by either affecting sperm quality or impairing female genital immunoregulation using an animal model of experimental autoimmune prostatitis. C57BL/6 male mice were immunized with prostate antigens (PA) or saline (C) on days 0 and 15 and the specific immune response, prostate histopathology and infiltrating leukocytes were assessed on day 26. At day 24, males were mated with BALB/c female mice and uterine immune changes that occur after insemination and different fertility parameters were analyzed. Prostate-antigen-immunized mice showed chronic pelvic pain development and the induction of PA-specific Th1/Th17 responses associated to prostate and semen inflammation, and reduced sperm quality. Interestingly, female mice mated with PA-immunized mice showed significantly decreased fertility indexes and higher embryo loss rates with respect to controls. Remarkably, either early after copulation or during the peri-implantation window, significantly increased infiltrates of macrophages, dendritic cells, NK cells and $CD4^+$ Teff cells, whereas altered number of Tregs, were observed in the uterine mucosa from these females with respect to controls, showing a shift towards uterine inflammation. Our results indicate that PA-specific Th1/Th17 immune responses underlie chronic prostatitis development and causes semen inflammation. Of clinical interest,

prostatitis significantly impairs fertility by reducing the fertilizing ability of sperm, altering the uterine immune response triggered after insemination, and increasing embryo loss.

Tu178. Isolation of Amnion Cells from Human and Non-human Primate Placenta for Flow Cytometry and Transcriptomics

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Intrauterine infection/inflammation (IUI) is a frequent complication of pregnancy leading to preterm labor and fetal inflammation. How inflammation is modulated at the maternal-fetal interface is unresolved. The amnion is a thin layer of fetal origin in contact with the amniotic fluid which plays a key role at the feto-maternal interface during pregnancy. Here, we present a detailed protocol for isolation of human and Rhesus macaque amnion cells as well as two complementary methods (flow cytometry and RNAseq library preparation and analysis). Of note, a deep characterization of these cells via different approaches may provide a new understanding of altered immunological pathways during intrauterine infections to develop new therapeutic strategies.

Stem Cell and Organ Transplantation

Th173. Altered Intestinal Barrier and Immunoregulatory Gut-derived Metabolites Contribute to Acute Rejection in Renal Transplantation

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Introduction: Long-term graft survival in renal transplantation remains a challenge. Gastrointestinal microbiota can impact extra-intestinal health. We aim to identify the interplay between the gut microbiota and recipient immunity in renal transplantation. Our hypothesis is that increased gut permeability and reduced availability of bacterial-derived metabolites associated with immunoregulation promotes a less tolerogenic environment that increases the risk of acute rejection (AR). Methods: Transplant recipients (n=92) and live-donors (n=23) were recruited into a longitudinal study, with urine, stool and blood samples collected at baseline and up to 12-months after surgery. Flow cytometry was used to assess B-regs ($CD45^+CD19^+IL10^+$). Gut permeability was assessed by measuring plasma intestinal fatty acid binding protein (i-FABP). 16s rRNA sequencing and mass spectrometry was used to assess the gut microbiome and metabolome. Results: Recipients with biopsy-proven AR had evidence of increased permeability before transplantation and decreased indole derivatives. In AR, a decreased IL-10:TNF ratio in $CD19^+$ B-cells was observed, particularly within the transitional B-cell compartment ($CD45^+CD19^+CD24hiCD38hiIL10^+$), at 3-months (0.14±0.107 vs 0.08±0.04; $p< 0.05$) and 6-months (0.11±0.05 vs 0.07±0.05; $p< 0.05$) compared to non-rejectors. Furthermore, we observed an increased frequency of B-regs in non-rejectors after transplantation when compared to baseline (2.66%±1.86% vs 4.62%±1.99%; $p=0.01$), and at 6-months when compared to rejectors (2.96%±1.69% vs 1.75%±1.25%; $p< 0.05$). Discussion: Increased gut permeability and reduced immunoregulatory metabolites are associated with reduction in $IL\text{-}10^+$ B-regs, predisposing patients to AR. We postulate that decreased responsiveness or availability of indoles and SCFAs may impact B-reg generation and maintenance resulting in reduced immunological tolerance that contributes to AR.

Th174. Human Tolerogenic Dendritic Cells Used in Clinic Regulate CD8⁺ T Cell Population

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Our study addresses the critical issue of allograft rejection in kidney transplantation, where current immunosuppressive drugs pose significant risks. We propose a novel approach to cell therapy using autologous tolerogenic dendritic cells (ATDC) to promote graft acceptance. Based on our encouraging results in rodents, our team developed a GMP-compliant ATDC manufacturing process, and this cell therapy was evaluated in a first-in-man phase I/II clinical trial. *In vitro* characterization of ATDC highlighted that these cells exhibit enhanced glycolytic activity, and the abundant produced lactate was responsible for the suppression of CD4⁺ T cell proliferation and activation. In our trial, reduced CD8⁺ T cell activation and increased FoxP3 expression were observed in the circulating immune cells of kidney transplant recipients treated with ATDC. Focusing on the regulation of CD8⁺ T cells by ATDC, our *in vitro* experiments demonstrated that ATDC-derived metabolites suppress naive, effector, and TEMRA CD8⁺ T cell proliferation. Unlike CD4⁺ T cells, lactate was not involved in this suppression. However, the critical role of indoleamine-2,3-dioxygenase produced by ATDC to suppress CD8⁺ cells was evidenced using a specific inhibitor, establishing tryptophan deficiency as a mediator of CD8⁺ T cell proliferation inhibition. Metabolic and transcriptomic analyses highlighted that the ATDC environment promotes oxidative phosphorylation in naive CD8⁺ T cells, associated with their reduced activation. Moreover, ATDC decreases their migratory capacity through a contact-dependent mechanism. In conclusion, our findings support the efficacy of ATDC as a therapeutic strategy in kidney transplantation, particularly in suppressing CD8⁺ T cell-mediated allograft rejection.

Th175. Intracellular Membrane Attack Complexes Function as Non-cytolytic Alarmins in Transplant Rejection

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Complement membrane attack complexes (MACs) are well known mediators of cell death, but these structures are frequently detected within inflamed tissues following solid organ transplantation in the absence of widespread necrosis. As a basis for these observations, we describe an alternate immune function for MACs as non-cytolytic, intracellular alarmins. MAC⁺ amyloid deposits form in cohorts with antibody-mediated rejection, and MAC⁺ protein aggregates inducibly form *in vivo* and in human tissues. Following their assembly, surface-bound MACs become internalized into Rab5⁺ vesicles whose intraluminal milieu causes C9, a MAC-associated protein, to form insoluble aggregates. C9 aggregates subsequently stimulate aggrephagy to induce NF-□B-dependent inflammatory genes. Our lab recently discovered a novel Rab5 effector protein called ZFYVE21. We newly generated endothelial cell-specific ZFYVE21 knockout mice and found that these mice show attenuated C9 aggrephagy and reduced alloimmune tissue injury. Our findings describe a novel immune function for MACs as non-cytolytic, intracellular alarmins.

Th176. MHC II-specific Chimeric Antigen Receptor Regulatory T Cells for Transplantation Tolerance

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Solid organ transplantation is a critical intervention for end-stage organ failure. However, transplant recipients require lifelong immune-suppressive therapy to prevent immune-mediated allograft rejection. Cell therapy with regulatory T cells (Tregs) expressing a donor antigen-specific chimeric antigen receptor (CAR) is a promising alternative approach for promoting allograft tolerance. CAR-Tregs targeting ubiquitously expressed donor MHC I (HLA-A2) have entered clinical trials for transplantation. However, donor MHC II stimulates alloreactive $CD4^+$ T cells, which facilitate B cell and $CD8^+$ T cell responses, promoting allograft rejection. We therefore hypothesize that MHC II-specific CAR-Tregs could also promote allograft tolerance. To study the effectiveness of MHC II-specific CAR-Tregs in immunocompetent mouse models of C57BL/6xBalb/c (F1) to C57BL/6 skin and heart transplantation, we generated Tregs expressing a CAR specific for the BALB/c MHC II allele I-Ed. I-Ed-CARs selectively bound target I-Ed tetramers, with no cross-reactivity towards their C57BL/6 counterpart I-Eb or other MHC alleles. In response to $CD11c^+$ dendritic cells (DCs) from BALB/c or F1, but not C57BL/6 mice, I-Ed-CAR-Tregs upregulated CD69, LAP and PD-1 and Ki-67, and proliferated. In *in vitro* suppression assays, I-Ed-specific CAR-Tregs inhibited proliferation of C57BL/6 $CD4^+$ and $CD8^+$ T cells following allogeneic stimulation with BALB/c or F1 DCs, and suppressed CD80, CD86 and MHC II expression on DCs. Preliminary *in vivo* work demonstrates persistence and sustained FOXP3 expression of I-Ed-specific CAR-Tregs >30 days post-infusion. Overall, I-Ed-specific CAR-Tregs are antigen-specific and potently suppressive *in vitro*. Ongoing experiments are testing the *in vivo* efficacy of I-Ed-specific CAR-Tregs in mouse models of skin allograft transplantation.

Th177. The Role of Recirculating Regulatory T Cells in Thymic Regeneration

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T cells play a crucial role in adaptive immunity and rely on a functional thymus. Although the thymus harbor's endogenous ability to regenerate itself, it is highly sensitive to damage and acute involution which contributes to treatment-related morbidity and mortality. We have identified a specific population of Tregs that undergo expansion after injury (sublethal total body irradiation (SL-TBI, 550 cGy), cyclophosphamide, dexamethasone, LPS, and MCMV). These cells were observed to mediate thymic regeneration in depletion models using Foxp3DTR mice, and adoptive transfers of Tregs in injury models. Using RAG2GFP mice in injury, we found that the expansion was primarily due to RAG2GFP- cells. Parabiotic RAG2GFP to RAG2GFP models confirmed that these RAG2GFP- cells were actively recirculating after injury and adoptive transfer of RAG2GFP- Tregs had superior regenerative capacity compared to $RAG2GFP^+$ Tregs. We applied machine learning preprocessing methods to single-cell RNA sequenced RAG2GFP- thymic cells, revealing distinct gene regulatory networks enriched for factors of regeneration and cell stability. One of the top factors of regeneration was amphiregulin. Using a Foxp3CreYfp-Amphiregulinfl/fl mouse model, we demonstrated a Treg-specific Amphiregulin-dependent impairment in thymic regeneration after injury. In the human thymus, we observed an analogous recirculating population of Tregs with expanded TCR clonotypes using single cell sequencing. CITEseq identified CD39hiICOShi as markers of these putatively analogous human recirculating Tregs—markers that could be used to identify these human Tregs by flow cytometry, demonstrating their Amphiregulin secretion upon stimulation. Overall, our findings reveal a conserved Treg-amphiregulin axis mediating thymic regeneration in both mice and humans.

Th178. Unraveling Human Hematopoietic Progenitor Cell Diversity Through Association with Intrinsic Regulatory Factors
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Expression of CD34 is a hallmark of human hematopoietic stem and progenitor cell (HSPC) identity. Still, $CD34^+$HSPC, regardless of their origins, represent a heterogeneous mixture of phenotypic identities and functions. We hypothesized that quantification of evolutionarily conserved molecular regulators (i.e. transcription factors and chromatin proteins) with a deep phenotypic analysis of human HSPCs would better organize the functional diversity of the CD34 cell compartment. Leveraging single-cell mass cytometry we captured the expression of >40 protein-level phenotypes and functional regulators on three million $CD34^+$ cells spanning primary human hematopoietic tissues, including adult bone marrow (BM), mobilized peripheral blood (mPB) (G-CSF and Plerixafor), and cord blood (CB). Data-driven organization revealed a molecular-regulator-centric identity across the progenitor cell spectrum while, at the same time, grounding this information within the standard model of human hematopoietic definitions that allow for prospective identification. To validate our new model of human hematopoietic organization, we prospectively isolated newly identified E/Meg and myeloid populations, interrogating their epigenetic potential (i.e., chromatin landscapes) and associated clonal differentiation capacity *in vitro*. Overall, our study demonstrates the utility of molecular regulators in enhancing identifying and separating cellular identity and functional potential. Using this new rubric to align human BM progenitors with mPB and CB HSPC corroborates the expansion of the multipotent progenitor cells, specifically HSC and MPPs, within fetal tissues. Altogether, we provide new insight into the earliest events of human hematopoietic immune development, new definitions for prospective isolation of HSPCs, and a reference framework for further study and manipulation of these tissues.

Tu179. Comparative Analysis of Wild-type and Triple Gene-knockout Pig Red Blood Cell Transfusions in Non-human Primates: Therapeutic Efficacy and Immunological Responses
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Hallym University College of Medicine

Background: Blood donation shortages have led to research on pig red blood cells (pRBCs) as substitutes for human RBCs. This study evaluates the effectiveness and risks of wild-type (WT) and triple gene-knockout (TKO) pRBC transfusions in non-human primates. Methods: O-type WT and TKO pig blood was processed and transfused into ten monkeys, with groups receiving either WT or TKO pRBCs (n=4 each) or saline (n=2). Hematologic, biochemical, and immunological responses were monitored before, after transfusion, and at intervals. Results: Both WT and TKO pRBC transfusions effectively increased RBC count, hematocrit, and hemoglobin levels in the post-transfusion one day compared to the saline group. However, transfusions led to elevated ALT and total bilirubin levels in transfusion groups and cholesterol decrease in the WT group. Crossmatch tests showed variable aggregation; post-transfusion, profound antibody responses were observed in both transfusion groups. A second transfusion resulted in reduced hematological benefits and more adverse reactions. Conclusion: WT and TKO pRBC transfusions initially improved hematologic parameters, but rapid pRBC clearance followed. TKO pRBCs had less impact on liver function than WT. Despite initial benefits, strong antibody responses and potential adverse reactions highlight risks in xenotransfusion. The study suggests careful consideration for clinical applications of pRBC transfusions (Grant No. 22-CM-EC-18).

Tu180. Developing an Immunological Toolset to Investigate the Roles of T Cells in Rejection and Infection, and to Facilitate Rejection Diagnosis After Human Lung Transplantation

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Rejection and infection significantly limit lung transplantation (LuTx) success. Despite the importance of T cells in driving alloresponses and immune defense, their dynamic repopulation, clonal distribution, alloreactivity, and anti-microbial reactivity after LuTx are largely unknown. We utilized flow cytometry for chimerism determination and T-cell phenotyping, and a combination of high-throughput sequencing with mixed lymphocyte reactions to define and track alloreactive T cell receptors (TCRs) in graft-vs-host (GvH) and host-vs-graft (HvG) directions. Pathogen-reactive TCRs were identified by integrating public databases. In our cohort (n=13), recipient $CD14^+$ cells showed rapid infiltration in bronchoalveolar lavage (BAL) post-Tx, suggesting a role in priming GvH response. Much higher levels of HvG compared to GvH clones were associated with faster recipient T cell repopulation in the BAL. Recipient graft-infiltrating T cells gradually acquired tissue-resident memory features after LuTx. High levels of HvG-reactive TCRs among recipient-mappable repertoires were detected in BAL, but not paired PBMCs, during early rejection. In patients with EBV or CMV infections, we observed corresponding increase in EBV- or CMV-reactive TCRs in the BAL that were enriched for non-alloreactive clones. In some other patients with high levels of HvG TCRs in BAL, pathology was read as negative for rejection, despite complications indicative of rejection occurring later, suggesting that cellular and clonal changes may have already happened in lung allografts when histology is not diagnostic of rejection. Developing an immunological toolset at the chimeric, alloreactive clonal and phenotypic levels is expected to facilitate rejection diagnosis and mechanistic understanding of rejection and infection after human LuTx.

Tu181. Donor γδ T Cells May Contribute to Lymphohematopoietic Graft-versus-host Responses After Human Intestinal Transplantation

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Innate- and adaptive-like features of human γδ T cells are associated with $Vγ9^+δ2^+$ and non-Vγ9δ2 clonotypes, respectively. Despite their presence in the blood and many organs, the role of γδ T cells in transplantation is unclear. We performed phenotypic and clonal tracking of donor-derived γδ T cells after human intestinal transplantation (ITx) in blood, intestinal graft, and bone marrow (BM) by integrating flow cytometry and sequencing platforms. We previously demonstrated that patients with high levels of blood T cell chimerism had less rejection due to the migration of graft-derived graft-versus-host-reactive donor αβ T cells into the recipient blood and BM to mediate lymphohematopoietic graft-versus-host responses (LGVHR) which counteract the host-versus-graft response. Here we found that the level of blood γδ T cell chimerism correlated with total T cell chimerism. Donor γδ T cells were detected in recipient BM between post-operative day (POD)105–357. Single-cell RNA profiling of BM-infiltrating donor γδ T cells from 3 patients with pediatric donors revealed both Vδ1- and Vδ2-dominant clonotypes with cytotoxic effector phenotypes. In one patient, the top-dominant donor clone (Vγ8Vδ1) detected during peak blood T cell chimerism (POD8–20) was also predominant in recipient BM on POD126, with high cytotoxic (GZMB/PRF1/GNLY) but low proliferation (MKI67) and BM-homing (CXCR4) gene expression. BM-infiltrating donor $δ2^+$ T cells were dominated by sequences with zero N-additions that likely originated during fetal life and were shared across pediatric, but not adult donors, suggesting an age-related distribution and migration pattern. γδ T cells may influence LGVHR and blood chimerism after ITx.

W185. Donor-specific T Cell Activation and Expansion Following a Pig-to-human Decedent Kidney Transplant

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Introduction: Pig-to-human decedent transplantation provides a unique window into human anti-pig xenoresponses. We analyzed donor-specific T cell immunity in the context of a long-term (61-day) pig-to-human decedent thymokidney transplant. Materials and Methods: Blood and kidney biopsy samples were obtained pre-transplantation and at POD14, 28, 33 and 49. To identify donor-reactive T cell clones (DRTCCs), we performed direct and indirect mixed lymphocyte reactions pre-transplant and on POD 28. We FACS sorted proliferating CFSElow CD4 and CD8 T cells, extracted DNA and performed high-throughput TCRβ CDR3 sequencing (Immunoseq, Adaptive). Donor-reactive T cell clones (DRTCCs) were identified by expansion compared to sequenced unstimulated T cells. Kidney biopsies underwent digestion and lymphocyte isolation followed by single-cell RNA sequencing with TCR sequencing (10X). Results: Mild AMR on POD33 was successfully reversed. No pathological evidence for cell-mediated rejection was observed up to Day 61, when the experiment was terminated. DRTCCs were expanded in PBMCs at POD 33 and 49, when CD8 DRTCCs formed >15% of the total CD8 repertoires. In rejection-free biopsies (POD14 and 28), scRNA sequencing did not reveal activated T cells or NK cells. At POD33, scRNA sequencing revealed activated T cells and NK cells containing effector gene transcripts, including GZMB, PRF1, granulysin, IFN-γ, CCL4 and CCL5. Activated antigen-presenting cells with upregulated proinflammatory genes were also evident. DRTCCs were detected among graft-infiltrating T cells; transcriptomic analysis is in progress. Conclusion: Our results suggest a role for activated T cells and NK cells in apparently mild antibody-mediated porcine xenograft rejection in humans.

W186. High Dimensional Proteomic Analysis of Immune Cell Populations Following Vascularized Composite Allotransplantation in Mice

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Vascularized composite tissue allotransplantation (VCA) represents a promising therapeutic approach for individuals that have incurred debilitating injuries to the face or limbs. However, the complexity of tissues within VCA allografts presents unique challenges to our understanding of the host immune response. To address this, syngeneic (C57BL/6 to C57BL/6) and allogeneic (BALB/c to C57BL/6) hind limb transplants were performed in mice, and spleen cells collected on post-transplant days 1, 3, 5, and 7 (n=4-6 mice/per group/day). Custom panels of metal-conjugated antibodies to characterize myeloid populations (54 markers), T cell subsets and cytokines (24 markers) were established and validated. Cells were stained with antibodies and analyzed by mass cytometry. By UMAP dimensionality reduction, we identified seven myeloid cell clusters including a $F4/80^{+}Ly6ChiCD11b^{+}CCR2^{+}$ subset, that rapidly expanded in the spleen of allogeneic hind limb recipients and was significantly elevated compared to syngeneic recipients by day 5 ($P < 0.001$). Three distinct $CD8^{+}$ $TCRa/b^{+}$ effector/effector memory subsets (Tem) were elevated in the allogeneic group. In particular, the $CD8^{+}CD62lowCD44hiCD25hiCCR7-$ Tem subset was evident by day 3 and was significantly increased in the allogeneic group compared to the syngeneic group on days 5 and 7 (both $P < 0.001$). This Tem subset expressed the highest levels of cytokines (IFN-g, TNF-a, IL-6, and IL-10), granzyme B, and perforin. In summary, we identified both specific myeloid and Tem populations associated with graft rejection in VCA. Deep profiling and high dimensional analysis of VCA resulted in unique targets that will lead to more targeted and effective immunosuppression.

W187. Immunophenotypic Profiling Reveals Novel T Cell Subset Associated with "Tolerance" in Pediatric Transplant Recipients

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Achieving allograft health while minimizing chronic immunosuppression remains a challenge in pediatric solid organ transplantation. In a recent study we performed multilineage, single-cell analysis of the immune cell composition in pediatric solid-organ transplant recipients who received a heart, liver or kidney allograft (n=52); 24 children had stable graft function while 28 children experienced a rejection episode. Hierarchical clustering of the mean cell type proportion across allograft types and health states showed that a distinct subpopulation of CD4 T cells (CD45RA-,$CD25^{+}$,$CD5^{+}$,CD38-) was significantly ($p< 0.05$) associated with stable graft function, and were termed TOT cells. The cosine similarity analysis of proteomics data from PBMC showed that TOT cells are phenotypically most similar to Tregs and least similar to $CD4^{+}$ TEMRA. To further characterize the TOT cells, we developed an 8-marker flow cytometry panel (CD3, CD4, CD45RA, CD45RO, CD25, CD38, CD127, CCR7) to specifically sort Treg and TOT populations and performed 10x single-cell RNA-seq and TCR-seq with subsequent analysis in R and Loupe Browser. Differentially expressed genes that were specifically associated with TOT cells included ANXA1, IL7R, TCF7, THEMIS, NELL2, KLRB1 and CD40LG while Treg expressed FOXP3, IKZF2, CTLA4, IL2RA, and RTKN2, consistent with their known function in immune suppression. TCR analysis indicates unique expansions of T cell clonotypes in TOT cells not seen in Treg. Further investigation into the immunoregulatory capacity of TOT cells could be instrumental in developing new strategies for enhancing graft survival.

W188. Improving Lymphocyte Purity for Flow Cytometric Crossmatch Assays: A Comparison of Cell Isolation Methods

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Introduction: The flow cytometric crossmatch (FCXM) assay is used to determine whether donor specific HLA antibodies (HLA-DSA) are present in recipient sera. Isolated cell purity is a widely recognized critical factor in the test outcome. Our goal was to compare the performance of two cell isolation methods in FCXM assays carried out with peripheral blood from living donors for kidney transplant. Materials and methods: FCXM assays carried out after processing donor samples by erythrocyte aggregation and total lymphocyte enrichment through negative magnetic selection (EA&TLE) and gradient density centrifugation (Ficoll) were compared and correlated with HLA-DSA detection using the Luminex single-antigen bead assay (LSA). Results: The mean lymphocyte percentage of samples processed using EA&TLE was 94% (95% confidence interval [95% CI] 89% to 97%), with 24 to 50% of the lymphocytes present before isolation retained. Analysis of 65 FCXM assays performed with EA&TLE and 66 with Ficoll demonstrated that EA&TLE results in higher sensibility (67% EA&TLE vs 43% Ficoll) and higher kappa correlation coefficient (0.61, 95% IC 0.318 to 0.90 EA&TLE vs 0.40, 95% IC 0.01 to 0.82 Ficoll) with HLA-DSA detection by LSA. Furthermore, FCXM assays performed with EA&TLE showed a lower detection threshold for class II HLA-DSA in comparison to FCXM assays performed with Ficoll (lowest mean fluorescence intensity of 5193 for EA&TLE vs 10289 for Ficoll). Conclusions: Implementation of EA&TLE for cell isolation from whole blood increased lymphocyte purity in the samples, improved FCXM assay sensitivity and resulted in a greater correlation with HLA-DSA identification by LSA.

W189. Intra-bone Bone Marrow Transplant from Human CD47 Transgenic Swine as an Approach to Tolerance in Nonhuman Primates

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Introduction: Tolerance will be essential to optimal clinical xenotransplantation. Mixed xenogeneic chimerism achieves T cell, B cell and NK cell tolerance in human immune system mice. We aim to achieve mixed xenogeneic chimerism and tolerance using non-myeloablative intra-bone bone marrow transplantation (IBBMT) in baboons. Since pig bone marrow cells are rapidly destroyed by baboon macrophages, donors were transgenic swine expressing human CD47 (hCD47), which binds to SIRP-alpha on macrophages, transmitting a "don't eat me" signal. Methods: Four baboons (papio hamadryas) received IBBMT from hCD47/hCD55 transgenic GalT-KO swine following low-dose total body irradiation and thymic irradiation and induction with anti-CD20mAb and thymoglobulin. Maintenance immunosuppression was MMF, FK506, and anti-CD40mAb. Two animals (#3 and 4) also received anti-CD2mAb (LoCD2b) and cobra venom factor (CVF). 1-2x10^9 BM cells/kg were administered intravenously and intra-bone. Chimerism was determined by flow cytometry on peripheral blood, and stained for baboon CD45, pCD45, pSLA1, pSWC3a, pCD3, pCD4, and pCD8. Non-Gal anti-swine antibody levels, mixed lymphocyte reactions (MLR) and ELISPOT responses were followed. Results: Animals 1 and 2 demonstrated chimerism (>0.1% of $pCD45^{+}bCD45^{-}$) for < 48 hours. Animals 3 and 4 demonstrated multi-lineage chimerism for approximately 20-30 days. MLR and ELISPOT did not demonstrate donor hyporesponsiveness, but anti-swine antibody levels decreased in animal 3 from the pre-transplant levels. Studies in animal 4 are ongoing. Conclusion: Enhancing addition cell depletion with anti-CD2mAb and inhibiting with CVF prolonged the duration of porcine chimerism in baboons.

W190. Intra-clonal Diversity of Donor Specific B Cell IgH is Associated with Pathogenic Allo-immunity

Marilia Cascalho[1], Matthew Cusick[1], Mayara de Mattos barbosa[1], Mona Doshi[1], Evan Farkash[1], Nicholas Lesniak[1], Jeffrey Platt[1] and Eline Prak[2]
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Donor-specific antibodies (DSA) are thought to be pathogenic as DSA correlate with risk of rejection and graft loss. However, recipients with DSA often do not reject at time of detection and conversely antibody-mediated rejection is often detected in recipients lacking appreciable DSA. This could reflect two confounding processes (i) non-pathogenic DSA and (ii) DSA absorbed to grafts. We reasoned that sequencing Ig variable region genes (IgH) on donor-specific B (DSB) cells, to assess clonal evolution might offer insights to distinguish innocuous from pathogenic responses. Donor-derived primary fibroblast lines and recipients PBMCs were obtained before and after transplantation. DSBs were isolated by panning PBMC using fibroblasts as targets. DSBs specificity were verified by antibody binding to donor, self and third-party cells. IgH sequences from individual DSB were obtained by NGS. Before transplantation, all 10 unselected recipients had DSB; 3.1 DSB in 10^4 blood B cells, on average. DSBs increased 6-12M after transplantation by an average of 24-fold in recipients not given induction and by 80-fold in recipients given induction. DSB IgH sequences of recipients with allo-immunity accumulated SHM and the intra-clonal distribution of SHM was notably skewed owing to effective selection. DSB IgH in recipients lacking any evidence of allo-immunity had decreased or unchanged SHM and unselected intra-clonal distribution of SHM. Our results indicate that ascertaining the properties of DSB could make it possible to assess pathogenic DSB before rejection. We propose that increased intra-clonal competition favors healthy outcomes while decreased competition owing to clonal narrowness favors disease (Figs. A-B).

W191. The Role of Recipient γδ T Cells in Graft Rejection After Human Intestinal Transplantation
Nathan Suek, Zhou Fang, Kevin Crosby, Katherine Long, Tyla Young, Zicheng Wang, Constanza Bay Muntnich, Alaka Gorur, Wenyu Jiao, rebecca Jones, Qi Yan, Yufeng Shen, Prakash Satwani, Joshua Weiner, Mercedes Martinez, Tomoaki Kato, and Jianing Fu
Columbia University

The role of γδ T cells in intestinal transplantation (ITx) is unclear. We performed phenotypic and clonal tracking of recipient-derived γδ T cells after human ITx in blood and intestinal grafts by integrating flow cytometry and sequencing platforms. We previously demonstrated that donor T-cell blood macrochimerism (peak level ≥4%) is associated with less rejection and slower replacement of donor T-cells in the intestinal graft by the recipient. With γδ T cells, we found that, regardless of macrochimerism status, turnover dynamics were more rapid in patients with younger donors. Graft-repopulating recipient γδ T cells showed effector (Teff) phenotypes early post-Tx and gradually acquired resident-memory (TRM) phenotypes with "private" non-Vγ9δ2 clonotypes. Single-cell profiling of recipient γδ T cells from 2 quiescent and 4 rejecting intestinal mucosal specimens late post-Tx were enriched for interchangeable Teff/TRM clusters, with higher cytotoxic Teff gene expression during rejection. In one patient, the top-dominant Vδ2 sequence (mainly Vγ5δ2) in the blood during quiescence was found at low frequencies in early quiescent graft samples but as the top-dominant sequence in later rejecting samples, suggesting active exchange of γδ T cells between the blood and graft during rejection. TCR distance analysis using the tcrdist3 algorithm suggested that this top-dominant sequence unlikely recognizes MICA or CD1d, given high distance scores (>150) from MICA- or CD1d-specific TCRδs, but has structural similarity with some TCRδs apparently stimulated by cytokines produced by autologous αβ T cells, with distance scores as low as 48. Taken together, recipient γδ T cells may affect ITx outcomes.

Systems Immunology

Th179. Single-cell TCR α/β and TCR γ/δ Immune Receptor Analysis and Immunotyping in a 96-well Plate
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This study describes an easy-to-run, low-throughput, single-cell immune profiling methodology by FACS sorting single cells in a 96-well plate to assess the clonotype repertoire diversity, paired-chain information, and cell phenotypes using a multiplex RT-PCR-based assay without any specialized equipment. This is an alternative cost-effective and convenient workflow to conventional microwell arrays or droplet microfluidics-based single-cell workflows. The 96-well plate is pre-loaded with primers for T-cell receptor (TCR) α/β or γ/δ, alongside 30 key T-cell markers. T cells from PBMCs are sorted in a single cell per well format. We conduct RT-PCR library prep using the DriverMap™ AIR Profiling Assay and sequence the CDR3 region. The results show clonotype frequencies, chain pairing details for α/β and γ/δ chains, and T-cell subtype classifications based on gene-expression profiling. This approach facilitates in-depth analysis of TCR gene rearrangements at the single-cell level, enhancing the understanding of T cell development, proliferation, and clonality, essential in studying cancer, immunodeficiencies, and autoimmune disorders. Additionally, we can effectively identify and characterize γδ T cells by integrating single-cell TCR sequencing with RNA sequencing data to potentially develop γδ cancer immunotherapies. In summary, this method provides a cost-effective and convenient workflow to analyze paired clonotypes and immunophenotypes in a single 96-well plate assay.

Th180. Single-cell Transcriptomes Reveal Unique CD8 T Cell Atlas Induced by Cytomegalovirus Viremia in Kidney Transplant Recipients

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Cytomegalovirus (CMV) infection is a risk factor for graft loss and mortality in solid-organ transplant and impacts the phenotype and function of T cells in kidney transplant recipients (KTRs). We performed single-cell RNA-sequencing of blood CD8 T cells from CMV seropositive (S/P) and seronegative (S/N) KTRs at Baseline (BL, 3-mo post-Tx), 1wk post-CMV viremia (1W), and long-term (LT, 1yr post-Tx), and propensity matched PCR- control groups to assess the temporal dynamics of CD8 T cells before and after PCR detection of CMV in KTRs experiencing CMV primary infection (S/N PCR^+) and reactivation (S/P PCR^+). CD8 T cells were markedly impacted by CMV serostatus and viremic episodes: late-differentiated cells were enriched in S/P patients at BL and LT, and at 1W S/N PCR^+ KTRs were separated from S/P PCR^+ KTRs. Pseudotime trajectory analysis of CMV viremia revealed cells from S/N PCR^+ KTRs showed limited transcriptomic variation over-time with continuous enrichment in naïve and T stem cell memory phenotypes. Meanwhile, S/P PCR^+ patients have greater longitudinal transcriptomic changes consisting of early (1W) expansion of terminally differentiated, senescent-like CD8 T cells with restricted capacity for cell proliferation, cell cycle progression, protein translation but high cytotoxicity, related functions needed for acute viremic control. Primary infection also resulted in poor CMV recall response post-Tx compared to reactivation. Our results suggest that CMV memory response can be difficult to establish and maintain after primary infection under immunosuppression, and strategies such as CMV vaccine could be helpful to induce pre-Tx CMV specific immunity and prevent CMV disease.

Th181. Spatially Exploring RNA Biology in Archival Formalin-fixed Paraffin-embedded Tissues

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[1]Yale University, [2]Children's Hospital of Philadelphia

Spatial profiling of different RNA species throughout the life cycle in formalin-fixed paraffin-embedded (FFPE) tissues holds transformative potential for pathology research. Herein, we develop Patho-DBiT that combines in situ polyadenylation, deterministic barcoding in tissue using microfluidic devices, and computational innovations for spatial whole transcriptome sequencing in clinically archived FFPE samples. Taking advantage of the inhibitory impact of formalin fixation on endogenous endonuclease activity, Patho-DBiT even outperforms fresh frozen tissue spatial transcriptomics and further allows for the profiling of a full spectrum of RNA species. Patho-DBiT permits spatial co-profiling of gene expression and alternative splicing, unveiling region-specific isoforms in the mouse brain. High-sensitivity transcriptomics is constructed from 5-year archived T-cell lymphoma tissues, with cross-validation conducted using super-resolution spatial phenotyping technology (CODEX). Furthermore, genome-scale single nucleotide RNA variants are captured to autonomously distinguish malignant from non-malignant cells in human B-cell lymphomas. Patho-DBiT also enables spatially resolved co-profiling of large and small RNAs, facilitating the analysis of a microRNA-mRNA regulatory network within clinical biopsies and elucidating their roles in tumorigenesis. With superior intronic read capture efficiency, Patho-DBiT spatially mapped RNA splicing dynamics associated with the developmental trajectory of tumor B cells. High resolution Patho-DBiT with a 10-μm spot size reveals the heterogeneities of human lymphomas within a spatial neighborhood and traces the spatiotemporal molecular kinetics driving tumor progression at the cellular level. Patho-DBiT represents a first-of-its-kind technology, enabling the spatial exploration of rich RNA biology in FFPE tissues to aid pathology diagnosis.

Th182. Target Identification by Comparative Analysis of Gene Association Networks Using Single Cell RNA Sequencing Data

Nima Nouri and Virginia Savova
Sanofi

Single-cell RNA sequencing (scRNA-seq) data has revolutionized our understanding of systemic perturbations to organismal physiology, revealing gene expression at the level of individual cell types. However, despite the increased information content and dimensionality of single-cell data, the relevance of genes to a perturbation is still commonly assessed through differential expression analysis. This approach provides only a one-dimensional, high-level perspective of the transcriptomic landscape, risking the oversight of tightly controlled genes characterized by modest changes in expression but with profound downstream effects due to strong connectivity with other genes (e.g., transcription factors or signaling molecules). In this study, we develop a novel in silico quantitative method to identify perturbation-relevant genes. In the context of disease, such genes may be interpreted as possible drivers of disease phenotypes and/or valuable targets for therapeutic intervention. Our approach, Gene Expression Network Importance eXamination (GENIX), is a gaussian graphical-based model that infers cell-type-specific gene association networks to uncover condition-relevant gene programs and target genes through comparative analysis. To demonstrate the effectiveness of GENIX, we utilize a publicly available dataset. In particular, we analyze influenza vaccine-induced immune responses in peripheral blood mononuclear cells (PBMCs) collected longitudinally from recovered COVID-19 male and female patients. This analysis recapitulates and sheds light on the mechanistic underpinnings of gender differences in response to immunization. In summary, our methodology represents a promising avenue for identifying novel targets that could assume pivotal roles in systems biology, thereby expanding the scope of perturbation analysis and target discovery beyond differentially expressed genes.

Th183. The Futuristic Cure of Type 1 Diabetes

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Problem: Type 1 Diabetes is an autoimmune disease where Beta cells are destroyed resulting in life long dependency on insulin therapy and blood sugar monitoring. At the terminal end of the disease spectrum of Type 1 Diabetes— when there is already a substantial damage to multiple organs such as in eyes and kidneys, the patient is offered Islet cell transplant as a potential cure for Type 1 Diabetes. The cure in fact is traded with iatrogenic complications by introducing immune suppressive drugs to preserve functioning of fragile islet cells. The immune suppression of the host expose him to increased risk of infection and cancers. Islet cells often don't survive long enough. This requires repeated islet cell infusion potentially in portal veins that contribute to adverse effects such as Induction of instant Blood-Mediated Inflammatory Reactions (IBMIR). This happens as a result of activation of thrombosis and complement pathways. This is the sole reason for post-transplantation islet loss with its adverse effects. Solution: New approach to cure Type 1 Diabetes and its vascular complications are based on targeting amplifying loop of C3 that control 80% of complement activation that cause immune pathogenesis of Type 1 diabetes and islet cell transplant failures. Endocrine Technology has developed a small molecule that targets immune- inflammatory aspects of Type 1 diabetes. Appropriate formulation and clinical trials are needed for futuristic cure of Type 1 Diabetes.

Th184. The Influence of Age and Alzheimer's Disease Risk on Immune Cell Gene Expression Trends and T Cell Clonal Expansion

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Columbia University

Age is the biggest risk factor for Alzheimer's disease (AD). While efforts to investigate immunological changes in aging and age-related disease are accelerating, comparatively few studies have comprehensively catalogued immune phenotypes across the adult lifespan. Understanding changes to immune cell types, gene expression, and antigen specificity over time may help identify early signs of pathology in the blood. We performed single-cell RNA sequencing (scRNA-seq) paired with single-cell T cell receptor (TCR)-seq and generated >200,000 immune cell transcriptomes from a genotyped multi-ethnic sample of 100 individuals ages 29-80. We investigate T cell clonal expansion, the abundance of common and rare immune cell types and the interactions between them, differentiation trajectory, and gene expression changes across the lifespan. We also explore the influence of individual AD risk variants and overall genetic risk for AD on these omics data. Our findings are uniquely positioned to reveal early immunophenotypic changes linked to genetic risk for AD, prior to the onset of dementia. We therefore detect age-related transcriptomic and T cell repertoire changes that may arise during the ideal therapeutic window for preventive strategies against neurodegeneration, enabling further opportunities to delay onset or slow progression of age-related neurological disease.

Th185. Unraveling the Dynamics of Time, Division, and Cell Cycle with Primary Human T Cell Differentiation in Drug Treatment and CAR-T Models Using Single-cell Proteomics

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Stanford University

Cell cycle (CC) progression and division are tightly linked to cell fate decisions across development of the hematopoietic and immune systems. Activated T cells enter the CC and rapidly proliferate into a deluge of cellular phenotypes with diverse functional capacity, which is crucial to establish protective immunity. Using Cytometry by Time of Flight, we combine high-dimensional proteomics, CFSE-derived division id annotations, and trajectory inference to computationally reconstruct CC timing across multiple generations in millions of asynchronously dividing primary human T cells. Leveraging our highly-resolved model of CC dynamics across divisions, we deconvolve the interplay between time since activation, CC, and division with cell state in *ex vivo* T cell expansion. We further characterize the link between CC and T cell receptor (TCR) stimulation by targeting CC and TCR signaling in drug perturbation experiments, and studying a tonically signaling CAR-T cell model of exhaustion. CC slowing with CDK4/CDK6 inhibition, and signaling inhibition by irreversible interleukin-2–inducible kinase (ITK) blockade both induce fate-skewing and proliferative effects. CC slowing partially explains the effects of ITK blockade, with combination treatment having mixed additive and antagonistic effects on T cell state. Finally, tonically signaling exhausted CAR-T cells have more aberrant CC states and skew in late SG2/G2M phases. We propose that our integrated experimental and computational technology generates a highly-resolved model of CC timing across divisions that enables granular study of the link between cell fate with CC and division for use in T cells and other cell systems.

Tu182. A Structured Schema to Represent Human Immunological Studies

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Immunological studies yield increasingly extensive and detailed datasets, making capturing and storing this data in a well-structured way, accessible for both humans and machines, essential. The Human Immunology Project Consortium (HIPC) [PMID: 23648045] has been committed to enabling the reproducibility and reuse of its findings since inception, and a dedicated Coordinating Center (HCC) is now tasked with the creation, maintenance, and expansion of a publicly accessible online resource, ImmuneSpace (immunespace.org). The ImmuneSpace platform has recently been transformed and expanded to include a centralized Knowledgebase and application programming interface (API) to promote utilization by the greater scientific community. ImmuneSpace builds on the data model developed by the Immunology Database and Analysis Portal (ImmPort) [PMID: 29485622] but implements additional standardization following the HIPC Data Standards initiative [PMID: 22343568, 26861911, 31272390, 32283555] and multiple ontology-based community data standards to support the consistent use of terminology and promote data compatibility. Upon transfer into the ImmuneSpace Knowledgebase, the data's internal consistency is validated, and terms are standardized using uniform ontology-based labels. Study components stored in different repositories, un-parsed raw data, and computationally inaccessible study elements are curated and integrated. The study data is restructured to generalize the components found across all human immunology studies to be easily translatable into a graph-based format. In addition, ImmuneSpace provides multi-omics modules and signatures that result from systems-level profiling studies [PMID: 28842433, 36266291]. The HIPC Project and ImmuneSpace platform verify the feasibility and huge benefit of a structured approach to representing human immunological studies in deciphering system-level phenomena.

Tu183. Accelerating Target Discovery in Immunology Using Target Immune Engine

Cora Ames, Travis Ahn-Horst, Nadine Biesemann, Corneliu Bodea, Clement Chatelain, Emanuele de Rinaldis, Giorgio Gaglia, Johanna Harrison, Shameer Khader, Samuel lessard, Dongyu Liu, Hamid Mattoo, Nima Nouri, Franck Rapaport, Leonardo Rodrigues, Virginia Savova, Heming Xing, and Lu Zhang
Sanofi

The exponential growth of biological data offers unprecedented opportunities for understanding and discovering new treatments for immune-mediated diseases, but these can only be realized with a strategy that can seamlessly analyze and integrate multiple data layers. To meet this challenge, we have developed the Target Immune Engine (TIE), a machine-learning-based framework for integrated analysis of several orthogonal data layers, and automated prioritization of novel immune drug targets. TIE integrates for each gene of the human genome and across nine immune conditions (Asthma, Atopic Dermatitis, COPD, Crohn's Disease, Psoriasis, Rheumatoid Arthritis, Systemic Lupus Erythematosus, Type 1 Diabetes, and Ulcerative Colitis), a large set of orthogonal data layers extracted from over 1000 studies, 500,000 samples, and 1400 features, totaling 135 million data points. These include genetic evidences from genome-wide associations and expression quantitative loci, single-cell and bulk transcriptomics data, proteomics data, along with functional information, druggability and gene-disease association from knowledge graph. Through combined use of machine-learning algorithms informed by retrospective clinical data and a rule-based weighting system, TIE implements automated target assessment scores, enabling discovery of novel target hypotheses that would be hidden through single-layer data analysis. In this presentation, we will introduce the general data and methodological framework, along with its target predictive performance and a few examples of novel target candidates.

Tu184. Analysis of Healthy Adult Immunotypes Reveals Young Female Cohort with Elevated Inflammatory Response to Truculture Stimulation

Megan Smithmyer[1], Alex Hu[1], Matthew Dufort[1], Alyssa Ylescupidez[1], Alice Wiedeman[1], Carolina Acosta-Vega[1], Sheila Scheiding[1], Grace De Castro[1], Thomas Bumol[2], Lynne Becker[2], Troy Torgerson[2], Xiaojun Li[2], Qiuyu Gong[2], Claire Gustafson[2], Jane Buckner[2] and Cate Speake[1]
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The frequencies of many circulating immune cells are stable over time but vary between healthy individuals, suggesting the presence of diverse stable immunotypes. The relationship between immunotype and immune response kinetics and character is poorly understood. We measured circulating immune cells in a cohort of 100 adults with no history of chronic disease, aged 25-35 and 55-65. These individuals were followed approximately every three months for two years, allowing us to characterize between- and within-subject variation in immune cell frequency. We identified cell populations with an intraclass correlation coefficient above 0.5, and used them to cluster individuals revealing four immunotypes dominated by older adults (n=30), younger adults (n=19), CMV^+ adults from both age cohorts (n=31), and, surprisingly, a group of young female participants (n=20). This cluster was defined by low levels of NK cells and high levels of naïve $CD4^+$ T cells and central memory $CD8^+$ T cells. Compared to other young adults, they had elevated levels of neutrophils and basophils. To investigate the relationship between immunotype and immune response, we performed RNAseq on whole blood samples stimulated with LPS, SEB, PolyI:C, R848, and $IL1\beta^+TNF\alpha$. We found that participants in the young female cluster had elevated inflammatory responses to LPS, $IL1\beta^+TNF\alpha$, and R848. These findings highlight the diversity of immunotypes and their role in driving immune responses even among healthy adults and may be useful in understanding the influence of sex, age, and immune history in response to infection.

Tu185. CyNET- a Network Analysis Framework for Holistic System Level Analysis of Immune Response

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Modern flow-cytometer and mass-cytometer (CyTOF) allow deep immune phenotyping and identification of various immune cell subsets in an unbiased manner. The established statistical procedures are used to find the perturbed subsets in comparative pathophysiological conditions. However, approaches that consider immune response at system levels are lacking. In real in-vivo conditions, various immune cell subsets interact and influence each other's functions. The coordinated interactions between these cell subsets determine whether the outcome is a normal physiological state or pathological condition. Established statistical procedures completely ignore the interactions between subsets and rely on statistically significant changes in the frequency of cell subsets. We developed CyNET (Cytometry Network)— an analysis platform based on a network science approach to understand the immune system holistically. In CyNET we quantified the systems level property and subsets level property. We used the CyNET to analyze the immune development with age. Peripheral blood cells from Healthy newborns (cord blood), adults (20 to 55 years), and old (70 years and above) human subjects were further used for validation using single-cell transcriptomics. We found that network edge density, degree centralization, and assortativity score reflect the maturation and development of the immune system at the age axis. Change in the centrality of the nodes (Immune subsets) better reflects the biological function than changes in frequency. In conclusion, our network analysis approach enables the summarization of immune response holistically and allows the identification of key or hubs of the immune cell network

Tu186. Data Integration Across Different Mass Cytometers: Implication in Data Sharing and Re-use for Discovery

Nicholas Khoo[1], Martin Wasser[2], Katherine Nay Yaung[2], Dianyan Guo[2], Su Li Poh[2], Judith Wong[2], Thaschawee Arkachaisri[3], Joo Guan Yeo[2] and Salvatore Albani[2]
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Recently, emphasis has been placed on the acquisition of Big Data using multi-parametric cytometer to study complex diseases, and their sharing through web portals such as ImmPort for integration and reuse for novel discovery. However, issues such as batch effects hinder this process and strategies for its mitigation lacking. To address this unmet need, we interrogated multiple vials of cryopreserved peripheral blood mononuclear cells (PBMCs) from a single preparation of an adult healthy blood donor (technical replicate, n=10) and healthy volunteers (children and adults, n=52) over six months using two different mass cytometers (Helios and XT). A standardised operating procedure (SOP) for thawing, stimulation and staining was performed. Batch-scaling, clustering and depiction of the degree of similarities of the cell frequencies between the technical replicates using t-SNE and PCA were done. With batch-scaling, our technical replicates (n=10) clustered in close proximity with no data segregation by cytometer (Helios or XT) used. Additionally, the frequencies of major immune lineages such as $CD4^+$ (Coefficient of variation, CoV: 8.0%) and $CD8^+$ (CoV: 4.6%) across both cytometers showed an acceptable CoV, indicative of data reproducibility. Importantly, our healthy donors' data (n=52) segregated by age with a distinct age gradient with the healthy adults clustered closer to the technical replicates. Our findings suggest that the concerns regarding data integration from various cytometers can be mitigated by using batch-scaling with a SOP to handle experimental batch effects. Translationally, repurposing and integrating Big Data to boost statistical power with larger sample sizes can hasten novel discovery.

Tu187. Development of NULISAseq™ Inflammation Panel 250 for Comprehensive Immune Profiling

Xiao-Jun Ma, Ishara Ariyapala, Joanne Beer, lei Fang, Wei Feng, Qinyu Hao, Sean Kim, Dwight Kuo, Anna Lee, Yuling Luo, Vasudha Murlidhar, helena Sun, Ming Yu, and Bingqing Zhang
Alamar Biosciences

Comprehensive profiling of cytokines and chemokines in blood can provide deeper insights into the mechanisms underlying this highly complex and heterogeneous group of diseases. However, many of these proteins are present at very low concentrations in plasma, below the limit of detection of current immunoassays. We recently developed an automated multiplex immunoassay technology, NULISA™, capable of attomolar-level sensitivity and high levels of multiplexing, and we demonstrated the performance characteristics of a 200-plex assay and its correlation with existing platforms1. Here we report the development of an expanded inflammation-focused panel called NULISAseq™ Inflammation Panel 250 targeting 200 cytokines/chemokines and their receptors and 50 other important inflammation and immune response-related proteins. The assay demonstrated high sensitivity, detecting 96.4% of the targets in at least 50% of plasma samples, and high precision, with median intra-plate CV of 8% and inter-plate CV of 11%. It also demonstrated the ability to quantify proteins of a wide dynamic range without diluting samples. Comprehensive testing for target specificity demonstrated that 93% of the assays in the panel had < 1% cross reactivity to nonspecific targets. In summary, with ultrahigh sensitivity and the most comprehensive coverage of inflammatory cytokines/chemokines, the Inflammation Panel 250 assay provides a powerful discovery tool for inflammation and immunology research at the systems level, which may lead to new diagnostic biomarkers and therapeutic targets. 1. Feng, W. et al. NULISA: a proteomic liquid biopsy platform with attomolar sensitivity and high multiplexing. Nat Commun 14, 7238 (2023).

W192. Epigenetic Signature and Key Transcriptional Regulators of Human Antigen-inducible Regulatory T Cells

Alma-Martina Cepika, Laura Amaya, Rosa Bacchetta, Howard Chang, Pauline Chen, Michelle Mantilla, Maria Grazia Roncarolo, and Colin Waichler
Stanford University School of Medicine

Regulatory T (Treg) cells are essential to immune tolerance. Classical Tregs arise in the thymus and are skewed to recognize self-antigens, while type 1 Tregs (a.k.a. Tr1 cells) arise from peripheral $CD4^+$ T cells in response to extra-thymic antigens, such as alloantigens or allergens. We can recreate Tr1 cell differentiation *in vitro* to rapidly make clinical-grade Tr1 products for induction of antigen-specific tolerance. Alloantigen-specific Tr1 cells are already in phase I/Ib clinical trials in allogeneic hematopoietic stem cell transplantation (allo-HSCT), with promising early results. However, the identity of lineage-defining transcription factors (TFs), which regulate the differentiation, phenotype, and functions of human antigen-specific Tr1 cells, remains unresolved. Here, we first demonstrated that human antigen-specific Tr1 cells are clonally and transcriptionally distinct from classical Tregs and conventional $CD4^+$ T cells on a single-cell level. Next, we identified a TF motif signature unique to antigen-specific Tr1 cells, and predicted that IRF4, BATF, and MAF act as Tr1 lineage-determining TFs. The roles of these TFs in regulating various aspects of Tr1 differentiation, function, and phenotype were validated using functional genomics. Finally, we used the Tr1-specific TF signature to identify Tr1 cells in peripheral blood T cells of healthy donors and recipients of allo-HSCT and adoptive Tr1 cell therapy, and in T cells from solid tumors. Identification of the TF signature and key transcriptional regulators of human antigen-specific Tr1 cells will guide the development and optimization of Tr1 cell therapies, identification of human Tr1 cells *in vivo*, and mechanistic studies of Tr1 cell biology.

W193. High-dimensional Spectral Cytometry Panels for Fixed Whole Blood Immune Phenotyping

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Many clinical trials and translational studies involve a high number of blood samples that cannot be systematically collected and delivered to research facilities in time for immediate analysis. This may be due to geographical distance or challenging timings of the sample collection. For that reason, the fixation of whole blood before frozen storage for later analysis can be a choice of interest. Another advantage is that in this way, samples could be analyzed at the same time avoiding potential batch effects. One important limitation is that not all immunological assays are adapted for the treatment of fixed samples, including high-dimensional flow cytometry specifically developed for fresh blood samples. Recently, we developed two high-dimensional spectral flow cytometry panels that allow deep characterization of innate and adaptive whole blood immune cells (35 and 34 fluorescent markers, respectively) and standardized the protocol for sample handling, staining, acquisition and data analysis. This permitted the reproducible quantification of up to 200 immune cell phenotypes through standardized immunophenotyping at a single site applied to the Milieu Intérieur cohort and patients with diverse autoimmune diseases. In this work, we have adapted these two panels for whole blood samples fixed with the Cytodelics Whole Blood Cell Stabiliser, for preserving cellular proteins for later analysis. With the help of EasyPanel version 2 software, new antibody clones were chosen and tested for application in fixed blood samples. The new panels were tested in whole blood of healthy donors and samples from patients with diverse autoimmune diseases from our previous study.

W194. Latent Cytomegalovirus Infection Is Associated with Distinct Single Cell Immune Signatures and Diminished Anti-viral Responses in Older Adults

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Cytomegalovirus (CMV) is one of the most common chronic viral infections worldwide. Although largely asymptomatic, it is known to be associated with worse outcomes of respiratory infection and vaccine responsiveness in older adults. To date, no systemic studies have described CMV seropositivity-related transcriptional signatures at the single-cell (sc) level in both resting and *ex vivo* stimulated peripheral blood mononuclear cells (PBMCs). In this study, we employed scRNAseq to investigate the impact of CMV seropositivity on PBMC composition, transcriptional profiles, and cellular responses within an age, sex, and race-matched cohort of healthy adults aged 60 and older (n=27). Our findings confirmed known CMV-associated cell compositional changes, such as the expansion of the $CD8^+$ T effector memory compartment including TEMRAs and $GZMK^+$ cells, and revealed CMV-associated expansions in other populations including age-associated B cells, gamma-delta (γδ) T-cells, and CD56dim NK-cells. Additionally, we uncovered elevated expression of cytotoxicity related genes (GNLY, PRF1, NKG7) in CMV seropositive individuals (n=14) compared to CMV seronegative donors (n=13) in γδ T-cells, TEMRAs, $GZMK^+$ CD8, and CD56dim NK-cells. Interestingly, upon *ex vivo* stimulation with live influenza virus, cells from CMV^+ donors had significantly reduced antiviral responses compared to CMV- donors across all immune subsets, suggesting that CMV infections might negatively affect viral immune resilience. As a whole, our comprehensive single-cell level analysis sheds new light on the complex interactions between CMV and the aging host immune system and the immune responses to the influenza virus.

W195. Modeling the Effect of Immune Cell Infiltration on Tissue Function Reveals Cellular Rewiring Driving Human Thyroid Autoimmunity

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Thyroid autoimmunity is the one of the most common autoimmune disorders, in which the gland can become totally dysfunctional due to infiltration of immune cells. Yet, we currently lack a comprehensive understanding of the underlying processes that lead to functional impairment. Here we leveraged inter-patient variability to investigate how the cellular ecosystem changes during thyroid autoimmunity. We generated an unprecedented single-cell RNA sequencing atlas paired with T- and B- cell receptor (TCR/ BCR) repertoires and measurements of 204 surface proteins (CITE-seq) of human thyroid (375,337 cells) and paired blood (228,739 cells) generated from patients with Hashimoto's thyroiditis (n=8), Graves' disease (n=9) and control patients (n=6). Our analyses revealed a total of 89 immune, stromal, and epithelial cell states in the thyroid, including a unique epithelial cell population we termed "immuno-thyrocytes". Immuno-thyrocytes upregulates immune-modulating and co-inhibitory genes and their frequency is correlative with gland functionality. We pinpointed specific CD8 T cell subsets that interact with immuno-thyrocytes. TCR clonal analysis revealed CD8 T cell subsets homing from the blood into the thyroid and defined a T cell differentiation trajectory for the most expanded tissue CD8 T cell clones. We identified additional critical players sustaining autoimmune disease, such as germinal center B cells, follicular helper CD4 T cells and inflammatory fibroblasts and modelled their co-dynamics. Finally, we propose a novel categorization of thyroid autoimmunity patients based on the level of immune cell infiltration rather than classifying them based on their autoimmune disease, which may prove to be relevant to other autoimmune disorders.

W196. Peripheral Immune Signatures and Patient Stratification in Osteoarthritis: Application of Machine Learning Approaches to High-dimensional Immune Cytometric Data

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Osteoarthritis (OA) is a prevalent joint condition that is especially common among older people. It is recognised that OA pathogenesis involves a complex interplay of joint damage, poor cartilage regeneration, and inflammation. In addition to the lack of approved disease-modifying drugs available for OA, clinical management is complicated by variable disease progression and limited treatment response. This suggests disease heterogeneity with subtypes of different disease trajectories and treatment responsiveness. We employed mass cytometry time-of-flight (CyTOF) to investigate peripheral blood mononuclear cells (PBMCs) obtained from patients with knee OA (n = 57) and matched healthy controls (HCs) (n = 33). Using immune clusters derived from the high-dimensional CyTOF data, a combination of statistical and machine learning algorithms was used to extract disease-associated peripheral immune signatures for patient stratification. We identified an inflammatory OA subtype, characterised by enrichment of monocytes and effector T/T-like cells subsets, associated with increased pain, stiffness, poorer function. Furthermore, we also identified another group of patients exhibiting milder pain and stiffness where their immune profile suggests immune-modulation as seen in the increase in Treg and exhausted Th immune subsets in their peripheral immunome. In conclusion, our application of machine learning approaches to high-dimensional CyTOF data identified OA subtypes of distinct clinical phenotype and disease-associated peripheral immune signatures. By uncovering the underlying heterogeneity of peripheral immunome changes in OA, our findings may have applications as a precision medicine tool in OA management where individualized therapeutic decisions can be made according to patient's peripheral immunophenotype.

W197. Single-cell Multiomic Evaluation of Mechanisms Driving Dysregulation of the Adaptive Immune System in Preclinical Autoimmune Disease State

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Background. A loss of tolerance to self-antigens leads to increased levels of autoantibodies against nuclear components (ANA) prior to clinical disease onset. Only 4-8% develop autoimmune disease. Patients with incomplete lupus erythematosus (ILE) exhibit some clinical symptoms with most never progressing to Systemic Lupus Erythematosus (SLE). This study investigates whether alterations in lymphocyte populations and activation of cellular processes affect preclinical autoimmunity development. Methods. PBMCs from 64 subjects, divided evenly among disease groups: healthy (ANA-), healthy with autoantibodies (ANA^+), ILE, SLE, were used for 5'scRNA-seq/137-plex Total-seq, BCR/TCR repertoire to identify disease-associated clusters, differential gene signatures, dysregulated pathways. Serum soluble biomarkers levels were obtained via Olink Proximity Extension Assay (Explore HT). Results. We obtained profiles for ~650,000 cells across all PBMCs. Analysis of differentially expressed genes in T, B cells revealed importance of metabolic processes - oxidative phosphorylation, autophagy, mitochondrial dysfunction and dysregulation of MAPK signaling with disease progression. Cytokine storm signaling was downregulated in T cells of ANA^+. These findings were confirmed by protein. We also identified a cytotoxic $CD4^+$ T cell population in individuals with higher levels of IFN genes and increased antiviral response. VDJ analysis indicates cytotoxic T cells have largest fraction of expanded clonotypes, associated with higher expression of TXNIP, TMSB4X, HLA, GZMB and shared among ANA^+ and ILE individuals. Conclusions. These findings suggest that alterations in the adaptive immune system, in particular dysregulation of signaling in T and B cell activation related to changes in metabolic and apoptotic pathways play important role in preclinical autoimmunity trajectory.

W198. Single-cell RNA-seq Analysis of PBMCs of Three Cameroonian Populations Reveals Differences in Cell Type Distribution as Well as Differentially Expressed Genes

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Current understanding of the human immune system is mostly based on studies of individuals of Eurasian ancestry in westernized settings. Sub-Saharan African populations with great genetic diversity are underrepresented, which leads to gaps in our understanding and treatment of immune-based diseases in individuals of African ancestry. To address this disparity, we performed single-cell RNA-seq analysis on unstimulated and stimulated peripheral blood mononuclear cells (PBMCs) of 160 individuals from three diverse indigenous populations in Cameroon: the Baka/ Bagyeli rainforest hunter-gatherers (RHGs), Fulani pastoralists and Tikari agriculturalists. First results of our study reveal differences in cell type distribution at baseline, for example an expanded cytotoxic T cell population in the RHGs, who live in a high pathogen environment, compared to the Fulani and Tikari. Additionally, we identify quantitative and qualitative differences in differentially expressed genes (DEGs) in PBMCs. For instance, in age-associated B cells at baseline we find 187 DEGs between RHGs compared to Tikari compared to 104 DEGs between Fulani and Tikari. The specific DEGs with the greatest fold change differ, between RHGs and Tikari - LGMN is upregulated in the RHGs, whereas between Fulani and Tikari TMEM163 is upregulated in the Fulani. Our findings delineate variation in immune cells at baseline between populations and emphasize the need to take ancestry into consideration when treating individuals with immune diseases or using immune-based therapeutics. We are currently investigating the genetic variants that contribute to this expression variation by linking epigenetic features (ATAC-seq) with gene expression in the same cell (RNA-seq) in PBMCs.

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