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ABSTRACT SUPPLEMENT

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Allergy

Th100. Characterization of a Novel Casein-specific Anaphylactic Mouse Model

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Rationale: Cow's milk allergy, caused by ingestion of proteins such as casein, is one of the leading causes of anaphylaxis in children. Mouse models that mimic anaphylaxis in humans are needed to evaluate potential new therapies for food allergy. Since severe eczema is associated with food allergy in children and may be the portal of sensitization, we established a mouse model in which the mice are transdermally sensitized. Methods: Six- to eight-week-old male and female C57BL/6 mice (n=3-5) were exposed via gently abraded skin to dissolved casein or PBS weekly for six weeks. Two weeks after the last sensitization, all mice were challenged intragastrically with 15mg of dissolved milk powder. The challenged mice were placed in an observation chamber with live and infrared cameras to assess clinical symptoms and skin surface temperature. Following sacrifice, tissue samples including spleen, mesenteric lymph nodes, Peyer's patches, inguinal lymph nodes, and axillary lymph nodes were assessed. Results: Following casein sensitization, we detected casein-specific IgE in all sensitized female mice which peaked on day 28. IgE+ B cells were significantly increased in the inguinal lymph nodes of sensitized female mice compared to controls. We also observed signs of moderate anaphylactic reactions including continuous scratching and decreased reactivity in the female casein-sensitized mice. Conclusion: These findings suggest that four weekly transdermal sensitizations were sufficient to induce systemic reactions in intragastrically challenged female C57BL/6 mice. This mouse model can serve as a preclinical model to determine the efficacy of potential treatments for food allergy.

Th101. Characterization of Novel Nematode Pan Allergen (NPA)

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In this study, we identified and characterized a pan allergen, a homologous molecule that is present in multiple nematode parasites with significant IgE reactivity. The molecule, WbNPA, was first identified from the human parasite, Wuchereria bancrofti by screening a phage displayed cDNA expression library of the microfilaria larval stages of the parasite with human monoclonal IgE antibodies prepared from individuals showing clinical symptoms of Tropical Pulmonary Eosinophilia (TPE), a hypersensitivity allergic reaction seen in subjects with W. bancrofti Infection. After five rounds of biopanning, 13 clones were picked and sent out for sequencing. Our results showed that the sequences belonged to a gene (Bm2855) that was not annotated previously. A phylogenetic tree analysis showed that Bm2855 is highly conserved among nematode parasites and showed over 95% similarity in several nematodes with no homology to the human. Therefore, we named this gene as Nematode Pan Allergen (NPA, accession # ON023112). Subsequent analysis showed that the NPA gene is highly expressed in the microfilaria stage and adult female stages of W. bancrofti compared to all other lifecycle stages. Similarly, sera samples from TPE, and microfilaremic subjects had high levels of antibodies against NPA compared to chronic pathology (CP), and endemic normal (EN) control subjects. Further characterization studies showed that WbNPA is a histamine releasing factor and can induce inflammatory cytokines from alveolar macrophages. Thus, we report here a novel pan allergen that is present in several nematode parasites, and can induce allergic inflammation.

Th102. derp2 and TLR2 Ligand Fusion Protein Ameliorates Allergic Immune Responses in derp2-sensitized Mice

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Derp2, an antigen of house dust mites, appears to induce excess T helper 2 (Th2) associated immune responses and thus results in allergic rhinitis and asthma. Toll-like receptors (TLR) and their signaling pathways have gained attention for their therapeutic potential against inflammatory diseases. This study aims to evaluate whether Derp2 and TLR2 ligand fusion protein (lipo-Derp2) is able to ameliorate allergic inflammation and investigate the therapeutic mechanisms of Derp2-induced allergic animals. First, the recombinant Derp2 and lipo-Derp2 as well as the Derp2 extract from house dust mites were recognized by anti-Derp2 antiserum. The lipo-Derp2 appeared to activate bone marrow-derived dendritic cells (BMDC) and release interleukin (IL)-12. Remarkably, early exposure to lipo-Derp2 led to significant suppression of the Th2-associated features in the mice receiving Derp2-sensitization. Most importantly, a supplement of lipo-Derp2 was able to reduce the allergic responses, including serum levels of Derp2-specific IgE and eosinophil infiltration in the lung lesions of Derp2-induced allergic animals. Moreover, there appeared to be an elevated serum level of Derp2-specific IgG2a and elicited IFN-γ and IL-17 responses in the mice receiving lipo-Derp2 treatment, indicating provoked Th1, Th17, or both immune responses. These findings suggest that the lipidated allergen platform has great potential in allergy prevention as well as therapy.

Th103. Histamine Receptors Control B Cell Germinal Center Responses and Production of Antigen-specific Ige

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Histamine is a potent downstream mediator in allergic reactions, and yet histamine receptors are also expressed on cells important for the development of allergic immunity, including T cells, dendritic cells, and B cells. Previous studies in animal models have demonstrated that histamine alters antibody production via Histamine Receptor 4 (H4R) on dendritic cells or H1R and H2R on T cells, but direct regulation of B cells by histamine is unclear. Here, we demonstrate that human and mouse B cells express H1R and H2R and that both receptors are required for competent specific antibody production to in vivo immunization with ovalbumin. The strongest impact was exerted over the regulation of IgE, with deficiency in both H1R and H2R rendering a complete loss of specific IgE and poor germinal center formation. B cell subsets were largely unaffected in H1R/H2R deficient mice, and adoptive transfer of congenic cells into immunodeficient recipients established that histamine exerts effects on both the B and T compartments for optimal induction of IgE responses in vivo. A B cell intrinsic defect in IgE was observed in H1R/H2R deficient B cells using in vitro assays of IgE production, and molecular analysis of these B cells showed defects in expression of genes involved in the germinal center response and cell survival. Collectively, these findings support a role for histamine, released during IgE-mediated activation, in regulating the further development of IgE and antigen-specific antibodies and sustaining the allergic phenotype.

Th104. Iggenix Antibodies Enable Less Frequent Administration and Rapid Protection Against Accidental Exposure, Addressing the Challenges of OIT in Peanut Allergy

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Current therapy for peanut allergy using OIT calls for daily ingestion of peanut protein which is associated with significant side effects. Successful OIT is a protracted process taking months to reach desensitization. These challenges hinder patient adherence to OIT protocols and discourage patients from seeking treatment. IgGenix is

developing a novel therapy employing engineered high-affinity IgG antibodies targeting immunodominant peanut proteins. IGNX001, a two-antibody combination (IGX-0107 and IGX-0109), was tested in a C3H/HeJ mouse model of orally-induced peanut allergy. Following sensitization with intragastric (IG) peanut powder + cholera toxin, mice were treated with IGNX001 (10mg/kg SC), vehicle or isotype IgG control. Forty-eight hours later, animals were challenged with 20mg peanut protein (IG). IGNX001 showed significant protection against anaphylaxis. Following peanut protein challenge, the maximal drop in rectal temperature was reduced from -2.34±0.46 (vehicle) to +0.11±0.18°C (IGNX001); anaphylaxis score fell from 3.0±0.27 (Vehicle) to 0.5±0.27 (IGNX001) and serum MCPT-1 levels decreased from 167±32 (vehicle) to 33±13ng/mL (IGNX001) (P< 0.001). Responses to isotype control IgG were not significantly different from vehicle. The pharmacokinetics of the two components of IGNX001 were evaluated over 28 days following SC administration in cynomolgus monkeys. IGX-0107 and IGX-0109 had half-lives of 15.9 and 11.2 days, respectively. Using conventional allometric scaling these data predict human half-lives of 33.6 and 23.6 days. IGNX001 promises rapid onset of action and extended period of protection against accidental ingestion of peanuts in allergic patients, overcoming the challenges associated with OIT protocols and offering an attractive option to individuals who might overwise avoid treatment.

Th105. Impact of Blood Specimen Age on Cell Reactivity and Possible Strategies to Implement Basophil Activation Testing in Multi-centric Studies

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Background. Basophil Activation Testing (BAT) is a flow cytometry functional approach that enables the ex vivo characterization of basophil reactivity against allergenic molecules. The main focus revolves around democratizing this tool. After having recently developed a streamlined approach to BAT, we wanted to compare two possible strategies for implementing BAT in multi-centric studies: to store blood before or after sample stimulation and processing. Method. Fresh heparin and EDTA blood samples were processed in parallel following two workflows: “collect, store, process & analyze” or “collect, process, store & analyze”. Storage times between 0 and 7 days at temperatures of 18-25°C or 2-8°C were considered. Basophil reactivity to anti-IgEs was assessed using CD45, CD3 and CD294 as gating markers and CD203c and CD63 as activation markers. Results. The “collect, store, process & analyze” workflow showed that blood can be kept at least 2 days at 18-25°C or 2-8°C before further processing without impact on basophil reactivity but significant upregulation of CD203c and slight CD63 decrease after 2 days of storage. The “collect, process, store & analyze” workflow demonstrated that blood can be processed and then kept at least 5 days at 18-25°C or 2-8°C before being analyzed with no significant impact on CD203c or CD63 expressions. Conclusions. The “collect, store, process & analyze” workflow remains a simplified logistical approach but depending on the time available between clinical centers and reference laboratories. The “collect, process, store & analyze” workflow can constitute a workflow improvement to provide significant flexibility.

Th106. Multigenerational Effects of Maternal Bisphenol S (BPS) Exposure on the Development of Experimental Asthma

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We have reported the effects of maternal exposures to bisphenol A (BPA) on the development of experimental asthma. BPS has been used as a BPA substitute, but the effects on immune development have not been examined. The purpose of this study was to test the hypothesis that maternal exposure to environmental estrogens (EEs), including BPS, enhances the allergic sensitization that underlies childhood asthma. Female BALB/c mice (F0) received 10 g/ml BPS in their drinking water from one week before pregnancy to the end of the study. The pups were sensitized with low doses of ovalbumin (OVA) or PBS control on a postnatal day (PND) 4. On PND 22, serum IgE anti-OVA levels were quantified, and airway inflammation and hyperresponsiveness were assessed by enumerating eosinophils in bronchoalveolar lavage fluid and response to methacholine. Non-sensitized female pups were saved and mated with non-exposed male mice at eight weeks of age for the next generation. The resulting pups were sensitized, and the asthma phenotype was examined up to F4. Pups exposed to BPS displayed an asthma

phenotype in response to their "suboptimal" sensitization. OVA-specific IgE, eosinophilic airway inflammation, and airway hyperresponsiveness were significantly increased in the F1-F3 pups derived from BPS-exposed F0 dams than in the pups derived from non-exposed dams. Maternal exposure to EE, including BPS, promotes the development of experimental asthma. The immune alterations may be epigenetically perpetuated, causing multigenerational effects.

Th107. Multiple Patterns of Binding Within the 63DPYSPOHSQDPYS72 Sequential Epitope on the Immunodominant Peanut Allergen Ara H 2

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In food allergy, polyclonal IgE recognition of sequential epitopes is highly prevalent; however, little is known about the specific clonal recognition of these epitopes. Greater diversity of sequential epitope recognition by allergen-specific IgE is associated with increased severity and persistence of food allergy. For peanut allergy, the sequential epitope 63DPYSPOHSQDPYS72, located on a loop region of Ara h 2 and is identified in the serum of over 50% of peanut-allergic patients. We hypothesize that antibodies recognize multiple patterns of binding within this region of the immunodominant peanut allergen Ara h 2. To investigate this hypothesis, we utilized twelve monoclonal antibodies cloned from peanut-allergic, oral immunotherapy-treated patients to characterize the binding patterns of antibodies to synthetic variants of 63DPYSPOHSQDPYS72. Using alanine substitutions, we were able to identify three distinct patterns of amino acid residues engaged in epitope-paratope interactions within the 63DPYSPOHSQDPYS72 loop region of Ara h 2. Of these three patterns of binding, the most common binding pattern involves the hydroxyproline. Not only do these three groups of antibodies have distinct epitope-paratope binding patterns, but they also inhibit IgE binding to polyclonal serum differently on ELISA. In conclusion, we have identified three distinct binding patterns within the loop region of Ara h 2 which influences their ability to functionally disrupt IgE-allergen interactions. Therefore, previously identified sequential epitopes instead contain multiple overlapping epitopes. These variations in epitope-paratope interactions and their functional relevance is an essential component of antibody-mediated tolerance in IgE-mediated food allergy.

Th108. Serological Assessment of Patients Undergoing Peanut Oral Immunotherapy: The Role of igg4

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Peanut oral immunotherapy (OIT) aims to increase the safety of individuals with peanut allergy by gradual exposure to small, measured amounts of peanuts. Many studies use a maintenance dose of 300mg to achieve clinical desensitization. OIT is limited by frequent dose-related adverse reactions, leading to participant withdrawals. Data on immunologic parameters associated with maintenance doses lower than 300mg are limited. Seventeen peanut-allergic children aged 1 to 17 years were enrolled in a trial (NCT03532360) of low or high-maintenance dose peanut OIT. Patients were randomized to 300mg (N=9) or 30mg (N=8) groups. Blood was drawn at three timepoints: oral food challenge, post-escalation challenge and exit challenge. Serum samples were used for quantitative detection of total peanut- and Arah2-specific immunoglobulin (Ig) E and IgG4 levels by ELISA. After a median escalation phase of 15 months, peanut-specific-IgG4 significantly increased from a median baseline of 148.55 µg/mL (IQR 68.95-243.41) to 989.09 µg/mL (IQR 392.55-1905.33) ($p< 0.05$) in the 300mg group and from 58.13 µg/mL (IQR 20.54-303.92) to 277.91 µg/mL (IQR 64.85-634.99) in the 30mg group ($p< 0.05$). Peanut-specific-IgE did not significantly decrease in

either group. The IgG4/IgE ratio significantly increased over the course of peanut OIT in both groups ($p< 0.05$). From the low-dose and high-dose groups, 6/8 and 6/9 subjects were clinically desensitized respectively. Both low-dose and high-dose OIT maintenance doses resulted in significant increases in peanut-specific IgG4. Our findings suggest that changes in IgG4, rather than IgE, better reflect clinical desensitization. Large-scale studies are required to establish the role of IgGs versus IgE in OIT.

Th109. Sialic Acids on House Dust Mite (HDM) Allergen, Der P 2, Shift Immune Responses Towards a Regulatory Profile

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House Dust Mite (HDM) allergy affects more than 50 million people worldwide and is one of the most important risk factors for the development of asthma. Available allergen immunotherapy which consist of administering incremental doses of HDM allergen over a period of 3-5 years to induce tolerance, is lengthy and carries a high risk of severe side effects. Safer therapeutic options that can rapidly induce tolerance are therefore warranted. One of such therapeutic options includes the use of carbohydrate-based protein conjugates to target immunomodulatory receptors on the immune cells that are involved in the development of allergy. Sialic acid–binding immunoglobulin-like lectin-9 (Siglec-9) is an immunomodulatory receptor found predominantly on immune cells. It is an important negative regulator of acute inflammatory responses and is a potential target for the treatment of HDM allergy. We describe a Siglec-targeting platform consisting of an allergen of HDM, Dermatophagoides pteronyssinus (Der p 2), decorated with a natural Siglec-9 ligand, (α2→3) N-acetylneuraminic or sialic acid. Treatment of human peripheral blood mononuclear cells (PBMCs) with this glycoconjugate suppressed the production of allergy-associated inflammatory cytokine, interleukin-5 (IL-5) and augmented the expression of anti-inflammatory cytokine, IL-10. The glycoconjugate also suppressed the activation of CD4 T helper (Th) cells and induced the expansion of Tr1 regulatory T cells, known to suppress the function of pathologic Th cells. Collectively, these results demonstrate a promising potential of targeting Siglec receptors with glycan-based constructs for the rapid induction of an anti-inflammatory state in immune cells for short-lasting allergen immunotherapy against HDM allergy.

Th110. Understanding the Plausible Implications of Dysregulated Interferon Signaling on Esophageal Epithelial Cell Function

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Eosinophilic esophagitis (EoE) is a chronic, allergic inflammatory disease of the esophageal mucosa. Type 2 cytokines (i.e., interleukin (IL)-13) are predominant, although treating type 2 inflammation alone in EoE is not successful. Interestingly, EoE biopsy transcriptional signatures reveal upregulation of type I and II interferon (IFN) response, dysregulated immune signaling and downregulation of esophageal-specific genes in EoE patients. However, the role of interferon signaling during EoE remains unresolved. RNA-sequencing was performed using epithelial cells isolated from pediatric patient biopsies following dissociation and removal of CD45+ cells. IFN-γ response was identified as the most significant upregulated pathways in EoE epithelium. To determine the functional implications of interferon exposure, we used in-vitro cell culture models of immortalized esophageal epithelial cells, EPC2-hTERT. IFN-γ-pSTAT1 stimulation significantly increased epithelial cell death via apoptosis ($p < 0.001$); decreased transepithelial electrical resistance (TEER) ($p < 0.05$) and increased paracellular permeability to FITC-Dextran ($p < 0.05$) in air-liquid interface (ALI) culture. Esophageal epithelial organoids treated with IFN-γ exhibited reduced formation rate ($p < 0.001$), size ($p < 0.001$), and abnormal pattern of proliferation-differentiation gradient

(Ki67-IVL). Further, the treatment triggered upregulation of several genes, including tissue differentiation, epithelial barrier and apoptotic genes replicating the active EoE mucosa. In conclusion, IFN response gene signature enriched in the epithelium of active EoE suggests that upregulated interferon signaling is part of the dysregulated mucosal immune process present in EoE. Beyond EoE, our findings offer new insights into esophageal disorders which may involve increased IFN-γ signaling, including gastroesophageal reflux disease, and Barrett's esophagus.

W101. Omeprazole Downregulates Inflammatory Cytokines and Eosinophil Activation Markers in the Co-culture of Eosinophil and Esophageal Epithelial Cells

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Eosinophilic esophagitis (EoE) is a chronic allergic disease, characterized by esophageal dysfunction, type-2 cell-mediated inflammation, and esophageal epithelial eosinophilic infiltrate. Proton pump inhibitors (PPIs) are used as first-line EoE therapy however bidirectional communication between eosinophils and epithelial cells and the role of PPIs in the milieu is yet to be understood. Here we use monolayer or co-cultures to understand the mechanism of action of omeprazole. Human esophageal epithelial cells were grown in a monolayer and treated with 100 ng/ml of IL-13 and/or 50 µM acid-activated omeprazole for 24 h. Culture supernatant was collected for cytokine analysis, and cells were lysed to screen STAT 6 level. For co-culture, eosinophils isolated were added to the EPC2 cells monolayer in a 1:1 mixture of RPMI with 10% FBS and keratinocyte serum-free media. Cytokines were measured in culture supernatant and activation markers in cells by flow cytometry. IL-13 treatment significantly increased the phospho-STAT6, eotaxin-3, and IP-10 levels in monolayer which was downregulated by omeprazole. Similarly, in co-culture, eotaxin-3, IP-10, and MIP-1 beta were elevated in presence of IL-13 and omeprazole reduced the level back to normal. Eosinophils had 76% and 73% cell viability in coculture after 24h and 48h respectively whereas the viability was as low as 38% and 18.5% in eosinophil-only culture. Co-culture with EPC2 activated eosinophils with higher CD69 and siglec8 which was downregulated by omeprazole. In conclusion, omeprazole attenuates the EoE-associated inflammation by downregulating the inflammatory cytokines and eosinophil activation markers in the co-culture of eosinophils and esophageal epithelial cells.

W102. PD-L2 Maintains Metabolic Function and Stability of Regulatory T Cells

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Regulatory T (Treg) cells are central to limit immune responses to allergens. Our studies show that PD-L2 deficiency prevents the induction of tolerance to ovalbumin and control of airway hyperreactivity, in particular by limiting peripheral Treg (pTreg) numbers and function. Ex vivo, PD-1/PD-L2 interactions increase iTreg numbers and stability, notably by promoting metabolic activity, demethylation of the TSDR region and Foxp3 expression. In mice lacking PD-L2 we find lower numbers of splenic pTregs at steady state, producing less IL-10 upon activation and with reduced suppressive activities. In particular, there were no differences in thymic Treg progenitors nor differences in thymic Treg (tTreg) numbers, suggesting for a role for PD-L2 exclusively in the periphery. Remarkably, the numbers of splenic pTregs are restored by adoptively transferring PD-L2high dendritic cells to PD-L2KO mice, without affecting tTreg numbers. Functionally, our transcriptomic, epigenetic and bioenergetic studies show that activated pTregs isolated from mice lacking PD-L2 have lower Foxp3 expression, higher methylation of the TSDR region and a decreased TCA cycle associated with a defect in mitochondrial function. Consequently, adoptively transferred WT induced Tregs (iTregs) to PD-L2KO recipient mice had a lower survival rate compared to controls, confirming that exogenous PD-L2 controls pTreg stability in vivo. Our metabolic studies further show that pyruvate treatment of PD-L2KO mice partially restores IL-10 production and airway tolerance. Together, our study highlights the importance of the PD-1/PD-L2 axis in the control of metabolic pathways regulating pTreg Foxp3 stability and suppressive functions,

opening up avenues to further improve mucosal immunotherapy.

W103. Prolonged Antihistamine Usage Causing Murine Uterine Pathology
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Cimetidine is a histamine-2 (H-2) receptor blocker that was FDA approved in 1979 through clinical trials that were predominantly male. While originally approved for short-term prescription use, cimetidine has been available over the counter since 1995. Once sold solely under the brand name Tagamet, cimetidine remains available globally in generic formulations. Daily consumption of this antihistamine for periods of 4 months or longer in mice leads to significant uterine abnormalities by gross pathology and histopathology including severe disfigurement, swelling, and cysts. Female post-menopausal mice also showed endometrial hyperplasia. Furthermore, combination of exposure to this antihistamine concurrently with a bacterial infection that colonizes the uterus leads to hemorrhaging and/or pyometra in the female mouse. We are actively investigating common antihistamines on the market of both H-1 and H-2 receptor blocker types to evaluate their impact on the murine female reproductive tract. We are also investigating epidemiological associations between the use of these antihistamines and gynecological diseases. These data will help us better understand the role of histamine in the female reproductive tract. Furthermore, this study highlights the importance of more inclusive clinical trials and perhaps the need to revisit current FDA approved drugs that were not sufficiently tested in females.

W104. Rhinovirus Infected Epithelial Cells Drive Genetic Susceptibility to Childhood-onset Asthma
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Asthma is a complex disease caused by genetic and environmental factors. Epidemiological studies show that rhinovirus (RV) infection in children, increases the risk of developing asthma. However, why only some individuals develop asthma remains an area of active investigation. Genome-wide association studies (GWAS) have identified hundreds of genetic variants contributing to asthma susceptibility. While most genetic variants are non-coding and many affect gene regulation in T cells, a role for airway epithelial cells (AECs) in mediating genetic susceptibility to asthma remains under-studied. Here we hypothesized that gene by environment interactions occurring in AEC could play an important role in asthma development. We compiled transcriptomic (bulk and single-cell RNA-seq) and epigenomic datasets of AECs subjected to different stimuli. We applied methods that use GWAS data to characterize AEC states that mediate asthma genetic risk: single-cell disease-relevance score (scDRS) and Linkage Disequilibrium Score-regression in Specifically Expressed Genes (LDSC-seg). First, using immune cell datasets we validated that T cells mediate significant genetic susceptibility to adult-onset and childhood-onset asthma, with a distinct signal for Th2. Then, we found that AECs infected with RV significantly mediate genetic susceptibility to childhood-onset asthma. In contrast, Sars-Cov2-infected or cytokine-stimulated AECs do not. Furthermore, the RV-specific signal was exclusive to later points of infection (24-42 hours post-infection). Additionally, we found that RV-infected AECs from asthmatic patients showed a stronger enrichment for asthma risk compared to those of healthy individuals. Our results indicate that rhinovirus infection combined with a high genetic burden increases the risk of asthma development via AECs.

W105. The Effect of CCD Inhibition on Antigen-specific Ige Test Results in Multiple Allergen Simultaneous Test Using Advansure Alloscreen max108 Panel

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Introduction: We investigated how Cross-reactive carbohydrate determinants (CCD) inhibition actually affects various allergen-specific IgE results in a newly introduced multiple allergen simultaneous test, AdvanSure AlloScreen MAX108 Panel (LG CHEM, Korea).

Methods: Sera from 31 patients with positive CCD antigen (grade ≥ 1) in AdvanSure AlloScreen Max108 panel was included. Patients were stratified based on sIgE to CCD classes 1-3, (n=27, CCD-low) vs. classes 4-6, (n=4, CCD-high). All samples were treated with ProGlycAn CCD-blocker (ProGlycAn, Austria) at a concentration of 20 ug/ml and additionally at 40 ug/ml in high CCD.

Results: All 27 CCDs in CCD-low sera negatively converted, but none of CCDs in CCD-high sera when treated with 20 μg/ml. The positive rates for foodstuffs and pollens were significantly higher in CCD-high than CCD-low (foodstuffs 55.0% (110/200) vs 25.1% (279/1113), pollens 97.7% (125/128) vs 62.7% (511/815), $p < 0.0001$). The rates of the sIgEs turned negative after CCD inhibition showed a significant difference only in pollens (32.8% (41/125) in CCD-high vs 75.0% (383/511) in CCD-low ($p < 0.0001$). In CCD high group, there was no significant difference in negative conversion rates when treated with the concentration of 20 ug/ml and concentration of 40 ug/ml for all allergens (46.3%, 119/257 vs 54.1%, 139/257). However, the pollen group showed a significant difference (32.8%, 41/125 for 20 ug/ml vs 45.6%, 57/125 for 40 ug/ml, $p = 0.0386$).

Conclusions: CCD-blocker treatment in sample can reduce the false positive response in CCD-low sera, but, the negative conversion rates after CCD inhibition was different depending on the allergens.

W106. The Impact of Age on Lyme Disease-associated Atopic Dermatitis

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Borrelia burgdorferi are bacteria that cause Lyme disease (LD) after infecting a susceptible host. Immune responses to the bacteria are highly variable and host specific, both in mice and humans. The murine substrain, C3H/HeJ, is a frequently utilized model for LD because it develops two key features of the human infection. The murine tail skin shares critical features with human skin, and could provide an important and underutilized tool to probe dermatological manifestations of the infection. Age and sex-matched C3H/HeJ mice aged 5-8 weeks, 1 year, or 2 years were infected with 105 B. burgdorferi for a minimum of 8 weeks, up to a maximum of 2 years. Features of the skin were evaluated by gross examination and histology. Key features of atopic dermatitis were present in infected mice after 1 year of age, including ulceration, hemorrhaging, flaking, hair loss, and dark lesions as well as spongiosis and acanthosis. Furthermore, mice infected with B. burgdorferi containing luciferase were istudied over a 6-month long time course using an In Vivo Imaging System, and were found to have significant infection in their tails. We then identified 5,248 individuals with LD and 17,233 with atopic dermatitis in FinnGen and find that also in human epidemiological data LD associates with atopic dermatitis (OR = 1.91 [1.68 -2.37], P < 2e-16). We demonstrate the onset of murine atopic dermatitis with LD, which is further exacerbated by host age at time of infection, and likewise

report a similar association in humans.

Autoimmune Diseases

Th111. BCG Vaccinations Modulate Immune Training of B cells in Type 1 Diabetes: Human Clinical Trial Data
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Bacillus Calmette-Guérin (BCG), a >100-year-old vaccine derived from Mycobacterium bovis, is known for its broad protective abilities against diverse infectious and autoimmune diseases. Recent studies show that BCG also promotes long-lasting blood sugar control in individuals with type 1 diabetes (T1D) and protection against COVID-19 with high vaccine effectiveness. BCG efficacy in humans is dependent on BCG strain and multiple BCG vaccinations change the immune system to provide clinical benefits including infectious disease protection and durable glucose control. Although previous studies have attributed these benefits to acute monocyte modifications through innate immune "training," we now show that BCG treatment is associated with immune training of B cells in individuals with T1D. In this open-label study, we administered 2 doses of BCG (1 × 10^5 CFU; strain BCG Japan) to 31 T1D patients and longitudinally measured changes in the B cell compartment using flow cytometry studies. B cells and B cell markers (CD19+, CD21+, CD10+, and CD319+) were studied. Over the course of two years, we identified a significant and sustained increase in the Median Fluorescence Intensity (MFI) of CD10 and CD319 on the surface of B cells, markers that are present on early-/pre-B cells and plasma B cells, respectively. The percentage of CD10+ and CD319+ B cells was also significantly increased compared to both patients' baseline levels and to nondiabetic controls. The previously reported broad protection of BCG vaccination for diverse infectious diseases might be attributed to BCG priming the B cell compartment.

Th112. Correlation of Urine Immune Cells with Renal Histological Features in Lupus Nephritis: Lessons from the Accelerating Medicines Partnership (AMP) in SLE Consortium
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Lupus nephritis (LN) is a complex autoimmune disease with unknown cause that is associated with severe morbidity and mortality. Recent research suggested a benefit of serial kidney biopsies in managing LN. Studying immune cells in the urine of LN patients offers the prospect of minimizing the need for invasive kidney biopsies. We performed analysis of single cell RNA-sequencing data of urine immune cells collected from patients with active LN. We identified 101,787 high quality immune cells from 146 patients including myeloid, dendritic, B, T, NK, and dividing cells. The median number of cells per patient was 315 (IQR 42-1,071). The majority of cells were myeloid (91,215 cells, 89.6%); Still, most immune cell subsets identified in the kidneys of LN patients, including lymphocyte subsets, were also found in the urine. Approximately 39% of urine samples yielded higher numbers of immune cells compared to the corresponding kidney biopsies. Proliferative and mixed nephritis were associated with higher percentages of phagocytic myeloid cells (Kruskal Wallis q value < 0.001). The NIH Activity Index positively correlated with phagocytic myeloid fractions (Spearman rho=0.40, q value=0.002) and negatively correlated with that of dendritic cells (Spearman rho=-0.29, q value=0.030); while the NIH Chronicity Index correlated positively with B cells (Spearman rho=0.32, q value=0.012), negatively with phagocytic myeloid cells (Spearman rho=-0.28, q value=0.035), and positively with inflammatory myeloid cells (Spearman rho=0.32, q value=0.012). This work describes the complex immune cells present in urine during LN, and paves the way for additional work to predict renal histological features from urine samples.

Th113. Deep Immunophenotyping Reveals Cellular Phenotypes in Peripheral Blood Associated with Specific Patterns of Kidney Injury in Lupus Nephritis

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Lupus nephritis (LN) is a severe complication of systemic lupus requiring renal biopsy to guide treatment decisions. Defining LN heterogeneity using a non-invasive approach is a pressing need to improve treatment. We applied mass cytometry using four 48-marker panels to characterize peripheral blood mononuclear cells (PBMC) from 140 patients with biopsy-proven LN and 40 controls enrolled in the Accelerating Medicines Partnership RA/SLE Network. We projected cells in a reduced dimensional space and implemented covarying neighborhood analysis (CNA) to identify cell 'neighborhoods' associated with clinical features. Compared to controls, LN patients displayed significant enrichment of cells expressing interferon (IFN)-induced proteins including MX1 and Siglec1, reflecting a cytometric detection of an IFN signature. Comparing patients with proliferative LN (class III/IV+/-V, n=94) versus membranous LN (class V, n=46), we observed enrichment of a specific naive B cell population characterized as CD23- and CD21dim, in proliferative LN patients (p.adj=0.02). These cells were distinct from CD11c+ B cells and were also associated with increased histologic NIH activity index and with anti-dsDNA antibody, even after adjusting for immunosuppression. To further understand LN heterogeneity, we clustered patients and controls based on proportions of immune cell subsets using k-means clustering. We identified two major clusters: cluster1 included only LN patients, and cluster 2 included controls and 26 LN patients. Patients from cluster2 had significantly lower clinical extrarenal activity (p.adj=0.01) and IFN-induced protein expression (p.adj< 0.001) and higher histologic chronicity (p.adj< 0.001). These results nominate potential biomarkers associated with LN heterogeneity and highlight altered naive B cell activation in proliferative LN.

Th114. EBV in Multiple Sclerosis: Molecular Mimicry Between EBV EBNA1 and Glialcam
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Infection with Epstein-Barr virus (EBV) is associated with multiple sclerosis (MS), but the molecular mechanisms that explain this connection remain incompletely understood. Here we identified a cross-reactive antibody that binds the EBV transcription factor EBNA1 and the glial cellular adhesion molecule GlialCAM with high affinity, and we propose this molecular mimicry to be a mechanistic link that connects EBV and MS on a molecular level. The identified antibody binds EBV EBNA1 at an epitope known to elicit high antibody reactivity in MS patients. We provide structural evidence for the evolution from an unmutated B cell binding only EBNA1 to a cross-reactive antibody that also binds GlialCAM. Cross-reactivity is facilitated by a post-translational modification of GlialCAM. EBNA1-GlialCAM cross-reactive antibodies are more prevalent in MS patients than in healthy controls. Functional evidence for the impact of EBNA1/GlialCAM molecular mimicry was obtained from EBNA1-immunizations of the mouse model of MS, experimental autoimmune encephalomyelitis (EAE). Anti-viral antibodies in the CSF of MS patients were identified by single-cell B cell repertoire sequencing, followed by recombinant expression of selected clonally expanded antibody sequences. Antibodies were tested against a spectrum of viruses implicated in MS pathogenesis using protein microarrays. Binding and high affinity were confirmed by ELISA and biolayer-interferometry. Structures of antigen-antibody complexes were solved with X-ray crystallography. EAE mice were immunized with EBNA1 to investigate its effect in-vivo. Our results suggest that molecular mimicry between EBNA1 and GlialCAM is a relevant pathomechanism and provides a long sought mechanistic link for the association between MS and EBV.

Th115. Effects of Latent EBV on Responses to Anti-cd3 Mab (teplizumab) in Type 1 Diabetes
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Teplizumab has been approved for delay of Type 1 diabetes (T1D) in individuals at-risk. We aimed to study factors that affect responses to treatment. Latent DNA viruses may affect adaptive immune responses. In two T1D trials (for prevention (TN10) and treatment (AbATE)), EBVsero+ individuals had a more robust clinical response to teplizumab: in TN10, in EBVsero+ a delay in diagnosis of T1D of 52.8 (p=0.01) vs 28.7 mos (p=0.08) in EBVsero- with a 42.3 mo difference in sero+ vs- among teplizumab treated participants (p=0.08). We found an increased frequency of CD8+ T

cells expressing TIGIT and KLRG1 ("partially exhausted") in the EBV sero+ in the two trials by flow cytometry($p< 0.0001$ in both). To identify the effects of EBV, we performed scRNAseq on peripheral blood in the prevention study. In EBVsero+ at enrollment, there was a greater number of CD8+ effector and effector memory cells after teplizumab in EBV+ at approximately 3 mos and 18 mos ($p=0.03$ and 0.06). Importantly, teplizumab led to reduced expression of genes associated with cell signaling (NFkB, $z=-4.12$ $p< 0.01$, mTOR, $z=-3.3$ $p= 0.003$) that was maintained to 18 mos (NFkB, $z=-3.9$, $p< 0.01$, mTOR, $z=-3.1$ $p= 0.002$) in the EBVsero+ vs - participants. Moreover, in B cells we found reduced B cell receptor signaling ($z=-4.3$, $p< 0.01$). These results suggest that teplizumab treatment in patients with prior infection with EBV expands effector and effector memory CD8+ T cells with impaired signaling. There are also effects on B cells that may enhance the therapeutic responses.

Th116. Genetic Variants Associated with Immune Cell Population Abundances in Single-cell Data

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Multiple sclerosis (MS), an autoimmune disease of the nervous system affecting 2.8 million individuals worldwide, has well-established genetic risk factors. However, mechanisms mediating these genetic effects and their interactions with MS environmental risk factors remain unclear. To better understand immune-related diseases like MS, we developed a novel approach to flexibly identify fine-grained immune cell states that increase in abundance with the allelic dose of a genetic variant. When applied in a genome-wide survey, our method identifies a spectrum of immune states under genetic influence, building on previous studies using flow cytometry. However, our approach uses single-cell data without pre-specifying candidate cell types in order to characterize genetically-associated cell states with more flexibility and statistical power. In data from 969 Australians of European ancestry (the OneK1K cohort), our method reveals >150 independent genome-wide significant loci associated with changes in T, B, NK and Myeloid cell populations, including individual variants associated with simultaneous shifts across multiple cell types. One of these loci, in the major histocompatibility complex region, contained the strongest associations genome-wide. Fine-mapping within this region reveals that most of this MHC-region signal is explained by HLA-DRB1*15:01, the major MS genetic risk allele. We characterize shifts in B ($P=4e-182$), T ($P=1e-14$) and dendritic cell ($P=2e-16$) population abundances associated with increasing allelic dose of HLA-DRB1*15:01. These shifts appear in individuals without MS disease, and may illuminate how HLA-DRB1*15:01 interacts with pathogenic exposures like EBV to set the immunological stage for MS.

Th117. Leveraging Single Cell Transcriptomics to Identify Pathogenic Cell Lineages

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The abnormal physiology of disease can be conceptualized as driven by a subset of cells either acquiring novel functions or losing canonical traits. While in cancer this is a well-established model for disease pathology, its application to autoimmune and neurological diseases is still under debate. In these diseases, a crucial missing step is identifying the lineages that drive disease, or "pathogenic" cell types. Here, we started from the basic assumption that genetic disease modifiers would have an increased impact on cell types that actively expressed the gene, and in turn that a pathogenic cell would be more likely to express genes that have a causal link to disease. In key autoimmune diseases, we combined single cell transcriptomics and human genetics to rank cell types by their likelihood of pathogenicity. We leveraged scRNA-seq data to identify cell type specific genes in each disease and intersected them with the disease's casual gene modifiers. We found that the gene expression of genetic modifiers broadly clustered cells by their lineage, with several modifiers exclusively expressed in a single lineage. Moreover, we were

able to rank cell types by their pathogenicity in atopic dermatitis, lupus nephritis, type 1 diabetes and inflammatory bowel syndrome, and validated the scoring by comparing it to an extensive literature review effort. The data-driven pathogenicity score was overall able to predict the pathogenic cell types cited in the literature and find new cell types potentially driving disease.

Th118. Molecular and Cellular Dissection of the Nephrotic B-cell Response Reveals a Prominent Atypical B-cell Signature in Relapses of Idiopathic Nephrotic Syndrome

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The search for glucocorticoid (GC)-sparing treatments for idiopathic nephrotic syndrome (INS), the most common relapsing-remitting glomerular disorder in children, identified B-cell depletion as a successful approach in maintaining long-term remission from proteinuria. While this positioned B-cells as the central mediators of pathogenesis, the nature of the nephrotic B-cell response, including whether antibody-dependent or independent functions were involved, remained unknown. To dissect this, we carried out single cell RNA-sequencing (scRNA-seq) and high-parameter flow cytometry on peripheral blood B-cells from pediatric INS patients and healthy controls (HC). By scRNA-seq (N=4/group), we identified an expanded memory subset in INS characterized by a transcriptional profile consistent with atypical B-cells (atBCs) – a population associated with autoimmunity, viral infection, and aging. The atBCs in INS preferentially expressed genes associated with B-cell activation, antigen presentation, and immunoglobulin production. We confirmed the expansion of atBCs in INS using the flow-based unsupervised clustering algorithm FlowSOM (N>15/group). Moreover, we identified that this, alongside a marked expansion of plasmablasts, represented the predominant perturbation in peripheral B-cells in INS. While treatment with GC (N=15) diminished circulating atBC and plasmablast numbers, all memory B-cells were ablated in patients maintained in remission with the B-cell depleting biologic rituximab (RTX, N=14). As RTX-treated patients relapsed (N=4 longitudinally), scRNA-seq showed that the atBC subset partially rebounded and the pathogenic B-cell transcriptional signature was re-established. Our work proposes a central pathogenic role for atBCs in pediatric INS and thereby supports the understanding of INS as an autoimmune condition with relapses frequently taking place following viral infections.

Th119. Post-translationally-modified Trichohyalin Epitopes: Potential Triggers of Autoimmunity to Hair Follicles in Alopecia Areata

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Alopecia Areata (AA) is a non-scarring immune mediated hair loss disorder. Induction of ectopic immunocompetence in the lower hair-follicle (HF), followed by T-cell receptor recognition of exposed HF autoantigens by CD8+ T-cells is considered to play a central role. We propose that Trichohyalin (TCHH), a major structural protein of the inner root sheath produced in the anagen HF could be a potential autoantigen. TCHH undergoes significant anagen-mediated citrullination, a post-translational modification (PTM). Hence, we evaluate here TCHH's putative autoantigenic role in AA and identify post-translationally-modified immunoreactive TCHH epitopes. Ultrastructural TEM immuno-gold analysis of normal human anagen scalp HFs revealed that TCHH is expressed in the most external epithelial hair-bulb adjacent to the immunocompetent vasculature indicating that TCHH epitopes are accessible to peri-follicular sentinel immune cells. We extracted TCHH from human scalp anagen HFs and mapped for the immunologically-sensitive PTM-citrullination. Mass-spectrometric and in-silico analyses revealed that TCHH harbors several citrullinated residues; several of which exist within sequences predicted to be immunologically relevant. A total of 6 putative antigenic peptides harboring citrullinated residues were synthesized, along with their native forms, and

subjected to immunoassays. Cell-mediated autoimmune reactivity against putative epitope peptides was assessed using PBMCs of AA patients & healthy-controls by ELISpot. Humoral autoimmune reactivity was assessed using serum samples by ELISA. In summary, we demonstrate that earliest TCHH epitopes occurs in the most peripheral anagen hair bulb, the site of immune cell attack in AA. This site harbors immunologically-relevant citrullinated TCHH residues that could render TCHH a major autoantigen in AA.

Th120. Protective Association of HLA-DRB1*04 Subtypes in Neurodegenerative Diseases Implicates Acetylated Tau PHF6 Sequences

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Objective To explore genetic association between human leukocyte antigen (HLA) and neurodegenerative diseases and investigate mechanisms behind the association. Background Pathophysiology of Alzheimer's disease (AD) and Parkinson's disease (PD) involves accumulation of tau (neurofibrillary tangles) and amyloid-β-rich (Aβ, amyloid plaques) aggregates in AD. Consensus is growing that the adaptive immune system and human leukocyte antigen (HLAs) presentation of tau/Aβ antigens plays a role in AD. Design/Methods We analyzed HLA associations in ~176,000 individuals with PD or AD versus controls across ancestry groups. We next screened 448 overlapping tau peptides including the most frequent post translationally modifications (PTM) against multiple HLA subtypes. We further examined tau restricted T cells using tetramer HLAs and performed single-cell RNA sequencing. Results A shared genetic association was observed across AD and PD at rs601945 (AD: OR=0.91[0.89; 0.93]; p=1.8x10-22). Hierarchical protective effects of HLA-DRB1*04 subtypes best accounted for the association, strongest with DRB1*04:04/DRB1*04:07, intermediary DRB1*04:01/DRB1*04:03 and absent for DRB1*04:05. The same signal was associated with decreased neurofibrillary tangle density postmortem and was more associated with lower tau levels than Aβ42 level changes in the CSF. Further, protective DRB1*04 subtypes strongly bound the aggregation-prone tau PHF6 sequence, but only when acetylated at K311, a modification central to aggregation. T cells recognizing this epitope were identified and T cell receptor clones were characterized, showing relevance of this immune response in patients with neurodegenerative disorders and suggesting a protective role of HLA DRB1*04. Conclusion An HLA-DRB1*04-mediated adaptive immune response, decreases AD risk, offering the possibility of new therapeutic avenues.

Th121. Protein Cargo of Plasma-derived Extracellular Vesicles Drives Innate Mediated Inflammation in Dermatomyositis

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Extracellular-vesicles (EVs) have been implicated in autoimmune disease pathogenesis. Plasma-derived DNA containing EVs have been shown to induce STING-mediated proinflammatory responses in dermatomyositis (DM), but their protein content is not well characterized. We collected EVs from plasma of 16 DM patients and 5 controls. Protein profile was generated by mass spectrometry and differentially expressed proteins were assessed. Findings were used to train a machine learning model (random forest), which achieved 90% accuracy with AUC = 0.92 in correctly predicting disease state. Sixty-seven proteins were uniquely detected in the patient cohort. Thirty-five proteins were significantly differentially expressed, of which 13 were upregulated and 22 downregulated. Over representation analysis found the unique and upregulated proteins enriched for myeloid mediated immunity, glutathione metabolism, nucleic acid synthesis and vesicle transport pathways. EVs were enriched with USP15, TMED2, STK4, ABCC1, PDMS14 and MMP8, all of which participate in induction of innate inflammatory cascades. Downregulated proteins were enriched for the classical and lectin complement pathways. The diminution of complement components in vesicles may reflect its abundance in target tissues but may also reflect host inability to circulate these molecules to damaged tissue, which in a chronic stage of disease may have a protective role. Finally,

surfactant protein B was expressed almost exclusively in patients with lung disease, rendering it a possible marker for pulmonary involvement. Taken together, proteins carried by EVs appear to be markers of DM, particularly pulmonary manifestations, and may have a role in inflammation in these patients and may indicate potential therapeutic targets.

Th122. Rank Wise Effect of HLA-DQ5 Explains Risk for the Development of anti-iglon5 Disease

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Background Anti-IgLON5 disease is a rare, but likely underdiagnosed type of autoantibody encephalitis displaying a heterogeneous clinical phenotype, including sleep, movement and brainstem dysfunction. Its pathophysiology remains elusive, although strong association with HLA-DRB1*10:01-DQB1*05:01 supports an autoimmune basis. Methodology A multicentric cohort of 62 patients and 433 controls matched by ethnicity using principal component analysis was included. 4-digit resolution HLA imputation on genome-wide association data was used, with selected 8-digit resolution validation typing. Association with individual alleles and haplotypes was tested using stepwise generalised logistic models. Furthermore, we computationally predicted binding of IgLON5-derived peptides to risk-associated HLA-molecules. Results Our results indicate a rank wise effect of HLA-DQA1*01:05~DQB1*05:01 (heterozygotes: OR 46.6), HLA-DQA1*01:01~DQB1*05:01 (homozygotes: OR 26.9; heterozygotes: OR 2.5) and HLA-DQA1*01:04-~DQB1*05:03 (homozygotes: OR 30.9; heterozygotes: OR 5.6), in order of descending relative risk predisposition. HLA-DR effects are likely explained by linkage disequilibrium with DQ, as sequences of associated DR alleles widely diverge, whereas differences between encoded DQ heterodimers are minimal, suggesting a common function. Computational binding predictions support similar, high binding affinity for specific IgLON5 peptide segments in a post-translationally modified (N-deglycosylated) form by all three predisposing HLA-DQs. Conclusion Our results indicate a primary role of HLA-DQ versus HLA-DR in anti-IgLON5 disease, with higher reactivity against modified versus physiological peptides, in line with reduced T cell tolerance against these epitopes.

Th123. Real-life Retrospective Study of Long-term Glucocorticoid Side Effects and Efficacy in Patients with Systemic Lupus Erythematosus

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Introduction Glucocorticoids are used to treat flare-ups and provide rapid symptomatic relief in patients with systemic lupus erythematosus (SLE). However, long-term use of glucocorticoids is associated with numerous adverse events ranging from mild to severe. The objective of this study is to evaluate after real-life follow-up the incidence of side effects related to the long-term use of glucocorticoids in SLE patients and to assess the efficacy of immunosuppressive therapies. Methods We included 83 patients followed at the University Hospitals of Geneva and fitting the EULAR/ACR 2019 criteria for SLE. Clinical and biological data were collected from computerized records. Statistical analyses were performed with R software. Results 87% of the included patients were women with a median age of 34 years. The median initial dose of prednisone was 40 mg/d. Side effects occurred in 86.75%. Immunosuppressive therapies were used in 74.69%. Long-term glucocorticoid-related side effects occurred in 86.75%. The most common adverse events were weight gain (30%), infections (23.17%), and osteoporosis (15.66%). Patients with multiple risk factors and cardiovascular comorbidities at the time of SLE diagnosis were most likely to develop adverse events. Survival analyses (Kaplan-Meier), between-group comparisons (t-test), and correlation analyses (Spearman) showed a benefit on SLE disease activity (decreased number/incidence of relapses; decreased SLEDAI score) of long-term immunosuppressive therapy compared with long-term glucocorticoid use alone. Conclusion This real-life study confirms the harmfulness of glucocorticoids in long-term use with a dose-

dependent effect depending on comorbidities. Immunosuppressive treatments reduce the incidence of adverse effects while controlling SLE evolution.

Th124. Regulatory T Cell Fitness as Biomarker for Disease Activity in Juvenile Idiopathic Arthritis

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Juvenile Idiopathic Arthritis (JIA) affects about 1 in 1000 children worldwide. The majority of cases are oligoarticular and rheumatoid factor negative (RF-) polyarticular JIA, characterised by repeated flares of joint inflammation without skin/systemic involvement leading to pain, fatigue, and ultimately joint destruction and disability. However, no reliable biomarker exists to predict the erratic disease course. In JIA the immunoregulatory balance is broken with altered regulatory T cells (Tregs). Thus, monitoring changes in Tregs could offer novel biomarker potential of subclinical disease activity. Utilising full-spectrum flow cytometry and unbiased analysis, we identified three Treg clusters increased in peripheral blood (PB) Tregs from clinically active JIA patients (active joint count, AJC ≥1), including CD226highCD25low effector-like Tregs, while a 4-1BBlowID2intermediate Treg cluster predominated in inactive JIA (AJC=0). The ratio of these Treg clusters correlated to clinical Juvenile Arthritis disease activity score (cJADAS) and predicted inactive individuals that flared by 6-month follow-up. Moreover, machine learning biomarker discovery on nanoString Treg gene signature plus data from sorted Tregs (CD4+CD25hiCD127low) could successfully differentiate active JIA Tregs from healthy control (HC) Tregs (AUC=0.9875). Biomarker scores of Tregs from clinically inactive individuals (cJADAS< 0.5) were significantly different (p=0.011) from samples with higher cJADAS (≥0.5), but not different from HC Tregs. Thus, our data indicates altered Treg fitness as an important factor for disease progression versus remission. Validation of these PB Treg-derived biomarkers could lead to more personalised disease management, for example starting preventative treatment if a flare is likely or withdrawing medication safely when sustained remission is indicated.

Th125. Regulatory T Cells Within the Inflamed Liver of Mice with Primary Biliary Cholangitis Are Ineffective in Controlling the Disease

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Primary biliary cholangitis (PBC) is a chronic autoimmune disease where the immune system attacks the small bile ducts in the liver. CD8 T cells are known to play a significant role in destroying the bile ducts. However, regulatory T cells (Tregs), a type of immune cell that regulates the immune response, have also been found to accumulate in the liver of patients with PBC. This study used a xenobiotic-induced mouse model of PBC-like disease to test the idea that Tregs in PBC are not functioning correctly. In our study, female and male mice treated with 2-octynoic acid-conjugated ovalbumin (2-OA-OVA) developed autoimmune cholangitis (AIC). However, female AIC mice had worse liver inflammation and damage than male mice. Additionally, there were higher levels of CD8 T cells and their chemoattractants, CXCL9 and CXCL10, in the livers of female AIC mice. There were also more Tregs in the livers of mice with the PBC-like disease, but these Tregs did not have normal levels of CTLA-4 and TGF-β that help them control the immune response. Moreover, although treating the mice with low-dose IL-2 increased the number of hepatic Tregs, these Tregs could not rescue the pathology of AIC. Overall, these results suggest that Tregs in PBC were dysfunctional, and transferring with normal Tregs may be a better way to treat PBC.

Th126. Rheumatoid Arthritis and Older Age Are Associated with Lower Humoral and Cellular Immune Response to COVID19 Mrna Vaccines

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Background: People with autoimmune disease have worse outcomes when infected with SARS-CoV2. At the same time they have lower antibody responses to COVID19 vaccine. Immune suppressive medications used to treat rheumatoid arthritis (RA) are associated with lower vaccine responses, though contributions of comorbidities, T-cell immunity and age are less clear. Methods: We evaluated serum samples for anti-spike specific IgG by bead-based assay before and after doses of COVID19 mRNA vaccine, as well as Wuhan and Omicron neutralizing antibody levels, IFN-g and IL-2 production by ELISPOT assay, and immune cell subsets by flow cytometry in 51 RA and 95 non-autoimmune control participants. Results: RA participants had lower total spike-specific IgG and Wuhan-strain neutralizing antibody levels, and lower spike-specific IFN-g producing peripheral blood mononuclear cells (PBMC) after the vaccine doses. Neutralizing antibody levels against Omicron strain were low in both groups. IFN-g production correlated with IL-2 production and neutralizing antibody levels, while older age negatively correlated with spike-specific IL-2 and IFN-g and IgG levels. Lower antibody levels were driven by age and RA associated factors while lower T cell response was driven by older age. Conclusions: These data indicate lower COVID19 mRNA vaccine-induced antibody levels in persons with RA compared to controls, perhaps in part attributable to medications. At the same time older age associates with lower antibody and cellular immune response to vaccines. Further study is needed to clarify links between older age associated cellular immune defects and RA associated humoral immune defects in host response to COVID vaccine series.

Th127. Role of fam83h in Immune System Homeostasis

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FAM83H is expressed mainly in epithelial cells and has been suggested to be responsible for intracellular transport, regulation of cytoskeletal networks, and enamel formation. Deficiency of FAM83H was reported to be the cause of amelogenesis imperfecta (AI), a soft enamel disease. Interestingly, two AI patients from one family in Czechia with confirmed FAM83H mutation developed a juvenile rheumatoid arthritis. To understand the function of FAM83H in immune system homeostasis, we generated Fam83h knock-out (KO) and mutant mouse (Fam83htg/tg). Both mutant and KO animals exhibit decreased body size, sparse and scruffy coat, scaly skin, weakness and hypoactivity. While we have not observed dentin-related phenotype, mutant and KO pups show severe swelling of their forepaws accompanied by bone deformation at as early as 3 weeks. However the soft tissue lesions are being resolved and peripheral blood leukocytes return back to normal levels at 7 weeks of age. Moreover, mutant juvenile animals have increased neutrophils as well as G-CSF and inflammatory cytokine levels in their peripheral blood. Additionally, the development of leukocytes including T, B and NK cells is severely impaired in Fam83h mutants. Specifically, T cell development is arrested at the double-negative stage, B cells at the pro- and pre-B cell stages, and NK cells at the immature NK cell stage. Altogether, our findings advocating for the importance of FAM83H role in the hematopoietic niches and leukocyte development will contribute to the unraveling of the role of Fam83h in the onset of arthritic lesions and, in general regulation of the immune system.

Th128. Safety and Pharmacodynamic Effects of Novel Fully Human Anti-thymocyte Polyclonal Igg Antibodies in an IND Enabling GLP Toxicology Study

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SAB-142 is a first-in-class fully human polyclonal anti-thymocyte immunoglobulin (ATG) produced in SAB Biotherapeutic's proprietary DiversitAb platform. As a fully human antibody treatment, SAB-142 is expected to be non-immunogenic and well tolerated when used in humans, as previously demonstrated by several clinical phase investigational products produced in the DiversitAb platform. In contrast, other anti-thymocyte agents currently on the market, such rabbit-ATG, are known to cause serum sickness and severe hypersensitivity reactions in humans. Transchromosomic bovines that produce fully human polyclonal antibodies underwent a series of immunizations with human thymocytes. Post immunization plasma was collected and purified into an anti-thymocyte fully human IgG immunotherapeutic (SAB-142). In vitro characterization of SAB-142 demonstrated binding to multiple human PBMC populations. In an IND-enabling GLP NHP Toxicology study, a single infusion of SAB-142 was administered at 1, 5, and 10 mg/kg (N=6 per dose) and an FDA approved rabbit-ATG was administered at 5 mg/kg (N=6). All 24 animals were assessed for safety and pharmacodynamic effects with clinical assessments, clinical labs, and PBMCs collected at various timepoints. Analysis of peripheral blood lymphocyte populations post-treatment showed dose-dependent depletion of CD45+ CD3+ pan-T lymphocytes, CD45+ CD3+ CD4+ T helpers, and CD45+ CD3+ CD8+ cytotoxic T lymphocytes through day 28. Our data suggest that SAB-142 demonstrates safety and dose-dependent pharmacodynamic effects in NHPs. These results support IND applications for human clinical trials to prevent and/or treat various diseases and conditions, including type 1 diabetes, other autoimmune diseases, and transplant indications.

Th129. Serum Neurofilament Light Chain (NfL)and CSF Glial Fibrillary Acidic Protein (GFAP) Together Improve the Prediction of Long-term Clinical Progression in Multiple Sclerosis

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Objective To evaluate the individual and combined prognostic utility of the protein biomarkers NfL and GFAP in serum and CSF from samples obtained in early MS to predict disability progression (CDP). Methods Paired serum and CSF samples within 5 years of MS onset with >15 years of follow-up were included. NfL and GFAP were measured by SiMOA. We converted raw sNfL to age-adjusted percentiles using normative Swiss data from >5000 controls. CDP was defined by sustained EDSS score worsening between 6 monthly follow-up visits. Results: Sixty patients were included and followed for a mean of 15.8 years (s.d. 2.5). 25 developed CDP. Serum NfL was strongly correlated to CSF levels (r=0.86, 95% CI 0.78-0.92), while serum GFAP had a weaker correlation with CSF (r=0.05, 95% CI 0.29-0.67). In ROC curve analysis, age-adjusted serum NfL (AAsNfL) and CSF GFAP (cGFAP) were the two strongest predictors of CDP at 15 years (ROC curve AUC 0.77, p=0.0004 and AUC 0.64, p=0.001). Serum GFAP was not predictive. In a Cox Proportional Hazard Regression, each percentile increase in baseline AAsNfL and cGFAP was associated with a 2.5% and 1.4% increased risk of developing CDP after adjustment for baseline EDSS score. Comparing the 13 patients in the highest quintile for both AAsNfL and cGFAP with the 17 patients in the lowest quintiles, there was a 7.3x risk of developing CDP (95% CI 1.4-10.9) (log-rank p=0.00093). Interpretation: The combination of sNfL with cGFAP improved the prediction of CDP compared to either marker considered in isolation.

Th130. SH2B3 Risk Allotype Is Associated with Increased Vascular Inflammation in Induced Pluripotent Stem Cells from Type 1 Diabetes Donors

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Genome wide association studies have identified a single nucleotide polymorphism within exon 3 of SH2B3 (rs3184504 (C784T)) as a genetic element associated with increased risk for Type 1 Diabetes (T1D). SH2B3 encodes lymphocyte adaptor protein (LNK), a potent negative regulator of inflammatory signaling in leukocytes and endothelial cells. rs3184504 results in a non-synonymous change, R262W, with LNK262W associated with elevated disease risk. We hypothesize that LNK262W fails to restrain inflammatory signaling leading to increased vascular inflammation contributing to the development of T1D. Induced pluripotent stem cells (iPSC) from donors with T1D

(n=4) were edited using CRIPSR/Cas9 at rs3184504 (to 784C (LNK262R) or 784T (LNK262W risk)) and differentiated into endothelial cells (iEC). A single donor gene-edited iEC was treated with TNFα in static culture and the expression of adhesion molecules assessed by flow cytometry. At 500U/mL for 4 hours, LNK262W (risk) iEC exhibited elevated adhesion molecule expression necessary for T cell transendothelial migration compared to common allotype (LNK262R) expressing HUVEC (CD54 = 3.88 fold-change; CD106 = 3.19 fold-change p=0.0032) and iEC (CD54 & CD106 = 1.65 fold-changes each). These results demonstrate that LNK262W fails to restrain inflammatory signaling in EC, which may contribute to the resulting mobilization of autoreactive T cells into the islets of Langerhans to promote beta cell destruction. Follow-up studies involve longer more physiologically relevant treatment times, and results from these studies will contribute to our understanding of how the risk allotype of LNK262W influences mechanistic disease progression and clinical outcomes by regulating vascular inflammatory functions and phenotypes.

Th131. Single Cell Sequencing Reveals Shared CD8+ T Cell Clones Between Skin and Synovial Tissue in Psoriatic Arthritis

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IL-17 producing CD8+ tissue resident memory T-cells (TRM) are implicated in both psoriasis and psoriatic arthritis (PsA), suggesting common inflammatory processes. This raises the possibility that T-cells migrate from skin to joints to cause arthritis. Using CITE-seq, we investigated whether the CD8+ T-cell infiltrate from paired biopsies of inflamed skin, synovial tissue +/-synovial fluid (n=4-6 PsA patients) is linked in terms of T-cell clonality and phenotype. Synovial tissue and fluid CD8+ T-cells clustered together and had similar T-cell receptor (TCR) repertoires and therefore were pooled. There were marked differences in the frequency and phenotype of TRM cells between skin and joint: 66% skin vs. 22% synovial CD8+ T-cells resided in TRM cell clusters (defined as upregulation of ITGAE +/-CD103 protein). 94% skin and 33% synovial TRM cells resided in IL-17+ TRM clusters; whereas most (67%) synovial TRM cells resided in IL-17A-GZMK+ TRM clusters. Within each patient, select clones were present in both skin and synovium, comprising on average 7% skin and 8% synovial CD8+ TCR repertoires. These shared clones tended to fall in TRM rather than non-TRM cell clusters (odds-ratio 3.3, $p< 0.0001$). In PsA, inflamed skin contains a higher proportion of CD8+ TRM cells and a stronger IL-17 signature than inflamed joints. However, CD8+ T-cells of the same clonotype are shared between both sites and shared clones have a TRM phenotype. These data indicate that the CD8+ T-cell infiltrate in skin and synovium is linked in terms of CD8+ TRM cell clonality. Supported by MRC and NIHR-BRC at KCL/GSTT

Th132. Single-cell Multi-omic Evaluation Reveals Differences in Metabolic Pathways Across B Cell Populations in Progression Toward Systemic Lupus Erythematosus

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Background. Systemic Lupus Erythematosus (SLE) is an interferon-related autoimmune disease which the control of B cell signaling seems to be abnormal. Preclinical disease is characterized by autoantibody production and immune dysregulation, but only about 4-8% of anti-nuclear antibody positive individuals develop an autoimmune disease. Metabolic pathways play important role in B cells development and proliferation. Methods. PBMCs from 32 subjects (ANA-, ANA+, incomplete lupus (ILE) and SLE patients) were sorted using NanoCellect Wolf microfluidic flow cytometer to remove dead and dying cells. Viable cells were tested in multi-omics single-cell analyses using 10X

Genomics 5' scRNA-seq/137-plex Total-seq multiomics using the 10X Chromium X fluidics system. Data were analyzed for distinct cluster identification and differential gene signatures in Python. Pathway analysis was performed in Ingenuity Pathway Analysis (IPA). Results. Six distinct B cell clusters (Naïve, Activated Naïve, Transitional, Memory B cells, ABCs and plasma cells) were identified across all subjects. The proportion of cells varied by disease groups. Fractions of Naïve B cells were higher in ANA- controls and decreased moving toward disease development. Memory B cells are higher in ANA+. IPA analysis suggest the involvement of pathways related to oxidative phosphorylation (downregulated in ANA+; upregulated in SLE), apoptosis (upregulated in ANA+), PI3K signaling (upregulated in ANA+) and autophagy (downregulated in SLE). Conclusions. We found differences in pathways associated with oxidative phosphorylation, autophagy and apoptosis in ANA+ individuals. These results suggest that dysregulation of those pathways might affect preclinical autoimmunity development trajectories.

Th133. Single-cell Multi-parametric Analysis of the Enthesitis-related Arthritis (ERA) Synovial and Circulatory Immunomes Evidences a Pathological Chemokine Gradient That Directs CD4+ T Cell-mediated Synovial Inflammation

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ERA, a common subtype of JIA, has a poor prognosis. ERA immunopathogenesis remains unclear, especially the ontogeny of synovial pro-inflammatory T cells. We compared, using a high-dimensional approach, the synovial and circulatory immunomes of active ERA patients. We also characterized CD4+ T cell subsets strongly associated with synovial inflammation. We interrogated, using mass cytometry, synovial (n=10) and peripheral (n=30) CD45+ immune cells from ERA patients with active joint inflammation, as well as cells from blood of healthy pediatric controls (n=30). To evaluate CXCR3+CCR6+CD4+ T cells, we sorted them from active ERA SFMCs and PBMCs and conducted in-vitro maturation and Treg suppression assays. Inflammation is localized to the synovium, marked by a deranged immune architecture suggestive of DC-mediated CD4+ T cell priming with diminished NK and B cell involvement. Functional derangements in the CD4+ T cell landscape are dominated by Th1 and Th17 cells. The synovium has been shown to be enriched with CXCR3 and CCR6 ligands, and high-dimensional analysis identified a pro-trafficking CXCR3+CCR6+ CD4+ T cell population that migrates into the synovium where they mature into producers of Th1 and Th17 cytokines. The synovial CD4+ Treg landscape displays a suppressive phenotype and is shaped by the same chemokine gradient. CXCR3+CCR6+CD4+ Tregs are bona fide Tregs that are expanded in the synovium and suppress their Teff counterparts, but Teffs may be resistant to suppression in-vivo. The results suggest a pathogenic chemokine gradient that directs the localization of disease in ERA, whereby Teff activation in the synovium overcomes regulation and perpetuates inflammation.

Th134. Single-cell Sequencing of PBMC Characterizes a Distinct Inflammatory Monocyte Subset in Lyme Disease Patients with Post-treatment Lyme Disease Syndrome (PTLD)

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Lyme disease (LD) is caused by Bacteria Borrelia and is transmitted through a bite of an infected tick. LD has become the most prevalent tick-borne disease in North America. LD usually can be treated by antibiotics, but still, around 20% of LD patients develop chronic joint inflammation (arthritis), fatigue, and musculoskeletal pain which is characterized as Post-Treatment Lyme Disease Syndrome (PTLD). Therefore, PTLD has been suggested to be triggered by an autoimmune response but how the immunological reality of PTLDs is not well known. In this work, we identified an increase of CD8+ Tregs as we found in various autoimmune diseases. Importantly, we also identified a

population of inflammatory CD14+ monocytes dominant in PTLD patients, compared to return-to-health patients and healthy patients through single cell sequencing analysis. This monocyte cluster displayed distinct inflammatory gene signatures such as CCL3, CCL4, IL1B, CXCL2, CXCL8, and others. Similar inflammatory macrophages have been observed recently in RA, and so both these results firmly place Lyme disease in the realm of autoimmunity, which has not been very clear previously. Tracking this inflammatory monocyte subset could lead to a more accurate prediction of PTLD and identify molecular biomarkers and pathways for therapeutic intervention.

Th135. Sodium Perturbs Mitochondrial Respiration and Induces Dysfunctional Tregs

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FOXP3+ regulatory T cells (Tregs) are central for peripheral tolerance and their deregulation is associated with autoimmunity. Dysfunctional autoimmune Tregs display pro-inflammatory features and altered mitochondrial metabolism but contributing factors remain elusive. High salt (HS) has been identified to alter immune-function and to promote autoimmunity. By investigating longitudinal transcriptional changes of human Tregs, we identified that HS induces metabolic reprogramming, recapitulating features of autoimmune Tregs. Mechanistically, extracellular HS raises intracellular Na+, perturbing mitochondrial respiration by interfering with the electron transport chain (ETC). Metabolic disturbance by temporary HS-encounter or ETC blockade rapidly induces a pro-inflammatory signature, leading to long-term dysfunction in vitro and in vivo. Our results indicate that salt could contribute to metabolic reprogramming and that short-term HS-encounter perturb metabolic fitness and long-term function of human Tregs with important implications for autoimmunity.

Th136. T Cell Auto-proliferation in Multiple Sclerosis

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Introduction: Multiple sclerosis (MS) is an inflammatory disease characterized by distributed functional changes from MS susceptibility variants across all immune cell types and T and B cell modulation in the clinical setting. Autoproliferation of T cells has been reported as one dysfunctional response in MS. Here, we aim to assess the auto-proliferative capacity of cells from untreated MS patients in comparison to both treated patients and healthy donors. In addition, we are expanding our study to samples from at-risk MS patients' family members to assess for the presence of auto-proliferative phenomena prior to disease onset. Methods: Cryopreserved PBMCs from untreated and treated MS patients, healthy donors, and at-risk MS patient family members were thawed, stained with CFSE and cultured for 7 days in AIM-V media without activation. PBMCs were collected and stained for CD3, CD4, CD54RA, CD27, HLA-DR, CD20, using multiparametric flow cytometry (LSR II) we T cell proliferation was measured. Results: Untreated RRMS patient samples have significantly higher frequency of auto-proliferating CD3+CD4+HLA-DR+ T cells than healthy donors. Treatment with Glatiramer Acetate (GA) appears to diminish the auto-proliferation pattern observed in RRMS. The remaining samples are currently being processed and will be presented at the conference. Conclusion: We have replicated the reported auto-proliferative capacity of T cells in MS. The underlying immune dysfunction for the auto-proliferation appears to be reversible with GA, which has been reported to shift T cell phenotypes and enhance Treg function. We therefore set the stage for a more detailed, mechanistic investigation of autoproliferation in MS.

Th137. T Cell-specific Deletion of rab4a Leads to Hepatic Mtor Activation, Liver Inflammation, and Reduction of Regulatory T Cell Expression in Systemic Lupus Erythematosus

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HRES-1/Rab4 (Rab4A) is a GTPase that mediates the accumulation of oxidative stress-generating mitochondria and activation of the mechanistic target of rapamycin (mTOR) in T cells of systemic lupus erythematosus (SLE) patients and livers of SLE-prone mice. We investigated the impact of Rab4A on liver disease, mTOR activation, and immunity in SLE-prone mice. Constitutively active Rab4A (Rab4AQ72L) and T cell-specific deletion of Rab4A (Rab4ACD4KO) were generated on lupus-prone SLE1.2.3. B6-triple congenic (B6.TC) mice. Rapamycin 3μg/g (or carboxymethylcellulose vehicle control) was administered to 27-week-old B6.TC mice 3 times weekly for 14 weeks. Liver chunks were homogenized for Western Blot analysis or fixed in formalin for histopathology and splenocytes were analyzed by flow cytometry. Among vehicle-treated mice, B6.TC.Rab4ACD4KO livers had more inflammatory foci/mm2 (0.39±0.092) compared to B6.TC.Rab4AQ72L livers (0.13±0.027; p=0.0498). B6.TC.Rab4ACD4KO livers had elevated p-4EBP1, a target of mTORC1, compared to B6.TC.Rab4AQ72L livers (p=0.0428). B6.TC.Rab4ACD4KO livers had more CD3+ pixels/mm2 (664126±113298) compared to B6.TCWT (270447±68656; p=0.019) and B6.TC.Rab4AQ72L (135154±10457; p=0.042) and more B220+ Pixels/mm2 (19837±973) compared to B6.TC.Rab4AQ72L mice (5683±2169; p=0.004). CD3 and B220 clustered together within the inflammatory foci of the B6.TC.Rab4ACD4KO livers. Flow cytometry of B6.TC.Rab4ACD4KO splenocytes showed a 65% decrease in the populations of FoxP3+CTLA-4+ CD4+ T cells (p=0.0486) and a 45% decrease in FoxP3+Helios+ CD4+ T cells (p=0.033) compared to B6.TC.Rab4AQ72L mice. T cell-specific deletion of Rab4A promoted inflammation, mTOR activation, and T and B cell clustering in the livers of lupus-prone B6.TC mice, which may be driven by defects in regulatory T cell populations.

Th138. TARGETING INSULIN RESISTANCE IN TYPE 2 Diabetes and Morbid Obesity

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Problem: Over activation of Factor H and Factor D is linked with insulin resistance and metabolic abnormalities in Type 2 diabetes and morbid obesity (Moreno-Navarrete J. M et al in Diabetes 59:200–209, 2010). Method: A balanced inhibitor of Factor H and Factor D is developed using nano sulfonic polymers. Result: Inhibition of alternative complement activation in omental adipose tissue is feasible therapeutically to improve insulin sensitivity and reduce insulin resistance. Discussions: Early immune modulation of dysfunction of Alternate complement system will provide additional therapeutic benefits in Type 2 diabetes and obesity.

Th139. th2 Cell Subsets Predict Disease Progression in Type 1 Diabetes: A Role for th2 Clonal Expansion in Remission?

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Many people newly diagnosed with Type 1 Diabetes (T1D) experience a partial remission that can last from a few weeks to two years. Length of remission (LoR) is associated with residual beta-cell function and a reduced risk of secondary complications. CD4+CD25+CD127hi (127-hi) cells are a novel T cell population identified by our group, whose frequency at diagnosis correlates with LoR. Although 127-hi cells are a mixed population of naïve and memory anti-inflammatory Th2 and pro-inflammatory Th1 cells, compared to other CD4 subsets they have an enhanced Th2 profile. These data have led us to hypothesize that 127-hi Th2 cells actively prolong partial remission. To this end we have quantified cell subsets within the 127-hi cell population from the pediatric ExTEND study and found that it is the frequency of Th2 effector memory (EM) cells drives the correlation with LoR. Paired scRNAseq

with TCR sequencing of 127-hi cells has identified unique Th2 cell clusters within this compartment that clonally expand. Th2 clusters are identified by classical (GATA3) and non-classical (CRTH2) Th2 genes, compared to other memory CD4 T cells. A typical Th2 cluster shows upregulation IL17RB, IL9R, TNFRSF11A, CCR4, IL4R, GATA3-AS1 along with downregulation of Th1-like genes GZMK, GZMA, IFNG-AS1, EOMES. Interestingly, these Th2 clusters are absent in other memory CD4 subsets. Our combined data show a link between LoR and the clonal expansion of 127-hi Th2 EM. Additional analysis will reveal whether the patients that experience 127-hi Th2 EM clonal expansion have a longer partial remission.

Th140. The Association with HLA-DRB1*11:01 in anti-caspr2 Syndromes Is Exclusive to Limbic Encephalitis
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Background and Objectives: Antibodies against contactin-associated protein-like 2 (CASPR2) are associated with three neurological phenotypes: limbic encephalitis (LE), acquired neuromyotonia and Morvan syndrome. We analyzed human leukocyte antigen (HLA) subtypes in these three phenotypes versus controls. Methods: Four-digit resolution HLA genotypes were imputed from available Genome Wide Association data, and carrier frequencies compared between anti-CASPR2 patients (n=61) and controls (n=338), stratified by phenotype (LE=45, Morvan=9, neuromyotonia=7). Fisher exact tests and logistic regressions controlled by principal component analysis were used. Results: No HLA association was observed in neuromyotonia and Morvan syndrome. In contrast, LE was strongly associated with DQA1*05:05 (OR=5.4, 95% CI 2.5-11.5, p=1.5e-04), DQB1*03:01 (OR=4.3, 95% CI 2.0-9.4, p= 2.1e-03), DRB1*11:01 (OR= 8.5, 95% CI 3.9-18.7, corrected p=1.7e-06), and DRB3*02:02 (OR= 4.9, 95% CI 2.2-11.4, corrected p=3.4e-04) in the context of the common DR11~DQ3 haplotype. Importantly however, DQA1*05:05, DQB1*03:01 and DRB3*02:02 were no longer significant after controlling for DRB1*11:01, whereas DRB1*11:01 was still significant after controlling for DQB1*03:0 and DRB3*02:02, but not DQA1*05:05, indicating a primary DRB1*11:01 association. No secondary associations were found in non-DRB1*11:01 carriers or across DRB1*11:01 heterozygotes. DRB1*11:01 homozygosity was associated with a 10.9-fold increased risk versus heterozygotes (p=1.3e-03). Conclusions: HLA association is specific to DRB1*11:01 and to patients with LE, with a strong effect of homozygocity. DRB1*11:04, another frequent subtype with only one aminoacid difference, is not associated. The specific association to LE suggests a different pathogenesis versus other anti-CASPR2 phenotypes.

Th141. The Cellular Metabolism of SLE NK Cells Is Primarily Altered at the Level of Mitochondrial Homeostasis
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Systemic lupus erythematosus (SLE) is an inflammatory autoimmune disease, in which the role of natural killer (NK) cells remains poorly understood. In patients with SLE, NK cells are decreased in number, exhibit reduced cytotoxicity and impaired cytokine production. Herein, we examined the metabolic alterations of human SLE NK cells, compared to healthy controls. Real-time metabolic assay showed that SLE NK cells mitochondrial respiration (OXPHOS) is significantly increased, while glycolysis is similar to healthy controls, suggesting alterations in mitochondrial homeostasis. Accordingly, we examined the mitochondrial fitness of SLE NK cells. We observed that mitochondrial mass is increased, while mitochondrial activity is decreased, suggesting an accumulation of dysfunctional mitochondria. Mitochondria of SLE NK cells displayed increased levels of superoxide, thereby predisposing them to mitochondrial damage, defective debris clearance, and apoptosis. There was no difference in mitochondrial DNA quantification between SLE NK cells and healthy controls, suggesting that mitochondria are increased in size but not in number. Transmission electron microscopy showed mitochondrial cristae disorganization in SLE NK cells. Quantitative proteomic analysis revealed that SLE NK cells express high levels of proteins associated with

mitochondrial synthesis (TOM6) and OXPHOS (ATP synthase F(0), NADH deshydrogenase1 C1). In parallel, we observed a reduction in key proteins for mitochondrial clearance (such as E3 ubiquitin ligases: RNF181, MARCHF5), and lysosomal acidification (Proton-transporting V-Type ATPase). These results suggest an alteration in mitochondrial degradation (mitophagy) in SLE NK cells and suggest that impaired mitochondrial function and homeostasis represent a major feature of SLE pathogenesis.

Th142. Discovery of a Novel High Affinity Anti-human CD122 Antagonist Monoclonal Antibody (ANA033) That Abrogates IL-2 and IL-15 Signaling for the Treatment of T Cell-mediated Inflammatory and Autoimmune Diseases

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T cells play a crucial role in the development and progression of autoimmunity by regulating self-tolerance and participating in inflammation. Signaling through CD122, the beta subunit of the IL-2 and IL-15 receptors, is essential for the function of CD4, CD8 T cells and NK cells. CD122 signaling is also essential for tissue resident memory T cell (Trm) development and survival. Blockade of CD122 leads to elimination and/or reduction of the proliferation of pathogenic T cells in autoimmune diseases. We discovered a high affinity monoclonal antibody, ANA033, that binds CD122 and blocks IL-2 and IL-15 signaling. In vitro, using human peripheral blood, ANA033 inhibited IL-2 and IL-15-mediated primary human CD4, CD8 T cells, and NK cell proliferation. In vivo, we used a xenogeneic graft versus host disease (X-GvHD) model, in which human PBMCs were transferred into immunodeficient mice that constitutively expressed human IL-15. Over-expression of IL-15 in this GvHD model accelerated PBMC engraftment, enhanced weight loss and lethality and promoted the expansion of T cells and NK cells. Importantly, belatacept (CTLA-4 Ig), a reference standard of care that demonstrates survival efficacy in standard GvHD models, failed to maintain survival of the mice. Administration of ANA033 improved survival and suppressed expansion of T cells and NK cells, demonstrated a profound survival benefit superior to belatacept treatment, and continued to maintain survival even after cessation of dosing. Here, we propose that blockade of CD122 may provide great therapeutic value in the treatment of T cell-mediated inflammatory autoimmune disorders.

Th143. The Promise of Machine Learning: Using Seismic's IMPACT Platform to Design Igg Cleaving Enzymes for Chronic Treatment of Autoimmune Diseases

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Proteases derived from human pathogens can specifically cleave IgG into F(ab')2 and Fc fragments. This unique trait suggests a novel opportunity to use these molecules to treat auto antibody mediated disease. IdeS, an IgG cleaving enzyme derived from Streptococcus pyogenes has shown clinical proof of concept and is approved for use before kidney transplant. Due to the immunogenic nature of these proteases, the dosing regimen is impacted by pre-existing antibodies and the induction of anti-drug antibodies after dosing. To mitigate the impact of the immune system on our novel enzyme, we employed our IMPACT platform leveraging machine learning to reduce T and B cell epitopes and to ensure that our molecule exhibits desirable drug like properties while maintaining enzymatic activity. To extend the pharmacokinetics (PK) of our molecule, we fused it with a Fc domain. To evaluate IgG protease PK and pharmacodynamics (PD), intravenous immunoglobulin (IVIg) was injected at different time points after protease treatment and IgG levels were quantified by MSD. As expected, the addition of the Fc increased the molecule's half-life that resulted in a PD effect at later time points than observed with a control enzyme without a Fc. Taken together, our IMPACT platform leveraging machine learning demonstrates that we can optimize drug-like properties and reduce the immunogenicity of a molecule while maintaining function, resulting in a potential novel treatment for

chronic autoimmune diseases.

Th144. Thyroid Cells from Normal and Autoimmune Thyroid Glands Suppress upon Contact T Lymphocyte Proliferation -but Not Cytokine Production- revealing a Inhibitory Activity Independent of PD-1/PD-L1

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Immune Checkpoint Receptors include a number of inhibitory receptors that limit tissue damage during immune responses; blocking PD-1/PD-L1 checkpoint receptor axis led to a paradigm shift in cancer immunotherapy but also to autoimmune adverse effects, prominently thyroid autoimmunity. Although PD-L1 expression by human thyroid follicular cells (TFCs) of autoimmune glands was previously reported by our group, the effects of the direct interaction between T cells and thyroid cells expressing PD-L1 was not been investigated. Here we report that autologous primary TFCs, but not transformed TFCs, strongly inhibit CD4 and CD8 T cell proliferation but no cytokine production. Cocultures using of 0.4 µm pore transwell inserts and/or saturating concentrations of anti-PD-1 blocking antibody (Nivolumab, Opdivo®) demonstrated that this effect was not mediated by PD-1/PD-L1 pathway. Beta galactosidase analysis excluded culture-induced senescence as an explanation. High resolution flow cytometry demonstrated that autologous TFC/T cells coculture induces the expansion of several subsets of double negative (DN) T cells characterized by high expression of activation markers and negative immune checkpoints. scRNA-seq demonstrated that dissociated TFC express numerous candidate molecules for mediating this immune regulatory activity, including CD40, E-Cadherin, LGALS3 or TIGIT ligands. These ligands directly or through the generation of a suppressor population of DN T cells are most likely the responsible of TFC in vitro immunosuppressive activity. These results contribute to reveal the complex network of inhibitory mechanism that operate at the tissue level to restrain autoimmunity but also point to pathways, other that PD-1/PD-L1, that can contribute to tumor evasion.

Th145. Tolerogenic Bone Marrow-derived Dendritic Cells Prevent Autoimmune Diabetes by Inhibiting th17 Differentiation Through PI3K/C/EBP Signaling

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Dendritic cells (DCs) are key regulators of the adaptive immune response. Tolerogenic dendritic cells play a crucial role in inducing and maintaining immune tolerance in autoimmune diseases such as type 1 diabetes in humans as well as in the NOD mouse model. We previously reported that bone marrow-derived DCs (BM.DCs) from NOD mice, generated with a low dose of GM-CSF (GM/DCs), induce Treg differentiation and are able to protect NOD mice from diabetes. We also found that the p38 MAPK/C/EBPβ axis is involved in regulating the phenotype, as well as the production of IL-10 and IL-12p70, by tolerogenic GM/DCs. Here, we report that the inhibition of the PI3K signaling switched the cytokine profile of GM/DCs toward Th17-promoting cytokines without affecting their phenotype. PI3K inhibition abrogated the production of IL-10 by GM/DCs, whereas it enhanced their production of IL-23 and TGFβ. Inhibition of PI3K signaling in tolerogenic GM/DCs also induced naive CD4+ T cells differentiation toward Th17 cells. Mechanistically, PI3K inhibition increased the DNA-binding activity of C/EBPβ through a GSK3-dependent pathway, which is important to maintain the semimature phenotype of tolerogenic GM/DCs. Furthermore, analysis of C/EBPβ−/− GM/DCs demonstrated that C/EBPβ is required for IL-23 production. Of physiological relevance, the level of protection from diabetes following transfusion of GM/DCs into young NOD mice was significantly reduced when

NOD mice were transfused with GM/DCs pretreated with a PI3K inhibitor. Our data suggest that PI3K/C/EBPβ signaling is important in controlling the tolerogenic function of GM/DCs by limiting their Th17-promoting cytokines.

Th146. Treatment of Autoimmune Diseases by Neuromodulation

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Neuromodulation has been proposed as a potential therapeutic option for autoimmune diseases. Indeed, the autonomic nervous system can inhibit inflammation via the binding of catecholamine and acetylcholine to their respective cognate receptors expressed by immune cells. Clinical studies showed that vagus nerve stimulation (VNS) can be beneficial for patients with rheumatoid arthritis and Crohn's Disease. However, this approach lacked efficacy in some patients and VNS had adverse effects presumably because the vagus nerve contains both afferent and efferent fibers, which regulate the function of multiple organs. We hypothesized that applying electrical stimulation to exclusively efferent nerves proximal to target organs could potentially be a more efficacious and specific therapy. We tested the therapeutic efficacy of splenic nerve electrostimulation (SNES) in collagen-induced arthritis (CIA) and pancreatic nerve stimulation (PNES) in spontaneous type 1 diabetes (T1D) in mice. SNES treatment of CIA mice significantly reduced clinical scores compared to sham treated animals in a β- adrenergic receptor dependent manner. After SNES treatment, the numbers of autoantibody-secreting cells were reduced, as well as serum levels of TNF and anti-collagen antibodies. In a spontaneous mouse model of T1D, PNES inhibited disease progression in diabetic mice. PNES resulted in β-adrenergic receptor-mediated-accumulation of B and T cells in pancreatic lymph nodes (pLNs) and reduced production of pro-inflammatory cytokines. Autoreactive T cells showed reduced proliferation in pLNs of mice receiving PNES as compared to sham controls. In conclusion, our findings provide a rationale to further explore peripheral nerve neuromodulation as a treatment for autoimmune diseases.

Th147. Tuning Peptide Specificity for T Cell Tolerance in Type 1 Diabetes

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Type 1 Diabetes (T1D) is a classical T-cell mediated autoimmune disease, and fundamental data have implicated insulin as the dominant autoantigen in T1D disease. In the NOD mouse model of T1D, notable studies have found that mice lacking native insulin expression but with an altered insulin sequence to maintain blood glucose levels are protected from insulitis and diabetes. Growing evidence also indicates how the insulin peptide is bound within MHC Class II (peptide register) is essential in determining the strength of interactions and recognition of autoreactive T-cells. We have uncovered a unique peptide binding characterized by the dominant insulin epitope InsB:9-23. The majority of InsB:9-23-specific CD4 T-cells in the periphery recognize insulin bound in the unusual register 3, and knocking in this single amino acid variation into just one copy of the insulin gene in NOD mice, confers protection against T1D. In addition, these mice were protected from T1D, even when treated with checkpoint inhibitor blockade, unlike their wild-type littermates. Introducing this superagonist epitope into the NOD mouse has allowed us to address several key questions surrounding pathways of peptide generation, presentation by MHC molecules, and recognition by auto-reactive pathogenic T cells. In addition, we have been able to model how central and peripheral tolerance mechanisms can be altered when major epitopes in the Insulin gene are mutated, allowing us to explore means of dominant tolerance to understand the potential for translation into treatments for T1D.

Th148. Type I Interferon-dependent and -independent TRIF Signaling Are Required for Autoantibody Generation in an Induced Model of Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) is an autoimmune condition characterized by the production of autoantibodies to cellular and nuclear self-antigens, with damage to multiple organs. Immunization with the SLE autoantigen β2-glycoprotein I (β2GPI) and TLR4 ligand lipopolysaccharide (LPS) induces a murine model of SLE characterized by the generation of autoantibodies to multiple SLE antigens. LPS induces both inflammatory cytokines and type I interferons (IFNs) by signaling through the adaptor proteins MyD88 and TRIF, respectively. We investigated the mechanisms of LPS-dependent signaling on the induction of murine SLE following β2GPI/LPS immunization. Immunization of mice deficient in MyD88 or TRIF with β2GPI/LPS revealed that LPS-dependent TRIF signaling preferentially promoted autoantibody production in this model, compared to MyD88 signaling. Furthermore, SLE-specific autoantibody production was induced by TLR3 or TLR4 agonists, in combination with β2GPI, but not by agonists of TRIF-independent TLRs. RNA-sequencing of dendritic cells and macrophages isolated from wildtype (WT) and TRIF-deficient mice immunized with β2GPI/LPS revealed differential expression of both type I IFN-dependent and -independent genes. Moreover, type I IFN receptor (IFNaR)-deficient mice immunized with β2GPI/LPS showed diminished autoantibody production compared to WT mice, but to a lesser extent than TRIF-deficient mice. We conclude that LPS-dependent TRIF signaling is required for the generation of autoantibody production in our induced SLE model, both through type I IFN-dependent and -independent effects.

Th149. Ultrasensitive Immune Profiling of Autoimmune Diseases with 200-plex Nulisaseq Inflammation Panel

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Cytokines and chemokines are critical components of the immune system and play important roles in autoimmune diseases. Comprehensive profiling of proteins 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 a novel automated multiplex immunoassay technology, NULISA™, capable of attomolar-level sensitivity, and a 200-plex inflammation-focused panel targeting 124 cytokines/chemokines and 80 other important inflammation and immune response-related proteins. We analyzed 21 plasma samples from patients with rheumatoid arthritis (n=5), Sjögren's syndrome (n=5), systemic lupus erythematosus (n=5), and ulcerative colitis (n=6), and 79 healthy donor samples using the 200-plex NULISAseq™ Inflammation Panel and the Olink Explore 384 Inflammation assay. Differential expression analysis using linear models identified 115 and 94 significant targets at a 5% false discovery rate in the NULISAseq and Olink Explore datasets, respectively. Among the 92 targets shared between these two platforms, 54 and 43 significant targets were identified by NULISAseq and Olink, respectively, with 36 in common. Many of the targets identified only by NULISAseq were low-abundance targets that were poorly detected by the Olink assay (IL4, IL5, IL20, IL17A, IL17F, IL33, and IL2RB) but have important roles in autoimmune diseases. In summary, with improved sensitivity and the most comprehensive inflammatory cytokine/chemokine panel, NULISA promises to be a powerful discovery tool for autoimmune disease research, which may lead to new diagnostic biomarkers and therapeutic targets.

Th150. Immunophenotypic Abnormalities Similar to Established Rheumatoid Arthritis Are Present in ACPA(+) At-risk Individuals

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RA-associated autoantibodies (RF, ACPA) are detectable before the onset of clinically-apparent RA (i.e. “Clinical RA”), defining a state known as ‘At-Risk’ for future RA. However, the immunophenotype of this At-Risk state remains incompletely understood. Using a cross-sectional methodology, we analyzed plasma proteomics, PBMC compositions by flow cytometry, and PBMC transcriptomes by scRNA in a cohort of ACPA(+) At-Risk individuals and age, sex and BMI matched ACPA(-) controls. We observed significant differences in 138 plasma proteins between ACPA(+) individuals vs. ACPA(-) controls, including IL-6, IL1B, CCL3, and additional elevated inflammatory chemokines and cytokines. From flow cytometry and scRNA, we observed numerous PBMC phenotypic changes, notably increases in IgD- CD27- B cells and Tregs. A global enrichment of the oxidative phosphorylation pathway, characterized by the upregulation of the ubiquinone oxidoreductase supernumerary subunits (NDUF) genes, was observed across multiple immune cell types including T, B, and monocytes. Furthermore, IgD- CD27- B cells from ACPA(+) individuals expressed significantly lower levels of genes previously shown to be increased in IgD- CD27- B cells or autoimmune processes, including SAMHD1, ARID3A, and ZC3H12D, suggesting dysregulation of this compartment at the preclinical stage. ACPA(+) At-Risk individuals demonstrate numerous immune-related changes that are similar to changes in established Clinical RA, suggesting the “At-Risk” state is on a continuum with Clinical RA. These findings provide insights to potential biomarkers and immunopathologic mechanisms that may impact how we define the ‘At-Risk’ state and its development into RA, and suggest targets for interventions or intermediate endpoints in trials for RA prevention.

Th151. Upregulated PD-1 Expression in Tregs of Thymoma-associated Myasthenia Gravis with Immunodeficiency

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Introduction: Immunodeficiency associated with thymoma, known as Good's syndrome (GS), is characterized by recurrent severe infections and hypogammaglobulinemia/ B lymphopenia. Involvement of both B cell and T cell dysfunction has been reported but the molecular mechanism of impaired response to infection remains unclear. Purpose: To elucidate the role of immune checkpoint molecules in the pathogenesis of immunodeficiency in GS. Method: Peripheral blood mononuclear cells from thymoma-associated myasthenia gravis (TAMG) patients (n=18) were evaluated. TAMG patients with at least two previous episodes of infections requiring hospitalization (GS) and those without (non-GS) were compared to age-matched disease-free controls. Results: PD-1 expression on CD4+Foxp3+ cells (Tregs) was increased in GS patients (n=8) compared to controls (n=10) but not in non-GS patients (n=10) ($p< 0.05$, Mann-Whitney U test). PD-1+Tim-3+ Tregs were also increased in GS. The mean fluorescence intensity of PD-1 on Tregs was significantly higher in GS than in controls and was negatively correlated with B cell frequency ($Rs=-0.81$, $p=0.01$, Spearman's correlation coefficient). Cell viability assay on B cells by anti-CD40 antibody stimulation revealed that co-culture with autologous Tregs increased Annexin-V negative necrotic cell number ($p< 0.01$, Wilcoxon signed-rank test). A partial reversal of the effect was observed by anti-PD-L1 antibody, however, it was not statistically significant. Discussion/Conclusion: The role of check point molecules on Tregs is still under debate. We propose that the upregulation of PD-1+ on Tregs is involved in dampening immune response in

TAMG. Suppression of B cell survival by Tregs may be a factor contributing to B cell dysfunction in GS.

Th152. Virus Infection Causes Intestinal Dysbiosis to Promote Type 1 Diabetes Development

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Environmental factors including enterovirus infection and microbiome dysbiosis have been independently linked to type 1 diabetes in both humans and in mouse models. However, it is unknown whether these risk factors are able to engage in cross-communication to influence predisposition to diabetes development by skewing host physiology and immune homeostasis. Non-obese diabetic (NOD) mice were infected with coxsackievirus B4 (CVB4) and microbial community profiling using 16S rRNA sequencing as well as targeted metabolomics was used to determine how infection longitudinally alters the intestinal microbiome profile. Upon infection, CVB4 induced rapid onset of diabetes, immune infiltration into the pancreatic islets, an increased abundance of autoreactive CD8+ T cells, as well as reorganisation of the intestinal microbiota community composition. The dysbiosis resembled that of mice which spontaneously developed diabetes and resulted in reduced production of short chain fatty acid metabolites. Accompanying this dysbiosis, disruption of intestinal barriers caused increased intestinal permeability and bacterial translocation to promote inflammation. Furthermore, host antibody IgG and IgA responses to commensal bacterial antigens were increased both systemically and within the gut of infected mice. Finally, performing a fecal microbiome transfer (FMT) of the CVB4-induced “diabetogenic” microbiome to antibiotic-depleted NOD recipients was sufficient to promote diabetes onset in the absence of virus infection by reducing regulatory T cell responses in the intestinal environment. These findings signify virus infection can drive dysbiosis and disrupt intestinal immune homeostasis in a way that contributes to diabetes autoimmunity.

Th153. Whole Blood Based Functional Flow Cytometry Assays for Pre-clinical Research in Rheumatoid Arthritis

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Background Alternatives to animal, cell lines or isolated cell models are wanted to provide higher relevance and less ethical concerns for pre-clinical research. Despite extended capabilities, we believe that whole blood is not frequently enough considered. Easy to collect and applicable to functional studies, it contains many components (cells, proteins, metabolites, etc.) and enables to capture donor heterogeneity. Rheumatoid arthritis (RA) is a chronic inflammatory disease for which many treatments, such as anti-TNFs, exist. Still, they are not fully satisfactory since 30% of patients are non-responders, yielding a need for better pre-clinical models and approaches to patient characterization and stratification. Objective We propose a whole blood-based functional assay applicable to characterize therapeutic molecules and RA samples. Methods Whole blood from control or RA individuals fulfilling ACR/EULAR 2010 criteria was incubated with LPS alone or combined with anti-TNFs. Activation marker expression was measured using a 10-color flow cytometry panel following a streamlined workflow. Results Anti-TNF mechanisms of action were assessed following CD69, CD16, tmTNF and CD54 expressions in control samples. When considering untreated RA patients, higher basal activations through dysregulated CD69, CD11b or CD62L levels in a variety of cell subsets were observed together with impaired capabilities to up/downregulate these markers upon LPS activation. Finally, untreated and treated RA patients exhibited differences in activation marker expressions, providing a certain degree of segregation according to different clinical questions. Conclusion In conclusion, we believe whole blood based functional assays could be relevant and valuable tools to open new perspectives for RA pre-clinical research.

Tu101. 18F-FDG PET/CT Mapping of Functional Microbial Niches to Understand Host Glucose Regulation After BCG Vaccinations

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When the bacillus Calmette-Guérin (BCG) vaccine (live, avirulent Mycobacterium bovis) is introduced as experimental therapy into human hosts with underlying type 1 diabetes (T1D), the BCG bacillus gradually shifts energy metabolism in host blood lymphoid cells from oxidative phosphorylation to aerobic glycolysis, drawing more glucose out of the blood to fuel intracellular metabolism. This may underlie the BCG bacillus' therapeutic benefit: systemic, long-term reduction of excess blood glucose in TID patients. The organ-specific niches where the BCG bacillus alters metabolism and establishes persistent residence are not known. In a human clinical trial, we mapped organ niches for the BCG-induced shift to aerobic glycolysis using fluorine-18 fluorodeoxyglucose (18F-FDG) positron emission tomography (PET) and x-ray computed tomography (CT). This allowed us to identify organs with heightened glucose uptake in T1D participants (n=6) over a 2-year period after BCG vaccination versus prior and confirmed earlier work that BCG vaccination gradually lowers blood sugar levels without contribution from endogenous insulin. We also tested BALB/c mice (n=17) for the presence of BCG colonies in particular organ niches before and after vaccination. Results from the human and murine studies concurred that the major anatomic site of functional metabolic change and residence is the spleen. The BCG bacillus also transiently mapped to the bone marrow, liver, circulating lymphocytes in the descending aorta, and lungs. These findings support the spleen as the niche for the BCG vaccine's functional improvement of metabolism, and it is a lymphoid organ large enough to explain BCG's systemic benefits in T1D.

Tu102. A Bioluminescent Homogeneous Immunoassay to Detect Binding and Neutralising Anti-interferon Gamma antibodies Using Nanobit® Technology

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Endogenous antibodies that neutralize interferon-γ are a cause of severe adult-onset immunodeficiency with susceptibility to mycobacteria and other opportunistic infections. The syndrome was first recognised in 2012 in a cohort of Thai and Taiwanese patients with an AIDS-like immunodeficiency without evidence of HIV infection. A homogeneous immunoassay using NanoBiT® (Promega) was developed that demonstrates anti-interferon-γ antibody binding and neutralization of interferon-γ in vitro. NanoBiT® involves splitting the bioluminescent NanoLuc enzyme into a Large (18kDa) and Small (11 amino acids) BiT. Conjugation of Large and Small BiT to antibodies enables specific analyte detection with reconstitution of the functional enzyme when components are brought into proximity. Serum from patients known to have the AIDS-like immunodeficiency caused by the presence of anti-interferon-γ antibodies is added to a solution of interferon-γ. Binding of these antibodies to interferon-γ is demonstrated by the addition of human anti-interferon-γ conjugated to Large BiT and anti-human IgG. Reconstitution of NanoLuc occurs in the presence of an interferon-γ/anti-interferon-γ IgG immune-complex. The neutralising ability of these antibodies was demonstrated by measuring recovery of interferon-γ in 50µL of a 90000pg/mL solution. Interferon-γ levels were measured by detection antibodies to interferon-γ conjugated to Large and Small BiT. Patients with the AIDS-like immunodeficiency caused by the presence of anti-interferon-γ antibodies repeatedly demonstrated >99% neutralisation of interferon gamma in solution. These assays demonstrate the presence and functional significance of autoantibodies to interferon-γ in the aqueous phase, enabling rapid assessment without cumbersome wash steps. The methodology could be utilized to demonstrate the presence of any autoantibody.

Tu104. Additive Efficacy of a Novel Bispecific anti-tnf/il-6 NANOBODY® Compound in Translational Models of Rheumatoid Arthritis

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Rheumatoid Arthritis is one of the most common autoimmune diseases affecting primarily the joints. Despite successful therapies including antibodies against TNF and IL-6R, only 20-30% of patients experience remission. We studied whether inhibiting both, TNF and IL-6, would result in improved efficacy. Using backtranslation from single cell RNASeq data from RA patients, we hypothesized that TNF and IL-6 act synergistically on fibroblast-like synoviocytes (FLS) and T cells. We could prove this hypothesis in a coculture of FLS from RA patients with T cells, both on disease-driving pathways and biomarkers. This translated into superiority of a combination of anti-TNF and anti-IL-6 antibodies in collagen-induced arthritis (CIA) mouse models including sustained long-term remission, improved histology scores and effects on bone remodeling pathways. These promising data initiated the development of a novel anti-TNF/IL-6 bispecific NANOBODY® compound, with similar potencies against both, TNF and IL-6. We observed also additive efficacy of this NANOBODY® compound in a FLS/T cell coculture affecting arthritis and Th17 pathways. The NANOBODY® transcript signature inversely overlapped with described RA patient endotypes, indicating a potential efficacy in a broader patient population. Finally, we developed a quantitative systems pharmacology model for Rheumatoid Arthritis. The QSP model highlighted an increased efficacy versus the current monospecific therapies blocking either TNF or IL-6R, even with a lower dose. In summary, we showed superiority of our bispecific anti-TNF/IL-6 NANOBODY® compound over monospecific treatments in in vitro, in vivo and system pharmacology disease models and expect improved efficacy in current clinical studies.

Tu105. Adult-onset Still's Disease in a Young Adult Patient Presenting as Fever of Unknown Origin Treated with Tocilizumab: A Case Report

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Adult-onset Still's disease (AOSD) is a rare systemic inflammatory disorder characterized by quotidian pattern of fever in the absence of infections, malignancies, and rheumatic diseases. Other features include evanescent rash, arthralgia, sore throat, lymphadenopathy, splenomegaly, leukocytosis, liver dysfunction, and negative anti-nuclear antibody (ANA) and rheumatoid factor (RF). AOSD has been associated with markedly elevated serum ferritin concentrations. We report a case of a 29-year-old female presenting with fever with maximum temperature of 40C for 1 month duration, evanescent rash, sore throat, myalgia, arthralgia, and rash. Laboratory examination showed leukocytosis (WBC 18,400), transaminitis (ALT 175, AST 281), and hyperferritinemia (63,281ng/mL). Extensive work-up to look for infectious and malignant causes were unremarkable. ANA and RF were both negative. She was able to fulfill the Yamaguchi classification criteria for the diagnosis of AOSD. Glucocorticoid therapy was started, however, she remained to be symptomatic. Increased levels of interleukin-6 (IL-6) and other pro-inflammatory cytokines have been shown in AOSD. Tocilizumab is an IL-6 receptor antagonist monoclonal antibody that can be used as an alternative to anakinra. The patient received tocilizumab at a dose of 8 mg/kg with immediate relief of symptoms. Serum ferritin and transaminase levels decreased to 5105ng/mL and 126 U/L, 85 U/L, respectively. In this case report, tocilizumab is an effective and well-tolerated treatment option for patients with AOSD with predominantly systemic symptoms.

Tu106. An Efficient Functional Assay Platform of Autoimmunity Risk Alleles Using CRISPR Genome Editing

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Large-scale genetic association studies have identified hundreds of variants associated with rheumatoid arthritis development. The risk variants are enriched in regulatory regions of activated CD4+ T cells more than resting CD4+ T cells, suggesting that they involve in context-specific cis-regulatory machinery. However, previous functional genetics studies failed to conduct functional assays with sufficient variations of immunological context and were unable to detect their allelic effects on gene expression regulations. The failure is partially because the previous studies relied on the natural genetic variants that we possess from our birth. The major draw-back in using natural variants is its low-efficiency; we usually need to recruite huge numbers of donors to test allelic effects, and this requirement restricted the search space of immunological contexts. To overcome this limitation, we have established a novel experimental platform consisted of two components: (i) artificial variants generated by CRIPSR prime editing in human CD4+ T cells, and (ii) pooled target-assay for transposase-accessible chromatin with sequencing (pooled target-ATAC-seq). CRIPSR prime editing enables us to generate human primary immune cells with any variants with high efficiency. Using pooled target-ATAC-seq, we selectively amplify only tagment DNAs harboring specific risk alleles with unique molecular indexes, and sequence multiple samples simultaneously with customized barcorded transposome. Our novel platform requires only a few donors to evaluate risk variant's allelic effect on the chromatin accessibility, and with this high efficiency we can test allelic effects in variety of immunological contexts. Our platform contributes to better understanding of the genetic etiology of autoimmune disease.

Tu107. An Endogenous Metabolite, Itaconate Ameliorates Collagen-induced Arthritis by Inhibiting Fibroblast-like Synoviocytes Proliferation and Migration

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Introduction: Fibroblast-like synoviocytes (FLS) are critical players in the pathogenesis of rheumatoid arthritis (RA). A significant concern in the management of RA is increased susceptibility to infections. Itaconate (ITA) is an endogenous metabolite with anti-inflammatory and antimicrobial effects. This study aimed to identify the effect of ITA on FLS and its potential to treat RA. Methods: FLS were isolated from synovial tissue of patients with RA. TNFα-treated FLS were cultured with ITA or PBS. The proliferation potency of FLS was evaluated by tetrazolium/formazan and BrdU assay. Cell migration was measured by scratch assay. An extracellular flux analyzer was utilised to evaluate mitochondrial oxidative phosphorylation. We performed RNA sequencing and metabolomics using TNFα-treated FLS with or without ITA. Finally, intraarticular injections of ITA or PBS into ankle joints were performed on rats with type II collagen-induced arthritis (CIA). Bone erosion scores were assessed by micro-CT. Results: The proliferation of FLS measured by tetrazolium/formazan assay ($p< 0.01$), as well as BrdU assay ($p< 0.01$), was reduced by ITA. ITA also inhibited cell migration ($p< 0.05$). ITA decreased the maximal oxygen consumption rate. The results of RNA sequencing and metabolomics indicated that ITA altered FLS metabolism. Intraarticular injection of ITA reduced clinical arthritis score ($p< 0.01$) in the CIA model (two-way ANOVA test). The bone erosion score was reduced in the ITA-treated group (mean; 1.6 vs. 0.1, $p< 0.01$). Conclusions: ITA inhibited the proliferation and cell migration of FLS and ameliorated CIA model. ITA could be a novel therapeutic agent of RA with a low risk of infections.

Tu108. An Inducible Mouse Model of Primary Cicatrcial Alopecia Exhibits Loss of CD200R

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Primary Cicatricial Alopecias (PCAs) are a group of autoinflammatory disorders characterized by scarring of the hair follicle. As a result, irreversible hair loss occurs. CD8+ T cells attack epithelial hair follicle stem cells (eHFSCs), and fibrosis ensues. We developed an inducible mouse model of alopecia that exhibits skin fibrosis using adoptive transfers of OT1 T cells into an autoimmune prone host mouse that expresses ovalbumin under the control of a keratin 5 promoter. We performed skin biopsies for histology, NanoString inflammation and immunology target gene expression and flow cytometry quantification of immune filtrates from mouse skin and compared these to human datasets. Comparison of gene expression in PCA mouse model to published human datasets revealed overlap with LPP, CCCA and DLE. Flow cytometry analysis of affected mouse skin exhibited significantly reduced CD200R expression. Previous studies suggest that the CD200-CD200R signaling pathway is immunoregulatory and protects the hair follicle from immune attack. We plan to continue examining hair follicle immune privilege breakdown as it pertains to autoinflammatory T cells, with the goal of identifying novel treatment targets for scarring alopecia.

Tu109. ANA$^+$ igg Memory B Cells Are Hyperreactive in SLE

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In systemic lupus erythematosus (SLE), IgG antibodies directed against nuclear antigens (ANA) are pathogenic. Healthy individuals (HC) harbor a similar frequency of autoreactive mature B cells, without producing pathogenic IgG antibodies. While overall B cell hyperresponsiveness has been described in SLE, differences in behavior between ANA+ and ANA- cells in SLE patients and HC have not been explored. The aim of this study was to measure the intensity of the response to BCR activation in IgG+ memory B cells in SLE patients and HC. B cells from seven SLE patients and five HC were isolated by negative magnetic selection and activated with F(ab)'2 fragments of anti-IgG antibodies. Calcium flux was measured using indo-1 AM. The response was quantified by area under the curve (AUC) using the bound/unbound ratio of the marker for the duration of 5 minutes after activation. Values were compared using Wilcoxon rank sum test. In SLE patients, ANA+ IgG memory cells have a stronger calcium signaling response than ANA- cells (AUC for ANA+ vs ANA-: 89.46±18.51 vs 76.17±12.65; p=0.05). This difference is not present in HC (AUC for ANA+ vs ANA-: 88.02±16.51 vs 89.52±14.49; p=ns). These results show that ANA+ IgG memory B cells are hyperreactive in patients with SLE. The data also suggests a mechanism for the breach in tolerance that is observed in SLE. Further studies are warranted to explore the mechanism and how this affects plasma cells differentiation and autoantibody generation in SLE.

Tu110. anti-cd45rc Mab Treatment Improves Experimental Inflammatory Bowel Disease (IBD)

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IBD needs new treatments that could better control disease. Anti-CD45RC mAb-mediated depletion of Tconv and B CD45RChigh cells as well as increased CD45RCneg/low Treg numbers and function. These effects prevent tissue destruction while preserving protective immune responses in animal models of organ transplantation, GVHD and APECED, as well as with human cells in in vivo mouse immune humanized models and in vitro. In a model of colitis by transfer of CD4+CD45RChigh T cells into immunodeficient rats, anti-CD45RC mAb treatment resulted in significant survival at day 50 vs. isotype control-treated rats (8/9 animals vs. 0/7, respectively, with death before day 32). Treatment was associated to significant decreases in T cells vs. controls for both blood and spleen (8.3 and 2.7-fold, respectively) as well as for colon lesions' scores (1±0.4 vs. 3.2±0.5, respectively). In blood from Crohn's disease (CD) and ulcerative colitis (UC) patients CD45RChigh and CD45RClow/neg cells were observed in the T and B cell compartments in proportions roughly comparable to the ones in healthy controls (HC). Anti-CD45RC mAb induced apoptosis in vitro of T and B CD45RChigh (but not of CD45RClow/neg) cells from CD and UC patients comparable to the ones for HC. Immunohistology analyses of gut biopsies from CD and UC patients showed the presence of CD45RC+ cells among both T and B cells (~25 and ~80 % of them, respectively). In conclusion, animal models and clinical data support the potential of anti-CD45RC mAb for treatment of IBD patients.

Tu111. Antigen-driven B Cell Expansion Differentiates Patients with Non-infectious Uveitis
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Background: Rituximab, a B cell-targeting drug, is effective in some uveitis patients, suggesting that B cells play a role in uveitis. Understanding their role will help identify patients who would benefit most from rituximab. Here we analyzed both gene expression and B cell receptor (BCR) sequences to characterize ocular B cells in human uveitis. Methods: We performed single cell RNA and VDJ sequencing on ocular and peripheral blood immune cells from 23 uveitis patients and two controls. Results: B cells comprised 5% (range 0-43%) of aqueous immune cells in our cohort. A subset of patients had highly increased B cell numbers, comprising more than 20% of the ocular infiltrate. B cell clonal expansion was also greater in these eyes compared to blood, suggesting that local antigen-driven expansion of B cells may occur in some patients with uveitis. Ocular B cells were also enriched in gene expression consistent with B cell activation/differentiation, including FCRL4 and FCRL5, important for tertiary lymphoid organs; AICDA, a driver of somatic hypermutation; and CXCR3, which promotes differentiation to plasma cells. Conclusion: Taken together, these results suggest that B cells are enriched in a subset of uveitis patients and may locally expand and differentiate in response to antigen. B cells may drive ocular inflammation in a subset of patients and thus, analysis of ocular B cells may help identify patients who would benefit from B cell-targeted therapies.

Tu112. CD47 Antibody Therapy Protects Circulating Cells from Immune Destruction
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Senescent erythrocytes are phagocytosed by the macrophages that make up the reticuloendothelial system, a network of myeloid cell rich tissues responsible for filtering circulating cells. The principal effector tissue of the reticuloendothelial system is the splenic red pulp, whose principal effector cells are resident red pulp macrophages. During their lifespans, erythrocytes are protected from non-specific destruction by red pulp macrophages by their expression of CD47, a 'don't eat me' signal that binds to macrophage expressed SIRPa to inhibit phagocytosis. However, as monoclonal antibodies have been developed to block the CD47-SIRPa interaction for cancer immunotherapy, it has been necessary to overcome the toxicity associated with the off-target destruction of erythrocytes. It was found that a small loading dose of CD47 antibody confers to erythrocytes protection against much greater subsequent dosing. Here we identify the mechanism of this protection in mice. We find that a loading dose of CD47 antibody protects erythrocytes by impairing antibody binding and phagocytosis by red pulp macrophages. We begin by demonstrating that CD47 antibody induces FcgR-mediated pruning of erythroid CD47,

with global concomitant FcgR loss by reticuloendothelial myeloid populations. We then show that CD47 antibody therapy impairs red pulp macrophage phagocytosis of erythrocytes. Finally, we show that CD47 antibody therapy protects erythrocytes and platelets from antibody-mediated destruction and thus may have therapeutic potential for autoimmune hemolytic anemia (AIHA) and immune thrombocytopenia (ITP), disorders caused by autoantibody-mediated destruction of erythrocytes and platelets, respectively.

Tu113. Characterizing Skin Resident and Recirculating Memory T-cells in Human and Murine Cutaneous Lupus Erythematosus

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Cutaneous lupus erythematosus (CLE) is a severe autoimmune skin disease. Autoreactive T cells differentiate into skin resident memory T cells (TRM) in CLE lesions and persist even when patients are in remission. We postulate that targeting molecules necessary for autoreactive T cells' residency and function will serve as a novel therapeutic approach to effectively prevent flare-ups. To study TRM in CLE, we used a mouse model and human blister biopsies to analyze T cell characteristics using flow cytometry. Most skin TRM expressed CD69 (70%) in CLE mice. However, many of these cells were capable of recirculating (TRCM) based on their expression of CD62L. In human blister biopsies, we found that skin CD8+ T cells were CD69+CD103+, but not CD4+ T cells, which tended to express CD122 instead. As expected, many blood T cells are negative for these markers. We noted high variability in CD122/CD103 expression; therefore, we analyzed patient samples based on clinical subtype, specifically whether or not they experienced cutaneous (SCLE) or systemic lupus (SLE). We found that SCLE patients had CD122+ CD4 T cells in lesional/nonlesional skin, whereas their CD8 skin T cells expressed primarily CD103. Strikingly, CD4/CD8 T cells in the blood of patients who also experienced SLE had CD122, which was very low in SCLE patients. Examination of spatial transcriptomic data from CLE skin biopsies confirmed the expression of TRM and TRCM markers in CD3+ regions of interest. Together, these data suggest that the ability of TRM to recirculate may predispose systemic involvement in CLE patients.

Tu114. CUE-401: A Novel il-2/tgf-beta Fusion Protein for the Induction of CD4+ FOXP3+ Regulatory T Cells

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Strategies that increase the number and/or function of regulatory T cells, including IL-2 therapies and Treg cell-based therapies, are being developed to treat autoimmunity and chronic inflammatory diseases. We have developed a first-in-class biologic, CUE-401, that is comprised of attenuated IL-2 and TGFβ variants linked to an Fc molecule. Co-delivering signals through both IL-2 and TGFβ receptors represents a novel approach to not only expand existing Tregs but to induce new subsets of iTregs specific for disease related antigens. In vitro data demonstrate that CUE-401 effectively induces de novo FOXP3-expressing iTregs from both mouse and human CD4+ T cells, while also expanding existing Tregs. These iTregs are functionally suppressive and phenotypically indistinguishable from iTregs induced with recombinant TGFβ and IL-2. In vivo, CUE-401 expanded FOXP3+ Tregs in mice with ongoing autoimmunity without inducing proliferation of autoreactive T cells. Single cell RNA-Seq analysis revealed that CUE-401 induced a subset of Tregs that express signatures similar to those described for TGFβ-induced iTregs. Finally, administration of CUE-401 led to long-term suppression of organ specific autoimmune inflammation in a well-established T cell transfer model of autoimmunity. The ability of CUE-401 to potently induce iTregs and suppress autoimmune inflammation represents a novel therapeutic approach. Bias toward iTreg induction rather than

manipulation of the limited number and repertoire of existing nTregs may provide significant opportunities for the treatment of patients with various autoimmune diseases as well as treatment of GVHD.

Tu116. Divergent Effects of JAK Inhibitors on B Cell Activation, Maturation and Function

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Janus kinase (JAK) inhibitors have been established for the treatment of various immune-mediated diseases (IMIDs), such as rheumatoid arthritis (RA), and are currently under investigation for numerous other IMIDs. Nonetheless, comprehensive studies to examine the impact of various JAK inhibitors on B cells are lacking. This study thus aimed to explore the effect of JAK inhibition on the B cell compartment by comparing the specific outcomes of different JAK inhibitors, including tofacitinib (pan-JAK), baricitinib (JAK1/2), ruxolitinib (JAK1/2), upadacitinib (JAK1/2), and filgotinib (selective JAK1), on B cell activation, proliferation, class switch recombination, and involved pathways in vitro. In an in-vitro model of T-cell-independent B cell activation we observed a dose-dependent decrease in total B cell numbers as well as an altered B cell differentiation under JAK inhibition with a profound reduction of switched memory B cell formation, especially with JAK1/2 inhibition, as well as a significant increase in MZ-like B cells. Additionally, JAK inhibition resulted in reduced STAT3 expression and phosphorylation and altered cytokine secretion. To investigate whether the effects of JAK inhibition on B cell activation and proliferation were durable, we investigated B cells from RA patients treated with JAK inhibitors ex-vivo. Here, we observed increased switched memory and plasmablast development as well as increased antibody secretion upon drug withdrawal, highlighting the reversibility of the observed effects. The results showed that JAK inhibition has a significant but reversible effect on B cell activation and differentiation. The study further highlights the differential effects of different JAK inhibitors on B cell homeostasis.

Tu117. Dynamic Regulatory Elements in Single-cell Multimodal Data Capture Autoimmune Disease Heritability and Implicate Key Immune Cell States

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In autoimmune disease, an individual's immune system destroys host tissues1-3. Developing a precise understanding of the fine-grained cell states that mediate the genetics of autoimmunity is critical to uncover causal disease mechanisms and develop potentially curative therapies. Here, we leveraged multimodal single-nucleus (sn) snRNA-seq and snATAC-seq data across 28,678 cells from the inflamed synovium of 12 donors with arthritis to identify accessible regions of the genome that covary with gene expression patterns across cells. For 12 immune-mediated and seven control traits, we discovered that cell-state-dependent ("dynamic") peaks disproportionately captured autoimmune heritability in immune cell types, compared to cell-state-invariant ("invariant") peaks. These dynamic peaks implicated peripheral helper and regulatory T cell states in rheumatoid arthritis (RA), and dendritic and STAT1+CXCL10+ myeloid cell states in inflammatory bowel disease (IBD). Our study shows that leveraging dynamic regulatory measurements in single cells can pinpoint cell states enriched for disease-critical genetic variation.

Tu118. Early Immune Responses to sars-cov-2 Mrna Vaccination in the Skin of Patients on B-cell Depletion Therapy

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In contrast to robust immune response to SARS-CoV-2 mRNA vaccination in healthy individuals, patients on B-cell depletion therapy often fail to mount antibody responses but make robust T cell responses. We aim to describe early innate and adaptive immune response in B-cell depleted individuals to better inform clinical decision making in these vulnerable patients. Here 22 subjects (10 B-cell depleted and 12 controls) received secondary mRNA-based vaccines against COVID-19 and underwent skin biopsies near the vaccination site and on the contralateral arm 1-3 days post yielding 42 total samples. Skin tissue was cryopreserved and underwent single cell RNA-sequencing with VDJ repertoire immune profiling. Samples were grouped by vaccination and immune status. Integration of all samples showed 22 clusters of 169,361 total cells with no significant difference in the proportion of vaccinated to unvaccinated cell subpopulations between groups. Dendritic cells in vaccinated skin across all groups demonstrated increased genetic expression of genetic regulator STAT1, complement factor C1QA, maturation markers IDO1, high affinity immunoglobulin gamma Fc receptor FCGR1A and IFN response related genes GBP1 and GBP2. Similarly, macrophages demonstrated increased gene expression of STAT1, GBP1 and GBP4, and FCGR1A in vaccinated skin. These markers are consistent with activated gene profiles in antigen presenting cells in post-vaccinated skin. Furthermore, B cells in vaccinated skin of B-cell depleted patients exhibited increased TNF signaling relative to healthy counterparts. The prevalence of B-cells was comparable to healthy controls. Analysis of T cell receptor repertoire and cell-cell interactions are ongoing and will further elucidate local responses post-vaccination.

Tu119. Effect of AHSCT on Inflammatory Mediators in Serum and CSF of RRMS Patients in the HALT-MS Study

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Autologous hematopoietic stem cell transplantation (AHSCT) is a treatment option for highly active multiple sclerosis (MS). In the HALT-MS study, twenty-four participants underwent AHSCT, of which seventeen achieved durable remission. Remission following AHSCT is thought to be mediated by shifts in the reconstituted immune system favoring immunoregulation over inflammation, which we hypothesized would be reflected in cytokine and chemokine expression. We analyzed soluble markers in serum and cerebrospinal fluid (CSF) from HALT-MS participants before and after AHSCT. Objectives included determining effects of AHSCT, associations with clinical parameters, relationships between serum and CSF, and prognostic value of baseline data. CSF levels of IgG, IgM, NfL, and GFAP, biomarkers of MS activity, were significantly altered by AHSCT at 24 months, consistent with reduced disease activity. In serum, AHSCT resulted in persistent changes in NfL but not GFAP. Serum NfL, CSF NfL, and CSF GFAP correlated with clinical activity, including EDSS and MRI measures. Consistent with declining neuroinflammation, AHSCT decreased intrathecal levels of inflammatory cytokines and chemokines at 24 months, including IL-12/23p40, IFN-gamma, IL-6, IL-10, CCL19, CCL22, and ICAM1. In serum, AHSCT resulted in many acute changes consistent with immune ablation and reconstitution, but few persistent effects. Only two serum cytokines were significantly decreased at 24 months: TNF-alpha and MIP1-beta. Using repeated measure modeling of serum biomarkers across all timepoints, the strongest associations with disease activity were TNF-alpha and MIP1-beta. These results demonstrate that AHSCT-induced immune reconstitution reduced serum and CSF levels of cytokines and neuroinflammatory markers, which correlated with reduced disease activity.

Tu120. Efficient Control of Experimental Rheumatoid Arthritis (RA) by anti-cd45rc Mab Therapy

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RA is a chronic relapsing/remitting disease characterized by synovitis, joint deformity, loss of function and increased mortality. While T-cells are a major component in the pathogenesis, B-cells are also pathogenic. Anti-CD45RC mAb treatment depletes B and T CD45RChigh cells, increasing the ratio of Treg:Tconv and has demonstrated efficacy in experimental models of diseases. Analysis of RA patients showed significantly more CD4+ and less CD8+T cells vs healthy controls (HC), and no differences in the proportions of CD45RChigh/low/neg. Analysis of synovial tissues using IHC demonstrated that non-stabilized patients have more leukocyte infiltration (T and B cells) and more CD45RChigh cells than stabilized or responding patients with methotrexate (MTX) and biologicals. Analysis of the potency of the anti-human CD45RC mAb demonstrated comparable apoptosis in vitro of CD45RChigh T and B from blood cells from RA patients and HC. Treatment with an anti-rat CD45RC mAb in a rat model of collagen induced arthritis efficiently prevented weight loss, mean arthritis severity, maximum score and AUC, similarly to the positive control MTX. Anti-CD45RC mAb completely inhibited anti-collagen antibody and GM-CSF detection in the sera in contrast to MTX and negative control groups. Efficacy of anti-CD45RC mAb correlated with an efficient depletion of CD45RChigh T and B cells as soon as d3 (>94%) and until sacrifice with conserved Treg numbers. Altogether our study demonstrates that anti-CD45RC mAb treatment is a potent immunomodulatory agent to reduce joint inflammation and disease activity in RA and a potential alternative for first line DMARD.

Tu121. Emergence of HLA-DR^{+} CD15^{+} 'hybrid' Cells in Active Rheumatoid Arthritis (RA)

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Most rheumatoid arthritis (RA) patients carry a particular 'shared' sequence of the HLA-DRB1 beta chain, implicating antigen presentation by HLA-DR as a key pathogenic mechanism in this inflammatory disease. We developed a gating strategy (Cytek Aurora) to comprehensively profile blood HLA-DR+ cells (isolated by Ficoll density centrifugation) in RA. Spectral cytometry showed a marked increase in HLA-DR positive cells resembling low-density neutrophils (LDN, CD15+CD16+CCR3-). The same cells also expressed CD303, traditionally associated with plasmacytoid dendritic cells (pDC). In contrast, CD123, expected in pDC, was undetectable, supporting that these cells are not simply LDN or pDCs but instead are better conceptualized as 'hybrid' cells. Successful RA treatment reduced 'hybrid' cells to negligible levels, whereas in treatment-resistant RA they remained much higher than in healthy donors. 'Hybrid' cells from patients with high disease activity formed a dominant HLA-DR+ cluster in active RA, which — along with very high levels of CD303 — co-expressed CD83 and CD275 (ICOS-L). The emergence of HLA-DR+CD15+ 'hybrid' cells in active RA highlights the potential clinical relevance of these cells that — given their expression of CD15 — may be of myeloid origin. Their simultaneous expression of CD303 and HLA-DR suggests that the cells may be involved in the capture and ultimate presentation of RA self-antigen. Their expression of ICOS-L (CD275) and CD83 lends support for potentially pro-inflammatory co-stimulation. Subsequent studies, aimed to confirm these functional characteristics, are pending.

Tu122. Enrichment of PD-1^{+}TIGIT^{+} CD4 Effector Memory Cells After Alefacept Treatment in Type 1 Diabetes

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Determining optimal dosage remains a challenge for the development of immunomodulatory therapies in heterogenous patient populations. Type 1 diabetes (T1D) is a polygenic autoimmune disease in which pancreatic insulin-producing beta cells are destroyed. Two 12-week courses of alefacept, an LFA3-Ig fusion protein that binds to CD2, preserved residual beta cell function over 2 years, demonstrating clinical efficacy in T1D subjects in the Immune Tolerance Network T1DAL study. Treatment depleted CD2 high effector memory T cells (TEM) via antibody-dependent cellular cytotoxicity. Concomitant preservation of CD2 low regulatory T cells (Treg) resulted in a >75% increase in the Treg:TEM ratio in 82% of treated subjects at week 12. This is consistent with the proposed mechanism of action of alefacept. We also found that CD4 TEM co-expressing the inhibitory receptors PD-1 and TIGIT increased following this depletional therapy. PD-1+TIGIT+ TEM increased as a proportion of TEM by 20% at 12 weeks in 80% of subjects and persisted for over a year (30 treated, 12 placebo, $p \leq 0.011$). In vitro coculture of PD-1 negative CD4 T cells with alefacept did not induce PD-1 expression. In contrast, PD-1+TIGIT+ TEM were CD2 low with increased proliferation (Ki67) after treatment. Both before and after treatment, PD-1+TIGIT+ TEM secreted low levels of pro-inflammatory cytokines IL2, IFNgamma, and TNFalpha. Together these data suggest that alefacept may promote expansion of hyporesponsive PD-1+TIGIT+ TEM. Thus, increases in two complementary regulatory phenotypes (Treg:TEM ratio and PD-1+TIGIT+ TEM) may reflect pharmacodynamic effects of alefacept treatment and could guide dosage decisions for CD2-depleting therapies.

Tu123. Exacerbation of Sensorineural Hearing Loss (SNHL) After COVID -19 Infection and COVID-19 Vaccination in AIED Patients

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The association between COVID-19 and its effect on autoimmune inner ear disease (AIED) has not been explored. We investigated the exacerbation of sensorineural hearing loss (SNHL) after COVID -19 infection and COVID-19 vaccination in these patients. Our prior research in AIED demonstrated that peripheral blood mononuclear cell (PBMC) from corticosteroid-resistant AIED patients had increased interleukin-1β (IL-1β) and IL-6 levels. In this study, we compared IL-1β, IL-1Ra and IL-6 mRNA production and release between AIED patients with worsening SNHL after COVID-19 vaccination or infection (Covid (+)) (n= 7) with AIED patients with worsening SNHL unrelated to Covid (Covid (-)) (n=7). In these two groups, we treated isolated PBMCs with increasing dose of SARS-CoV-2 spike protein and compared responses to stimulation with myelin basic protein (MBP), and LPS samples. In Covid (+) AIED patients, their PBMCs exposed to 12 ug/ml spike protein had almost 5-fold greater release of IL-6 as compared to Covid (-) AIED patients, and 4-fold greater for 12ng/ml and 12pg/ml of spike protein. In Covid (-) AIED patients, a greater induction of IL-1RA was observed in response to all concentrations of spike protein as compared with Covid (+) AIED patients, suggesting a stronger anti-inflammatory effect. Minimal differences were observed for IL-1 β release with spike protein. Notably, no sequence homology is observed between spike protein and cochin, the inner ear protein implicated in AIED. Further studies are underway to get more definitive answer to understand the relationship between the COVID-19 infection or COVID-19 vaccination and SNHL.

Tu124. Functionally Stable and Suppressive Regulatory T Cells Are Expanded with a Novel Engineered IL-2 Mutein

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Regulatory T cells (Tregs), a CD4+ T cell subpopulation critical to immune homeostasis, play a role in self-tolerance, allograft tolerance and prevention of fetal rejection during pregnancy. Interleukin 2 (IL-2) is a pleiotropic cytokine which drives proliferation of both Tregs and conventional T cells via the heterotrimeric IL-2 receptor (IL-2R, CD25/CD122/CD132). Enhancing IL-2 selectivity to maximize Treg activity while minimizing other effects is of therapeutic interest. MK-6194, an IL-2 Fc fusion mutein, has enhanced binding to the high-affinity IL-2R and reduced CD122/CD132 dependency, favoring Treg expansion. As previously described, Tregs from humanized MK-6194-treated mice have demonstrated FOXP3 and CD25 expression, characteristic of enhanced function and stability. To further characterize the behavior of a selective IL-2 mutein, we tested the effects of an MK-6194 surrogate molecule in an established model of alloantigen-specific Treg expansion in which ex vivo mixed lymphocyte reactions (MLRs) using HLA-mismatched PBMCs from healthy donors are performed in the presence of co-stimulatory blockade. MK-6194 mutein-supplemented MLRs showed greater Treg expansion. These Tregs had demethylated Foxp3 epigenetic signatures characteristic of functionally suppressive cells. Flow cytometric assessment of Treg markers including Foxp3, Helios and CD161 suggested these were functional and stable Tregs. Further, MK-6194 surrogate-expanded Tregs efficiently suppressed autologous CD8+ T cell proliferation, activation (HLA-DR+/CD25+) and cytotoxicity (Granzyme B+/Perforin+). Together these results indicate that the MK-6194 family of IL-2 muteins can expand functional, stable and immunosuppressive Tregs. These data support further evaluation of a role for MK-6194 in achieving transplant tolerance and in treatment of autoimmune and inflammatory diseases.

Tu125. Granzyme K Activates a New Complement Pathway

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The role of T cells as major drivers of rheumatoid arthritis (RA) pathogenesis is well established, but nearly all research has focused on CD4+ T cells. We have recently identified a new effector CD8+ T cell population that is abundant in inflamed RA synovium. Unlike cytotoxic T lymphocytes, these CD8+ T cells have low cytotoxic potential, expressing low levels of granzyme B and perforin. Instead, these CD8+ T cells express high levels of granzyme K (GzmK), a granzyme that does not drive apoptosis of target cells. Similar GzmK+ CD8+ T cells are also highly enriched in many other inflamed tissues, although their function remains unclear. Here, we report that GzmK activates a new complement pathway by cleaving C4 and C2 to generate C3 convertases (C4b + C2a) that cleave C3 and evolve into C5 convertases (C4b + C2a + C3b). In doing so, GzmK elicits activation of the entire complement cascade, generating the anaphylatoxins C3a and C5a, the opsonin C3b, and the membrane attack complex. Further, we demonstrate that synovial fibroblasts are the dominant source of complement in the synovium, and GzmK cleaves fibroblast-derived C4 and C2 to activate the complement cascade. Our findings illustrate the existence of a fourth pathway of complement activation, where GzmK secreted by CD8+ T cells cleaves complement components derived from fibroblasts and other cells. This novel pathway is likely to be important at sites of inflammation where GzmK-expressing CD8+ T cells are enriched, where it may contribute to the perpetuation of local tissue inflammation.

Tu126. High-throughput Western Blot for *in Vitro* and *in Vivo* Assessment of Human Ig Specific Cleaving Enzymes

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Antibodies play a vital role in the body's immune defense mechanism. However, pathogenic Ig antibodies are thought to contribute to several autoimmune conditions and transplant rejection. Proteases derived from human pathogens can specifically cleave IgG into F(ab')2 and Fc fragments, thus allowing for rapid clearance of IgG. This unique trait suggests a novel opportunity to use these molecules to treat autoantibody mediated diseases. Using our IMPACT platform leveraging machine learning, we have engineered Ig proteases that specifically cleave IgG, and treatment with these proteases causes cleavage below the hinge region, which can result in the generation of the following degradation products: 1) Fab'2 and Fc fragments lacking effector function; or 2) single chain cleaved fragments with reduced effector functions. To detect these species, we developed a method using JESS, an automated high throughput western blot instrument. Using this system, we can detect intact, single cleaved and fully cleaved IgG in a time and dose dependent manner in both in vitro and in vivo assays. In conclusion, we successfully optimized a high throughput assay format that is highly specific and sensitive in evaluating the cleavage activity of different Ig proteases and could be a powerful tool to assess efficacy of IgG cleaving enzymes in the clinic.

Tu127. HLA Class II Association of Autoantibodies Against GAD65 in North Indian Type 1 Diabetes Patients

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AIM AND OBJECTIVE: This study was planned to evaluate the genetic association of HLA class II genes/haplotypes with the presence of autoantibodies against GAD65 (GADA) in study subjects from North India. METHODS: GADA in the sera of 252 T1D patients and 230 healthy controls were quantified using ElisaRSR™GADAb kit (RSR Ltd., UK). Genomic DNA was extracted using a modified ammonium acetate salting-out method. HLA genotyping was performed using HystoType SSP DR/DQ Combi Pack kit (BAG Healthcare, Germany). RESULTS: Out of 252 T1D patients screened for the presence of GADA, 128 (50.79%) patients were GADA+, while only 2 out of 230 healthy subjects were GADA+ (0.87%; p=2.09e-34). Prevalence of GADA was found to be comparable in recently-diagnosed (RD) versus long-standing (LS) patients (58.18% vs 48.73%) as well as in patients with age at disease onset (ADO) < 18 years versus ≥18 years (55.41% vs 44.23%). GADA positivity was also comparable in DR3+RD versus DR3+LS patients (50.91% vs 440.61%) as well as in DR3+ patients with ADO < 18 years versus ≥18 years (47.30% vs 38.46%). HLA-DRB1*03 and -DQB1*02 frequencies were significantly increased in GADA+ versus GADA– patients {91.41% vs 66.13; Pc=1.11e-5; OR=5.45(95%CI=2.67-11.08) and 94.53% vs 73.39%; Pc=2.19e-5; OR=6:27(95%CI=2.70-14.49), respectively}. Further, our data revealed a significantly increased frequency of DRB1*03-DQB1*02 haplotype in GADA+ versus GADA– patients {60.55% vs 41.94%, P=3.94e-5; OR=21.3(95%CI=1.49-3.03)}. CONCLUSION: Our study points to a central role of DRB1*03 and DQB1*02 genes in initiating and driving GAD65-mediated autoimmune response that possibly led to progressive loss of β-cells and development of T1D.

Tu128. HLA, KIR and Genetics of Anti-nmdar Encephalitis

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Objective To explore genetic association between anti-NMDA-receptor encephalitis (anti-NMDARE) and human leukocyte antigen (HLA), killer cell immunoglobulin-like receptors (KIR) and other loci. Methods GWAS in 511 (non-post herpetic) cases and 2,827 PCA-matched controls and allelic KIR and HLA sequencing in 449 cases and 1,577 ethnically matched controls. Results GWAS significant loci include ACP2/NR1H3 and DMXL2 genes. ACP2/NR1H3 locus is in high LD with LRP4 and MADD (rs11039155; p=10-13; OR=2.1). The lead variant is a strong expression quantitative trait (eQTL) for all of these genes (p< 10-10) across various tissues. The second locus is in the vicinity of DMXL2 on chromosome 15 (lead variant rs76144151; p=1.1x10-8; OR=1.58) and regulates expression of DMXL2 in various tissues. Of note, LRRK1 is not significant. These results suggest involvement of the rab3-associated synaptic release or lysosomal acidification pathways. Strikingly, there is a weak, but significant signal in HLA class II after controlling for population stratification, suggesting predisposing effects of DQA1*01:01~DQB1*05:01 (OR=2.2). Using KIR sequencing, we found independent associations with specific KIR2DL4 and KIR3DL3 alleles (OR=1.98 and OR=4.29, respectively), loci interacting with potential checkpoint inhibitor genes HLA-G and HHLA respectively. Primary expression of these KIR genes in CD56bright NK-cells and decreased CD56bright NK-cell numbers in patient vs control suggest involvement of NK cells in anti-NMDARE. Conclusion This study is the, so far, largest genetic study on anti-NMDARE. Our results suggest involvement of innate immunity, HLA and KIR in this disease. Follow-up on the functional roles of these loci and validation in a larger cohort is needed.

Tu129. Human T Cell Behavior in Lymphopenic Environments: Contribution of Lymphopenia-induced Proliferation to Autoimmunity

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Considerable evidence suggests a role for lymphopenia-induced proliferation (LIP) of T cells in the initiation or facilitation of autoimmunity in both mice and humans. TCR interactions with p.MHC are central to the development of autoimmunity. However, the relative contribution of cytokines vs antigen recognition to the LIP that promotes autoimmunity and the role of LIP in human autoimmunity is poorly understood. Using adoptive transfer of T cells in human immune system (HIS) mice, we now demonstrate that both naïve and memory human T cells undergo rapid proliferation in T cell-deficient HIS mice and promote the development of autoimmune disease. We demonstrate that T cells recognizing p.HLA of selecting thymic epithelium undergo faster LIP and induce faster autoimmunity upon adoptive transfer. In the absence of interactions with p.HLA:TCR present in the thymus, IL-7 does not induce T cell survival and proliferation. Interaction of T cells undergoing LIP with myeloid cells presenting the p.HLA that the T cells were selected on led to maturation of the myeloid cells. TCR sequencing confirmed the clonal expansion of T cells with increased hydrophobicity of amino acids at their CDR3Bs predictive of high peptide recognition. Through the adoptive transfer of different T cell subsets, we established that non-Treg CD4 T cells were mainly responsible for the observed autoimmune disease. These studies shed light on mechanisms of T cell activation and proliferation in lymphopenic environments and LIP-induced autoimmunity.

Tu130. Humanized *in Vivo* Models Supporting the Identification of Novel Inhibitory Receptor Agonists for the Treatment of Immune-mediated Diseases

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Evidence in both animal models and patients demonstrate that inhibitory receptors (IRs) play an important role in limiting autoimmunity. Similarly, blockade of checkpoint inhibitory receptors has been proven to be efficacious for the treatment of cancer and often associated with new onset of immune-related adverse events. Since the loss or blocking of inhibitory receptor pathways can result in inflammation, we hypothesized that agonism of these pathways should restore normal immune homeostasis. However, the generation of agonistic antibodies is often challenged by

the lack of inhibitory receptor mouse orthologue cross-reactivity thus limiting our options to evaluate putative therapeutic agonists pre-clinically. Here we will survey the promise and limitations of using humanized animal models to interrogate efficacy and mechanism of action of novel inhibitory receptor agonists for the treatment of immune-mediated diseases.

Tu131. Hybrid Insulin Peptides Activate Autoreactive T Cells Restricted by a Non-risk HLA Haplotype

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Hybrid insulin peptides (HIPs) formed through covalent cross-linking of proinsulin fragments to other secretory granule peptides have been detected within murine and human islets. HIPs were shown to be recognized by pancreas infiltrating T cells from nonobese diabetic mice and human pancreatic organ donors with type 1 diabetes (T1D). A recent study probed T cell reactivity in new onset patients, documenting HIP-specific responses in most subjects and isolating several unique HIP-responsive T cell clones. Surprisingly, one T cell clone responded vigorously to a C-peptide/WE14 HIP (HIP9, the human equivalent of murine 2.5HIP) in the context of DRB1*11:03 (DR11). Since DR11 is a non-risk haplotype, we reasoned that T cell responses with this HLA restriction could play either pathogenic or protective roles. Therefore, we recruited subjects with T1D and healthy controls with DR11 haplotypes and utilized an HLA class II tetramer to characterize HIP9-reactive CD4+ T cells. HLA class II tetramer staining revealed higher frequencies of HIP9-reactive T cells in subjects with T1D than the HLA-matched control subjects. Furthermore, in DR11+ subjects who had heterozygous haplotypes that carry the disease-associated DRB4 allele, HIP9-reactive T cell frequencies were higher than those observed for an immunodominant proinsulin epitope (PPI 9-28). Finally, phenotypic analysis indicated that a subset of HIP9-reactive T cells exhibit a Th1-like phenotype. These results provide the first direct evidence that a HIP containing a ChgA sequence is relevant in human disease and suggest a mechanism that may contribute to the rising incidence of T1D among individuals with non-risk HLA haplotypes.

Tu132. Identification and Characterization of Inhibitory Receptor Agonists for the Treatment of Autoimmune and Inflammatory Diseases

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The development of autoimmune diseases is the result of a dysregulated immune response against self-antigens. Upon activation by antigens, lymphocytes upregulate inhibitory receptors as a feedback mechanism to limit their effector activity. Inhibitory checkpoint receptor deficiency is associated with autoimmunity in mice and humans. Similarly, blockade of inhibitory receptors with antagonist antibodies, that are efficacious for the treatment of cancer, is often associated with new onset of immune-related adverse events. Therefore, if loss or blocking of inhibitory checkpoint receptor pathways can result in inflammation, agonism of these pathways may restore normal immune homeostasis. However, generating agonistic antibodies can present some challenges. For instance, it is known that to elicit strong receptor agonism, super-clustering, typically mediated by the binding of the constant region (Fc) of an antibody to FcγRs, must occur. FcγRs are widely expressed on antigen-presenting cells (APCs) and are classified, based on their function, as activating or inhibitory. Of note, FcγRIIb is the only inhibitory Fc receptor, and dendritic cells derived from mice deficient of this receptor exhibited exaggerated cytokine release. Many agonist antibodies currently in clinical development contain effector-competent Fc and by binding activating Fc receptors can potentially trigger an unwanted production of pro-inflammatory cytokines. To mitigate such a liability, we designed novel agonistic antibodies that selectively bind FcγRIIb. These molecules support superior agonism of checkpoint inhibitory receptors while simultaneously preventing APC activation.

Tu133. Identification and Characterization of Selective Fcγriib Binders and Their Potential Of impacting Immune-mediated Diseases

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Biologics targeting multiple pathways and/or multiple cell types simultaneously have the potential to restore normal immune homeostasis. Inhibitory checkpoint receptor agonism by antibodies benefits from higher order clustering for strong activation, which can be achieved by antibody Fc binding to Fc gamma receptors (FcγRs). However, many of the current agonist antibodies non discriminately bind activating FcγRs. This has the potential to trigger inflammatory cytokines by antigen presenting cells (APCs) or cause antibody dependent cellular cytotoxicity (ADCC). Clustering by Fc binding to the inhibitory Fc receptor, FcγRIIb has the potential to provide superior clustering by avoiding inflammatory cytokine responses and limiting APC activation. However, FcγRIIb is 93% homologous to the activating receptor FcγRIIa, and designing molecules that specifically bind FcγRIIb presents a challenge that our IMPACT platform can address. Using integrated machine learning, structural biology and traditional antibody campaigns, we have identified selective FcγRIIb binding molecules, and characterized their potential for IMPACTing immune-mediated diseases.

Tu134. Identifying Next Generation Ig Selective Cleaving Enzymes for Treatment of Autoimmune Diseases Using IMPACT Platform

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Dysregulated humoral immune mechanisms result in immunoglobulin production that is pathogenic and contributes to a wide range of autoimmune diseases and transplant rejection. Proteases derived from human pathogens can specifically cleave these immunoglobulins (Ig) and can potentially eliminate circulating, cell-surface and immune-complex Ig classes and modulate Ig-mediated autoimmunity and inflammation, providing a novel opportunity to treat antibody-mediated diseases. We describe here the discovery, generation, and functional characterization of Ig specific proteases. These proteases were identified using Seismic Therapeutic's IMPACT platform and tested for their ability to cleave intact Ig subclasses and Ig isotypes in a high-throughput CE-SDS cleavage assay. Additionally, we found that these enzymes cleave soluble and cell bound Ig in human blood, dramatically reducing effector function. We believe that these subclass and isotype specific cleaving proteases will provide an opportunity to develop novel targeted therapies for various autoimmune diseases where autoantibodies are the key drivers of the disease. In addition, we believe that they will be potentially better tolerated compared to current therapeutic interventions such as plasma exchange and other immunosuppressive therapies.

Tu135. Immune Cell Heterogeneity in Lupus Nephritis Kidneys and Its Relation to Histopathological Features: Lessons from the Accelerating Medicines Partnership (AMP) in SLE Consortium

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Lupus nephritis (LN) is characterized by considerable variability in its clinical manifestations and histopathological findings. Understanding the cellular and molecular mechanisms underlying this heterogeneity is key for the development of personalized treatments for LN. Droplet-based single-cell RNA-sequencing was applied to the analysis of dissociated kidney samples, collected from 155 LN patients with active LN and 30 healthy controls. 73,440 immune cells passing quality control were identified, spanning 134 cell subsets, representing various populations of tissue-resident and infiltrating leukocytes, as well as disease-related activation and differentiation. Principal component analysis (PCA) was used to characterize the variability in cell subset frequencies across the LN patients, and its relation to histopathological features. We found that the main source of variability in leukocyte composition (PC1) reflected the balance between lymphocytes and monocytes/macrophages, and was significantly correlated with the Chronicity index, such that patients with a higher lymphocytes-to-monocytes/macrophages ratio had a higher Chronicity score (rho = -0.439, p-value < 0.001). The second source of variability (PC2) represented the degree of macrophage differentiation to an alternatively activated phagocytic profile, and was positively correlated with the Activity index (rho = 0.495, p-value < 0.001). Furthermore, a higher degree of macrophage differentiation was associated with proliferative or mixed histology class, compared to pure membranous nephritis (p-value = 0.001, Kruskal–Wallis test). Network analysis revealed a complex structure of interactions associated with different modes of immune responses. These results provide important insights into the immune mechanisms underlying LN.

Tu136. Immune Responses to Gut Bacteria Associated with Time to Diagnosis and Clinical Response to T-cell Directed Therapy for Type 1 Diabetes Prevention

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Immune-targeted therapies have demonstrated efficacy for treatment of several auto-inflammatory diseases. For example, a single treatment course with the T cell-specific anti-CD3 monoclonal antibody teplizumab delayed disease onset in participants at high risk for Type 1 Diabetes (T1D) in the TN-10 trial. However, heterogeneity in therapeutic responses observed in TN-10 and other immunotherapy trials identify the need to improve prediction of disease progression and treatment responses. The intestinal microbiome is a potential source of new biomarkers, but extensive bacterial sequencing studies have not identified either single, or consortia of species associated with future T1D diagnosis in at-risk cohorts. In contrast, we reported that antibody responses to gut commensal bacteria were associated with T1D diagnosis suggesting that immune reactivities to these microbes may help predict disease onset. Here, we examined anti-commensal antibody (ACAb) responses against cultured intestinal bacterial species in serum sampled from TN-10 participants before and after randomization into teplizumab or placebo treatment arms. We identified IgG2 responses to three taxonomically distinct species that were associated with time to T1D diagnosis and with treatment responses. These ACAb are the first biomarkers linking human intestinal bacteria with T1D progression. Measures of these ACAb responses before teplizumab treatment added value to known T1D risk factors in predictive models. Thus, ACAb responses may help identify individuals who will benefit from teplizumab, a drug recently approved by the U.S. FDA for use in delaying T1D onset.

Tu138. Impact of Liraglutide Treatment on Gene Expression in Peripheral T-cell Populations from Adults with Recently Diagnosed Type 1 Diabetes

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Type 1 diabetes is an autoimmune disease characterized by attack from immune cells infiltrating the pancreas and loss of functional beta-cell mass, requiring exogenous insulin treatment. Novo Nordisk has conducted a randomized, double-blind, placebo-controlled, phase 2 trial assessing whether the combination of GLP-1R agonist liraglutide and anti-IL-21 antibody could preserve beta-cell function. A collaboration with Immunai was established, leveraging a single cell multiomic approach to assay mRNA, protein, and TCR sequences with the 10x genomics platform, using select PBMC samples from the placebo (n=13) and liraglutide (n=11) arms of this trial, at baseline, (WK0) and end of treatment (WK54). Immunai employed proprietary computational techniques to process sequence data, integrate sample and clinical metadata, annotate cell types, and compare cell type abundances and differential gene and pathway expression between WK0 and WK54 samples in the liraglutide and placebo arms. Downstream analysis focused on genes and pathways that changed from WK0 to WK54 in the liraglutide arm but did not show statistically significant changes from WK0 to WK54 in the placebo arm. Compared to WK0, liraglutide-treated patients at WK54 have indicated decreased proliferation, cytotoxicity, and other markers of effector functions in NK and CD8+ T cells while indicating increased proliferation and suppressive function in Tregs. Of note, public human data repository analyses further substantiated a novel role for GLP-1R in Treg function, supporting the notion that GLP-1R might play a role in Tregs involved in autoimmunity and thereby guiding future clinical trials assessing GLP-1R agonists in type 1 diabetes.

Tu139. Impacting Autoimmunity Through Dual Targeting of Antigen Presenting Cells and T Cells via Inhibitory Receptor Agonism

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Autoimmune diseases are triggered by a critical break in peripheral tolerance, thereby allowing the immune system to target and destroy host tissues. Tolerance mechanisms, such as inhibitory receptor (IRs) on T cells, can greatly influence autoimmune disease progression and outcomes, representing an opportunity for therapeutic intervention. Indeed, evidence in both animal models and patients suggests that IRs play a major role in limiting autoimmunity. Immunotherapies targeting IRs have revolutionized the treatment of many cancers and provided evidence that IR modulating therapeutics can significantly impact patient outcomes. However importantly, analogous IR-targeted autoimmune therapeutics have just begun to be tested. Despite interest, IR agonist antibodies have proven difficult to generate. Optimal agonist antibodies require IR superclustering, which is not efficiently induced by Fc-null antibodies. Successful agonists will likely rely on simultaneous Fc (constant region) tethering on antigen presenting cells (APC), thereby allowing efficient IR superclustering and downstream signaling. Current agonistic antibodies targeting IRs contain an IgG1 wild-type Fc, which by binding both activating and inhibitory Fc receptors can trigger unwanted production of inflammatory cytokines by APCs. Here we show that antibodies binding selectively FcγRIIb, support superior agonism compared to IgG1 wild-type antibodies and mitigate potential liability of current IR agonists under clinical investigation. Indeed, dual targeting of the APC and T cells shows promising pre-clinical results and will be used to create novel IR agonists.

Tu140. Impaired Thymocyte Selection in Type 1 Diabetic Immune Systems

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The extent to which failure of thymic selection contributes to human Type 1 diabetes (T1D) is unknown. To address potential hematopoietic stem cell (HSC)-intrinsic abnormalities, we compared thymic selection in human immune system (HIS) mice generated from healthy control (HC) and T1D patient HSCs. Negative selection of an islet autoreactive TCR was diminished in T1D-derived compared to HC immune systems. Differentiation of thymocytes with this TCR to the T-regulatory (Treg) cell lineage occurred in HC thymocytes but was impaired in T1D systems. T1D-associated single nucleotide polymorphisms (SNPs) in SH2B3 and Erk/MAP kinase pathway-associated genes were associated with impaired thymocyte negative selection. Single cell gene expression analysis revealed that a major population of thymocytes apparently undergoing negative selection, with high expression of genes in the TCR signaling pathway and in pro-apoptotic pathways, was absent among T1D thymocytes. Additionally, T1D thymocytes demonstrated differential expression of multiple genes involved in thymic selection, including decreased expression of TCR signaling molecule Zap-70 and increased expression of anti-apoptotic genes compared to HC thymocytes. These studies provide the first direct evidence for impaired thymic negative selection and autoreactive Treg differentiation in T1D immune systems, and suggest genetic mechanisms through which selection is impaired, providing novel insights into T1D pathogenesis.

Tu141. In Depth Transcriptomic Analysis of Myeloid Populations from Health to Disease in Rheumatoid Arthritis

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Single-cell synovial-tissue suspensions from Rheumatoid Arthritis(n=41), individuals 'at risk' of RA (n=5) and healthy controls(n=11) were obtained through keyhole mini video-arthroscopy. Synovial-tissue macrophage subsets were examined using advanced flow-cytometric analysis, single-cell and bulk RNA-sequencing, metabolic and functional assays. Multidimensional analysis identifies enrichment of CD206+CD163+ synovial-tissue macrophages co-expressing CD40 in RA compared to healthy synovial-tissue, with CD206+CD163+CD40+ macrophage frequency associated with increased disease activity and treatment response. In contrast, CX3CR1-expressing macrophages enriched in healthy synovium are significantly depleted in RA. Importantly this signature is an early phenomenon, occurring prior to clinical manifestation of disease in individuals 'at-risk' of RA. Bulk RNAseq of RA synovial-macrophages identified distinct inflammatory, phagocytic and tissue-resident gene signatures paralleled by bioenergetically stable profiles as indicated by NAD(P)H emission. Functionally CD206+CD163+ RA macrophages are potent producers of pro-inflammatory mediators (reversed by CD40-signalling inhibition) and induce an invasive phenotype in healthy synovial-fibroblasts. Single-cell transcriptomic profiling of synovial-tissue cells from RA-patients and healthy individuals gave a unique myeloid atlas from health to disease. Nine distinct synovial-tissue macrophage clusters were further classified into four subpopulations: TREM2high, TREM2low, FOLR2high, IL-1Bhigh. IL-1B+CCL20+ and SPP1+MT2A+ which were identified as pro-inflammatory macrophage populations, are enriched in RA compared to healthy synovial-tissue and display heightened CD40 gene expression. Receptor-ligand interactions indicate that these populations interact with stromal/T-cells to induce pro-inflammatory mechanisms. These findings identify distinct pathogenic populations of synovial-tissue macrophage involved in shaping the immune response in RA. Crucially, inflammatory myeloid signatures are present pre-disease onset representing a unique opportunity for early diagnosis and therapeutic intervention.

Tu142. *In Vivo* PK/PD Assessment of Invisibilized Igg Cleaving Protease for Chronic Treatment of Autoimmune Diseases

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Pathogenic autoantibodies are key effectors of inflammation, promoting complement cascade activation, immune cell response, and the consequent tissue damage in autoimmune disease and transplant rejection. Proteases that cleave IgG antibodies present a novel therapeutic opportunity to treat autoimmune diseases and antibody mediated transplant rejection. Using our IMPACT platform leveraging machine learning, we engineered IgG proteases with reduced immunogenicity for acute and chronic treatment of antibody-mediated diseases. In addition, the invisibilized protease was fused to a Fc to extend its half-life. To evaluate these IgG proteases in vivo we developed a pharmacokinetics and pharmacodynamics (PK/PD) model. C57BL6 mice were dosed with intravenous immunoglobulin (IVIg) at different time points after protease treatment. Protease and IgG levels were quantified by MSD 15 minutes before and two hours after IVIg injection, respectively. IgG cleavage was detected at early time points which correlated with higher systemic drug levels. Additionally, the Fc-fused protease was detected at later time points with increased systemic enzymatic activity, as compared to the enzyme domain only. Finally, we evaluated the potential of the Fc-protease to cleave antigen bound IgG at the tissue level in a mouse anti–glomerular basement membrane (GBM) induced nephritis model. In summary, our in vivo studies established a PK/PD relationship between exposure and cleaved IgG while showing the benefit of fusing the protease domain to a Fc.

Tu143. *In Vivo* Reprograming of Stromal Cells for Tolerogenic Immunotherapy Using Mrnas with Restricted Expression

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The type of antigen-presenting cells (APCs) targeted by immunotherapy greatly influences its efficacy. Presentation of autoantigens by hematopoietic APCs may negatively affect their ability to induce tolerance and ameliorate autoimmune disease if they become immunogenic. Moreover, these APCs are reported to have impairments in inducing tolerance in Type 1 diabetes (T1D). In contrast, stromal cells (SCs) could favor tolerance as they lack costimulatory molecules and express various types of inhibitory molecules. However, SCs are not effective APCs due to low MHC expression levels. Overexpression of constitutively active STAT1 (STAT1c) in SCs, like exposure to IFN-γ, resulted in MHC and PD-L1 upregulation, but not CD80 and CD86. We transfected various cell types with GFP mRNA formulated into nanoparticles and confirmed that incorporation of miR-142 target (T) sequences into the mRNA restricts expression to SCs in vitro and in vivo. Delivery of autoantigen-encoding mRNA with miR-142T led to a limited CD4 T cell response in non-obese diabetic (NOD) mice, but codelivery with STAT1c-encoding mRNA returned the response to the level seen with autoantigen mRNA without miR-142T. Interestingly, SCs express higher MHC-II levels in pancreatic lymph nodes due to its more inflamed environment, and there, they elicited robust CD4 T cell responses without the need for STAT1c. Our findings show proof-of-concept that SCs can be selectively targeted and reprogramed in vivo to serve as effective APCs for immunotherapy of T1D. Further studies will aim to confirm the tolerogenic nature of T cell responses and the resulting effect on disease progression in NOD mice.

Tu144. Mechanisms of Type I Interferon-mediated UVB Sensitivity in Human Keratinocytes

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Photosensitivity is a characteristic of skin diseases such as cutaneous lupus erythematosus (CLE) and dermatomyositis (DM). The exact cause of photosensitivity is unclear, but it is believed that the sensitivity of keratinocytes (KCs) to UVB radiation may play a role, as the epidermis absorbs UVB radiation. Our single-cell RNA sequencing on skin samples of patients with CLE and DM revealed a robust type-I-interferon (IFN-I) signature in basal KCs, even in non-lesional skin, suggesting subclinical priming for subsequent inflammatory insults. ELISA analysis of interstitial skin fluid from DM patients showed elevated levels of IFN-beta. To investigate how IFN-Is affect KC death after UVB irradiation, in vitro assays were conducted. The study found that pyroptosis, not cell lysis, is the primary mechanism of UVB-induced inflammatory death in KCs, mediated through the host-encoded pore-forming protein gasderminE (GSDME). Furthermore, GSDME cleavage occurs in a caspase 3 (CASP3)-dependent manner. ZAK-alpha knockout KCs showed a significant reduction in cleaved CASP3 and GSDME, suggesting ribotoxic stress as a mechanism of UVB-induced KC pyroptosis. We also found that pretreatment of KCs with type I IFNs increased pyroptosis through a GSDME/CASP3-independent mechanism following UVB exposure, which was inhibited by pan-caspase inhibition. Bulk-RNA sequencing of KCs identified upregulation of apoptotic-related genes, including TXNIP, GCH1, and ATF3, after treatment with type I IFNs, suggesting them as targets that could prime KC sensitivity to UVB. These findings shed light on the underlying mechanisms of UVB photosensitivity in IFN-I-mediated skin diseases.

Tu145. Metabolic Regulation of Human B-cell Effector Cytokines in Health and Disease

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Among key antibody-independent B-cell functions is their capacity for context-dependent secretion of distinct cytokine profiles that shapes immune responses relevant in both health and disease, such as the CNS inflammatory condition multiple sclerosis (MS). Here we investigated fundamental mechanisms underlying regulation of the balance between human B-cell pro- and anti-inflammatory cytokine responses and their impact on CNS inflammation. Compared to anti-inflammatory cytokine producing B cells (IL-10+ B cells), the generation of pro-inflammatory cytokine-expressing B cells requires higher metabolic activity and limiting mitochondrial-respiration (but not glycolysis) mediates an anti-inflammatory B-cell cytokine shift, and dampens the ability of B cells to promote myeloid cell responses. Upon stimulation, B cells from MS patients exhibit higher mitochondrial respiration and inhibition of their mitochondrial respiration restores the balance of B-cell cytokines. Limiting B-cell mitochondrial respiration specifically in B cells decreases pro-inflammatory immune responses and diminishes clinical disease severity in EAE, a commonly used murine model of neuroinflammation. Mechanistic studies further reveal that mitochondrial derived ATP is involved in shaping B-cell cytokine responses through the purinergic receptor P2RX7. Blockade of P2RX7 reduces B-cell proinflammatory cytokine production both in vitro and in vivo, and ameliorates clinical severity of EAE in vivo. Together, our study reveals a fundamental mechanism involving metabolic regulation of B-cell pro- and anti-inflammatory cytokine responses; identifies ATP and its metabolites as a 'fourth signal' shaping B-cell responses; and indicates that non-depleting therapeutic strategies may restore a normal B-cell cytokine balance in human autoimmune disease by targeting B-cell metabolism.

Tu146. Metabolomics Analysis Reveals Common and Differentiating Metabolic Phenotypes in Peripheral Blood Mononuclear Cells from Type 1 Diabetes, Systemic Lupus Erythematosus and Healthy Participants

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Identifying the metabolic reprogramming associated with autoimmune diseases can generate novel insights of disease mechanism. We compared the intracellular metabolic profile and the metabolic responses to immune activation in peripheral blood mononuclear cells (PBMCs) from type 1 diabetes patients (T1D, n = 24), systemic lupus erythematosus patients (SLE, n = 22) and healthy controls (n = 26). PBMCs from each participant were subjected to T-cell stimulation by anti-CD3/CD28 dynabeads® or placebo for 24h. The intracellular metabolites were measured using UHPLC-MS metabolomics. Data was analyzed using a mixed effect model with stimulation, health group and stimulation × health group as fixed factors and participant as a random factor. Bonferroni correction was performed to account for multiple testing. Stimulation of PBMCs led to significant increase in intracellular lysophosphatidylethanolamine C16:0, phosphocholine, citric acid, hexose diphosphate, taurine, and uridine. Phenylacetic acid was higher (health group effect p-value = 0.003) and hippuric acid (p-value = 0.005) was lower in T1D and SLE compared with healthy, albeit not after Bonferroni correction. Isorhamnetin was reduced in T1D and SLE in response to stimulation compared with healthy participant (stimulation × health effect p-value = 0.003). Our findings show shifts in lipid and energy metabolism in PBMCs after stimulation regardless of the health status. Phenylacetic acid, hippuric acid and isorhamnetin, which represent a differentiating metabolism in T1D and SLE than healthy, are the intermediates in the metabolism of phenolic compounds and their changes suggest a shift in xenobiotic metabolism. Therefore, xenobiotic metabolism under autoimmune diseases warrants further investigations.

Tu147. Mitochondrial Defects Caused by Tissue Inflammation: New Hints from Spatial Transcriptomics in a Model of Myositis

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Inflammatory myopathies are autoimmune muscle disorders with unmet therapeutic needs. Their pathophysiology has remained difficult to unravel due to the paucity of relevant experimental models. ICOS pathway invalidation in NOD mice results in a switch of autoimmunity from diabetes towards myositis, providing a valuable murine model. Indeed, we show that Icos-/- NOD mice exhibit reduced muscle strength and muscle histopathological features including macrophage and IFNg-secreting CD4 T cell infiltration. Muscle holoproteome and transcriptome analyses suggested metabolic alterations and mitochondrial defects while elevated reactive oxygen species confirmed the presence of muscle oxidative stress. To demonstrate that inflammatory infiltrates are the cause of metabolic dysfunction observed in myofibers, we performed Nanostring spatial transcriptome analysis. Through segmentation with desmin and CD45, we analyzed the whole transcriptomic profile of myofibers adjacent or not to large immune cell clusters, and of muscle-infiltrating immune cells themselves. We found a strong downregulation of the skeletal muscle contraction machinery in myofibers from diseased Icos-/- NOD mice. The expression of genes encoding proteins involved in key mitochondrial metabolic processes, mitochondrial dynamics and structure stability was also strongly reduced. For many of these genes, the decrease was exacerbated in myofibers directly adjacent to large immune clusters. Histoenzymology, ex vivo oxidative phosphorylation assessment and electron microscopy confirmed severe mitochondrial defects in myofibers. N-acetyl cysteine therapy significantly ameliorated mitochondrial alterations and clinical signs of myositis. Together, our data show that the pathophysiology of myositis involves severe mitochondrial defects in myofibers caused by tissue inflammation that can be attenuated by

antioxidant therapy.

Tu148. Modulation of the T Cell Phenotype by Vitamin D Supplementation in MS

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system resulting from a complex interaction between genetic predisposition and environment. Among the latter, vitamin D deficiency is significantly correlated with increased MS risk and severity. However, whether vitamin D supplementation is able to improve MS is still debated. Herein, we analyzed the effect of vitamin D supplementation on the proportion of T cells and their phenotype in a small cohort of patients with CIS supplemented by either high dose cholecalciferol (100,000UI every 14 days, n=6) or placebo (n=6) for 3 months (D-lay MS clinical trial, NCT01817166). Unsupervised analyses showed a significant increase in the proportion of effector T cells in the vitamin D-treated group. Phenotypic changes could also be observed notably in adhesion molecules between the two groups. These preliminary data suggest a modulatory effect of Vitamin D on the phenotype and proportion of T cells in patients in CIS. Longer follow up of these patients will reveal whether these changes are related to clinical benefits. This work is funded by the ARSEP foundation and the Agence Nationale de la Recherche (ANR - 19 - CE14 - 0043).

Tu149. Multiparametric Comparison of the Adult-onset and Childhood-onset Systemic Lupus Erythematosus (SLE) Immunomes Reveals Multiple Derangements

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SLE is a complex autoimmune disease. Therapy is mainly immunosuppression, which has many side-effects. Using a holistic, multiparametric approach to understand disease mechanisms can improve theragnostics. Peripheral blood mononuclear cells(PBMCs) from 26 adult-onset SLE (timepoints:40, median age:40 years)/23 age-matched healthy subjects and 30 childhood-onset SLE (53,14 years)/17 age-matched healthy subjects underwent mass cytometry. Data was analysed using the Extended Polydimensional Immunome Characterisation(EPIC) machine learning platform. Normalization and FlowSOM clustering were performed with 43 immune markers. Unsupervised analysis revealed significant differences ($p < 0.05$,Mann Whitney U) between adult-onset and childhood-onset SLE immunomes. A CD11c+Tbet+CD21- B cell subset was significantly enriched in childhood-onset SLE (median:0.42%, interquartile range 0.16-0.72% of CD45+ PBMCs) versus adult-onset SLE (0.15%;0.09-0.31%;$p < 0.001$). These age-associated B cells(ABCs) increase with age and autoimmune disease. This may offer insights into differences between adult-onset and childhood-onset SLE; the latter tends to have more active disease. Transitional B cells (CD24+CD38+) were also significantly increased in childhood-onset SLE (0.91%;0.41-1.84%) compared to adults (0.3%;0.043-0.65%;$p < 0.001$). In T cells, an activated CD4+IL21+ subset was expanded in childhood-onset SLE (0.41%;0.23-0.68%) compared to adult-onset SLE (0.19%;0.1-0.38%;$p < 0.001$). IL21 is key to B cell activation and therefore of mechanistic interest. Another CD8+CD45RA+BAFF+ T cell subset was enriched in adult-onset (1.95%;1.24-2.86%) versus childhood-onset SLE (0.88%;0.45-1.37%;$p < 0.001$). B-cell activating factor(BAFF) supports autoreactive B cell survival in autoimmune disease and an anti-BAFF drug (belimumab) is FDA-approved for SLE. However, patient response varies, so BAFF inhibition alone may not adequately alter disease activity. Studying these mechanistically important cell subsets further may identify pathological networks to facilitate SLE theragnostic improvements.

Tu150. Multiple Sclerosis Patients Under Long-term Fingolimod Treatment Fail to Elicit Humoral and Cellular Immune Responses upon Repeated sars-cov-2 Mrna Vaccinations

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The effects of immunomodulation on the immune response to COVID-19 vaccination and the consequences for patients have not yet been conclusively determined. We here examined the effects of disease-modifying therapies (DMTs) on the immunogenicity of the SARS-CoV-2 mRNA vaccine in patients with multiple sclerosis (MS) in a prospective cohort study. We enrolled 126 MS patients of which 105 received either anti-CD20-based B cell depletion (aCD20-BCD), fingolimod, interferon-β, dimethyl fumarate, glatiramer acetate, teriflunomide or natalizumab, and 21 were untreated MS patients for comparison. In contrast to all other treated and untreated MS patients, a proportion of patients treated with aCD20-BCD or fingolimod exhibited defects and/or lack of any humoral response. In aCD20-BCD patients, the absence of humoral responses correlated with peripheral blood B cell numbers prior to the first vaccination and consequently with the last aCD20-BCD treatment time point. CD4+ T-cell responses were intact. However, in patients who received long-term treatment with fingolimod, we did not detect spike-reactive CD4+ T-cell responses, a defect that could not be dissolved by repeated booster vaccinations. The duration of fingolimod treatment (>29 months), rather than peripheral blood B and T cell counts prior to each vaccination, correlated with whether a humoral immune response was elicited. Our results suggest that patients treated long-term with fingolimod are at risk for severe SARS-CoV-2 infection despite booster vaccinations, which is important for clinical decision making and adapted protective measures.

Tu151. Myeloid-derived Suppressor Cells Showed Differential Recruitment in SLE Animals Contributing to the Pathology

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Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease that can affect various organs. The overall incidence rates are between 1.5 to 11 cases per 100,000 people, with a higher predisposition in women than in men. Myeloid-derived suppressor cells (MDSC) are a cell type involved in multiple pathologies, such as cancer or infections, where they have been characterized by promoting a state that is detrimental to the host. In patients with SLE, increased arginase 1 (Arg1) secretion by MDSCs has been reported to contribute to pro-inflammatory responses. To evaluate the contribution of the MDSCs during the development and progression of systemic lupus erythematosus, two murine models capable of acute and chronic disease development were evaluated. In both models, we identified changes in the recruitment of granulocytic MDSC (G-MDSC) and monocytic MDSCs (M-MDSC) populations in the different target tissues. Also, these changes were accompanied by a specific cytokines pattern expression. In addition, MDSCs presented a possible APC phenotype, which could also be related to changes in different adaptive cells. This work presents new background on how MDSCs influence the progression of systemic lupus erythematosus.

Tu152. rab4a Controls the Depletion of IL-2 in CD4$^+$ T cells via Enhanced CD38 Expression: Potential Involvement in Proinflammatory Lineage Development in Systemic Lupus Erythematosus

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Rab4A is a GTPase that is overexpressed in systemic lupus erythematosus (SLE) patients' T cells and enhances surface receptor recycling. Increased expression of CD38 and loss of IL-2 production have independently been associated with proinflammatory skewing in SLE. We investigated the impact of Rab4A on the expression of CD38 and the production of IL-2. Our lab created Rab4A-mutant Jurkat cell lines, containing GFP-expressing vector alone (control), doxycycline-inducible vectors that overexpress Rab4A (Rab4Ahi) or the dominant-negative mutant Rab4AS27N (Rab4ADN). Furthermore, we CRISPR knocked out (KO) CD38 in these cell lines. These cells were co-stimulated with anti-CD3 antibody and PMA. Cell surface markers and cytokines were analyzed by flow cytometry and protein levels by western blot. NAD+ levels were measured by LC-MS/MS. In the Rab4Ahi cells compared to the control, CD38 expression was upregulated (p=2.49x10-13), intracellular production and secretion of IL-2 significantly decreased (p=1.16x10-7 and p=0.0401, respectively), NAD+ concentration decreased (p=0.0039), while pSTAT3 levels increased (p=0.0318). In the Rab4Ahi CD38KO cells compared to the Rab4Ahi CD38WT cells, secretion of IL-2 significantly increased (p=0.0145) and pSTAT3 levels decreased. CD38 is an NAD+ hydrolase, which regulates Sirtuin-1 activity, a NAD+-dependent histone deacetylase that suppresses STAT3 activity. Elevated pSTAT3 levels may underlie diminished IL-2 production by binding to the promoter of FoxO1, which inhibits IL-2 production. The overexpression of Rab4A and CD38, increased pSTAT3, and diminished IL-2 production reflect changes observed in SLE patients. Our results suggest that increased expression of Rab4A may underlie the overexpression of CD38 and diminished secretion of IL-2 in SLE.

Tu153. Therapy of Atypical Hemolytic Uremia Syndrome(ahus)
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Problem: aTypical Hemolytic Uremia Syndrome (aHUS) is an extremely rare disease that contribute to life threatening disease. The human CFHR–Factor H gene cluster encodes are emerging complement and immune modulators. (Peter F. Zipfel, Thorsten Wiech, [...], and Christine Skerka "CFHR Gene Variations Provide Insights in the Pathogenesis of the Kidney Diseases Atypical Hemolytic Uremic Syndrome and C3 Glomerulopathy" Published online 2020 Jan 24. doi: 10.1681/ASN.2019050515 J Am Soc Nephrol. 2020 Feb; 31(2): 241–25). Results: The underlying disease is due to hyper activation of Complement and Coagulation cascade. Since both pathways can be blocked by proximal inhibition of complement system, Factor D inhibition offer an attractive molecular target for therapy. Discussions: Sulfonic polymer and its formulation methods are advanced as therapy of aHUS for better therapeutic outcome.

Tu154. Efficacy and Safety of Ebdarokimab, in Subjects with Moderate to Severe Ulcerative Colitis: Results from a Randomized, Double-blind, Placebo-controlled, Single-dose Escalation Phase Ib Clinical Study
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Background
Interleukin-12 (IL-12) and interleukin-23 (IL-23) are two essential cytokins involved in the immune-mediated inflammatory disorders of Ulcerative Colitis (UC). Ebdarokimab (AK101) is a fully human monoclonal antibody (mAb) targeting IL-12/IL23 pathway, and being developed for treating UC, has showed excellent safety and tolerability in pre-clinical studies.

Methods
A total of 34 UC subjects (male and female) with age ranging from 18 to 65 years were enrolled, 26 subjects received Ebdarokimab and 8 subjects received placebo. The study includes subcutaneous (SC) and intravenous (IV) groups. Subjects in the SC groups were given a single dose of 270mg or 540mg respectively. Subjects in the IV groups received 4mg/kg, 6mg/kg or 9mg/kg of Ebdarokimab at week 1 (D1) for the dose escalation phase of this study.

Results
26 subjects were treated with Ebdarokimab, 10(83.3%) of 12 subjects in the SC group experienced treatment emergent adverse events (TEAE); 9(64.3%) of 14 subjects in the IV group; 2(25%) of 8 subjects in the placebo group experienced TEAEs. Most TEAEs /TRAEs were mild and moderate. No death was reported.

The median Tmax of Ebdarokimab SC was approximately 7 days. The absolute bioavailability was approximately 59.4% in 270mg group. The mean Vz, CL, and half-life ranged from 10.3 to 11.7L,0.37 to 0.46L/day,17.4 to 21.4Day following a single dose IV, respectively. The exposure of Ebdarokimab increased dose proportionally in the dose range from 4mg/kg to 9mg/kg. There was no treatment-related ADA-positive in the evaluated subjects.

Conclusion
Ebdarokimab was generally safe and well tolerated, and indicated dose-proportional exposures.

W100. 17 Beta-estradiol and B-cell Intrinsic Type I Interferon Amplify Toll-like Receptor 7 Signaling Loop in B Cells of Female Lupus Prone BXD2 Mice

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While systemic lupus erythematosus (SLE) disproportionally affects women versus men, estradiol (E2) elevation alone is not sufficient to promote development of autoantibody producing B cells. The objective of this study is to determine if B-cell intrinsic mechanisms contribute to increased TLR7 response to E2 stimulation. We identified elevated circulating levels of E2 in young African American (AA) SLE patients (24-41yr-old), compared to older AA (42-56yr-old) and European American SLE patients (24-66yr-old). Circulating E2 levels positively correlated with levels of anti-Smith (Sm) and expression of IFN-beta in naïve B cells of SLE patients (n=39). Mouse studies were used to determine if E2 stimulates expression of IFN-beta in B cells, and if sex plays a role to influence B cell responses to E2. Serum levels of Sm/RNP and RNP autoAbs positively correlated with levels of E2 in female lupus prone BXD2 mice post-puberty (12wk-old) but not pre-puberty (4-6wk-old). At the post-puberty stage (>12wk-old), there were significantly elevated levels of anti-DNA and anti-Sm in female BXD2 mice, compared to males. This was associated with increased TLR7-induced expression of IFN-beta and CD69 in transitional stage 1 (T1: CD23-IgM+ CD93+) B cells in female BXD2 mice, compared to males. E2 stimulation promoted intracellular levels of IFN-beta and TLR7 in T1 B cells from female but not male BXD2 mice. Our results suggest that elevation of E2 in combination with increased B-cell susceptibility to E2 induction of IFN-beta may play a role in increased B cell responses to TLR7 stimulation in individuals predisposed to developing SLE.

W107. BCG Clinical Trial Programs in Advanced Type 1 Diabetes: 2023 Update

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The bacillus Calmette-Guerin (BCG) vaccine is being tested in clinical trials for treatment of diverse forms of autoimmunity. Multiple clinical protocols in type 1 diabetes (T1D) are underway at our institution testing various aspects of BCG's ability to lower HbA1c, reduce insulin requirements and regulate blood sugars: 1) Ten-year follow up of a Phase I study of BCG vaccination in adults with longstanding T1D to study the durability of lowered HbA1c values after treatment; 2) Randomized, double-blinded Phase II clinical trial testing the BCG vaccine in adults with longstanding T1D; 3) Radiologic study to identify organs and organ systems with higher sugar utilization after BCG treatment; 4) Study in adults with longstanding T1D evaluating two versus 6 doses of BCG over 5 years of observation; 5) A multicenter clinical trial of adolescents with >2 years since T1D diagnosis to evaluate potential benefits of BCG vaccination. Immune and metabolic mechanisms accounting for BCG's durable effects include

epigenetic modulation of glucose signaling pathways, reset of the Treg cellular subset and correction of underlying aerobic glycolysis defects in T1D lymphocytes, leading to regulated glucose control. To date, 295 of 500 planned patients are enrolled, including 272 BCG-vaccinated patients. Global studies show that the impact of multi-dose BCG vaccination in multiple sclerosis (MS) and T1D is seen with at least 2 years of follow up and that the effects appear durable without further treatment in both MS and T1D to 5+ years. BCG vaccine therapy may offer a safe, affordable intervention in longstanding autoimmunity.

W108. Blood TCR Signatures Classify Autoimmune Diseases and Predict Response to Low-dose IL-2
Encarnita Mariotti-Ferrandiz, Encarnita Mariotti-Ferrandiz, Pierre Barennes, Paul Stys, Roberta Lorenzon, Nicolas Coatnoan, Kenz Le Gouge, Hélène Vantomme, Michelle Rosenzwagj, Agnes Hartemann, jeremie Sellam, Francis Berenbaum, David Klatzmann, Encarnita Mariotti-Ferrandiz, Pierre Barennes, Paul Stys, Roberta Lorenzon, Nicolas Coatnoan, Kenz Le Gouge, Hélène Vantomme, Michelle Rosenzwagj, Agnes Hartemann, jeremie Sellam, Francis Berenbaum and David Klatzmann
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Autoimmune diseases (ADs) are chronic and debilitating diseases representing an important societal burden. They call for disease-specific and curative treatments as well as for better diagnostic and prognostic markers. ADs result from patient's T and B lymphocyte attacking their own tissues. While autoantibodies are used for AD diagnosis, T-cell are not considered for diagnosis in spite of their critical role in AD onset and maintenance. To tackle this major gap, we analyzed the T-Cell Receptor (TCR) repertoire by next-generation sequencing in various AD contexts. We sorted CD4 T effector cells (Teff) and CD4 T regulatory T-cells (Treg) from the blood and analyzed their TCRs using an innovative machine learning approach based on sparse least square discriminant analysis. When applied to type 1 diabetes (T1D) and rheumatoid arthritis (RA) patients, we identified Teff and Treg TCR signatures that i) classify these patients versus healthy volunteers with > 90% accuracy and ii) properly cluster T1D and RA patients separately. These signatures were shown to contain TCRs that were previously shown to relate to these conditions and were validated on external datasets. We also applied our analyses to sequential samples from lupus patients treated with low-dose IL-2. We identified a Treg TCR signature that could predict clinical response to treatment. Altogether, these results show that peripheral blood TCR repertoires contain relevant disease-specific information that could serve as biomarker to improve AD diagnosis, prognosis and patient care.

W109. Dermatomyositis Skin Inflammation Is Associated with Type-i IFN Response and the Presence of pd1-high, CXCR5-, CXCL13^{+} CD4^{+} T cells, Which Expands in Response to UVB Irradiation
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Dermatomyositis (DM) is a rare inflammatory skin disease associated with chronic pruritic rash and UVB photosensitivity. Using single-cell RNA sequencing and flow cytometry, we identified a distinct population of CXCR5-, PD1-high, CXCL13+ CD4+ T helper cells only in lesional skin, which exhibited a type I interferon (IFN-I) signature, relatively stronger compared to other subsets of T cells. Immunohistochemical analysis of lesional DM skin demonstrated that CXCL13 CD4 T cells tightly infiltrated around capillaries and small blood vessels in the dermis, but surprisingly no evidence of B or plasma cells aggregate. Using ELISA on interstitial skin fluid captured via suction blistering, we discovered a significant elevation of both IFN-β and CXCL13 in lesional DM compared to non-lesional DM and healthy skin. Spatial transcriptomics of lesional skin revealed that T cells near basal keratinocytes (KCs) exhibited a stronger IFN-I signature. Primary KCs obtained and cultured from DM skin exhibited an increased expression of IFN-I following exposure to UVB compared to healthy KCs, in vitro ($p< 0.01$). Further, we found that IFN-β induced a significantly higher expression of CXCL13 in CD4+ T cells isolated from the blood of DM patients compared to healthy, in vitro ($p< 0.001$). Moreover, UVB photoprovocation of clinically normal-appearing skin in a DM patient resulted in the expansion of CXCL13-producing T cells in vivo. Taken together, our findings suggest that a

pathologically enhanced IFN-I response following UVB exposure in DM KCs could lead to the expansion of CXCL13-producing CD4 T cells in the dermis and lead to skin inflammation.

W110. Immune Therapy of Sickle Cell Disease and Its Prophylaxis
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Problem: Sickle cell disease is an autosomal recessive genetic red cell disorder with a worldwide distribution. Alternate complement system is hyper activated contributing to acute sickle cell related vaso-occlusive crises. (Lombardi E. et al "Factor H interferes with the adhesion of sickle red cells to vascular endothelium: a novel disease-modulating molecule in Haematologica 2019 Volume 104(5):919-928) Method: Sulfonic nanopolymers are developed as inhibitor of Factor D to reduce host inflammatory reaction to hyper activated alternate complement system Discussions: Sulfonic nanopolymers in appropriae formulation strategies may prevent sickle cell vaso-occlusive crisis and may be used both for immune therapy and prophylaxis.

W111. Individual Myasthenia Gravis Autoantibodies Can Mediate Three Distinct Mechanisms of Pathology
Minh Pham[1], Gianvito Masi[2], Abeer Obaid[2], Rosa Patzina[2], Seneca Oxendine[2], Sangwook Oh[3], Aimee Payne[3], Richard Nowak[2], Kevin O',Connor[2], Gianvito Masi[2], Abeer Obaid[2], Rosa Patzina[2], Seneca Oxendine[2], Sangwook Oh[3], Aimee Payne[3], Richard Nowak[2], Kevin O' and Connor[2]

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Serum autoantibodies targeting the acetylcholine receptor (AChR) in autoimmune myasthenia gravis (MG) can mediate pathology via three distinct mechanisms: complement activation, receptor blockade, and antigenic modulation. However, it is unclear whether multi-pathogenicity is mediated by individual or multiple autoantibody clones. Using an unbiased B cell screening approach, we generated a library of 11 human-derived AChR-specific monoclonal autoantibodies (mAb) and assessed their pathogenic profiles. Five mAbs activated complement, 3 blocked the receptor, and 7 induced modulation. Furthermore, 2 mAbs were each highly efficient at all three mechanisms, demonstrating that pathogenic mechanisms are not mutually exclusive. Using novel cell lines individually expressing each AChR subunit (α2βδε), triple-pathogenic mAbs were determined to exclusively bind the α subunit, demonstrating a link between mAb specificity and pathogenic mechanism. These findings provide new insight into the immunopathology of MG, demonstrating that single autoreactive clones can efficiently mediate three modes of pathology. Therapeutic approaches targeting only one autoantibody-mediated mechanism may be evaded by mAbs with multifaceted pathogenicity.

W112. Influenza Antibody Titers in Recent Onset Narcolepsy
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Objective: Epidemiological studies have shown associations between pandemic H1N1 2009 Influenza-A infection and vaccination (only using Pandemrix®) and the onset of narcolepsy, an autoimmune disease associated with HLA-DQB1*0602. We tested whether patients with recent onset have increased flu antibodies versus matched DQ0602 controls. Methods: Serum samples of 82 recent onset type-1 narcolepsy patients (12 [1-25] months) and 84 healthy controls matched by sex, age, and year and season of sample collection were used. Samples were tested for Influenza-A and B antibodies using HAI assays against the dominant strains known to circulate at time of collection. HAI assays against H1N1 2009 were also tested independently in all subjects. Titers were log2 transformed, with zero being < 1/10 dilution, 1 being 1/20, 2 1/40 etc., so that every dilution represents an increment of 1 unit. Results: pH1N1 2009 titers were increased in 25/63 (39.7%) subjects collected after 2009. Increasing titers (doubling rate) of H1N1 2009 [OR=1.2962(1.0513, 1.5984), p=0.015], all H1N1 [OR=1.13(1.03-1.24), p=0.023] and Influenza-B Victoria

[1.37 (1.16-1.61) p=0.001] were associated with narcolepsy, whereas no association was found with H3N2 and Influenza-B Yamagata. Conclusion: Both Influenza-A H1N1 and Influenza-B Victoria, but not other strains, may trigger narcolepsy onset. This result is in line with a recent epidemiological study in Europe that reported a strong increase in narcolepsy onset in 2010 (following 2009 H1N1) and a secondary peak in 2013 following a season with primary Influenza-B Victoria infections.

W113. Interconnected Differentiation Trajectories of Exhausted and Nk-like CD8+ T cells Linked to Alefacept Therapy in Type 1 Diabetes

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Better response to Alefacept (LFA3-Ig) in type 1 diabetes patients was previously linked to increased exhausted CD8+ T cells (Tex) with either a CD57+ or PD-1+ phenotype. Lineage relationships between CD57+ and PD-1+ cells and their importance in Alefacept response remained unknown. Here we used bulk ATAC-sequencing (n = 4 Alefacept responders, 0 wk and 104 wk) and single cell RNA-seq (scRNA-seq) combined with T cell receptor (TCR) sequencing (n = 12, 6 responders and 6 non-responders, 104 wk) to elucidate the lineage relationships and diversity of these populations. Bulk ATAC-seq revealed PD-1+ and CD57+ populations are epigenetically distinct and share increased accessibility of exhaustion-related genes and Natural Killer Cell Cytoxicity pathway genes. CD57+ cells, however, were distinguished by increased inhibitory Killer Cell Immunoglobulin-like Receptor (iKIR) accessibility. scRNA-seq with TCR seq identified previously unappreciated gene expression heterogeneity in CD57+ cells, characterized by iKIR and Interferon pathway expression diversity. CD57+ and PD-1+ populations also displayed reciprocal Inhibitory Receptor (IR) expression despite shared chromatin accessibility of exhaustion genes. Analysis of TCR sharing between PD-1+ and CD57+ populations revealed they share a common lineage and differentiate using four trajectories; Tex-PD-1+, Tex-CD57+, Tex-Branching, and Tex-Fluid, suggesting precursor cells differentiate into either the PD-1+ or CD57+ phenotype, or both. This pattern was supported by finding CMV/EBV-specific cells with both CD57+ and PD-1+ phenotypes in scRNA-seq data and sorted tetramer-specific cells. These findings demonstrate interconnections between exhausted and NK-like CD8+ cell populations and prompt investigation of cues driving their differentiation and role in response to therapy.

W114. Islet Autoreactive Regulatory T cells in Type 1 Diabetes Are Clonally Expanded and Have a Unique TCR Repertoire

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Autoimmune type 1 diabetes (T1D) results from unrestrained destruction of pancreatic islets by autoreactive T cells, but little is known about islet autoreactive (IAR) regulatory T cells (Treg) in T1D. We analyzed IAR CD4 conventional T (Tconv) cells and Treg in the blood from 10 established DRB1*0401 T1D subjects and 10 matched healthy controls using a CD154-CD137 activation assay combined with single cell RNA-sequencing to capture the TCR repertoire and transcript profiles in parallel. We found no difference in the frequency of IAR CD4 Tconv or Treg between T1D subjects and healthy controls, nor was there a difference in Treg/Tconv ratio. However, the transcript profiles of IAR Treg from T1D subjects differed from controls, with reduced expression of genes involved in signaling, metabolism, biosynthesis, and regulation of cell proliferation. Clonal expansion of IAR Tconv and Tregs was detected within T1D and control donors, but the TCR repertoire was unique for each cell type. Sharing of TCRα chains between donors was observed primarily in IAR CD4 Tconv cells, however, TCRα chains from both cell types were shared with TCRs from pancreatic islets. Gene edited Tregs expressing an IGRP-specific Treg TCR mediated robust antigen-specific and bystander suppression of CD4 Tconv cells. Thus, circulating IAR Tregs numbers are not reduced in T1D donors

and show evidence of antigen driven expansion. While their TCR repertoire is unique from IAR Tconv cells, circulating IAR Treg share TCR chains with pancreatic TCRs and can mediate robust suppression.

W115. JAK Signaling in Keratinocytes and Synovial Cells (FLS): A Regulatory Role in the Pathogenesis of Psoriatic Disease

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In psoriasis and psoriatic arthritis (PsA) aberrant activation/migration of specific T cell subpopulations (Th17,Th9,MAIT cells) in the skin and joint synovium induce local inflammation and upregulation of cytokines such as IL-9, IL-17A/IL-17F and IL-22 for activation and proliferation of keratinocytes and synovial cells (FLS) which leads to plaque and pannus formation. Among these JAK-1,3 are activated by IL-9 and JAK1/TYK2 by IL-22. There are reports suggesting JAK/STAT signaling system is active in human keratinocytes and in the joint FLS the two key regulatory cells for the disease process of psoriasis and PsA. JAK/STAT signaling system on T cells is well established but its function in keratinocyte and FLS biology and its regulatory role on pannus/plaque formation remains unknown. Here we hypothesized that IL-9 and IL22 induced JAK/STAT signaling has regulatory role in the inflammatory/proliferative cascades of KC and FLS in psoriasis and PsA. In cultured FLS and KC we observed that compared to the media rIL-22 and rIL-9 induced increased phosphorylation of JAK1/TYK2 and JAK1/JAK3 respectively ($p < 0.01$). IL9/IL22 also activated STAT3/RORγt. The critical events induced by IL-22 and IL-9 in psoriasis/PsA such as cell proliferation; IL-6, IL-8 and MMP-3 production of KC and FLS were regulated by the respected JAK-STAT kinases associated with IL-22 and IL-9. Further pan-JAK inhibitors and other specific JAK-1 and TYK2 inhibitors could effectively block these effects ($p < 0.001$). These data provide a novel insight about the role for JAK-STAT signaling in the pathogenesis of psoriatic disease and provide mechanisms of actions of specific JAK inhibitors.

W116. Lymphoma Driver Mutations Drive Pathogenic B Cell Clones in Autoimmune Hcv-associated Cryoglobulinemic Vasculitis

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The pathogenesis of autoimmune disease remains unclear. It has been proposed that some aspects of autoimmune disease overlap with lymphoid cancer pathogenesis. The autoimmune syndrome, mixed cryoglobulinemia, and its more severe form, cryoglobulinemic vasculitis, almost exclusively arises secondary to hepatitis C virus (HCV) infection. HCV infection is associated with an increased risk of developing B cell lymphoma. Here, we interrogate the autoreactive pathogenic B cell clones in four patients with HCV-associated cryoglobulinemic vasculitis, for the presence of somatic gene lesions. Using a combination of whole genome sequencing and single cell genome amplification techniques, we reveal the pathogenic autoreactive B cell clones harbour somatic gene mutations (KLF2, TRAF3, NOTCH1) and chromosome aberrations (11q22-23 del, trisomy 12) recurrently detected in B cell lymphomas. The somatic gene lesions are predicted to cause hyperactivation of the NF-kB signalling pathway. The discovery of functional somatic mutations linked to hyperactivation of NF-kB signalling pathway, not only explains the capacity of the B cell clone to expand and persist in the patients even following clearance of HCV, but also demonstrates how the pathogenic B cell clones and their self-reactive precursors are able to evade B cell tolerance

checkpoints. These findings provide evidence for a shared pathogenesis between autoimmunity and lymphoid cancer which may be relevant to the pathogenesis of other autoimmune diseases.

W117. Nanodisc Technology for Inducing Antigen-specific Immune Tolerance Against Type 1 Diabetes

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Type 1 Diabetes (T1D) is an autoimmune disease characterized by the T cell mediated destruction of insulin-producing β-cells. Antigen-specific Tregs are efficient regulators in suppressing autoimmunity, making them attractive targets for tolerogenic therapy. However, it remains unknown how to efficiently induce antigen-specific Tregs in vivo. Here, we report the development of a novel synthetic high-density-lipoprotein-based nanodiscs (ND) for the delivery of autoantigens and induction of immune tolerance in mouse T1D models. ND was prepared by lyophilization approach [1]. Chromogranin A-derived mimetopes (p31 and 2.5mi) were incorporated onto ND. By using BDC2.5 and NY8.3 adoptive transfer models of T1D, we optimized the dosing regimen and improved the efficacy in protecting against disease. Subcutaneous treatments with p31-ND completely prevented diabetes in NOD.SCID mice transferred with preactivated BDC2.5 splenocytes. In a BDC2.5 + NY8.3 co-transfer model, treatment with p31-ND delayed the disease progression from 8 days to an average of 4 weeks, indicating bystander suppression. Mechanistically, one single dose of 2.5mi-ND induced 2.5mi-specific T cells that exhibited anergic phenotypes. In NOD.SCID mice, p31-ND treatment after co-transfer of BDC2.5 CD4 T cells and NY8.3 CD8 T cells enriched antigen-specific anergic CD4 T cells and Foxp3+Treg cells in the pancreas and prevented CD8 T cell activation and infiltration. Collectively, these results show that ND induces antigen-specific Tregs that dampen ongoing autoimmunity, potentially through recognizing the same tissue-specific self-antigens or through bystander suppression. The novel antigen-specific therapy may have implications for development of immune modulating treatment for T1D patients.

W118. Natural Killer Cells as Immunological Signatures of Long-term Stable Remission in Rheumatoid Arthritis

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With the use of modern therapeutics and improved clinical management strategies achieving long-term stable clinical remission in patients with rheumatoid arthritis (RA) is readily achieved. To prevent complications associated with long-term use of immuno-suppressants, drug tapering or complete withdrawal is recommended. However, due to the lack of robust tools in defining a true biological remission state, disease flares remain a frequent outcome, highlighting the need for a more precision medicine-based approach. Utilising high dimensional immune phenotyping platforms, we set out to define immunological signatures of deep RA remission. longitudinal disease activity scores (DAS28) and clinical information from the REMission in RA (REMIRA) cohort were used to define distinct patient trajectory groups. Patients who sustained clinical remission over time (denoted as 'stable remission') were compared to patients who fluctuated between remission and states of low and moderate disease activity (denoted as 'intermittent remission'). Differences in the immune system between remission groups was analysed in an un-biased fashion by mass cytometry (CyToF). Analysis identified a CD8+CD57+ NK cell population associated with stable remission, which was validated by spectral flow cytometry. Functional studies demonstrated that NK cells associated with stable remission are defined by minimal pro-inflammatory cytokine expression in response to in-vitro IL-2 priming and K562 target cell co-culture (even to below levels of healthy controls). NK cells from active disease demonstrate distinct phenotypic changes associated with enhanced pro-inflammatory cytokine expression. In conclusion, we have defined NK cell phenotypes and associated in-vitro functional responses as immune signatures of long-term stable

RA remission.

W119. Opposing Effects of the Aryl Hydrocarbon Receptor and Type I Interferon Control Generation of CXCL13^{+} T cells in Systemic Lupus Erythematosus

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Expansion of B cell helper-T cells, including T follicular helper (Tfh) and T peripheral helper cells (Tph) is a prominent feature of systemic lupus erythematosus (SLE), an autoimmune disease with broad autoantibody production. Human Tfh and Tph cells highly express the B cell chemoattractant CXCL13; however, regulation of CXCL13 production by T cells remains largely unknown. Here, mass cytometry immunophenotyping revealed a marked imbalance in CD4 T cell phenotypes in SLE patients, with expansion of CXCL13+ Tfh/Tph cells and specific reduction of CD96hi IL-22+ T cells. We hypothesized that a common regulator might drive this skewed balance. Using CRISPR screens of human CD4 T cells, we identified the transcription factor aryl hydrocarbon receptor (AHR) as a central regulator of an axis of T cell polarization with CXCL13+ and IL-22+ states at opposing ends. Transcriptomic, epigenetic, CUT&RUN, and functional studies demonstrated that 1) AHR activation drives T cells away from a CXCL13+ phenotype and towards an IL-22+ phenotype, and 2) AHR engages the AP-1 family member JUN to regulate CXCL13- and IL-22-associated phenotypes. Treatment of T cells from SLE patients with an AHR agonist reduced the frequency of PD-1+ Tph cells. In contrast, type I interferon (IFN-I), a central mediator in SLE, represses AHR activation in T cells and synergizes with AHR inhibition to boost CXCL13 production and promote a Tph cell phenotype. These results reveal AHR, JUN, and IFN-I as regulators of a previously unrecognized CXCL13 <—> IL-22 polarization axis and highlight an imbalance of this axis in SLE.

W120. Parenchymal Response to Interferon Regulates Murine Lupus Nephritis

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Background Lupus nephritis is the most common life-threatening end-organ complication of SLE. Interstitial infiltrates, specifically T cells, are major predictors of disease outcomes. We recently determined that kidney-infiltrating T cells (KITs) are suppressed after kidney infiltration. PD-L1 is upregulated on the parenchyma of lupus nephritis patients and lupus-prone mice. Therefore, we postulated that IFNγ induces a protective program mediated by PD-L1 which results in suppressed immune mediated destruction of the kidney in lupus nephritis. Methods To determine whether PD-L1 and IFNγ signaling on parenchymal cells regulates disease, we generated bone marrow chimeras by transferring congenically labeled WT immune cells into either wild-type (WT) or IFNγR-/- MRL.Fas/lpr or PD-L1-/- MRL.Fas/lpr recipients. Additionally, we have created similar IFNγR-/- recipient chimeras using a second lupus prone strain, the FcγR2b-/-.yaa model. Results/Discussion As hypothesized, the IFNγR-/- MRL.Fas/lpr recipient mice exhibited more severe and rapid disease onset than WT recipient controls. The IFNγR-/- recipients had significantly increased glomerulonephritis and interstitial disease. Consistent with these findings, IFNγR-/- recipients had reduced survival in both the MRL and FcγR2b-/-.yaa nephritis models. Interestingly, this IFNγR mediated regulation of disease occurred independently of PD-L1 as similar findings were not observed in PD-L1 deficient recipients. These experiments suggest that parenchymal IFNγR signaling results in upregulation of protective mechanisms which reduces kidney disease and alters T cell phenotypes. This data supports the hypothesis that IFNγ, and possible other inflammatory mediators, may have differential effects varying cell lineages. In all, these findings should be considered

when devising novel targeted therapies.

W121. Pathogenic Autoreactive Th Cells Are Identified in Autoimmune Neuroinflammation by Expression of neuropilin-1 (NRP1)

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Many autoimmune diseases are associated with self-reactive CD4+ T helper (Th) cells generating pathogenic responses against self-tissues. Autoimmune disease treatment is restricted by a lack of ability to directly identify harmful self-reactive Th cells from beneficial Th cells that respond to foreign antigens. We have reported in several mouse models of systemic autoimmune disease that populations of self-reactive pathogenic Th cells upregulate Neuropilin-1 (NRP1), a surface receptor usually expressed only by regulatory Foxp3+ Th cells in the steady state. The NRP1+Foxp3- Th cell level correlated with disease severity and these cells were pathogenic on transfer; additionally, treatment targeting NRP1-expressing cells reduced clinical systemic autoimmune disease (Raveney et al. EMBO Mol. Med. 2022). Immunization of mice with self-peptides, such as MOG35-55 which generates the autoimmune neuroinflammatory disease experimental autoimmune encephalomyelitis (EAE), induced novel expression of NRP1 by Foxp3- Th cells, whereas immunization with non-self-peptides did not ($p< 0.0001$). At peak EAE, NRP1+ Th cells formed more than 40% of CNS-infiltrating Th cells and changes in NRP1+ CNS Th cell proportion mirrored clinical disease. NRP1+ Th cells generated pathogenic responses upon restimulation with a MOG35-55 peptide, whereas NRP1- Th cells made only low responses. Further, the presence of circulating NRP1+ Th cells was significantly associated with multiple sclerosis patient groups ($p=0.004$, Fishers exact test). Together these data hint a common marker for self-specific Th cells that have developed into pathogenic autoreactive Th cells. NRP1 measurement provides exciting potential for identifying and targeting harmful self-reactive Th cells even when their cognate antigen is unknown.

W122. Pathophysiological Mechanisms and Molecular Indicators of IPEX Disease and Their Implications for the Development of New Therapies

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Immunedysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome is a prototypical primary immunoregulatory disorder caused by mutations in a FOXP3 gene, a transcriptional factor critical for the suppressive function of regulatory T cells (Treg). Atypical presentations of IPEX have recently increased the clinical variability and complicated the diagnoses. To facilitate early diagnoses and to better understand the disease's pathophysiological mechanisms, we studied the common molecular immune features of IPEX patients and the sources of autoreactive T cells. We identified a common Th-2 skewed cytokine profile in the patient's plasma and Th-2 skewed T cell phenotype. The epigenetic counting of immune cells showed expansion of the Treg compartment. Further studies of the IPEX's expanded Treg cells indicated that they are unstable and adopt Th2 skewed Teff-like phenotype. Since Treg are physiologically self-reactive, the unstable Treg together with expanded autoreactive effector T cells, as indicated by TCR analyses, are representing the sources of the autoreactive T cells. The frequency of expanded unstable Treg seems to increase with disease severity and duration, sometimes preceding the clinical worsening of the disease. These data are crucial to complement the diagnosis by gene sequencing and monitor response to treatment. Currently, we are in phase I of CD4LVFOXP3 cell therapy, in which autologous IPEX T cells are converted to Treg-like cells by lentiviral-mediated FOXP3 expression. Understanding the heterogeneity of the cellular input for cell product manufacturing is a crucial step also for the development of Treg-based therapies.

W123. Pilot Study for Simultaneous Multiomic Single-cell Profiling in T and B Cells from Type 1 Diabetes and Multiple Sclerosis Patients

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Single-cell profiling sheds light on heterogeneity and dynamics of individual cells that conventional bulk-population measurements cannot reveal. In this pilot study, our goal is to establish a pipeline using simultaneous single-cell profiling of gene expression, surface protein, and chromatin accessibility to generate data accurately and rapidly from clinical trial samples. We analyzed peripheral blood mononuclear cells collected prior to treatment from four participants in each of two Immune Tolerance Network (ITN) clinical trials: recently diagnosed Type 1 Diabetes patients from the AbATE clinical trial and multiple sclerosis patients from the HALT-MS clinical trial. We collected an equal number of sorted T cells (CD3+CD19-CD14-CD56-) and B cells (CD3-CD19+CD14-CD56-) from each subject and pooled together for droplet-based single-cell assays and downstream analyses. The multiplexed samples were sequenced through single-cell multiome ATAC+Gene Expression and CITE-seq (RNA/163 Proteins)+TCR/BCR repertoire. We performed demultiplexing and quality-control on the pooled samples based on dense genotyping of the subjects and obtained, on average, 918 cells per sample for the former platform and 4056 cells per sample for the latter platform. We performed canonical correlation analysis on the multimodal CITE-seq data and produced refined clusters using RNA and 45 informative protein markers simultaneously to identify global B and T cell populations, and subsets including naïve and memory T cells. The clusters were further merged with multiome data for linkage to epigenetic states. This work highlights the quality and potential utility of simultaneous multiomic single-cell profiling of T and B cell states and tracking changes over time during immune-modulating therapies.

W124. Potential of Innate Immune Memory by Retinal Pigment Epithelial Cells

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Studies of immune privilege mechanisms demonstrate that the ocular microenvironment is anti-inflammatory and the melanocortin pathway has a central role. Based on our findings that soluble factors produced by the retinal pigment epithelial cells (RPE) alter the response of macrophages to pro-inflammatory signals, we assayed for the potential of RPE regulation of innate immune memory. The conditioned media (CM) of RPE eyecups, intact RPE monolayer, choroid, and sclera of the posterior eye segment, from the eyes of C57BL/6 naive mice and mice with active experimental autoimmune uveitis (EAU) were assayed. Resting peritoneal macrophages were treated with the RPE-CM for 24 hours, washed, and rested for 48 hours. The macrophages were then stimulated with LPS for 24 hours, and the cultures were assayed for TNF-a and IL-10 by ELISA. To initially assess a potential role for the melanocortin pathway, macrophages were treated with the neuropeptide α-MSH instead of RPE-CM and assayed for NO• and IL-1 after LPS addition. The macrophages not pre-treated with CM significantly ($P < 0.001$) produced TNF-a and no IL-10. Pretreatment with naive RPE-CM had lower, but not statistically significant, TNF-a production; however, there was a significant ($P < 0.001$) elevation in IL-10 production. In comparison, macrophages pretreated with EAU RPE-CM had no significant change in TNF-a but had only background levels of IL-10. The a-MSH pretreatment of macrophages significantly ($P < 0.05$) suppressed NO• generation but not IL-1. The results suggest that the RPE can potentially mediate innate immune memory selectively.

W125. Pre-clinical Pharmacologic and Tolerability Characterization of MTX-101, a Novel kirxcd8 Targeting Bispecific CD8 Treg Modulator

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In healthy individuals, CD8 Treg activation leads to selective elimination of self-reactive CD4 T cells. The CD8 Treg network appears dysfunctional in autoimmune diseases and insufficient to kill self-reactive CD4 T cells, in part due to expression of inhibitory KIR2DL1/2/3; We have developed MTX-101, a bispecific CD8 Treg modulator targeting CD8 and KIR2DL(1/2/3) molecules. MTX-101 targets CD8 Treg, activating the cells, thereby reducing inflammation without increasing unwanted immune cell activation or pro-inflammatory cytokines. Our early data suggest that MTX-101-mediated enhancement of CD8 Treg function is a broadly applicable and a promising CD8 Treg specific therapeutic to restore immune balance for the treatment of autoimmune diseases, including gastrointestinal indications (eg., celiac disease, Crohn's and ulcerative colitis). We have evaluated the binding and specificity profiles of MTX-101 in vitro and in vivo, . No activation of immune cells or increased proinflammatory cytokines were observed in vitro and improved outcomes were demonstrated in an acute inflammatory GVHD model. In both Balb/c and in humanized CD34+-engrafted NSG(IL-15Tg) mice, the pharmacokinetic profile of MTX-101 was consistent with antibody-like molecules. We evaluated binding, impact to immune cell phenotypes, pharmacology and early tolerability of MTX-101 in humanized mice and observed detectable binding on immune cells in peripheral blood and terminal tissues, with no resulting activation. Importantly, no induction of proinflammatory cytokines in the serum was observed following a single dose of MTX-101. Evaluation of PK/PD and biomarker readouts in this model will inform clinical dosing and development of MTX-101 to improve treatment outcomes in autoimmune disease patients.

W126. Proteogenomic Immune Signatures Delineate the Landscape of Pediatric Acquired Demyelinating Syndromes

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Approximately 20-30% of children presenting with acquired inflammatory demyelinating syndromes (ADS) have multiple sclerosis (MS). Another 30% harbor serum antibodies against myelin oligodendrocyte glycoprotein and are referred to as having MOG-associated disease (MOGAD). While MS and MOGAD can have similar features, differences in response to immune therapies point to distinct underlying immune mechanisms. We aimed to assess potentially distinct immune mechanisms underlying MS and MOGAD and applied proteogenomics to high quality cryopreserved peripheral blood mononuclear cells collected from patients with ADS prior to institution of immune therapy, as well as from healthy controls. CITE-seq profiling recovered 104,200 single cells total from 24 children (6 healthy donors; 6 with ADS but neither MS or MOGAD; 6 with MOGAD; and 6 with MS, ascertained with long-term follow-up). Comparative analyses revealed features within the T-cell compartment that differed between children with MS and MOGAD. Specifically, the CD4 T-cell compartment in children with MS (when compared to MOGAD) was enriched for a memory population with a Th1-like phenotype and enriched for a Th17-like memory population expressing surface VLA-4 components. CD8 effector T cells in children with MS (when compared to MOGAD) were enriched for the checkpoint-molecule TIGIT. The CD4 T-cell and CD8 T-cell compartments in children with MOGAD (when compared to MS) were enriched for an interferon-response gene-expression signature. Overall, our study points to distinct features of circulating cellular immune profiles in children with MS and MOGAD and provides novel insights into early immune mechanisms that may be involved in each of these conditions.

W127. Single Cell Sequencing Suggests Enteric Origins of Putative Pathogenic T Cells in hla-b27-associated Inflammation

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HLA-B27 is associated with ankylosing spondylitis (AS) and acute anterior uveitis (B27AU), which cause joint and eye inflammation, respectively. As HLA-B27 is an MHC class I molecule, antigen presentation to CD8 T cells may be central to disease pathogenesis. We have recently identified clonal expansion of public T cell receptors (TCRs) that are shared across patients with AS and B27AU and enriched in the joint or eye over the blood. These TCRs recognize both bacterial and self-peptides presented by HLA-B27, suggesting a role for molecular mimicry in HLA-B27-associated inflammation. The main bacterial peptide recognized by these public TCRs is derived from yeiH, a gene present in E. coli, Shigella, Salmonella, and other enteric microbiota or pathogens. It remains unclear, however, whether these AS- and B27AU-associated T cells have had a prior antigenic encounter within the gastrointestinal tract. We therefore performed single-cell RNA sequencing (scRNAseq) and T cell receptor sequencing (scTCRseq) on immune cells from the eye and blood during B27AU (n=5) and non-B27AU (n=14). In this analysis, we found that the expanded T cell clones in B27AU contained higher expression of TNF and IFNG, suggestive of a pro-inflammatory transcriptional program. Moreover, we were able to identify an additional expanded TCR sequence that also recognized YEIH. Finally, expanded TCR clones were enriched for a transcriptional program associated with intestinal CD8 T cells. Collectively, our data suggest that, in HLA-B27-associated inflammation, the early antigen exposure and differentiation of putative pathogenic CD8 T cells may have occurred in enteric organs.

W128. TCR-MAP: A Novel, High-throughput T Cell Antigen Discovery Platform to Identify Viral- and Self-antigen TCR Reactivities in Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is a prevalent autoimmune disorder characterized by chronic inflammatory processes that lead to joint destruction. Immunologically, both CD4+ and CD8+ T cells are highly enriched in the synovium and contribute to the inflammation and pathology of the disease. However, the identities of the antigens that stimulate synovial T cells and how such reactivities relate to the molecular underpinnings of RA pathogenesis have remained elusive. Here, we present T Cell Receptor Mapping of Antigenic Peptides (TCR-MAP) a novel, synthetic antigen discovery platform that uses a TCR-stimulated circuit in immortalized T cells to activate sortase-mediated tagging of engineered antigen presenting cells expressing genetically encoded peptides on MHCs of interest. Tagged antigen presenting cells containing the cognate T cell epitopes can be directly purified for deconvolution by next-generation sequencing, enabling TCRs with unknown specificity to be queried against large, barcoded peptide libraries in a pooled screening context. TCR-MAP accurately captures self- or viral-reactivities with high-throughput and sensitivity for both MHC class I- (CD8+) and class II- (CD4+) restricted T cells. We have used TCR-MAP in combination with single-cell TCR profiling of T cells from patient synovial fluid to identify T cell antigens in RA. This revealed that clonally expanded synovial T cells can be highly specific or exhibit multi-antigen cross-reactivity to self- and/or viral-proteins. TCR-MAP is a powerful tool for mapping unknown TCR-antigen reactivities and further application of this technology has the potential to accelerate T cell antigen discovery efforts in the context of cancer, infectious disease, and autoimmunity.

W129. The Roles of $CD4^+$ and $CD8^+$ Regulatory T cells in Controlling Autoimmune Response in the Human Tonsil Organoid System

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Two regulatory T cells (Tregs) have emerged over the last several decades and proved their importance in maintaining immune response to foreign antigens and self-tolerance. One is the well-known CD4+ Treg, which

depends on the transcription factor, FOXP3. The second has only become clear in the last few years, CD8+ Treg, which is closely associated with the response to infectious diseases. However, the distinct and complementary roles of these two Tregs in regulating autoantibody responses and maintaining self-tolerance are poorly understood in humans. Here we analyzed how these two Tregs controlled cellular and humoral response to common autoantigens in our recently developed human tonsil organoid system using cell depletion and CRISPR/Cas9 genes knock-out. Knocking out FOXP3 in tonsillar T cells disrupted CD4+ Tregs functions and allowed autoantigen-specific B cells to expand and mature into antibody-secreting plasma cells in the organoids. In contrast, after disrupting CD8+ Treg functions by knocking out GZMB, the organoids had only a modest effect on autoantibody production but allowed autoreactive CD4+ and CD8+ T cells to expand. In addition, CD8+ Tregs regulated plasmablasts expansion required CD4+ T cell help. These results not only reveal essential aspects of how these two T cell types operate in different aspects of autoimmune response but also show that we can mimic critical features of autoimmunity in an entirely human, in vitro system.

W130. Unique Cellular and Autoantibody Signatures in Patients with Iraes Revealed by Longitudinal Immune Tracking

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The use of anti-PD-1 (aPD-1) immunotherapy has seen significant success in clinical practice, corresponding with a continued rise in clinical indications for multiple cancer diagnoses. Immune-related adverse events (irAEs) are a type of secondary autoimmune toxicities arising in the setting of cancer immunotherapy. They can cause significant morbidity and disruption of the treatment of oncologic patients. They also offer a controlled setting for dissecting the cellular and signaling networks of autoimmunity development. To better understand irAEs in the setting of aPD-1 immunotherapy, we established a prospective, longitudinal cohort at the University of Pennsylvania enrolling patients before immunotherapy and following them for a year. We stratified the responses in two groups: patients that developed at least one irAE event (irAE+) and patients that never developed an irAE event (irAE-). Using high-dimensional cytometry, we found that irAE+ patients had a larger increase in their activated CD4 T cells after PD-1 inhibition compared to irAE- patients. In addition, plasmablast generation following immunotherapy was higher for irAE+ patients. Using PhIP-Seq, an autoantigen screening assay, we found that irAE+ patients demonstrated robust enrichment in autoantibodies against various tissue antigens after immunotherapy with unique patterns for each patient. Finally, a large-scale proteomic analysis revealed that irAE+ patients at baseline have increased levels of circulating inflammatory mediators, including IL-1 and CCL17. These results indicate that there are distinct cellular and serological imprints of irAE+ patients that reflect their heightened autoimmune reactivity and can be used to uncover the underlying pathogenic mechanism of irAEs and design predictive algorithms.

Basic Science of Immunology - Adaptive Immunity

Th155. A Rheostat Governs Maturation and Regulation of in Utero Human Fetal T Cells

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We have previously published that human fetal T cells were susceptible to in utero microbial priming (Cell). This places in question the nature of fetal Treg dominance, and the scale of T cell maturation permitted during gestational development.To answer this question, we performed CyToF analysis of human fetal circulation, thymic and peripheral tissue (gut, liver, lung, mesenteric lymph nodes) compartments, to examine the profile of fetal T cells in a holistic manner. Comparing the fetal circulation against our age continuum immunome EPIC database (Nature Biotech), we detected a rare yet diverse memory spectrum. This spectrum of fetal T cell functional states originated from thymic imprinting but experienced memory expansion in peripheral fetal tissues. Restriction in TCR$\alpha\boldsymbol{\beta}$ CDR3 diversity in memory fetal tissue T effector cells indicated antigenic selection. Overall, this body of evidence supports the notion that the gestational environment is conducive for T cell maturation. Tissue fetal T effector maturation was functionally regulated by an early thymic-peripheral tissue wave of PD1+Ki67+Treg with strong suppressive capacity. Notably, memory bias in Th1 effectors across fetal tissues was correspondingly balanced by a specific regulatory arm of Tbet+Treg, with tissue infiltrations exceeding adult levels. Despite strong regulatory oversight, partial demethylation of the FoxP3 promoter locus in fetal Tregs points to limited lineage stability, suggesting a mechanism for residual programming for T effectors. Overall, beyond thymic imprinting, fetal T effectors in utero experiences antigenic memory expansion, that is precisely controlled by an immune regulatory rheostat.

Th155. Molecular Underpinnings and Significance of Regulatory T-cell Plasticity in Cancer

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Regulatory T-cells (Treg cells) are endowed with functional plasticity which facilitates their response to changes in environmental stimuli and tissue homeostasis. This plasticity becomes a liability in chronic inflammatory diseases such as in cancer. We found that Tregs isolated from colorectal cancer patients have enhanced T-cell suppressive properties but are compromised in suppressing inflammation. These changes correlated with elevated levels of □□-catenin. We used genetic mouse models to show that stabilization of b-catenin or loss of its DNA binding partner TCF-1 causes the activation of Tregs but compromises their ability to suppress inflammation. TCF-1 suppressed the transcription of genes that were co-bound by Foxp3. Single-cell RNA-sequencing analysis identified clusters of Treg cells with differential expression of memory and activation markers. TCF-1 deficiency did not change the core Treg cell transcriptional signature but promoted alternative signaling pathways whereby Treg cells became activated and gained gut-homing, T-cell suppressive, and TH17 characteristics. TCF-1-deficient Treg cells strongly suppressed T cell proliferation and cytotoxicity but were compromised in controlling CD4+ T cell polarization and inflammation. In mice with polyposis, Treg cell–specific TCF-1 deficiency enhanced tumor growth. Consistently, tumor-infiltrating Treg cells of patients with colorectal cancer showed lower TCF-1 expression and increased TH17 expression signatures

compared to adjacent normal tissue and circulating T cells. Thus, b-catenin/TCF-1 regulate Treg plasticity in colorectal cancer to promote pathogenic TH17 inflammation and suppress tumor-directed T cell cytotoxicity. These changes can determine colorectal cancer outcomes. This work was supported by National Institutes of Health (NIH) grants R01 AI 108682 and R01CA160436.

Th156. Neonatal Treg-ppar Axis Defines Melanocyte Stem Cell Function

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Early life is a critical period for the development of solid organs and the immune system. Regulatory T cells (Tregs) are present in the skin during the first week of life, but their function in the neonatal skin is unknown. A peak expression of Treg activation markers was observed on postnatal day 9 (P9). Depletion of early Tregs (eTregs) on P6 and P8 caused a 20% reduction in skin pigmentation on P28. By contrast, pigmentation developed normally after depletion of late Tregs (lTregs) on P10 and P12. Pigment-producing melanocyte marker genes, such as Dct, and PPARγ target genes were downregulated only after depletion of eTregs, and not lTregs. A PARγ antagonist, T0070907, in neonatal mice similarly leads to defective skin pigmentation (p=0.0017). Additionally, PPARγ agonist, GW1929, restores skin pigmentation to homeostatic level in Treg-depleted mice. scRNA-seq analysis revealed higher expression of PPARγ target genes in human neonatal melanocyte in comparison to adult melanocytes. Similarly, PPARγ target genes were elevated in healthy adult melanocytes in comparison to melanocytes in lesional skin of human vitiligo patients. We report a novel finding that P6-P8 represents a window of Treg-PPARγ axis that defines the melanogenic function of melanocytes. Disruption of this axis results in abrogation of homeostatic pigmentation in the skin. This signalling pathway is differentially regulated in human neonatal and adult melanocytes and is disrupted in vitiligo. Our finding suggests that defective Treg function during neonatal stage leads to disruption of the PPARγ pathway, with implications in vitiligo.

Th157. Neurocognitive Functions of Patients with Digeorge Syndrome

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Background: DiGeorge syndrome, the most common microdeletion syndrome, affects multiple organs, including the heart, the nervous system, and the immune system. In this study, we aimed to evaluate the clinical, laboratory, radiological, and neuropsychological findings of our patients with DGS. Methods: Clinical and laboratory data of fifty-two patients with DGS between June 2000- March 2022 were evaluated retrospectively. Brain MRI and neuropsychological tests were performed to assess the neurocognitive status of the patients Results: Fifty-two patients (28 maleand 24 female) were included in our study. Fifteen of them died during follow-up. All 37 patients under clinical follow-up had partial DGS. The median age of patients was ten years and seven months, and the median age at diagnosis was five years and four months. Bilateral conduction deceleration in the anterior visual pathways in 6 (20%) of 30 patients was determined by the VEP (Visual Evoked Potentials). The auditory brainstem evoked potential test (BAEP) showed sensorineural hearing loss in 11 out of 30 (36.6%) patients. Cranial MRI disclosed developmental brain abnormalities in 18 out of 25 (72%) patients. Impairments in executive functions, expressive language, and verbal memory were noted in 18 patients who were neuropsychologically assessed. Conclusion: It is important to keep in mind that patients with DGS may be presented with neuropsychiatric findings as the initial symptom. Therefore, a basic knowledge of this syndrome is crucial for pediatric and adult neurologists and psychiatrists who follow up with patients with epilepsy, movement disorder, or mental disorders.

Th158. Probing Intestinal Immunity Using Cryptosporidium Infection

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Many infectious and inflammatory diseases affect the gut and associated lymphoid tissue (GALT), which houses the largest number of lymphocytes in the body. It is therefore important to understand the regulation of tissue immunity to develop better therapies to manage disease. Cryptosporidium is a unicellular parasite that is restricted to intestinal epithelial cells (IECs) that can be used to understand immune regulation in the intestine. While infection and shedding of infectious oocysts is self-limiting in immunocompetent individuals, life-threatening chronic diarrhea and liver disease can occur in acquired and primary immunodeficiencies (AIDS, CD40L/CD40 deficiency, IL-21R deficiency). Because Cryptosporidium is restricted to the gut, it is a challenge to understand local T cell responses, though it has been previously established that sterilizing immunity requires CD4+ T cells and interferon gamma (IFN-γ). In order to characterize antigen-specific responses, transgenic C. parvum was engineered to express MHCII-restricted model antigens. Antigen-specific TCR-transgenic CD4+ T cells expanded in the gut and GALT in infected mice. These cells made IFN-γ and relied on type 1 conventional dendritic cells (cDC1s) for expansion and function. A prominent CD4+ T cell-dependent but IFN-γ-independent mechanism of control exists, as humans and mice deficient in IFN-γ are not susceptible to chronic infection. Our system revealed T cells produce IL-22 that limits infection, and therapeutic targeting using engineered cytokines suppressed infection. By using Cryptosporidium fecal oocyst shedding as a readout of gut immune function, this system can dissect immunity in the gut and probe therapies aiming to augment immunity within the tissue.

Th159. Quantifying How TCR Sequence Variation Affects T cell Fate at Single-cell Resolution

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Mucosal-Associated Invariant T (MAIT) and Natural Killer T (NKT) cells demonstrate that some T cell transcriptional fates are driven by the T cell antigen receptor (TCR). Though activation through the TCR is integral to T cell differentiation, the contribution of TCR sequence features to other T cell transcriptional fates remains yet to be defined. In this study, we identify how αβ V(D)J recombination affects T cell differentiation at single-cell resolution. By applying Canonical Correlation Analysis (CCA) to 340,557 TCR clones collected from 256 individuals, we define a set of TCR scoring functions that quantify transcriptional fate predispositions conferred by the TCR. Unsurprisingly, the strongest fate predispositions correspond to cognate peptide presentation molecules: MR1- or CD1d- restricted PLZFhigh innate-like (MAIT/NKT) transcriptional fate (72.6-fold increase in fate likelihood for top percentile compared to bottom percentile TCRs, AUC = 0.85) and MHC class I-restricted CD8 fate versus MHC class II-restricted CD4 fate (18.7-fold increase, AUC = 0.75). However, our results also uncover that hydrophobic CDR3 residues promote regulatory T cell transcriptional states in both the CD8 and CD4 lineages, and that TCR sequence features preferred by thymic positive selection continue to promote memory formation in the periphery. Applying our TCR scoring functions to 10x dextramer-labeled T cells reveals that not all antigen-specific TCRs are equally capable of mounting an effector response. Even among T cells that recognize the same antigen, biophysical variation in the TCR sequence modulates which T cells will undergo transcriptional reprogramming to form immunological memory.

Th160. Regulation of Fetal Tolerance by KIR^{+}CD8^{+} T cells During Human Pregnancy

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Immune responses during pregnancy need to be precisely regulated to protect the fetus from microbial infections and to maintain tolerance for the semi-allogeneic fetus. However, the mechanisms regulating fetal tolerance during pregnancy are not well understood. Recently, we have identified KIR+CD8+ T cells as a previously unappreciated subset with regulatory functions in humans to suppress harmful self-reactivity. Therefore, we asked whether KIR+CD8+ T cells also function to induce immune tolerance during pregnancy. We first observed an increased frequency of KIR+CD8+ T cells in the peripheral blood of pregnant women at second trimester compared to age-matched nonpregnant females. Moreover, those with a male fetus had an even higher level of KIR+CD8+ T cells than the ones with a female fetus. In vitro, we found that KIR+CD8+ T cells can suppress HY-specific CD8+ T cells only in mothers with a male fetus. Therefore, the higher induction of KIR+CD8+ T cells in mothers carrying a male fetus may suppress the additional allogenic immune responses triggered by the Y chromosome from the male fetus. As revealed by the longitudinal analysis, KIR+CD8+ T cells expand, lose memory features and become cytotoxic along gestation and their levels are maintained postnatally. Last, we found increase of KIR+CD8+ T cells correlated with pregnancy disorders (e.g., spontaneous abortion and pre-eclampsia). Taken together, our findings suggest an important role of KIR+CD8+ T cells in the maintenance of fetal tolerance by suppressing alloreactive fetus-specific T cells, therefore they may be useful as predictive biomarkers or drug targets for human pregnancy disorders.

Th161. Sex Differences in the Percentage of IRF5 Positive B Cells Are Associated with Higher Production of TNF-α in Women in Response to TLR9 in Humans

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Background: The clinical course and outcome of many diseases differ between women and men, with women experiencing a higher prevalence and more severe pathogenesis of autoimmune diseases. The precise mechanisms underlying these sex differences still remain to be fully understood. IRF5 is a master transcription factor that regulates TLR/MyD88-mediated responses to pathogen-associated molecular patterns (PAMPS) in DCs and B cells. B cells are central effector cells involved in autoimmune diseases via the production of antibodies and pro-inflammatory cytokines as well as mediating T cell help. Dysregulation of IRF5 expression has been reported in autoimmune diseases, including systemic lupus erythematosus, primary Sjögren syndrome, and rheumatoid arthritis. Methods: In the current study, we analyzed whether the percentage of IRF5 positive B cells differs between women and men and assessed the resulting consequences for the production of inflammatory cytokines after TLR7- or TLR9 stimulation. Results: The percentage of IRF5 positive B cells was significantly higher in B cells of women compared to men in both unstimulated and TLR7- or TLR9-stimulated B cells. B cells of women produced higher levels of TNF-α in response to TLR9 stimulation, and TNF-α production was positively correlated with percentages of IRF5 positive B cells prior to TLR stimulation. Conclusions: Taken together, our data contribute to the understanding of sex differences in immune responses and may identify IRF5 as a potential therapeutic target to reduce harmful B cell-mediated immune responses in women.

Th162. Single-cell Transcriptomic Analysis Further Delineates Human CD8$^+$ T Regulatory Cell Subsets

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Introduction: Although described in the 70's, CD8+ regulatory T cells (Tregs) remains incompletely understood and to date, although several markers are used to define them, they remain inconsistent. The identification of good markers, as it was done for CD4+ Tregs; remains an urgent task and a challenge to advance our understanding. Methods: Herein, we addressed the heterogeneity within total CD8+ T cells using single cell CITEseq and VDJ T cell receptor

sequencing. We included markers used to previously identify Tregs, in particular CD45RC described by our team and others to divide effector (CD45RChigh) and pro-regulatory (CD45RClow/-) CD8+ T cells. 7K non-stimulated CD8+ T lymphocytes, including CD8+CD45RClow/- Tregs and CD8+CD45RChigh T cells of 4 healthy volunteers were analyzed. Results: These analyses enabled the characterization of the transcriptomic heterogeneity at a single cell level from steady state total CD8+ T cells and allowed definition of regulatory CD8+CD45RClow/- Treg subsets. Functional analysis using cell sorting and suppressive assays highlighted the suppressive potential of the CD8+CD45RClow/-TNFR2+CD29low Tregs subset. Conclusion: To date, to our knowledge, this is the largest characterization study of human CD8+ Tregs, this huge data resource will help in the current revival of CD8+ Tregs in research, will improve our understanding of T cell heterogeneity and will help translate CD8+ Tregs to the clinic.

Th163. Successful Measurement of 13 Functional Readouts Including IL-5, IL-10, and IL-13 from Human Peripheral Blood Mononuclear Cells via Use a Cytof Intracellular Cytokine Staining Panel

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Measurement of functional signatures of immune cells in a comprehensive, cross-lineage manner would provide key insights into several facets of immunology. Intracellular cytokine staining (ICS) is ideal for this type of analysis, and with the advent of full spectrum flow cytometry and improvements in cell interrogation efficiency with CyTOF, we posited that one or both platforms are now able to measure a large number of functions from individual cells with new resolution. We evaluated stimulation-induced cytokine signal from peripheral blood mononuclear cells in large (31-color) ICS Aurora and CyTOF XT panels that include analytes known to be challenging for cytometric analyses, such as IL-5, IL-10, IL-13, IL-17A, IL-21, IL-22 and GM-CSF. Data collected from the both instruments showed well-resolved signal for many cytokines, including IL-4, IFN-γ, TNF-α, IL-17A, and Granzyme B. However, IL-5, IL-10, IL-13, and GM-CSF positive populations were clearly identified by the CyTOF XT in a stimulation-specific manner but could not be resolved by spectral flow cytometry (in these and/or previous experiments). The CyTOF results can serve as a gold standard to further functional readout capacity using spectral flow ICS panels; however, it is possible that inherent features of fluorescent cytometry may be prohibitive for achievement of equivalent results. Also, with the CyTOF's capability for further panel expansion (50+ parameters), additional functional readouts could be added to this panel. In sum, our data indicate that the CyTOF XT platform could serve as a catalyst for seminal discoveries germane to the cross-lineage functional diversity of many immune cell subsets.

Th164. Suppression of Type 2 Inflammatory Responses by Half-life Extended IL-27 in Ovalbumin Induced Delayed Type Hypersensitivity

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IL-27 (EBI3/p28) is a heterodimeric cytokine with well described regulatory effects on inflammatory T cell responses. These effects include direct suppression of transcriptional regulators such as GATA3 resulting in limited Th2 responses, the induction of coinhibitory receptors and the promotion of Treg specialization and function. To evaluate the regulatory role of IL-27 in vivo, we generated murine half-life extended (HLE)-IL-27. By sub cutaneous (sc) dosing the half-life of the reagent was determined to be approximately 40 hours and resulted in a dose dependent increase in the expression of PDL-1 on CD4 T cells. We evaluated the ability of this reagent to regulate Th2 inflammatory responses in an ovalbumin (OVA)-induced delayed hypersensitivity (DTH) model in BALB/c mice. HLE-IL-27 dosed sc Q2D significantly inhibited OVA induced ear swelling. In serum OVA DTH resulted in increased IL-13 and IgE that was robustly suppressed by HLE-IL-27 while simultaneously increasing IL-10. Profiling of Treg and Teff from draining

auricular lymph node demonstrated that HLE-IL-27 increased the ratio of Treg/Teff. Phenotypic modulation of Treg by in the increased expression of PDL-1, CTLA4 and CD39 could also be observed. HLE-IL-27 also decreased the number of GATA3+ CD44+ Teff. Collectively these data demonstrate the multiple suppressive activities resulting from agonism of IL-27R.

Th165. TCR Gene Rearrangements and a Novel Fate Mapping Mouse as Tools for Studying the Origins Fate of Innate Lymphoid Cells

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Innate lymphoid cells (ILCs) are a recently identified lineage of lymphocytes that play a central role in pathogen surveillance, modulation of immune response, and tissue repair. ILC1s, ILC2s and ILC3s show remarkable similarity to Th1, Th2 and Th17 T cells, respectively, in terms of their cytokine production, function and transcription factor dependence but have been regarded as thymic-independent due to their lack of expression of cell surface T cell receptor, their abundance in RAG-deficient mice and the identification of ILC precursor cells in the bone marrow. Recently, we made the striking observation that highly purified lung ILC2s, ILC3s and ILC precursors from normal adult mice exhibit frequent V-to-J gene segment rearrangements of TCR gamma chain loci and/or deletions of the TCR delta constant region locus consistent with failed T cell development within the thymus. These data challenge the bone marrow centric view of ILC development and instead argue for a thymic origin of some fraction of the tissue resident ILCs. Whether these thymic-derived ILCs represent an early developmental wave or a continued production of ILCs from the adult thymus remains an open question. To investigate this further, we have now generated a novel mouse strain (IL17Rb-CreERT2-IRES-GFP) that permits the highly-selective, vital, monitoring of all tissue ILC2s, and the pulse labeling of these cells for tracing their fate during development. The combine use of this novel, highly-selective, pulse-labeling strategy together with TCR gene rearrangements as a natural barcode now allows us to directly address ILC origins, fate and plasticity in tissues.

Th166. The Development and Function of Cytotoxic CD4$^+$ T cells in Human Disease

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There has been growing interest in the role of cytotoxic CD4+ T cells (CD4-CTLs) in human health and disease over the past decade. While commonly observed in patients with cancer and autoimmune conditions such as IgG4-related disease (IgG4-RD), they are also expanded in healthy supercentenarians and have been recently characterised in acute conditions including severe acute respiratory syndrome coronavirus 2 2019 (SARS-CoV-2) infection. Importantly, in patients with severe SARS-CoV-2 infection they are associated with bias away from protective antibody production. While a significant body of work has been done to understand these cells, there are still many questions about their developmental pathways, function and significance in a number of human diseases. To better understand these cells and how they influence adaptive immunity, we performed RNA sequencing, whole genome bisulfite sequencing (WGBS) and assay for transposase-accessible chromatin using sequencing (ATAC-seq) on FACs sorted CD4-CTLs, CD8-CTLs, Th1 CD4 cells, naive CD4 and CD8 T cells from the peripheral blood of patients with IgG4-RD and idiopathic pulmonary fibrosis. We have identified key epigenetic and transcriptional signatures associated with CD4-CTLs that help elucidate their development and provide insights into their roles in the adaptive immune system. Functional studies in mouse models are currently being performed to confirm molecular mechanisms. By understanding the key developmental pathways and function of CD4-CTLs in human disease, we may be able to manipulate their impact on adaptive immune responses to improve vaccine development and antitumor therapeutics, as well as limit their role in autoimmune and inflammatory conditions.

Tu155. A 47-marker Immune Profiling Flow Cytometry Assay to Enable Comprehensive Antigen-specific Immune Analysis

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The complex nature of the immune system requires deep interrogation at the single-cell level. Flow cytometry using CyTOF® technology employs antibodies tagged with isotopically enriched metals instead of fluorophores, resulting in discrete signals that facilitate highly multiparametric characterization on a single-cell level. CyTOF technology enables analysis of over 50 parameters simultaneously without the impediments of spectral overlap or autofluorescence, allowing for a reliable evaluation of immune responses. The Maxpar® Direct™ Immune Profiling Assay™ is a pre-titrated, dried-down, and validated 30-marker antibody cocktail for single-tube staining of human whole blood or PBMC and acquisition on CyTOF systems. Using the assay and Maxpar Pathsetter™ software, stained samples are automatically resolved into 37 immune populations. In this study, we highlight the customizability of the Maxpar® Direct panel by adding an array of 17 surface and intracellular parameters with the Maxpar Direct™ T Cell Activation Expansion Panel and live-cell barcoding. The simultaneous detection of key surface and intracellular markers in this expanded panel enabled comprehensive analysis of immune cell activation and antigen-specific recall responses to PBMC stimulated with a pool of immunodominant microbial peptides. Taken together, this 47-marker panel and automated analysis unlocked deeper immunophenotyping and functional profiling of activated immune cells and provided insight into the cell-mediated adaptive immune response to foreign targets. Such phenomena are hallmarks for research on infection, vaccine development, and immunotherapy. For Research Use Only. Not for use in diagnostic procedures.

Tu156. A High-throughput Solution for Building Tcr-antigen Maps at Single Cell Resolution

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Understanding T-cell receptor (TCR) antigen recognition is essential to understanding the adaptive immune response to disease. However, building a TCR-antigen map is a challenge due to the formidable diversity of TCRs and antigens. To address this we developed Barcode Enabled Antigen Mapping (BEAM), a technology fully integrated with the 10x Genomics Immune Profiling Solution enabling simultaneous profiling of peptide-MHC or antigen binding to CD8+ T or B-cells, along with paired receptor sequences and gene expression. Thereby presenting a comprehensive picture of the immune-receptor, its target, and phenotypic cell state. We demonstrate assay performance with a series of spike-in experiments with SARS CoV2 peptide-stimulated T-cells. The background was a complex mixture of 40% CMV-reactive T-cells, 10% Flu-reactive T-cells and HLA-matched human PBMCs. Cells were labeled with a panel of five viral pathogen peptide-loaded multimers along with a scrambled control peptide. The use of a negative control allows us to evaluate the specificity of binding and report antigen specificity scores for each cell. In each experiment we profiled ~10,000 antigen-enriched T-cells and were able to identify a rare cluster of SARS CoV2 reactive CD8+ T-cell clonotypes at 1% spike-in. While the experimental design screened a mixture of 5 peptides, we observed highly-specific binding of each peptide to its target receptors. We further tested the robustness of our protocol on a variety of samples, including mice splenocytes and dissociated tumor cells. We envisage single cell, high-throughput, and multimodal experiments such as these will underpin the rapidly evolving therapeutic and diagnostic landscape of tomorrow.

Tu157. Age Influences Immunotype Diversity in Healthy Adults

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Immune perturbations induce diverse responses in healthy adults that are not entirely predicted by demographics. We and others have shown that the frequencies of many circulating immune cells 1) can be predictive of response to vaccination or disease 2), are highly variable between healthy adults but stable over time within individuals, and 3) return to baseline after an immune challenge. Our goal was to interrogate variations in immune baselines among healthy adults across different age groups to understand the functional impacts of natural variation of the healthy human immune system. To do this, we modeled the breadth of immune phenotypes (immunotypes) in a cohort of 100 healthy adults, half aged 25-35 and half aged 55-65 years. We quantified circulating immune cell frequencies of 54 cell types measured using mass cytometry at 10 visits over two years. We defined immunotypes based on levels of relatively stable immune cell populations at unperturbed visits. Our study design also incorporated immune perturbations including response to influenza immunization. We quantified similarity of participants' immunotypes using Euclidean distances between participants in multidimensional immunospace at unperturbed visits. Immunotypes were more similar within our younger cohort and more diverse in our older cohort and this was reflected functionally in the response to polyIC stimulation (TruCulture) and flu vaccination. These findings indicate that immune experience may drive increased diversity of immunotypes with age. We are currently expanding this work to investigate the relationship between immunotype and response to environmental factors such as pollen and wildfire.

Tu158. Antigen-driven Epigenetic Rewiring Drives Rapid Exhaustion of Memory T Cells in Progressing Tumors

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When naive antigen-specific CD8 T cells encounter antigens in an acute inflammatory context (e.g. acute infection), T cells undergo clonal expansion and differentiate into cytolytic effector T cells. After pathogen or antigen clearance, most effector T cells die, but a small fraction survive and form long-lasting memory T cells. There are three functional hallmarks that distinguish memory T cells from their naïve counterparts: they are long-lived, able to self-renew, and have a lower activation threshold. We and others have shown that the memory T cell state is encoded epigenetically, through gene poising and transcriptional memory. Due to this epigenetically-imprinted superior functionality it is thought that memory T cells would retain their qualitative and quantitative superiority also in settings of persistent antigen encounter, such as in tumors. In fact, memory T cells are currently being selected as precursor T cells for the generation of gene-modified TCR- or CAR-transduced T cells for adoptive T cell therapy for the treatment of cancers. Here, we tested whether memory T cells could delay or resist differentiation to exhaustion and dysfunction during tumorigenesis. Surprisingly, we found that memory T cells became exhausted and lost effector function more rapidly and profoundly compared to their naïve counterparts, revealing unexpected limitations of memory T cells in settings of persistent tumor antigen. Here, we present novel insights in the epigenetic regulatory mechanisms, including genome-wide DNA-methylation modules, which drive rapid exhaustion of memory T cells in tumors with important implications for the development of cancer immunotherapeutic strategies.

Tu159. Characterizing Quantitative Properties of T-cell Repertoires Across Disease Categories

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High-throughput T-cell receptor sequencing is a rapidly growing technology that provides new data-driven insights into the adaptive immune system and its role in various diseases. While much of this work focuses on identifying antigen-specific clones, there is also interest in more general metrics that can be used to summarize differences between T-cell repertoires. For example, various measures of repertoire diversity have been shown to correlate with clinical outcomes and therapeutic responses in a variety of settings. However, there remains little consensus as to which quantitative metrics are the most robust and salient for use in various investigational settings. In this study, we

used the immunoSEQ® Assay (Adaptive Biotechnologies, Seattle, WA) to sequence and compare TCRB repertoires of healthy individuals with a variety of disease states including various solid and hematological malignancies, autoimmune, or neurodegenerative conditions. Repertoire properties were characterized using multiple antigen-agnostic metrics, including repertoire diversity metrics such as Simpson's clonality, clone distribution slope, and convergence. Each metric was evaluated for its robustness to sampling depth (based on input DNA and the number of sequences detected) and its correlation with the other metrics. We also characterized the variation of these metrics both within and between indications, disease states and tissues. This study establishes 1) the strengths and limitations of the currently available quantitative metrics used to summarize various attributes of immune repertoires; 2) the relationships between these metrics and the overlapping or complementary repertoire properties they quantify; and 3) the observed ranges of these metrics within specific disease settings.

Tu160. Clinical Outcome of Dominant Negative Mutation in Caspase Activation and Recruitment Domain Family Member 11 (CARD11)

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Caspase activation and recruitment domain family member 11 (CARD11) is a scaffold protein belonging to a family of membrane-associated guanylate kinases required for B-cell receptor and T-cell receptor signalling and subsequent activation of the nuclear factor κβ (NF-κβ) pathway. Aberrations in CARD11 have been shown to impact lymphocyte differentiation, proliferation, and survival, resulting in combined immunodeficiency. We previously reported a multi-generation kindred with novel heterozygous c.C887T variant in CARD11, resulting in amino acid change R30W. The variant lead to loss-of-function of CARD11 while exerting a dominant negative effect. Functional studies identified defects in canonical NF-κβ pathway signalling resulting in dampened IFN- and IL-2 secretion. In addition to combined immunodeficiency, these individuals presented with marked multi-organ atopy and autoimmunity. Objective: Describe the clinical progress of a kindred with CARD11 R30W 5 years after established diagnosis. Methods: A comprehensive retrospective chart review was performed. Results: During the follow-up period, 2 of the individuals (proband and daughter) exhibited worsening autoimmunity and atopy. The other 3 cases had well controlled asthma, eczema and food allergy. From an infectious standpoint, all 5 family members remained relatively well and did not develop any severe or recurrent infections. Three experienced mild COVID-19 and recovered without complication. In contrast, the proband developed colitis flare post-COVID-19 while her daughter developed severe atopic dermatitis not responding to conventional therapy. Conclusion: CARD11 R30W results in susceptibility to infection but with more prominent multi-organ atopy and autoimmunity symptoms. Longitudinal follow-up revealed worsening autoimmunity and atopy in 2 of the 5 affected individuals.

Tu161. Delay in Peripheral Blood Processing Depletes Monocytes While B and T Cell Populations Are Maintained

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Peripheral blood mononuclear cells (PBMC) are an important tool for human immunology. Isolation of PBMC within 24 hours of blood collection is commonly recommended to maximize cell viability and minimize phenotypic changes. However, when shipping blood from clinical sites to a processing facility, longer delays can occur. We systematically measured the impact of delays of up to seven days on cell isolation, recovery, and immunophenotype. The study included five healthy volunteers (HV) and two rheumatoid arthritis (RA) patients. Surprisingly, delays to day 7 did not impact recovery and viability (measured as proportion with intact cell membrane) of PBMC isolated from heparinized blood ($p>0.05$). However, by two days after collection, red blood cell (RBC) contamination was visible in the cell layer. Using CyTOF we identified global (B cell, T cell, NK cell, monocyte, dendritic cell) immune cell populations and rare and unstable subsets (Tfh, Treg, plasmablasts, etc). The frequency of monocytes decreased by day seven

(p=7x10^-5), with a corresponding increase of CD8 T cells (p=0.0005). CD4 T cells and B cells (p>0.05) appeared to be more stable. Rare T cell subsets were consistently detected from day 0-7. Detection of cDC and pDC subsets varied by day two, and detection of plasmablasts varied by day four in the RA subjects. The variation of the global subsets complicated analysis of variation in the rare subsets. This study demonstrates that delays in blood processing increase RBC contamination of PBMC, decrease monocyte recovery, and have limited impact on the recovery of B and T cells.

Tu162. Functional Overlap of Inborn Errors of Immunity and Metabolism Genes Define T Cell Immunometabolic Vulnerabilities

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Inborn Errors of Metabolism (IEM) and Immunity (IEI) are Mendelian diseases in which complex phenotypes and patient rarity can limit complete understanding of the full clinical impact of the molecular defect. The genetic overlap of recognized IEM and IEI is currently narrow, but immunometabolic demands of the immune system suggest functional overlap is underestimated. We performed CRISPR screens in mouse CD4+ T cells to test IEM genes for immunologic roles and IEI genes for metabolic effects and found considerable crossover. Analysis of IEM genes showed that N-linked glycosylation and the de novo hexosamine synthesis enzyme, Gfpt1, are critical for T cell expansion and function. Interestingly, TH1 cells were more reliant on Gfpt1 than TH17 cells, which expressed higher levels of Nagk for salvage UDP-GlcNAc synthesis. Conversely, screening IEI genes identified the transcription factor Bcl11b as a metabolic regulator. Bcl11b promotes CD4+ T cell mitochondrial activity and Mcl1 expression necessary to prevent metabolic stress. Notably, mitochondrial deficiencies were also observed in developing T cells in the thymus of Bcl11b conditional knockout mice, corresponding to impaired T cell development. These data illustrate a high degree of functional overlap of IEM and IEI genes and point to potential immunometabolic mechanisms for a previously unappreciated set of these disorders.

Tu163. Germline-encoded Amino Acid-binding Motifs Drive Immunodominant Public Antibody Responses

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Despite the vast diversity of the antibody repertoire, infected individuals often mount antibody responses to precisely the same epitopes within antigens. The immunological mechanisms underpinning this phenomenon remain unknown. Here, by mapping 376 immunodominant “public epitopes” at high resolution and characterizing several of their cognate antibodies, we conclude that germline-encoded sequences in antibodies drive recurrent recognition. Systematic analysis of antibody–antigen structures uncovered 18 human and 21 partially overlapping mouse germline-encoded amino acid-binding (GRAB) motifs within heavy and light V gene segments that, in case studies, are critical for public epitope recognition. GRAB motifs represent a fundamental component of the immune system’s architecture that ensures antibody recognition of pathogens and promotes species-specific reproducible responses that can exert selective pressure on pathogens.

Tu164. Group Similarity Analysis (GSA) Is a Visualization Method to Evaluate Batch Effects and Sample Stratification in High-dimensional Mass Cytometry Data

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High-dimensional mass cytometry or CyTOF (cytomtery by time-of-flight) measures over 50 proteins in single cells. We apply mass cytometry to profile immunome remodeling during healthy human development and disease. As data acquisition usually spreads over many experimental batches, it is essential to separate the contributions of biological and technical factors to measured immune variations. We created a two-step method, dubbed group similarity analysis (GSA), to visualize immune profiles obtained from unsupervised clustering. First, the multi-dimensional vectors representing the proportions of detected cell populations are projected into a two-dimensional embedding, such as UMAP, tSNE or PCA. Colorizing data points according to batch or biological properties reveals similarities between samples. Second, silhouette analysis of the embedding coordinates quantifies the similarity of samples to their respective groups. We illustrate how GSA helped us evaluate the benefits of batch normalization in two projects. In the EPIC reference immune atlas (Nature Biotechnology, 2020) that portrays the development of the healthy immune system from birth to old age, GSA demonstrated a significant improvement in sample stratification between different age groups. In an immune-oncology project, GSA showed the improved separation of hepatocellular carcinoma, adjacent non-tumor liver and peripheral blood. Other applications include the benchmarking of batch normalization and clustering methods and their parameters. For instance, we can show that increasing cluster numbers can aggravate batch effects. Furthermore, GSA can assist in the optimization of meta-clustering. In summary, GSA is a versatile tool in high-dimensional pattern discovery.

Tu165. Heterogeneous Regulation of IL-10 Expression in B-cell Subsets

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Regulatory B-cells (Bregs) are a heterogeneous cell subset of growing interest with the capacity to supress inflammatory responses. The most studied class of Bregs are those defined by the expression of interleukin-10 (IL-10), however B-cell subsets which express IL-10 and their mechanism of IL-10 induction remains unclear. Toll-like receptor activation is known to induce IL-10 expression in both mouse and human B-cells, with CpG – a TLR9 agonist – commonly used to induce Bregs in vitro. In this study, we use CpG to induce and study IL-10 regulation across mouse B-cell subsets, identify signalling pathways crucial for IL-10 expression, and explore transcriptomic relationships between IL-10+ B-cells derived from multiple B-cell subsets. Using primary cells from IL-10-reporter mice, we have found that mouse B-cell subsets differ in their capacity to express IL-10 in response to CpG stimulation. Splenic marginal zone (40% IL-10+) and splenic B1 cells (50% IL-10+), and peritoneal B1 cells (70% IL-10+) exhibit the greatest IL-10 competence compared to follicular B (10% IL-10+) and peritoneal B2 (25% IL-10+) subsets after 48 hours. Peritoneal B-cells were capable of upregulating IL-10 expression more rapidly than splenic B-cells, and IL-10 induction in splenic B-cells was p38 dependent. Breg gene expression significantly differed based on the originating subset. Our results show that cell-intrinsic heterogeneity contributes to Breg heterogeneity in behaviour and phenotype, significantly influencing IL-10-competence. This work highlights the necessity of deconvoluting heterogeneous cell responses to better understand mechanisms of gene regulation due to the significant differences between lymphoid and circulating B-cell populations.

W131. Activating the Cgas/sting Pathway Suppresses Regulatory T cell Function and Induces Antiviral Interferon Secretion in a Temperature-sensitive Manner

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There is an urgent need for improved vaccine adjuvants, as current FDA-approved adjuvants are generally ineffective at inducing T cell-mediated immunity necessary for many anti-viral or anti-tumor responses. Nucleic acid agonists have emerged as potential adjuvants that stimulate T cell responses by activating nucleic acid sensors, including the Stimulator of Interferon Genes (STING), to induce antiviral Type I Interferon (IFN-I) production by innate immune

cells. Importantly, STING is also highly expressed in T cells and can be activated by infection and cell-stress (e.g. hyperthermia) induced self-DNA leakage. However, the role of STING in regulatory T cells (Tregs) is not well-understood. Furthermore, although heat is a hallmark of inflammation, the effects of fever on Tregs remain unknown. Here, we report that STING induces a pro-inflammatory phenotype in murine Tregs, which normally exert anti-inflammatory, immunosuppressive functions. At physiological and fever-range temperatures (FRT, 39.5°C), STING activation significantly decreased Treg viability and expression of Treg markers CD25 and FoxP3 and immunosuppressive proteins TGF-β and CTLA-4. Surprisingly, STING induced high levels of IFN-I, suggesting that STING can promote antiviral responses by Tregs. Additionally, FRT decreased Treg suppressive function and significantly enhanced the effects of STING. In summary, these data provide the first evidence that STING agonists can induce IFN-I production by Tregs, suppress their anti-inflammatory phenotype, and that these effects are potentiated by fever. Thus, this study motivates further investigation of Treg function at FRT and suggests the potential for Treg-targeted STING agonists as a method to improve T cell responses to vaccines and immunotherapies.

W132. Human Regulatory B Cells Prevent Effector $CD4^{+}CD25^{-}$ T cell Proliferation Through a Mechanism Dependent from Granzyme B and Lymphotoxin Alpha

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Human Granzyme B (GZMB) regulatory B cells (Bregs) have suppressive properties on CD4+ effector T cells by a mechanism partially dependent on GZMB. Moreover, these cells may be easily induced in vitro making them interesting for cell therapy. Our aim was to study their profile, role and interactions in regard with their mechanisms of suppression in scRNAseq performed on induced Bregs, non-Bregs and T cells after coculture. Predicted receptor/ligand interactions on Bregs suppressive properties were then assessed by functional analysis. Bregs exhibit a profile of 149 highly differentially expressed genes, mainly associated with cell proliferation, apoptosis, metabolism, and altered antigen presentation capacity consistent with their differentiated B cells profile. When cocultured with Bregs, T cells display strong inhibition of genes of proliferation, activation, inflammation and apoptosis. T cells/Bregs interaction analysis evidenced Lymphotoxin alpha (LTA) as a key Breg ligand. LTA and GZMB are regulated by common transcription factors CREM directly modulated by LTA and BATF downstream IL-21. Finally, we demonstrated that LTA is involved in GZMB+Bregs suppressive properties with Pateclizumab, an LTA inhibitor, preventing their function. These data are the first to report on a role of LTA in the suppressive properties of Bregs as an up-stream enhancer of GZMB.

W133. Identification and Treatment of CD8 T Cell Exhaustion and Symptoms in Myalgic Encephalomyelitis/chronic Fatigue Syndrome (ME/CFS) and Long COVID with Inspiritol in a Case Based Study

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Patients with Long COVID, the new pandemic, have a symptom complex highly analogous to ME/CFS, suggesting that at least a subset may have the same disorder. There is an urgent need for diagnostic tools and treatment strategies for these two related poorly understood devastating disorders. Here, we hypothesize that Long COVID and ME/CFS maybe due to an aberrant response to an immunological trigger like infection, which results in a dysregulated immune system dominated by CD8 T cell exhaustion. We treated 4 ME/CFS and 4 Long COVID patients in an open-label study with Inspiritol, a compounded drug taken by nebulizer that has the potential to relieve oxidative stress, attenuate NF-κB signaling, and act directly against pathogens. First, we show that CD8 T cells were

functionally exhausted in production of IFN and TNF by intracellular cytokine assays in ME/CFS (n=12) and Long COVID (n=8) patients compared to healthy donors (n=10). Then we used this assay and our symptom severity questionnaire to track disease outcome during Inspiritol therapy. Here, we present two observations fundamental to the pathogenesis and treatment of Long COVID and ME/CFS: 1) both diseases are characterized by exhausted T cells with severe deficiencies in their abilities to produce IFN and TNF; 2) a new immune-modulatory and anti-oxidant reagent, Inspiritol, corrects this immune deficiency and improves health of the patients. This work provides evidence of a new treatment and a biomarker useful for diagnosis and tracking of treatment outcomes that can help expedite clinical trials in both Long COVID and ME/CFS.

W134. Identification of a Subpopulation of Human B Cells with Characteristics of Antigen Cross-presenting Cells

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Amongst the diverse functions of B cells, a small literature suggests that these cells have the capacity to drive CD8+ T cell responses via cross-presentation of exogenous antigens. In this study, we investigate whether alloantigen-stimulated human B cells demonstrate characteristics required for antigen cross-presentation. Using a combination of transcriptional, immunofluorescence and multi-color flow cytometric analyses of responder B cells in mixed lymphocyte reactions (MLRs), we identified and characterized the emergence of a subpopulation of activated (CD86+) B cells characterized by FcRL5 expression. FcRL5+CD86+ B cells show significantly higher expression of molecules associated with processing and presenting endocytosed antigens consistent with putative antigen cross-presenting functionality (pCP B cells). The proportion of such pCP B cells correlated with the proportion of granzyme B+ perforin+ cytotoxic CD8+ T cells in MLRs. Consistent with a requirement for uptake and processing of exogenous allo-antigenic material, pCP B cells demonstrate significantly higher phagocytic capacity in endocytosis assays. We also identified a subpopulation of B cells with these pCP B cell characteristics in disaggregated but otherwise unmanipulated human splenocytes, showing that such cells can be found in vivo. In conclusion, we have identified a distinctive subpopulation of human B cells with features consistent with a role in allo-antigen cross-presentation. Further investigation of this B cell subset could lay the foundation for identification of biomarkers and targeted therapeutics relevant to curbing the contributions of pCP-B cells to alloresponsiveness and minimizing morbidity in post-transplant patients.

W135. Identification of CD4^{+} T cell Antigens in Sjögren's Disease Using Tscan-ii

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CD4+ T cells play a fundamental role in orchestrating various aspects of immune responses and tissue homeostasis by recognizing a plethora of self and foreign antigens. However, our inability to unbiasedly associate various peptide HLA-II complexes with their cognate TCR has significantly hampered our understanding of CD4+ function and its role in the etiology of human disease. Therefore, we have recently developed TScan-II, a platform for unbiasedly discovering CD4+ antigens using genome-scale human and virome libraries. This platform simultaneously incorporates the endogenous HLA-II antigen processing machinery in APC cells and the endogenous T cell signaling in T cells for antigen discovery. We illustrate the adaptability of the TScan-II for multiplexed HLA and TCR screens by leveraging the platform for de novo antigen discovery of clonally expanded CD4+ T cells in the salivary gland(SG) of Sjögren's disease(SjD) patients. We identified self-antigens expressed on multiple HLA alleles that can readily activate their cognate TCRs. Furthermore, through spatial transcriptomics and single-cell sequencing of SG biopsies, we observed that the identified antigens are expressed within cells in the SG cells capable of processing and presenting HLA-II antigens. Collectively these observations implicate CD4+ in SjD pathogenesis.

W136. IL-12 Drives the Differentiation of Human T Follicular Regulatory Cells

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T follicular regulatory (Tfr) cells counteract the B cell-helper activity of T follicular helper (Tfh) cells and hinder the production of antibodies against self-antigens or allergens. A mechanistic understanding of the cues initiating the differentiation of T regulatory (Treg) cells into Tfr cells, which is instrumental for the therapeutic manipulation of diseases associated with a Tfr-imbalance, is still missing. Despite their opposed roles, Tfr and Tfh cell differentiation appears to be controlled by partially overlapping signals, including TCR stimulation and costimulatory molecules. However, it is still unknown if cytokines that have been shown to control the biology of human Tfh cells, including IL-12 and activin A, can influence the differentiation of Tfr cells. To address this question, we evaluated the impact of these two cytokines on the in vitro differentiation of Tfr cells. Herein, we report a role for IL-12 in driving, on activated Treg cells, the induction of molecules that belong to the Tfr cell program, including CXCR5, PD-1, BCL6 and ICOS meanwhile preserving Tfr regulatory function. Conversely, activin A presented a largely negligible effect on the in vitro differentiation of Tfr-like cells and only modestly strengthened the IL-12-driven differentiation of Tfr-like cells. Importantly, patients with inborn errors of immunity in IL12RB1 gene presented with a severe decrease in circulating Tfr cells and produced higher levels of anti-actin autoantibodies in vivo. Overall, this study unveils IL-12 as an inducer of Tfr cell differentiation in vivo and provides a novel approach for the in vitro generation of human Tfr-like cells.

W137. Improving Characterization of T-cell Lineage and Activation States in Scrna-seq with T-cell Annotator (TCAT)

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T-cells function as a computational nexus in the immune system: they detect antigens from all major pathogens, and based on environmental cues and prior experiences, execute gene expression programs (GEPs) to carry-out adaptive functions (e.g. proliferation, cytotoxicity, cytokine production). Painstaking flow cytometric analysis in controlled models has defined core mutually exclusive response programs – canonically Th1, Th2, and Th17 – that helper T-cells enact upon encountering antigen. However, dozens of contemporary scRNA-Seq experiments have revealed a continuum of T-cell states without distinct clusters corresponding to these subtypes. Here, we resolve this discrepancy by advancing an alternative analysis strategy -- consensus non-negative matrix factorization (cNMF), which learns GEPs from scRNA-Seq, representing each cell as a mixture of programs. Applying cNMF to seven datasets spanning over 2,000,000 T-cells from 700 individuals across tissues and diseases, we identify 60 GEPs including 50 that are reproducible across 2+ datasets and many associated with diseases including Covid-19 and cancer. We discover GEPs reflecting the core known functions of T-cells including proliferation, cytotoxicity, exhaustion, Th1/Th2/ Th17 effector states, and ten novel programs. Simultaneously quantifying the activities of all active GEPs in each T-cell, rather than the single strongest one (as in hard clustering), better reveals developmental lineages and activation states that underlie a cell's expression profile. We provide our GEP catalog and software – T-Cell AnnoTator (TCAT) – to infer their activities in new datasets. Our approach generalizes to all cell-types and provides quantitative measures of GEP activity for applications including eQTL analysis and disease associations.

W138. *In Vitro* Assay Development to Assess Successful Removal of T and B Cell Epitopes Through Seismic's IMPACT Platform

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Proteases derived from human pathogens can specifically cleave IgG into F(ab')2 and Fc fragments. IdeS, an IgG cleaving enzyme derived from Streptococcus pyogenes, has shown clinical proof of concept, and is approved for use before kidney transplantation. Due to the immunogenic nature of these proteases, dosing is limited by high prevalence of pre-existing antibodies and the induction of anti-drug antibodies after dosing. Therefore, to mitigate the impact of the immune system on our enzyme, we identify and remove putative T and B cell epitopes using Seismic's IMPACT platform leveraging machine learning. To experimentally validate in silico predictions, we developed two complementary in vitro assays using intravenous immunoglobulin (IVIg) to assess removal of B cell epitopes. First, we assess how mutating the wild type (WT) protease sequence impacts pre-existing antibodies in a competitive binding assay followed by evaluating global binding of pre-existing antibodies to our protease. Additionally, we developed an in vitro PBMC proliferation assay to determine the success of T cell epitope removal. Using flow cytometry, we quantitively assess CD4 proliferation via bromodeoxyuridine (BrdU) incorporation. We demonstrate examples of molecules designed through our IMPACT platform to reduce both T and B cell epitopes. The B cell epitope assays show a decrease in competition as compared to the WT protease while achieving a reduction in IVIg binding. These data suggest that these in vitro assays can be used to screen for successful invisibilization of molecules of interest.

W139. Induction of Regulatory Inkt Cells by Liposomes Containing Glycolipids Using Partially Humanized Mice for Inkt Cells

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Invariant NKT (iNKT) cells, a subpopulation of NKT cells, have attracted substantial attention because of their ability to be activated specifically by glycolipid antigens. The activation of iNKT cells (mainly NKT10 cells, a novel iNKT cell subset with IL-10-dependent regulatory function) with α-galactosylceramide (α-GalCer) can protect against inflammatory diseases. Nevertheless, the strong activation of iNKT cells elicited by α-GalCer exhibits limited therapeutic efficacy, mainly due to the induction of a mixed pro- and anti-inflammatory cytokine response and anergy. Since iNKT cells can be differentially activated by α-GalCer analogs, it is crucial to determine which α-GalCer analogues will expand NKT10 cells. In this study, we identified NKT10 cells in hCD1d-KI mice (a partially humanized murine model for NKT cell responses). Hence, we evaluated different experimental conditions, such as immunization schemes, glycolipid activators of iNKT cells, and the use of glycolipid delivery systems. We observed an expansion of NKT10 cells in hCD1d-KI mice treated with α-GalCer at seven days, similar to the expansion of NKT10 cells reported during the immunization scheme of 30 days. Finally, we incorporated glycolipids ligands of iNKT cells into liposomes; it was observed that the incorporation of glycolipids into stearylated octaarginine-modified liposomes effectively induced in vitro activation of iNKT cells hybridoma and remarkably increased the in vivo expansion of NKT10 cells, which was superior in the group of mice treated with the α-GalCer analog AH10-7 incorporated in liposomes. Therefore, AH10-7 contained in liposomes could be an excellent candidate for therapies that target NKT10 cells.

W140. Invariant Natural Killer T Cells Activation by a Novel Glycolipid-containing Nanoparticle Formulation Induces Expansion of Regulatory Immune Cells

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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 the differentiation of diverse immune cells 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 liposomal nanoparticles, in order to both activate iNKT cells and modulate the immune response in an antigen-specific manner. Liposomes were generated by thin lipid film hydration and extrusion, following OVA-anchoring to its surface. Physicochemical and functional characterization of the different liposomal formulations were assessed previous to the administration of the nanoparticles in OVA-sensitized mice. In vivo, we found that the immunization of glycolipid-containing liposomes led to the expansion, activation and production of a specific cytokines profile from iNKT cells. Furthermore, activation of iNKT cells led to significant expansion of regulatory B (Bregs), regulatory T (Tregs) and type 1 regulatory T (Tr1) cells. Additionally, the production of anti-inflammatory cytokine IL-10 was enhanced in the aforementioned cells, given by the activity of iNKT cells. Herein, we present a novel strategy to expand regulatory cells given by the activation of iNKT cells, induced by different nanoparticle formulations, becoming the first step to generate a cutting-edge strategy to reduce the inflammatory response and reestablish immunotolerance on different types of pathologies.

W141. Mapping Critical Regulators of Tissue-resident Memory T cell Temporal Development Across Skin and Intestinal Niches

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Tissue-resident memory T cells (Trm) play a critical role in peripheral immune-responses to pathogens upon infection, but the understanding of their development remains incomplete. To investigate this process, OT-I cells were adoptively-transferred to mice followed by intradermal VACV infection. Single-cell-RNAseq (scRNAseq) was performed on OT-I cells from skin and draining-lymph node across 10 timepoints (day 0 – 60) post-infection. Additionally, we obtained a publicly available scRNAseq dataset capturing adoptively-transferred P14 cells from the small-intestine intraepithelial-lymphocyte (IEL) compartment post-LCMV infection (8 timepoints: day 4 – 90). Linear modelling of each dataset captured genes significantly associated with Trm development whose expression steadily increased with their temporal trajectory. There were 166 Trm-associated genes unique to skin, including transcription factor Ikzf2/Helios and immune mediators Ccr1 and Gzmc. There were 270 Trm-associated genes unique to the IEL compartment, including transcription factors (Zeb2, Klf3, and Tox) and heat-shock proteins (Hspa1a, Hspa1b, Hsph1). We defined a consensus signature of 71 Trm genes found at both sites that included genes associated with hypoxia (Klf6, Tiparp, Rora) and those canonically associated with immediate-early responses (Fos, Fosl2, Nr4a2). Additionally, SCENIC regulon analysis demonstrated an up-regulation of genes with Fos/Jun transcription factor binding motifs in the Trm from skin and IEL compartments. ATAC-seq performed on OT-I cells 30 days post-infection confirmed open chromatin at known Fos/Jun binding sites. In conclusion, we find that while there are important distinct features at disparate sites, there is a consensus regulatory program driven by Fos/Jun transcription factors that is critical to Trm development across niches.

W142. Mapping Human Thymic Regulatory T cell Development Using Spatial Multiomics

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Regulatory T cells (Tregs) develop in the thymus and are crucial for immune tolerance and homeostasis. The thymic Treg developmental trajectory has been extensively studied in mice, but knowledge in humans is limited. To better

understand human thymic Treg development we used Co-Detection by Indexing (CODEX), Cellular Indexing of Trancriptomes and Epitopes by Sequencing (CITE-seq), and Assay for Transposase-Accessible Chromatin using Sequencing (ATAC-seq) to generate a spatial, multiomic dataset from age- and sex-matched postnatal thymuses (N=7). Flow cytometry for transcription factors and cell surface proteins were used to validate key observations. We identified several subpopulations of candidate Treg progenitors, including CD25hiFOXP3-, CD25-FOXP3lo/+, and CD4+CD8+FOXP3+ cells and mature thymic Tregs (tTregs), defined as CD4+CD25hiFOXP3hiCCR7+ cells. We also found a distinct population of mature, activated Tregs, termed resident/recirculating Tregs (rrTregs). These rrTregs, which were 5-14% of CD4+CD25+ cells, were distinct from tTregs as they lacked thymic emigration markers (CCR7, KLF2, S1PR1), and expressed proteins associated with tissue-resident (CCR8, CD39, ICOS, HLA-DR) and effector (BATF, PRDM1, MAF) Tregs. Cell-cell interaction analyses predicted interactions between IL1R2-expressing rrTregs and IL1B-expressing dendritic cells (DCs), suggesting that rrTregs may act as an inflammatory cytokine "sink", like their mouse counterpart. CODEX analysis showed that rrTregs are interspersed with tTregs and DCs primarily in the medulla. A future direction will be to sort thymic Treg subsets and conduct functional experiments. Understanding how thymic Tregs develop in humans will lead to novel insights into potential causes of autoimmunity and a better understanding of Treg biology for cell therapy applications.

W143. Mechanism of Action of anti-cd45rc Antibody in the Elimination of cd45rchigh T and B cells

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Administration of anti-CD45RC mAbs in preclinical models of transplant rejection, GVHD or APECED leads to amelioration and control of the disease. The anti-human CD45RC mAb (humanized IgG1) has a unique mechanism of action and induces CD45RChigh T and B cell death, while preserving and boosting CD45RClow/neg Tregs, inducing a long-lasting effect. Using in vitro studies, we demonstrated that anti-human CD45RC mAb induces CD45RChigh T and B cell death mainly by direct apoptosis through binding to CD45RChigh cells. Quantification of CD45RC surface level indicated that around 105 molecules per µm2 of membrane were expressed by CD45RChigh cells and 45 molecules per µm2 of membrane are required to reach EC50 of apoptosis. ADCC killing of CD45RChigh T cells mediated by NK cells and ADCP of CD45RChigh T and B cells mediated by monocytes also play an add-on role improving the effect of the anti-CD45RC mAb. Anti-CD45RC mAb does not induce CDC killing, as opposed to positive control Rituximab. We also showed improved apoptosis mediated by anti-CD45RC mAb in presence of cross-linking secondary antibodies. Finally, using in vivo studies in CD34+ immune humanized NSG mice, we showed killing of CD45RChigh T and B cells and not of CD45RClow/neg Tregs in a dose and time-dependent manner and we showed a dose dependent efficacy in a model of GVHD in PBMC-reconstituted NSG mice. Altogether our study demonstrates the mechanisms by which anti-human CD45RC mAb mediates CD45RChigh T and B cell death.

Basic Science of Immunology - Innate Immunity

Th1. Flow-based Multiplex Immunoassays for Measuring Human Acute Phase Response Proteins and Fibrinolysis Biomarkers

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BioLegend

The acute phase response is a complex reaction triggered by the onset of various critical conditions such as trauma or tissue damage, inflammation, stress, and neoplasia. Such response is characterized by an increase of liver-secreted proteins, known as acute phase proteins. One of them is fibrinogen, an important glycoprotein involved in coagulation. Fibrinolysis is a naturally occurring process that controls blood coagulation and the breakdown of clots to restore blood flow. BioLegend has developed two Multiplex Panels using flow cytometry for quantifying critical acute phase and fibrinolysis proteins. LEGENDplex™ Human Acute Phase Panel 1 is designed for the simultaneous quantification of 8 key acute phase molecules including α2-microglobulin, α1-Acid Glycoprotein, Haptoglobin, α1-antitrypsin, Ceruloplasmin, Fibrinogen, Prothrombin, and Serum Amyloid P Component in blood and cell culture supernatant samples. LEGENDplex™ Human Fibrinolysis Panel allows the measurement of 5 human proteins: Fibrinogen, Antithrombin, Plasminogen, Prothrombin, and Factor XIII in plasma samples. The antibody pairs used in these assays are highly specific and validated on biosamples for their ability to detect native proteins. The Panels are thoroughly validated for dilution linearity, cross-reactivity, and inter- and intra-assay precision, and offer greater efficiency and broader dynamic ranges than the conventional ELISA method. These panels are valuable research tools for studying the pathology of related diseases, development and testing of therapeutics, monitoring of the condition, and risk prediction in cardiovascular diseases and many other disorders.

Tu166. Human Femoral Atheroma Exhibit Enhanced Myeloid Inflammation-resolving Potential and Reduced Inflammatory Monocyte Mobilization Compared with Human Carotid Atheroma

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Femoral and carotid atherosclerotic plaques differ in morphology and clinical sequelae: carotid plaques are comparatively rupture-prone and implicated in acute atherothrombotic events, whereas femoral plaques are stable and not rupture prone. However, few cellular level insights exist into immunologic features underlying these differences. We performed single-cell ribonucleic acid sequencing (scRNA-seq) on 65,920 leukocytes from freshly excised atheroma of 13 unique individuals who underwent femoral (N=9; 35265 CD45+ cells analyzed) or carotid (N=4; 30655 CD45+ cells analyzed) endarterectomy, then validated plaque niche-level findings by flow cytometry and immunohistochemistry in 36 distinct femoral or carotid endarterectomy patients. Inflammatory foam cell-like macrophages and monocytes comprised 2.5- to 4-fold higher proportions of myeloid cells in carotid versus femoral plaques, whereas anti-inflammatory foam cell-like macrophages and LYVE1-overexpressing resident-like macrophages comprised 3.5- to 9-fold higher proportions of myeloid cells in femoral versus carotid plaque ($p< 0.001$ for all). Metabolic gene expression scoring revealed bias toward oxidative phosphorylation in femoral plaque macrophages. Flow cytometry of plaque and blood confirmed a significant comparative excess of CCR2+ (versus CCR2-) macrophages in carotid versus femoral plaque, as well as higher CCR2 expression in classical (CD14++CD16-) monocytes in the blood of patients undergoing carotid versus femoral endarterectomy. Lymphoid-focused analyses revealed comparative B cell bias and anti-inflammatory B cell transcriptional profiles femoral versus carotid plaque, whereas CD8+ T cells comprised a higher proportion of T cells in carotid plaque. Overall, our findings in 49 unique individuals undergoing femoral or carotid endarterectomy suggest novel, translationally relevant plaque

niche-specific leukocyte trafficking and differentiation cues.

Tu167. Hypoxia Stimulates Neonatal hif-1a Dependent Macrophage Secretion of Cardiomyocyte Mitogens
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Macrophages facilitate neonatal murine cardiac regeneration. How macrophages orchestrate regeneration remains vague and the neonatal myeloid response to hypoxia as well as the role of myeloid Hypoxia-Inducible Factors (HIFs) in regeneration are unknown. We hypothesized that post-ischemic regulation of regenerative programs are confined to neonatal cardiac macrophages and are lost in the adult. We also hypothesized hypoxia is a stimulus for neonatal macrophages to secrete mitogens in a HIF-dependent manner. To explore differences in regenerative vs reparative responses to injury, single-cell sequencing was performed on cardiac myeloid cells post myocardial infarction (MI) in wildtype neonatal and adult mice. The neonatal macrophage response to hypoxia was investigated by co-culturing supernatant from hypoxia stimulated neonatal bone marrow-derived macrophages from myeloid HIF-1a expressing vs HIF-1a genetically depleted mice with neonatal cardiomyocytes harvested from wildtype mice. Immunofluorescent staining was performed to quantify cardiomyocyte proliferation. Neutralizing antibodies were utilized to identify mitogens. Gene Ontology identified significantly upregulated genes in biological pathways associated with regeneration and HIF-1a stabilization in C1qa+TLF+ macrophages in the neonate but not the adult. Immunofluorescent staining of neonatal cardiomyocytes revealed a significant increase in mitotically active cardiomyocytes exposed to supernatant from hypoxia stimulated HIF-1a+ macrophages compared to HIF-1a- macrophages. IGF-1 was identified as a mitogen secreted from hypoxic macrophages. In summary, we have newly identified enrichment of regenerative and HIF-1a stabilization pathways specific to C1qa+TLF+ neonatal cardiac macrophages after ischemic injury. We also newly conclude hypoxia is a stimulus for neonatal macrophages to secrete HIF-1a dependent mitogenic factors such as IGF-1.

Tu168. Impact of slc11a1 Gene Variants on Host Response and Tissue Repair After Ti Implantation
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The role of host inflammatory immune response to biomaterials grafting remains unclear. Slc11a1 is a modulator inflammatory response intensity, being one of the factors responsible for the hyper/hyper-responsiveness of AIRmax/AIRmin mice strains. To address the role of host response to biomaterial implantation, Ti devices were implanted in the subcutaneous tissue of AIRmin and AIRmax mice, followed by histomorphometric and immunological analysis. When comparing the AIRmin vs AIRmax groups, a higher density of blood clot density was observed in AIRmax group at 1 and 3 days. AIRmin animals showed higher fibroblasts and collagen fibers density in 1 to 7 days period, while AIRmax strain present a higher density at 14 days. The connective tissue showed statistical difference in the periods of 7 and 14 days where AIRmin has higher density in 7 days and AIRmax in 14 days. Regarding inflammatory cells, AIRmax strain presented a higher density at 1,7 and 14 days, as well as for giant cells in the period of 3d. The immunohistochemical analysis revealed increased GR1 counts at 1 and 3 days in AIRmax strain, while CD3 and CD206 were upregulated in this group at 14d time point. Our results demonstrate AIRmax strain mice presents a stronger pro-inflammatory responsiveness to Ti grafting when compared with AIRmin strain. The exacerbated host response is associated with delayed tissue repair at the grafting area, while a moderate responsiveness results in a more favorable repair outcome, suggesting that translational strategies to tune host response may be applied to improve biomaterials grafting outcome.

Tu169. Retinoic Acid-related Orphan Receptor Alpha Expression Differentiates Distinct Populations of Human Group 2 Innate Lymphoid Cells

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Group 2 innate lymphoid cells (ILC2s) are innate lymphocytes with central roles in host defense and tissue homeostasis. ILC2s are defined by expression of transcription factor GATA-3 and production of type 2 cytokines IL-4, IL-5, IL-9, and IL-13. A subpopulation of ILC2s has also been shown to produce IL-10. Recently, these IL-10 producing ILC2s (ILC210) were shown to limit T cell responses in contexts including islet transplant, helminth expulsion and allergic rhinitis. Transcription factors that promote protective or pathogenic functions of human ILC2s are not well defined, particularly those that underlie IL-10 expression by ILC2s. Murine studies have demonstrated a crucial role for RORA in ILC2 development, yet the role of RORA in differentiated human ILC2 function is unknown. Here we show that ex vivo expanded human ILC210 from peripheral blood express RORA by single cell RNA sequencing and flow cytometry, but exhibit little to no GATA3 expression, whereas ILC2s that lack IL-10 production express GATA3. In ex vivo expanded ILC2s, protein level RORA expression positively correlates with IL-10 production, with ILC2s still maintaining high expression of IL-4, IL-9, and IL-13. Using a lentiviral gene transfer system, we are currently examining effects of enforced RORA expression on differentiated ILC2s, including downstream gene programs and expression of ILC-associated cytokines compared to effects of ILC1 and ILC3-associated transcription factors TBET and RORC2. Collectively, these efforts aim to define the role of RORA in the function of differentiated human ILC2s and to better understand transcriptional programs that underlie IL-10 production by ILC2s.

Tu170. TRPV2 Plays a Critical Role in Hepoxilin a3-medited Neutrophil Transepithelial Chemotaxis

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Purpose: Neutrophil transepithelial migration and accumulation at mucosal surfaces is a hallmark of many inflammatory conditions. Recent studies have demonstrated lipids secreted from the luminal surface of intestinal epithelial cells to regulate this event with N-acyl ethanolamine-type (NAE) endocannabinoids (eCBs) suppressing and the eicosanoid hepoxilin A3 (HxA3) activating the chemotaxis of neutrophils across this mucosal barrier. We hypothesized that directional chemotaxis of neutrophils mediated by HxA3 should involve cell-surface receptor(s). Here, we identify a role for the transient receptor potential cation channel subfamily V member 2 (TRPV2) in HxA3-mediated chemotaxis that drives neutrophil transepithelial migration. Methods: Human promyelocytic cells (HL-60), differentiated (dHL-60) into a neutrophil-like phenotype, were used in a surface internalization-biased protocol, and proteomic analysis of internalized materials following HxA3 exposure identified receptor candidates. Cell migration assays were performed using dHL-60 cells, followed by the addition of chemoattractant HxA3 or fMLP or 2-aminoethoxydiphenylborane (2-APB). siRNA-knock-down of CB2R and TRPV2 genes were performed for their ability to block HxA3- dHL-60 cell migration. Results: TRPV2 was identified in the proteomic screen. The CB2R agonists individually blocked HxA3 and 2-APB-induced migration of dHL-60 cells. Our data suggests TRPV2 interacts with CB2R, linking these two elements in a potentially coordinated chemotactic function that could be used to position neutrophils in the lamina propria in a state of readiness for transepithelial migration induced by the apical epithelial secretion of HxA3. Conclusions: We have identified TRPV2 as a promising candidate receptor for HxA3 to drive the transepithelial migration of neutrophils using the dHL-60 cell system.

W144. Activation of T Regulatory Cells During Allograft Tolerance-induction Requires cdc1 Mitochondrial Activation of TGFβ1

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Purpose: While survival after heart transplantation has improved acutely, chronic rejection and morbidity from immunosuppressant medication remains a challenge for patients. We investigated the influence of conventional dendritic cell 1 cells (cDC1s), a population described to attenuate inflammation in multiple disease models, on cardiac transplantation survival and tolerance induction. Methods: B6 and cDC1 knock out (KO) mice were subjected to tolerization regimen of donor splenocytes (DST) plus anti-CD40L costimulation blocking antibody (CoB) and heterotopic heart transplantation. Intrinsic cDC1 reprogramming during tolerance induction was evaluated by single cell RNA sequencing (scSEQ) of DC enriched splenic cells from B6 mice receiving DST CoB. Results: We observed decreased survival and impaired contractile function of cardiac allografts in cDC1 KO mice alongside a decrease in allograft and splenic T regulatory (Treg) cells following transplantation. There was a significant upregulation of cDC1 TGFβ-1 following DST CoB, thus we sought to identify cell intrinsic signaling for this response. scSEQ of splenic cDC1s revealed upregulation of electron transport chain (ETC) and TCR signaling pathways, implicating a metabolic link between cDC1s and Treg polarization. Culture of bone marrow derived DCs from mice deficient in ETC complex III in cDC1s with donor antigen revealed decreased cDC1 CD86 and TGFβ-1 expression. Conclusions: These data imply a role for cDC1s in cardiac allograft survival and suggest requirements for cDC1 mitochondrial metabolic activation of TGFβ1 for the induction of allograft protective Tregs. Continued interrogation of cDC1 metabolic polarization may provide alternative avenues for therapeutic targets to prolong allograft survival and minimize morbidity.

W145. Development of Knowledge Tree Based on Recent Advances in Genomics and Fundamentals of Immunology

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Problem: Self--Non--Self based models of cellular or adaptive immunity is used in Covid-19 both as vaccines and monoclonal antibody therapy. Both, are experiencing immune evasion, immune resistance due to SARS-CoV2 variants offering continuing risk of Covid-19 infection and future explosion of pandemics. Method: We identify "Alternate Complement System" as a key Self--Non--Self Fluid mediator that plays a prominent role in both fluid and cellular immunity. We identify in Pub Med Central 15221scientific articles as of 01/15/2023. They are related to "Alternate Complement system" Under "Factor H Term" https://www.ncbi.nlm.nih.gov/pmc/?term=Factor+H". Above articles are periodically examined and categorized to develop "Knowledge Tree" of medicine. This is described as subdivision of Tree such as A. Trunk represent the basic foundation of "Recent Advances in Genomics and Fundamentals of Immunology" that is narrowed down to Factor H and Factor D. B. Branches: Represent different medical specialities such as Neurology, ophthalmology, oncology, Endocrinology, Pandemics, Transplantation, Hematology and Obstetrics etc. C. Leaves represent different major life threatening diseases in above medical specialties. Result: We developed sulfonic nanopolymers for balanced inhibition of Factor H and Factor D. Novel formulation methods can be developed to target different major life threatening diseases in different medical specialities. Enhanced human safety, reduced risk of adverse effects, reduce risk of immune evasion and immune resistance are the potential future benefits with prospect of herd immunity at global level.

W146. Endothelial Micro RNA Mediates Surface Expression of interleukin-15

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Interleukin-15 (IL-15) is a potent cytokine that augments lymphocyte-mediated killing and is basally produced at low levels by many cell types, including endothelial cells (ECs). The regulation of IL-15 is extremely complex with multifaceted control of transcription, translation, protein trafficking, and proteolysis. In response to pro-inflammatory signals including tissue necrosis factor (TNF) and IL-1β, ECs can display IL-15 on the cell surface as a heterodimeric complex with the alpha chain of the IL-15 receptor (IL-15Rα), where it can signal to lymphocytes in a process called trans-presentation. Trans-presented IL-15 contributes to the activation of circulating alloreactive CD8+ and CD4+ effector memory T cells. In cultured human ECs, interferon-γ (IFN-γ) increases IL-15 and IL-15Rα transcripts, but surface IL-15 is not expressed. IL-1β has a much smaller effect on IL-15 and IL-15Rα transcript levels, but results in expression of surface IL-15/IL-15Rα complexes, with or without IFN-γ. Small interfering RNA (siRNA) targeting IL-15 and IL-15Rα prevent IL-1-induced surface complex expression. Surface expression also depends on protein translation and on both activation and nuclear import of the transcription factor p65/RelA (i.e. canonical NF-κB). However, inhibition of RNA polymerase II by DRB, assessed as inhibiting E-selectin, does not prevent the surface expression of IL-15 by ECs, suggesting NF-κB regulation of translation, perhaps through non-coding RNA. Dicer knockdown by siRNA, preventing miRNA processing, produces surface IL-15/IL-15Rα complexes, in the absence of cytokine treatment. These data suggest tonic inhibition of EC surface IL-15 by an unknown miRNA(s) that is reduced in response to canonical NF-κB stimulation.

W147. Trajectories of Immune Aging in Diverse Adults Reveal an Immunosenescent Cytotoxic NK Cell Population

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Immunosenescence (ISC) involves gradual changes in immune-system composition and function with advancing age. Clear multi-modal ISC trajectories remain unknown due to the limited number of large-scale studies that are mostly centered on individuals of European ancestry. Here, we examined age-associated phenotypic and functional changes of peripheral blood (n=257, age: 25-88 years) profiling participants of diverse ancestries, using multiparametric-cytometry profiles. First, we used PhenoGraph to empirically cluster PBMCs into 38 PBMC and 43 T-cell discrete clusters. We used linear regression adjusted for sex and genetic ancestry to identify age-associated clusters. We also created an immunological-age estimate using an elastic net regression, selecting for informative cell-populations. We report a novel CD56+PD-L1+GranzymeB+NK cell-subset increasing in frequency with age (p=0.0025). Moreover, we uncovered 10 cell-subsets newly associated with advancing age: naïve CD20+CD4+ (p=0.0028) and CD20+CD8+ (p=0,0032) T-cells, activated IgDlowCD4 central memory T-cells (p=0.0079) across all three populations. We confirmed previous findings: decreased naïve CD8 T-cells (p=5.56 x10-16), increased CD8 TEMRAs (p=3.97x10-9), decreased CD20+ B-cells (p=2.95x10-3) mostly reported in Hispanics and non-hispanic

whites (NHW) with only naïve and TEMRA changes being significant in African Americans. Using these data, our estimate of the immunological-age captured 58.4% of variance in chronological-age, and it appears to be related to brain atrophy on MRI. These findings shed light on ISC trajectories in diverse populations, which include novel ISC cytotoxic CD56+PD-L1+GranzymeB+ NK-cells, potentially contributing to higher cancer incidence in elderly. Establishing a robust immunological age measure in diverse populations will contribute to an ongoing effort to personalize immunotherapies.

W148. Unique Role of IFN-λ1 on Fetal Small Intestinal Function and Immunity

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Type-III interferons (IFN-λs) are recently described family of IFNs. While sharing many properties with type I IFNs they are particularly important to epithelial and barrier surfaces. The human gene family is composed of four members IFNL1-4 and their unique roles are just starting to be illuminated. In adult tissue, IFN-λ expression is restricted to epithelial cells and participates in anti-viral immunity. However, how IFN-λs contribute to fetal intestinal immunity are largely unknown. We developed an extensive single cell atlas of small intestinal (SI) tissue across the human life span ranging from 8 weeks gestational age to adulthood (350,458 of cells). Surprisingly, there was a strong IFN signature in fetal T cells compared to post-natal SI. Mining the atlas, we determined that in utero IFN production was restricted to memory T cells and included both IFNG and unexpectedly IFNL1. Although IFNγ signature predominated in the SI immune cells, epithelial cells had a stronger IFNγ signature. Differential analysis of IFNLR1+ epithelial cells suggested an enrichment for antigen processing pathways and upregulation of MHCI and II. We next addressed the effects of IFNL1 in fetal SI organoids in vitro. Contrary to previously described effects of IFNL on adult derived organoids, IFNL1 treatment of fetal SI organoids promoted proliferation, reduced apoptosis, and enhanced MHC I expression while still inducing a strong IFNL signature consistent with the scRNAseq data. Taken together, our results suggest a unique function of IFN-λs in fetal intestinal homeostasis and immunity during fetal development.

W149. Using Machine Learning to Harness the Complexities of Inflammasome Biology for Novel Drug Discovery

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Inflammasomes are critical components of our innate immune system that convert internal and external danger stimuli into pro-inflammatory signals. Dysregulation of these complex pathways leads to hyperinflammation and tissue damage, linking them to pathogenesis in many human diseases. Traditional readouts of inflammasome activation cannot sufficiently distinguish the effects of complex or convergent pathways, necessitating tools that can resolve differences between various cellular states of health, activation, and inhibition. Machine learning and advanced multidimensional data analysis can strengthen inflammasome drug discovery screening efforts by facilitating analysis of complex, physiologically relevant data. Using primary human PBMCs under physiological inflammasome activation conditions we developed novel machine learning-based analytical tools for fluorescence microscopy images. Every image was analyzed using a mixture of automated quantification of targeted cell features, unbiased discovery of cell subpopulations, compound similarity metrics, and deep learning signatures specific to validated inflammasome cellular states. In parallel, we performed multidimensional cytokine analyses on supernatants from these same plates. We then scaled these experiments into a high content imaging compound screen using advanced lab automation. Multiple scoring rubrics were designed to categorize compounds into novel inflammasome inhibitor classes whose mechanisms of action can be uniquely matched to relevant human pathologies. Using this approach, we screened over 12,000 compounds and identified compounds from five

mechanistic classes that could be used to treat inflammasome-mediated diseases, such as sepsis and gout. This approach could also be applied to other, similarly complex and disease-relevant biological processes to drive multiple clinical paths forward from a single compound screen.

COVID-19

Th168. Effect of 3rd and 4th Doses of Mrna Vaccines on the Magnitude and Stability of Antibody and T Cell Responses to sars-cov-2 in Immunocompromised Patients with Immune-mediated Inflammatory Diseases
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The IMPACT (immune response after COVID-19 vaccination during maintenance therapy in immune-mediated inflammatory diseases) observational study investigated the immunogenicity of SARS-CoV-2 mRNA vaccines in 161 patients with immune-mediated inflammatory diseases (IMID: inflammatory bowel disease, rheumatic or psoriatic disease), with or without maintenance immunosuppressive therapies, compared to healthy controls (HC). IMID patients were untreated or treated with anti-IL-17, anti-IL-12/23, anti-IL-23, methotrexate/azathioprine (MTX/AZA), anti-TNF, or anti-TNF+MTX/AZA. Antibody and T cell responses and neutralization of wildtype (WT) and variants of concern (VOC) were assessed pre and post successive vaccine doses. Compared to HC, IMID patients exhibited accelerated waning of antibody and T cell responses after dose 2. A 3rd vaccine dose restored these responses, and there was reduced decay after 3rd and 4th doses. While IFNy responses maximized after 1 dose in all participants, IMID patients required a 3rd vaccine dose to maintain stability of IFNy responses. Maximal IL-2 responses required 3 doses and IL-4 production continued to increase after 4 doses. Neutralization of VOC was lower in IMID patients, while all patients exhibited robust T cell immunity to VOC. Anti-TNF treated patients had the lowest antibody and neutralization responses, even after 4 doses. The 4th dose subtly affected the magnitude of immune responses in all patients and stabilized the neutralization response against VOC in IMID patients. Our study demonstrates that IMID patients show greater waning of immunity to SARS-CoV-2 than HC, stressing the importance of 3rd and 4th doses of mRNA vaccines in the immunocompromised to stabilize and broaden responses to SARS-CoV-2.

Th169. Longitudinal Antigen-specific Immune Profiling During sars-cov-2 Breakthrough Infection Highlights Early Activation of Memory CD8 T Cells Followed by Later Activation of Memory B Cells
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SARS-CoV-2 infection of vaccinated individuals has become commonplace due to waning antibody and the evolution of immune evasive variants. Nevertheless, the risk of severe disease is lower in people who are vaccinated, likely due to the enhanced potency and accelerated kinetics of memory T and B cell responses. To date, there is little detailed data profiling the kinetics of memory T cell, B cell, and antibody responses during the early stages of infection. To evaluate the kinetics of recall immune responses during SARS-CoV-2 infection, we longitudinally sampled omicron-infected individuals during the days and weeks following symptom onset. The early response engaged Spike-specific plasmablasts and CD8 T cells, which were activated within 7 days of symptom onset. Activation of CD8 T cells was dominated by central memory cells but was observed in effector memory subsets as well, demonstrating that vaccination primes multi-functional memory CD8 T cells that respond during the first week of infection. Activation of Spike-binding memory B cells followed during the second week after symptom onset, marked by amplification of variant-binding and IgA class-switched memory B cells. Despite early detection of plasmablasts, neutralizing antibody titers did not increase until 15 days post-symptom onset. Responses targeting non-Spike

proteins from SARS-CoV-2 had delayed kinetics and lesser magnitude compared to Spike-specific responses, supporting the notion that vaccine-primed recall responses are more potent than primary responses. These data demonstrate the functionality of mRNA vaccine-induced memory B and T cell populations and highlight CD8 T cells as first responders during SARS-CoV-2 breakthrough infection.

Th170. Mapping the Impact of Vaccination and Viral Strain on sars-cov-2 Infection and Host Response in the Human Nasal Mucosa

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SARS-CoV-2 vaccines have been shown to ameliorate COVID-19 disease and reduce transmission suggesting their ability to shape host-viral dynamics at the site of infection. However, detailed knowledge as to how vaccines, novel variants, and their intersection shift host responses in the human nasal mucosa remains unclear. We performed scRNA-seq on nasopharyngeal swabs from 124 adult patients collected during the original, Delta, and Omicron waves of the COVID-19 pandemic in Mississippi, resulting in a dataset of 73,000 diverse cell types. To understand the impact of mRNA vaccination, we compared the cellular composition and transcriptional response in the nasal mucosa between vaccinated and unvaccinated patients. We identified signatures of macrophage recruitment and activation in vaccinated patients regardless of strain, suggesting that stimulation of mucosal myeloid cells may play a role in vaccine-induced protection. To investigate why some patients still become critically ill despite vaccination, we compared mild and severe cases. We found that an impaired epithelial-intrinsic interferon response is persistent in vaccinated patients with severe disease, emphasizing the need to further understand why some patients fail to mount this response. By aligning to a joint host-viral genome, we mapped which cell types and subsets are likely actively infected with SARS-CoV-2. We found that Delta samples have an increased abundance of viral RNA that was independent of ACE2 expression. These results will inform the identification of patients at risk of severe disease from SARS-CoV-2 infection, our understanding of how to optimally design novel mucosal vaccines, and potential therapeutic pathways for COVID-19.

Th171. Mucosal Vaccination to Improve Immunity to sars-cov-2

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Impressive progress has been made during the COVID-19 pandemic to deliver effective and safe vaccines for SARS-CoV2. However, while current vaccines can reduce disease severity, they are still not entirely able to prevent infection and transmission. In this context, immunity at barrier sites may play an important role. We are investigating mucosal vaccination approaches to improve the generation of tissue resident memory T cells (TRM) at the respiratory mucosa. Mice were immunized intramuscularly (i.m.) with Spike protein and poly I:C followed by an intranasal (i.n.) boost with the same formulation 3 weeks later and viral spike epitope-specific T cells analyzed using fluorescently labeled MHC class I and II tetramers for CD8+ and CD4+ T cells, respectively. After i.m. priming, we observed modest expansion of antigen-specific T cells in the iliac lymph nodes (iILN) as well as lower levels of expansion in the lungs, nasal mucosa (NM) and mediastinal and mandibular lymph nodes (medLN/mandLNs), but they all waned after 21 days. After i.n. boosting, spike-specific T cells increased in iILNs to levels similar to that during priming, while in the lungs, NM, medLNs and mandLNs, their expansion was much more robust, with the cells persisting in these tissues for at least 65 days. Most of the spike-specific CD4+ and CD8+ T cells had a TRM phenotype. Our data suggest that antigen exposure in the respiratory tract is necessary for the generation of local viral-specific T cells that will persist long term in the respiratory mucosa.

Th172. Similarities and Differences Between Myocarditis Following COVID-19 Mrna Vaccine and Multiple Inflammatory Syndrome with Cardiac Involvement in Children

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SARS-CoV-2 vaccines have been the most efficient measure in COVID-19 pandemic management, with a reduction in morbidity and mortality. Despite the multiple benefits, myopericarditis after COVID-19 vaccination have been reported. Considering male predominance and inflammation characteristic of myocarditis (MYO), we investigated androgens levels along with clinical, routine laboratory and proteomic analysis in MYO (n=15,mean age 15.72yrs), in comparison with age and gender-matched Multisystem inflammatory syndrome in children (MIS-C) (n=14, mean age 13.07yrs), SARS-CoV-2-infected children (n=21, mean age 15.16yrs) and healthy controls (n=31) (mean age 15.12 years). Correction for multiple comparisons was performed by False Discovery Rate method, considering statistically significant for those with an adjusted p-value< 0.05. Our analysis showed higher level of testosterone, DHEAS, DHEA, androstenedione and cortisone in MYO which persisted months after the acute event, suggesting a primary higher level and a potential role for these hormones in the pathogenesis. Proteomic analysis showed higher levels of proteins involved in generalized inflammation in MIS-C and SARS-CoV-2 compared to MYO, where the heart-related inflammation proteins appeared higher. Indeed, proteins related to systemic inflammation (IFN-gamma, CXCL9, CXCL10) were found higher in MIS-C compared to MYO, whereas a higher level in proteins related to cardiomyocyte apoptosis and myocardial injury (SIRT2, STAMBP, AXIN1) was found in MYO. In conclusion, we showed that a peculiar androgen profile in adolescents may favor myocarditis pathogenesis along with a specific heart-restricted inflammation following mRNA vaccination. Additional studies are needed to further confirm this hypothesis. Despite these findings, the benefits of the COVID19 immunization still outrage the risks.

Th173. The Underlying Mechanisms of Obesity-related COVID-19 Exacerbations

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Obesity has been recognized as one of the most significant risk factors for the deterioration and mortality associated with COVID-19. A certain proportion of COVID-19 patients experience marked elevations of inflammatory mediators termed “cytokine storm”, resulting in the deterioration of the respiratory condition. In the present study, we elucidated that the burden of high visceral adipose tissue (VAT) was more closely related to accelerated inflammatory responses and the mortality of Japanese COVID-19 patients than other obesity-associated markers, including body mass index and the burden of subcutaneous adipose tissue. After evaluating three different types of obese mice, we revealed that two C57BL/6 background obese mice, ob/ob mice and diet-induced obesity mice were extremely more vulnerable to mouse-adapted SARS-CoV-2 infection when compared to non-obese wild-type mice or even to similar obese db/db mice with slightly different genetic background. SARS-CoV-2 genome and proteins were more abundant in the lungs of ob/ob mice, engulfed in macrophages, resulting in increased production of inflammatory cytokine represented by IL-6. Both an anti-IL-6 receptor antibody treatment and the prevention of obesity by leptin replenishment improved the survival of SARS-CoV-2 infected ob/ob mice by reducing the viral protein burden and

excessive immune responses. Our results have proposed that the VAT dominant obesity would be prone to cytokine storm due to SARS-CoV-2 infection and that anti-cytokine strategies can provide more beneficial effects to COVID-19 patients with this type of obesity, at least in Japanese patients.

Th174. Therapy of Covid-19 Patient
Kumarpal Shah
Endocrine Technology, LLC, NY, NY

The Problem: Currently there are no approved drugs in Covid-19 infection to target immune pathogenesis of Covid-19 that includes dysfunction of alternate complement system (Jia Yu, Gloria et al "Complement dysregulation is associated with severe COVID-19 illness" Haematologica 2022; Volume 107(5):1095-1105 Method: We developed nano sulfonic polymers for balanced targeting of Factor D. Results: This will a. inhibit immune piracy of factor H; b. Inhibit hyper activated alternate complement system due to factor D and c. reduced adverse effects of factor h. Discussions: Based on extensive Human safety record generated - sulfonic polymers may provide an ideal immune modulation therapy. This can be rapidly integrated in to evolving standards of covid-19 therapies covering prevention, prophylaxis and therapy of Covid-19 infection.

Th175. Third Generation Nonbiological covid-19 Vaccine
Kumarpal Shah
Endocrine Technology, LLC, NY, NY
The Problem: Omicron and its sub variants have exposed the weakness of first generation biological vaccines. SARS-CoV-2 have rapidly adapted to host immune system and first generation vaccines. This increases the future risk of periodic burst of Covid-19 Pandemics at global level. Rationale: It is critical to target immune evasion, immune virulence and immune resistance part of immune pathogenesis of SARS-CoV2. The underlying molecular target is Factor H as shown in the development of biological meningococcal vaccine (Lisa K. McNeil, et al "Role of Factor H Binding Protein in Neisseria meningitidis Virulence and Its Potential as a Vaccine Candidate To Broadly Protect against Meningococcal Disease Microbiol. Mol. Biol. Rev. 2013, 77(2):234. DOI: 10.1128/MMBR.00056-12. (We characterize as Second generation Biological Vaccines) Method: We have developed nonbiological third generation vaccine targeting immune evasion, immune virulence, immune resistance and inflammation mechanism of Factor H involved in Covid-19 pandemics. Discussions: Sulfonic nanopolymers as inhibitors of Factor H and Factor D can be rapidly adapted to assist and improve first generation biological vaccine in Covid-19 and enhance the prospect of herd immunity at a global levels.

Tu171. A Novel Human Immune System Mouse Model for COVID-19
Amber Wolabaugh[1], Nichole Danzl[2], Vrushali Agashe[2], Mohsen Khosravi Maharlooei[2], Katherine Long[2], Christopher Parks[2], Moriya Tsuji[2], Megan Sykes[2], Nichole Danzl[2], Vrushali Agashe[2], Mohsen Khosravi Maharlooei[2], Katherine Long[2], Christopher Parks[2], Moriya Tsuji[2] and Megan Sykes[2]
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Both innate and adaptive immune responses contribute to the control of SARS-CoV-2 infection and may cause pathology. However, animal models do not allow investigation of the role of each human immune component. We have developed a novel human immune system (HIS) mouse model in which human B cells, T cells, and myeloid antigen-presenting cells are generated via engraftment of human fetal liver-derived CD34+ cells and human fetal thymus in NOD/LtSz-scid IL2R gamma null (NSG) mice engineered to express hACE2 under physiologic control. Using CRISPR, the human ACE2 gene was knocked into the endogenous murine ACE2 locus in NSG zygotes. We demonstrated that hACE2 is expressed in 14 different tissues in these mice and mirrors hACE2 expression in humans. Upon intranasal infection with SARS-CoV-2, the virus disseminated to eight different tissues throughout the body of the HIS hACE2 mouse, including the brain, heart, GI tract and pancreas. Transplanting human liver cells

without fetal thymus in thymectomized hACE2 NSG mice generated only B cells and APCs without T cells, allowing assessment of the effect of T cells on pathogenesis and viral control. Viral dissemination was not dependent on the presence of T cells but was markedly enhanced by the presence of a human immune system. Infected lungs contained macrophage clusters, thickened interstitium and inflammation around the airways, resembling human COVID-19 patient histology. This model will permit determination of the role of T cells and other human immune components in the pathogenesis of acute COVID-19 and post-acute sequelae of SARS-CoV-2 infection (PASC).

Tu172. Association of COVID-19 and Development of Type 1 Diabetes - A Danish Nationwide Register Study

Runa Nolsoe[1], Bochra Zareini[2], Kathrine Kold Sørensen[3], Pia Eiken[4], Thea Fischer[2], Peter Lommer Kristensen[2], Maria Lendorf[2], Ulrik Pedersen-Bjergaard[2] and Christian Torp-Pedersen[2]
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Aim To investigate whether the incidence of type 1 diabetes (T1D) increased during the COVID-19 pandemic compared to previous years and if there is an association between SARS-CoV-2 infection and the development of T1D. Methods We used Danish nationwide registers to include all Danish residents < 30 years of age without diabetes from 2015-2021 and followed them from 01.01.2015 or their birth until 31.12.2021, development of T1D, age of 30, emigration, onset of cancer, initiation of immune modulating therapy, or development of type 2 diabetes or other autoimmune diseases. We calculated incidence rate ratios (A-IRR) of T1D adjusted for age and gender. Subsequently, we matched persons with COVID with three controls (1:3) on gender, birth year, and vaccination status and estimated relative rate differences. Results Of a total of 2,381,348 persons (median age 12.8 years (interquartile range (IQR): 3.4-21.1) and 51.4% males), 3,795 developed T1D. A-IRR with 95% confidence interval in each quarter of the year in 2020 and 2021 compared to 2019 was: Jan-Mar-2020: 1.12 [0.89; 1.12], Jan-Mar-2021: 1.10 [0.87; 1.10], Apr-Jun-2020: 1.10 [0.84; 1.10], Apr-Jun-2021: 1.55 [1.22; 1.55], Jul-Sep-2020: 1.16 [0.92; 1.16], Jul-Sep-2021: 1.24 [0.98; 1.24], Oct-Dec-2020: 1.02 [0.82; 1.02], Oct-Dec-2021: 1.06 [0.85; 1.06]. We matched 338,670 COVID-19 cases with 1,004,688 controls (median follow-up: 42 days (IQR: 11-225)). Among cases, 31 individuals developed T1D, compared to 110 individuals among controls (hazard ratio 0.87 (95% CI: 0.59-1.29)). Conclusion The increase in T1D incidence during Apr-Jun 2021 compared to Apr-Jun 2019 could not be attributed to the SARS-CoV-2 pandemic.

Tu173. Behavioral Change in Response to COVID-19 Restrictions Among People with and Without Diabetes: A Retrospective Case-control Study in Denmark

Filip Gnesin, Mille Cajar, Runa Nolsøe, Maria Elisabeth Lendorf, Peter Lommer Kristensen, Christian Torp-Pedersen and Ulrik Pedersen-Bjergaard
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Introduction We aimed to investigate whether the COVID-19 restrictions enforced by the Danish government altered the behaviour of patients with diabetes compared to the background population. Methods This web-survey-based retrospective case-control study in the Capital Region of Denmark invited 25,000 cases and 100,000 matched controls tested positive or negative, respectively, for SARS-CoV-2 infection between November 2 and December 13, 2020. Controls were matched on test date, sex, age, and municipality. Participants were asked about known exposure to COVID-19, diabetes status, symptoms, and risk and precautionary behaviour during the period. Results Out of 47,826 included participants, 1,405 (3.0%) had diabetes and 11,591 (24.7%) tested positive for SARS-CoV-2. The differences in the behaviour of diabetics versus non-diabetics were largely independent of COVID-status. For risk behaviour, diabetics had fewer daily contacts, were less likely to travel by bus or train, participate in indoor sports, visit a gym, use a public swimming pool or sauna, do grocery shopping, and visit restaurants, cafés, or bars compared to non-diabetics. However, for precautionary behaviour, diabetics were less likely to sneeze in the elbow, stay at home, limit travel but more likely to avoid sports. The groups were similar in terms of wearing masks, avoiding handshakes, practicing physical distancing, avoiding public transports, and avoiding crowds. However, among cases,

diabetics were less likely to practice frequent handwashing and usage of hand sanitizers, but these differences were not present in the negative controls. Conclusion Diabetics generally exhibited less risk behaviour, but took fewer certain precautionary measures compared to non-diabetics.

Tu174. Characterization of a Poly (allylamine Hydrochloride) Nanoparticle-based Adjuvant That Provides a Rapid and Broad Mucosal Immune Activation

Guillermo DOCENA[1], Gaston Rizzo[2], Rodrigo Sanchez[3], Camila Chavero[2], Daiana Bianchi[2], Evangelina Dupuy[2], Eugenia Apuzzo[4], Santiago Herrera[4], Maximiliano Agazzi[4], Omar Azzaroni[4], sergio Costa Oliveira[3] and Smaldini Paola[2]

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Different functional nanoparticles (Np) can be designed based on different compositions, sizes, shape and surface properties for biomedical applications. This work aimed to characterize poly(allylamine) Np as a vehicle and adjuvant to be further used in vaccines. Self-assembly Np were characterized using several antigen-presenting cells (APCs), epithelial cells and mice. Cell interaction and activation was evaluated by fluorescence microscopy, flow cytometry and ELISA. Finally, Balb/c mice were systemically or mucosally immunized with Np-OVA and the pharmacokinetic and immunogenicity were evaluated. We found that APC internalized Np, which became activated, with a significantly increased expression of CD86 ($p< 0.05$) and IL-1β ($p< 0.01$). Using KO mice we demonstrated an NLRP3- and Caspase 1-dependent secretion of IL-1β. In vivo tracking experiments showed that native Np native protected OVA and reached the critical organs to promote immune activation. Then, a significant induction of serum OVA-specific IgG and IgG2a, increased secretion of IFN-γ by spleen cells and a high frequency of LT CD4+IFN-γ+ and LT CD8+IFN-γ+ cells were seen. In conclusion, we found that APC internalized Np and activated the inflammasome pathway with IL-1β secretion. Remarkably, this nanoparticle exhibited adjuvant properties for mucosal targeting to induce a Th1-dependent immunity that could be exploited in a vaccine.

Tu175. Characterization of the T Cell Responses Elicited by Four Doses of an Inactivated sars-cov-2 Vaccine in Adults

Susan Bueno[1], Constanza Mendez[2], Hernan Peñaloza[2], Felipe Melo-Gonzalez[3], Mariana Rios[2], Luisa Duarte[3], Alex Cabrera[4], Cristian Gutierrez[5], Alba Grifoni[6], Alessandro Sette[6], Daniela Weiskopf[6], CoronaVac03CL Study group, Jorge Soto[3], Leandro Carreño[5], Pablo Gonzalez[2] and Alexis Kalergis[2]

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Background. The SARS-CoV-2 Omicron variant has challenged the control of the COVID-19 pandemic even in highly vaccinated countries due to its ability to partially evade the immune response induced by vaccines. Here we studied whether a second booster of CoronaVac®, an inactivated vaccine, enhances the cellular response against the ancestral SARS-CoV-2 and the Omicron variant. Methods. In the context of a phase 3 clinical study, SARS-CoV-2 cellular immunity was analyzed in stimulated peripheral mononuclear cells of 46 volunteers by flow cytometry and ELISPOT. Findings. We observed a significant increase of CD4+ T cell expressing activate inducing markers (AIM+) and IFN-gamma producing cells after stimulation with a pool of peptides derived from the Spike protein at 2 and 4 weeks after the second dose of CoronaVac. We observed that the response remained stable after first and the second booster dose and no significant differences in IFN-gamma secreting cells or AIM+CD4+ T cells were observed. Importantly, CD4+ T cell response was equally increased when stimulation with a pool of peptides derived from the Spike protein from the Omicron variant. Furthermore, we observed an increase in the number of responder

individuals at 4 weeks after the second booster. Interpretation. A second booster of CoronaVac® contributes to stabilizing spike-specific CD4+ T cell responses against the ancestral SARS-CoV-2 strain and the Omicron variant.

Tu176. COVID-19 Vaccines as a Testing Agent for Allergy Diagnosis

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Severe allergic reactions to vaccinations, while rare, can occur and were recently brought to media attention with the novel mRNA SARS-CoV-2 vaccines. Previous case studies have found that excipient skin testing has a limited role when evaluating patients with mRNA SARS-CoV-2 anaphylaxis. In this single-site case series, a sample group was composed of 12 female and 3 male patients from South Bay Allergy and Asthma Group with ages ranging from 15 to 72 years old (median age, 45 years). Using a standardized technique for allergy skin testing, patients were tested percutaneously and intradermally with diluted residual doses of SARS-CoV-2 vaccines as well as with negative and positive controls (saline and histamine, respectively). Patients were contacted 24 hours after their initial visit and once more after being administered a SARS-CoV-2 vaccine, at a later date, to note if any new symptoms or reactions developed. Following testing, 8 of 15 patients were vaccinated. Seven patients presented with negative skin testing and no severe systemic reaction after vaccination. One patient who had a positive skin test experienced hives around their mouth temporarily following vaccination. Additionally, no anaphylactic reactions were reported for any patients during testing or following vaccination. Given the small sample size of this study, we cannot state that this testing method conclusively determines allergies to components of a SARS-CoV-2 vaccine. However, a larger sample size and further replication of this method may help confirm the accuracy of this diagnostic approach and could pacify patients who are worried about obtaining a vaccine.

Tu177. Features of Humoral Immunity to sars-cov-2 in Kidney Transplant Recipients

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The objective of the study – to compare the features of post-infection and post-vaccination antibody response to SARS-CoV-2 in kidney transplant recipients and immunocompetent individuals. The studies were carried out in recipients after COVID-19 (n=177) and recipients, immunized with Sputnik V (n=25) or Vero Cell (n=31) vaccines. COVID-19 immunocompetent convalescents (n=163) and persons, vaccinated with Sputnik V (n=257) or Vero Cell (n=160) were included. SARS-CoV-2 post-infection seroprevalence among kidney transplant recipients was 86.4% to S protein and 59.9% to N protein after 21 days COVID-19 symptoms manifestation. The same parameter to S protein was 91.5% and 86.3% to N protein in the immunocompetent individuals ($p < 0.001$). IgG to the S protein of SARS-CoV-2 were detected in 70.3% of recipients and only 24.5% of patients were positive for IgG to the N protein after 1 year of onset of COVID-19 symptoms (in immunocompetent individuals – 80.3% to S protein and 46.5% to N protein ($p < 0.001$). Seroprevalence in recipients with hybrid immunity after infection and vaccination with Sputnik V was 100%, after Vero Cell – 77.8%. High avidity IgG were observed in 33.3% of Sputnik V vaccinated recipients and were absent in Vero Cell vaccinated recipients (in immunocompetent individuals – 22.9% and 2.2%, respectively). In kidney recipients indicators of post-infection and post-vaccination humoral immunity to SARS-CoV-2 were lower in comparison to immunocompetent persons. High avidity IgG detection level towards SARS-CoV-2 was notably higher in the group of individuals vaccinated with Sputnik V in comparison to individuals vaccinated with Vero Cell

Tu178. Investigating Links Between Persistent Symptoms After COVID-19 and Longitudinal Changes in Memory T Cell Responses to EBV, CMV, HSV-1, and sars-cov-2

Jiabao Zhong[1], Erika Smith[2], Christina McConney[2], David Bean[2], Anna Belkina[2] and Jennifer Snyder-Cappione[2]
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Post-acute infection syndromes (PAISs) have long been observed following acute infections with bacteria, viruses or other microbes. Overlapping core symptoms across different PAISs suggest underlying mechanism(s) that is putatively independent of the causative agents. During the COVID-19 pandemic, accumulating reports of post-acute sequelae of SARS-CoV-2 infection (PASC), also known as “long Covid”, have provided new clues for the pathogenesis of PAISs, but the mechanistic drivers of persistent symptoms are still unclear. Here, we evaluated temporal changes in the memory T cell repertoire, in both frequency and functional signatures, of five individuals initially infected with SARS-CoV-2 in the spring of 2020 (three outpatient, two hospitalized), with three participants showing evidence of PASC. Longitudinal T cell responses were quantified as the frequencies of cytokine producing cells after overnight (ex vivo) stimulation of PBMCs with peptide pools from cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex virus type 1 (HSV-1) and SARS-CoV-2; cell readouts were obtained using intracellular cytokine staining (ICS). PBMCs were collected from multiple timepoints from acute illness to 12-25 months post-infection. Our unique dataset serves as an indirect readout of viral replication and provides insights to two possible explanations for PASC: the reactivation of latently infected viruses during and after the acute SARS-CoV-2 infection, and the persistence of SARS-CoV-2 antigens triggering chronic inflammation. For future studies, longitudinal T cell responses after acute infections with other microbes will be assessed in a larger cohort to determine the specificity of the findings to SARS-CoV-2 infection.

Tu179. Longitudinal Analysis After sars-cov-2 Infection and Vaccination in LA-SPARTA: A Cohort of High-risk Individuals

Meagan Jenkins[1], Eduardo Valli[2], Donna Phan Tran[3], Evelyn Flores[3], Deborah Kupferwasser[3], Harry Pickering[2], Ying Zheng[2], David Gjertson[2], Ted Ross[4], Joanna Schaenman[2], Loren Miller[5], Michael Yeaman[2] and Elaine reed[2]
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The correlates of risk and immune protection against SARS-CoV-2 infection and vaccination response are still largely unclear. Thus, we prospectively enrolled 200 participants with a high risk of SARS-CoV-2 occupational exposure, aiming to longitudinally analyze participant exposure risks, vaccination/infection status, and the the immune response to vaccination and natural infection. Symptoms of participants were assessed at 3, 6, and 12 months, along with blood and saliva collection. Quantitative ELISA assay was used to assess the serological response to the SARS-CoV-2 spike holoprotein (S), S-receptor binding domain (RBD) and nucleocapsid protein (NP). We found that 40 of 200 (20%) participants were infected via serology. No different in the infection incidence was noted in healthcare and non-healthcare occupations. 79.5% of infected participants seroconverted for NP, with 11.5% unaware they had been infected. The antibody response to S was greater than to RBD. Importantly, participants of Hispanic ethnicity had 2-fold greater incidence of infection despite vaccination. Booster vaccination significantly increased the quality of RBD-specific responses to variant spike proteins compared to the initial vaccination series. No differences in quality were found if a participant was boosted via vaccination or natural infection, via avidity analysis. Collectively, our results indicate that antibody quality and holoprotein response may reveal meaningful determinants of protection beyond RBD antibody titer alone. We also note that determinants of SARS-CoV-2 infection may include race/ethnicity, and remain independent of comorbidity and humoral response in this cohort.

W150. Longitudinal Measurement of Anti-viral and Autoimmune Antibodies for Two Years After sars-cov-2 Infection Reveals Uniquely Durable Vaccination Responses and Partial Waning of Self-reactive Igg

Christina McConney[1], Maria Jose-Ayuso[2], Erika Smith[2], Victoria Volfson Sedletsky[2], Manish Sagar[2], Alex Olson[2], Nahid Bhadelia[2] and Jennifer Snyder-Cappione[2]
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As temporal distance from the start of the COVID-19 pandemic increases, there is mounting concern of the long-term immunological consequences of SARS-CoV-2 infection(s). Abnormally high levels of a panoply of autoantibodies are found during acute COVID-19, and to date it is still unclear if these antibodies are sustained for a year or longer post-infection. Additionally, if there is improved longevity of vaccine-induced antibodies when administered against the immune backdrop of previous SARS-CoV-2 infection remains to be elucidated. To address these questions, we measured a series of anti-viral and autoimmune antibodies from samples taken at intervals from seven subjects infected with SARS-CoV-2 in the spring of 2020 (five outpatient and two hospitalized). Blood draws were performed beginning at acute disease and for an additional 10-25 months. Plasma IgG and IgA antibodies reactive with SARS-CoV-2 spike, RBD, and nucleocapsid, as well as CMV Mosaic, EBV GP 350, and Flu H1 were measured using the BU ELISA protocol, autoantibodies to self-antigens associated with connective tissue disease were measured using a commercial kit. Anti-viral antibody responses induced by vaccination were sustained for more than one year post SARS-CoV-2 infection in multiple subjects. Autoantibody trajectories varied, with many positive 'hits' falling below baseline after 1 year, and others remaining high for all time points measured. Future work will include increasing subject numbers to a larger cohort with differing clinical presentations and outcomes to define connections between serological signatures, protection from re-infection, and PASC.

W151. Mapping Immune Perturbations Associated with sars-cov-2 Infection in Infants

Djamel Nehar-Belaid[1], Asunción Mejías[2], Zhaohui Xu[2], Radu Marches[3], Duygu ucar[3], Jacques banchereau[4], Virginia Pascual[5] and Octavio Ramilo[2]
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Differences between COVID-19-induced immune alterations in adults and children have been previously described. However, no systematic immune profiling studies have been conducted in infants. We applied a multidimensional approach (single cell transcriptome, cytokine measurements and antibody titers), to decipher the immune responses in SARS-CoV-2 infected infants (n=26; 10 subacute, 11 moderate, and 5 severe; median [interquartile range] age: 1.63 [0.93-7.59] months) and matched healthy controls (n=14; 2.01 [1.86-4.28] months). Single cell (scRNA-seq) profiling of PBMCs revealed that severe COVID-19 in infants was associated with: (i) a prominent interferon-stimulated gene (ISG) signature involving many blood immune cell types, especially CD14+, CD16+ monocytes and CD4+ T cells, which contrasts with the attenuated ISG signature reported in adults with severe disease, (ii) a high frequency of CD14+ monocytes co-transcribing ISGs and inflammasome-related molecules; and (iii) an expansion of ISGhi proliferating cytotoxic CD8+ T cells. Serum cytokine analyses showed increased serum concentrations of inflammatory cytokines such as IL6 and IL8, but also IFN and IFN-induced cytokines such as CXCL10 and CXCL11. Antibody responses to SARS-CoV-2 were consistently detected in infants without evidence of anti-interferon autoantibodies. Comparison with adult scRNA-seq PBMC data revealed a markedly stronger ISG signature within T and B cells of infected infants. Steroid treatment was associated with the expansion of a subset of circulating CD14+ monocytes exhibiting CD163 and IL1R2, in both infant and adult patients. Our findings shed insight into the distinct immune responses to SARS-CoV-2 in the first year of life.

W152. Massive Microthrombosis in the Heart Muscular Tissues with Decreased ADAMTS13 Activity in a Lethal Case of Acute Heart Failure Following Mrna Vaccination Against Covid-19

Yuki Masuo[1], Hiroyuki Yoshitomi[2], Akihiko Sugimoto[3], Sachiko Minamiguchi[3], Hironori Haga[3], Takamoto Hirota[4], Kinta Hatakeyama[5], Yoshihiko Ikeda[5], Keiko Ogo[5], Masanori Matsumoto[6], Hideki Ueno[2], Hiroyuki Yoshitomi[2], Akihiko Sugimoto[3], Sachiko Minamiguchi[3], Hironori Haga[3], Takamoto Hirota[4], Kinta Hatakeyama[5], Yoshihiko Ikeda[5], Keiko Ogo[5], Masanori Matsumoto[6] and Hideki Ueno[2]

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Myocarditis has been shown to be one of the rare complications following immunization with mRNA vaccines against Covid-19. Although symptoms are mostly mild and self-limiting, lethal cases have also been reported. The pathogenesis of vaccine-associated myocarditis remains largely unknown and lack of appropriate animal models necessitates human immunology approach using samples from real world patients. Here, we performed an in-depth analysis of a case of lethal acute heart failure after the third mRNA vaccination. A previously healthy male in his fifties developed cardiac arrest sixteen hours after vaccination and deceased thirteen days later. Autopsy revealed infiltration of inflammatory cells into myocytes, confirming the diagnosis of myocarditis. Single-cell RNA sequencing of the pericardial effusion revealed a large proportion of macrophages expressing proinflammatory cytokine genes, including IL-1β, IL-6, IL-18 and TNF-α. Elevation of serum inflammatory cytokines at onset as well as the presence of bone marrow hemophagocytosis at autopsy demonstrated the presence of cytokine storm. Infiltration of neutrophils carrying IFN responsive gene signature was evident within the cardiac tissue. Additionally, massively distributed microthrombi were noted within the heart muscle tissues. Serum ADAMTS13 activity at onset was significantly reduced, but autoantibodies against ADAMTS13 were absent. We suspect that low ADAMTS13 activity together with activated neutrophils and vascular endothelial damage caused by the cytokine-storm seemed to coordinately contribute to the formation of microthrombi, resulting in the myocardial ischemia and subsequent cardiac arrest. In conclusion, our study demonstrates a possible involvement of systemic hyperinflammation and thrombosis in a lethal type of vaccine-associated acute heart failure.

W153. Protective Humoral and Cellular Immunity Induced by an Inactivated sars-cov-2 Vaccine in Phase 3 Clinical Trials

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An inactivated SARS-CoV-2 vaccine was approved for emergency use with significant efficacy and immunogenicity reported in several trials. In a randomized, multicenter, and controlled phase 3 trial in healthy adults, volunteers received two doses of the inactivated SARS-CoV-2 vaccine separated by 2 (0-14 schedule) or 4 weeks (0-28 schedule), with boosters given at 6 and 12 months. Humoral and cellular immune responses were evaluated by measuring the neutralizing antibodies and T cell activation. Both schedules exhibited robust neutralizing capacities, but the 0-28 schedule showed better responses. No differences were found in the concentration of antibodies against the virus and different variants of concern between schedules. PBMC stimulation with peptide mega pools induced the secretion of IFN-γ and the expression of activation-induced markers in CD4+ T cells, and significant correlations were observed. Although a 2.5-fold increase in ancestral SARS-CoV-2 neutralization was observed after the second booster when compared with prior its administration a reduced neutralization against the Omicron variant was detected. Activation of CD4+ T lymphocytes remained stable after the second booster and, importantly, equivalent activation of CD4+ T lymphocytes against the Omicron variant and the ancestral SARS-CoV-2 were found. Although the neutralizing response against the Omicron variant after the second booster was slightly increased, these levels were lower than those observed for the ancestral SARS-CoV-2. In contrast, robust CD4+ T cell responses may confer protection against the Omicron variant. Immunization with this inactivated SARS-CoV-2 vaccine promotes robust cellular and humoral immune responses, with a better profile in the 0-28 schedule.

W154. Severe Ancestral COVID-19 Leads to Enhanced Inflammatory Lipids Production in Plasma, Activation of DNA Damage Pathways in Pbmcs, and Upregulation of Eicosinoid Synthesis Genes in the Nasopharyngeal Mucosa

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The impact of severe SARS-COV-2 infection on lipid metabolism is not fully understood. To further explore this question, we performed total fatty acid analysis on plasma and bulk RNA-seq on PBMCs from 10 individuals infected with ancestral SARS-COV-2 and 10 age and disease matched uninfected participants. We compared this data with our ancestral SARS-COV-2 nasopharyngeal (NP) sc-RNA-seq dataset (Cell. 2021 Sep 2;184(18):4713-4733.). Univariate analysis between groups (Mann-Whitney U-test) demonstrated that six (6) lipids were significantly increased in SARS-CoV-2 participant plasma. These included the inflammatory lipids, Arachidonic Acid (AA) and Eicosapentaenoic Acid (EPA) which are both key contributors to eicosanoid production. AA, EPA, and the fatty acids, DHA and DPA, significantly correlated with WHO disease severity score in infected participants. When stratified by WHO score, heatmap analysis of bulk RNA-seq data from PBMCs demonstrated distinct transcriptional profiles. Ontology, KEGG and Reactome analysis identified several key pathways and nodes which were enriched for genes related to innate immunity, interactions between lymphoid and nonlymphoid cells and interleukin signaling. EPA message correlated with heightened cell cycling and DNA damage pathways in participants with WHO score >5. Furthermore, our ancestral SARS-COV-2 NP sc-RNA-seq dataset revealed that genes implicated in eicosanoid synthesis including alox5, alox12 and alox15B were up-regulated in NP cells including goblet cells, particularly in individuals with WHO score >5. Our study demonstrates that specific inflammatory lipids including EPA activate DNA damage pathways in PBMCs and lead to upregulation of eicosanoid synthesis genes at the mucosal surface in the nasopharynx in severe COVID-19 infection.

Immuno-engineering and Cellular Therapies

Th176. A Biosensor of Immunologic Synapse Formation for Multiplexed T Cell Antigen Discovery
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T cells recognizing self peptides play an important role in the development and regulation of autoimmune diseases. Identifying the specific T cell antigens involved in autoimmunity may yield improved diagnostics, disease classifications, and therapeutic targets. However, discovering T cell antigens for autoimmunity is a significant challenge, partly due to the diversity of potential tissue antigens and limited tools for multiplexed antigen mapping. To address this challenge, we have developed a synthetic biosensor that allows for the detection of immunologic synapse formation at a single cell level. Engineered antigen presenting cells (APCs) that autonomously present genetically encoded peptide antigens to T cells are equipped with a surface receptor that detects the mechanical pulling force between activated LFA-1 and ICAM1 at the immunologic synapse. Biosensor engagement triggers expression of a selectable marker so that APCs presenting relevant peptides can be isolated and sequenced for antigen identification. We have demonstrated efficient multiplexed identification of TCR targets from among hundreds of MHC class I or class II presented peptides. Our technology has potential to facilitate autoimmune T cell antigen discovery to improve immunodiagnostics and pave the way for precision immunotherapies.

Th177. Crispr-mediated Deletion of PD1 to Enhance the Efficacy of Human CAR Tregs
Dominic Boardman[1], Sonya Mangat[2], Jana Gillies[2], Vivian Fung[2], Manjurul Haque[2], Majid Mojibian[2], Andrew Brown[2], Avery Lam, Megan Levings[2], Sonya Mangat[2], Jana Gillies[2], Vivian Fung[2], Manjurul Haque[2], Majid Mojibian[2], Andrew Brown[2], Avery Lam and Megan Levings[2]
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Regulatory T cells (Tregs) are being tested clinically as therapeutic tools for treating transplant rejection and autoimmunity. Pre-clinical studies have shown that the efficacy of Tregs can be enhanced by using chimeric antigen receptors (CARs) to confer specificity for disease-relevant antigens, such as allogeneic HLA molecules in a transplant setting. However, recent data has suggested that PD1 signalling limits the activation potential of Tregs and may contribute to Treg dysfunction. We hypothesised that CRISPR-mediated removal of PDCD1 (encoding PD1) would improve the sensitivity of CAR Tregs to antigen stimulation, thereby enhancing their therapeutic efficacy. Human CAR Tregs were generated using a previously characterised HLA-A2-specific CAR, PD1 was removed by CRISPR and successfully edited cells were identified by incorporation of a homology directed repair DNA template that encoded a truncated CD19 (ΔCD19) reporter. A high degree of gene editing was achieved with >50% of the CAR+ Tregs expressing ΔCD19. When stimulated with HLA-A2+PDL1+ K562 cells, PD1-deficient CAR Tregs expressed higher levels of activation markers than PD1-sufficient CAR Tregs, demonstrating a greater sensitivity to antigen stimulation. However, these cells maintained their characteristic expression of FOXP3 and Helios following transfer into HLA-A2-transgenic NSG mice, suggesting PD1-ablation did not compromise Treg stability. Upon co-culture with mature HLA-A2+ dendritic cells, PD1-deficient CAR Tregs suppressed the expression of MHC class II and co-stimulatory molecules more effectively than PD1-sufficient CAR Tregs, demonstrating PD1-ablation enhanced the suppressive potency of CAR Tregs. Future work will test the therapeutic efficacy of PD1-deficient CAR Tregs in a humanised mouse model.

Th178. GNTI-122 Is a Stable Engineered Regulatory T Cell Therapy for Type 1 Diabetes That Is Specific, Expandable, and Efficacious in *in Vivo* Models

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Regulatory T cells (Tregs) drive immunosuppression and are appealing to address unmet therapeutic needs in autoimmune disorders, including type 1 diabetes (T1D). However, sorted cultured Treg cell therapies face difficulties including lack of phenotypic stability, suppressive specificity, expansion, and engraftment support. GNTI-122 is an autologous engineered Treg therapy for type 1 diabetes designed to overcome these barriers. GNTI-122 is generated from bulk CD4 T cells through CRISPR-Cas knock-in of an MND promoter to stabilize FOXP3, replacement of endogenous TCR with an islet antigen-specific TCR, and insertion of a rapamycin-activated chemically induced IL-2 signaling complex (CISC). This results in phenotypically stable engineered Tregs that target pancreatic islets and expand in subclinical concentrations of rapamycin. GNTI-122 displays characteristic Treg markers such as CD25, CTLA4, EOS, CD39, CD27, TNFRII, low CD127, low CD70, elevated LAP and GARP expression, and reduced pro-inflammatory cytokines (IL-2, TNF-a, IFNγ). GNTI-122 also demonstrates potent polyclonal and islet antigen-specific suppression in vitro. GNTI-122 uses CISC for pSTAT5 signaling in response to rapamycin alone. This allows rapamycin-dependent rapid expansion, enrichment of edited populations to >90% purity during culture, and tunable engraftment in vivo. Additionally, murine surrogates of GNTI-122 (mEngTregs) preserve beta cell mass after onset of pancreatic inflammation in an adoptive transfer NSG model. mEngTregs specifically traffic to the pancreas where they persist for 9+ weeks and significantly reduce effector memory T cell populations. Collectively, GNTI-122 is an engineered Treg that expresses a stable immunosuppressive phenotype, can be expanded with rapamycin-mediated signaling support, and exerts antigen-specific suppression in mouse models.

Th179. Reprogramming cd8$^+$foxp3$^+$treg to Deal with an Inflamed Environment

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Cell therapy using Tregs has been shown to be effective for the treatment of several immune diseases in preclinical and clinical studies. However, their stability and persistence after adoptive transfer in an inflammatory environment is a matter of safety and therapeutic interest. Here, we explored the sensitivity of CD8+Tregs to the pro-inflammatory environment, specifically the role of FOXP3 for Treg stability. Challenge of CD8+Tregs with the combination of proinflammatory cytokines IL-1b, IL-6 and TGFb shifted their suppression mechanism towards cytolysis and disrupted their phenotype with a significant reduction in FOXP3 expression. Thus, we generated a lentiviral vector encoding FOXP3 to increase and stabilize the expression of FOXP3 in CD8+Tregs. Indeed, the modified FOXP3TgCD8+ Tregs had increased expression of FOXP3, but also of CTLA-4, CD28, GITR and granzyme B, as well as increased suppressive activity in vitro. Importantly, ectopic FOXP3 expression compensated for the decrease in its endogenous expression, the increase in expression of PD1 and 2B4 and the decrease in expression in CTLA-4 induced by pro-inflammatory cytokine challenge. Furthermore, RNAseq analysis after pro-inflammatory cytokine challenge showed 18 genes differentially expressed in FOXP3TgCD8+ Tregs versus 141 in unmodified cells. Finally, we identified upstream regulators of FOXP3, whose ectopic expression increased FOXP3 expression and confered resistance to pro-inflammatory cytokine challenge. Experiments are underway to assess the requirement of FOXP3 for CD8+Treg function, evaluate the function and persistence of FOXP3Tg CD8+Tregs in a model of humanized acute GVHD in NSG mice, and further investigate regulators of FOXP3 expression.

Th180. RICORS-TERAV: The Spanish Model for Collaborative Research in Academic Cell Immunotherapy, for Transfering Knowledge to the Clinic or the Benefit of Patient

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RICORS-TERAV is a public network of 32 research groups and more than 350 academic (basic and clinical) researchers in cell therapy promoted by the Instituto de Salut Carlos III of the Government of Spain to promote collaborative research in Advanced Therapies and the clinical translation of this knowledge for the benefit of patients (from 2022 to 2024). With 4 coordinated programs, this summary presents the main aspects related to program "IMMUNOTHERAPY AND CAR T CELL PROGRAM". This program 2 is also named "Immunocell" and it is mainly focused in antitumoral Immunotherapy, specially CAR proposals, for obtaining therapeutic products that could be used in the Spanish public health system. It is structurated in 3 work packages (aims): WP1: Identification, development and validation of new engineered IT products with increased efficacy and safety (basic and preclinical research). WP2: Improvemente of production strategies for delivering better ATMPs products (translational research and innovations) & WP3: Aplication of innovative clinical engineered IT programs for the treatment of solid and hematological tumors (Clinical production, RWD analysis and applied research). In these early years, several Academic CAR-T developments are already under clinical trials. The authorization for use (under the so-called "hospital exemption" rule) is the first option for translating these products, a concept that in many countries it is being named the "Spanish Model".

Th181. Safety and Feasibility of Intradermal and Intranodal Administration of Vitamin d3-tolerogenic Dendritic Cells in Two Coordinated Phase I Clinical Trials in Active Multiple Sclerosis Patients

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Background: Tolerogenic dendritic cell (tolDC) therapy is a promising strategy to treat autoimmune diseases since they have the potential to re-educate and modulate the pathological immune responses in an antigen-specific manner. We have developed an autologous antigen-specific cell therapy based on vitamin D3 (VitD3)-tolDC loaded with myelin peptides for treating multiple sclerosis (MS) patients. Objectives: To demonstrate safety and feasibility of VitD3-tolDC treatment in MS patients in two phase I, open-label, dose-escalation single –center clinical trials, investigating two routes of administration: intradermal (id, MS-tolDC, NTC02618902, Belgium) and intranodal (in,TOLERVIT-MS, NTC02903537, Spain). Methods: 18 active MS patients were included in a dose-escalation best-of-five design: Cohort 1 (5x10^6 VitD3-tolDC), Cohort 2 (10x10^6), Cohort 3 (15x10^6). Each cohort received 6 id or in (in cervical lymph nodes) administrations of myelin-loaded tolDC (first 4 every 2 weeks and last 2 every 4 weeks). Clinical, MRI and immunological monitoring were performed between 6 and 12 months after last administration. Results: In 17 out of 18 patients enough tolDC vaccines were generated. No statistical differences between the

number of adverse events (AEs) per route of administration (center) or per cohort were found. No Serious Adverse Events occurred. Remarkably, 16 out of 18 patients did not show either statistically significant disease progression or MRI activity (lesion volume) 12 months after the last toIDC administration. Conclusions: Treatment with intradermal and intranodal myelin-specific tolDC in MS patients is safe, feasible and well-tolerated. A phase II clinical trial is currently in preparation.

Th182. Stem Cell-based Correction Ofbruton's Tyrosine Kinase in X-linked Agammaglobulinemia Disorder
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Primary immunodeficiencies (PIDs) are a group of inherited, monogenic disorders characterized by recurrent, severe, chronic infections, autoimmunity, and inflammation. The most common PID to affect males is X-linked Agammaglobulinemia (XLA), which is caused by the inactivation of the Bruton tyrosine kinase (BTK) gene. Without treatment, XLA patients do not survive past childhood. The mainstream treatment is immunoglobulin replacement therapy (e.g., intravenous, or subcutaneous delivery of immuno-globulin), a life-long therapy that begins soon after diagnosis. However, it is not curative, leaving patients at risk of developing severe respiratory tract infections and chronic lung disease. Lentiviral- and gamma retroviral-based gene addition strategies have been attempted, but they are not feasible therapies for XLA since overexpression of the BTK gene is known to drive B cell malignancies. For these reasons, we are developing a 'universal gene targeting-based correction' as a novel therapy for XLA, where the underlying genetic mutation is corrected in a patient's hematopoietic stem and progenitor cells (HSPCs), in support of BTK gene endogenous levels of regulation and protein expression. We have developed highly efficient gene-targeting tools and achieved over 50% allelic modification at the BTK locus, using a therapeutic BTK codon-optimized (co)cDNA, demonstrating the feasibility of this approach. In upcoming studies, I plan to use OMICs–cytometry by time of flight, single-cell RNA sequencing, and Assay for Transposase-Accessible Chromatin–in addition to xenotransplantation studies to characterize the BTK-modified cells for the safety and efficacy of this new therapy and to potentially generate new insight into the pathophysiology of XLA disease.

Th183. Super-agnostic Anti-cd28 Antibody Facilitates Treg Expansion *in vitro*
Rebecca Nickle, Lisa Chou, Rebecca Nickle and Bijou De Jong
BioLegend Inc.

Signals through CD28 provide important co-stimulation for optimal T cell activation. Anti-CD28 antibodies have been widely used as T cell costimulation reagents in T cell studies and/or T cell expansion. We recently developed a panel of new anti-human CD28 monoclonal antibodies. Preliminary characterization demonstrated that these antibodies can provide effective costimulation for T cell activation/proliferation with suboptimal TCR stimulation. In addition, this effort also identified antibodies that can activate human T cells to proliferate without TCR stimulation, indicating super-agonistic function of these antibodies. In vitro culture experiments showed that these super-agonistic antibodies promoted T cell survival and proliferation. Importantly, FoxP3+ Treg cells proliferated to greater extend under super-agonistic anti-CD28 stimulation than under routine anti-CD3/anti-CD28 activation. This study is to further characterize the functions of these new anti-CD28 antibodies and their potential application in Treg cell expansion and Treg cell function.

Th184. Tackling Post-transplant Lymphoproliferative Disease - A New Model of Patient Derived Lung Organoids with Epstein-barr-virus Transformed B Cells as Test Platform for Epstein-barr-virus Specific T Cell Fighters
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Immunosuppressed patients after solid organ transplantation (SOT) are at high risk to develop malignancies. Post-transplant lymphoproliferative disease (PTLD) is one of the most severe complications after SOT, which manifests in the lung in up to 20% of the cases and treatment usually involves highly toxic agents. Anti-viral adoptive T cell therapy represents a novel probably less toxic therapeutical approach, which has shown promising results among other indications also for PTLD. However, to confirm the safety and efficacy of these EBV-specific T-cell products as a basis for clinical translation, we are in urgent need of a suitable test platform. We had the unique chance to set up a human co-culture platform of patient derived 3D lung organoids and patient derived EBV-transformed lymphoblastoid cells (LCLs) mimicking PTLD in the lung, as we have access to primary lung tissue and blood samples from the same patient. In order to generate this platform, we already implemented a co-culture of primary lung organoids and corresponding LCLs. We have found a suitable co-culture medium and investigated LCL interaction and infiltration into the organoid. Patient-specific EBV-reactive T cell product manufacturing is already established in a GMP compatible manner, and we are now integrating the antiviral T cell products into the PTLD platform for testing safety, efficacy and potential off-target activity. This autologous, human-derived platform holds the potential to yield valuable preclinical data regarding functionality and safety of human EBV-specific T cell products and will pave the way for clinical trials using EBV-specific T cell products.

Th185. Tackling the HIV Reservoir with Hiv-resistant Anti-pd-1 CAR-T Cells

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Background: A successful strategy to cure HIV would require eradicating its reservoir, mainly represented by PD-1+ T follicular helper cells. Herein, we hypothesize that chimeric antigen receptor (CAR)-T cells redirected against PD-1 may represent an effective approach to target the HIV reservoir. Methods: Second-generation CARs were cloned using the scFv of a blocking (bPD1-CAR) and a nonblocking (nbPD1-CAR) anti-PD-1 monoclonal antibody fused to an IgG4 hinge, CD28 transmembrane and 4-1BB signaling domains with a cherry reporter. Anti-CD19 CAR-T cells were used as controls. The killing activity was measured using luciferase-based assays and a real-time quantitative live-cell imaging platform. HIV resistance against R5 and X4 HIV strains was tested by selectively editing the CD4 receptor and/or CCR5 and CXCR4 co-receptors in different combinations using CRISPR-Cas9 technologies. Results: The PD-1 binding affinity and KD values of the two anti-PD-1 mAbs were similar. Both anti-PD-1, but not CD19-control, CAR-T cells caused delayed depletion of PD-1+ CD4+ cherry negative T cells in ex-vivo cultures. Interestingly, the bPD1-CAR but not the nbPD1-CAR mediated tonic signaling, which was efficiently prevented by editing the endogenous PD-1. Both anti-PD-1 CAR-T cells equivalently killed PD-1high transgenic cells, yet the nbPD1-CAR was significantly less efficient in killing wild-type PD-1low target cells. CD4 editing was sufficient to confer HIV-resistance against R5 and X4 HIV strains without impairing the killing activity. Conclusion: We successfully engineered two anti-PD-1 CARs with different functional activities. In-vivo experiments in humanized HIV-infected mice are undergoing.

Th186. Tcf1^{+}cd8^{+} T Cells Expanded by Orally Administered Microbial-based Prodrug Augment Immune Checkpoint Blockade Therapy

Youngseok Cho, Kai Han, James Moon, Kai Han and James Moon
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T cell factor-1 (TCF1) is a key transcription factor associated with stem-like CD8+ T cells, and tumor-infiltrating Tcf1+CD8+ T cells have been implicated in proliferative anti-tumor efficacy of the anti-programmed cell death protein-1 (α-PD-1) targeted immunotherapy. Here, we screened gut microbial metabolites and identified a unique metabolite that promotes stem-like T cells. The metabolite increased the level of central memory CD8+ T cells (CD44hiCD62Lhi) and promoted a subset of Tcf1+CD8+ T cells in vitro. To improve the pharmacokinetics of the metabolite and prolong its in vivo circulation, we have developed a long-acting "prodrug 201" and engineered it into an orally available formulation. Orally administered prodrug 201 improved the anti-tumor efficacy of α-PD-1 therapy, exerting potent antitumor efficacy in multiple tumor models including CT26 colon carcinoma and B16F10 melanoma. Tumor microenvironment analysis revealed that the prodrug 201 combined with α-PD-1 therapy significantly increased the levels of T cells in tumor and lymph nodes as well as the ratio of M1 to M2 macrophages. These findings revealed that the gut microbial metabolites as a source of unique modulators of CD8 T cells. Further studies are underway to delineate the mechanism of interaction for prodrug 201-mediated induction of Tcf1+CD8+ T cells.

Th187. The NOD Disease Improved by Grafting the Islets Labeled with Nanoparticles to Facilitate the Immunosuppression and Graft Tracking

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Islet transplantation has been proven to be effective in treating patients with insulin-dependent diabetes mellitus (IDDM). However, suppression of immune rejection and graft tracking appears to be the primary concerns. Recent works done by others and our groups suggest a non-invasive and quantitative in vivo imaging system of superparamagnetic iron oxide nanoparticles (SPION) and magnetic resonance (MR) imaging for clinicians to monitor grafted islets in a real-time manner. To enhance the survival of grafted islets, here we have further developed the above contrast agent that is equipped with immune modulators including IL-10 and PD-L1 to avoid rejection and autoreactive immunity. The results have demonstrated that the application of such specific nanoparticles has led to the success of allotransplantation by extending the survival of transplanted islets. Moreover, the elevated level of blood sugar in IDDM, which results from the damage of islets by the autoimmune T cells, was reduced in non-obese diabetes (NOD) mice. Also, we have shown that the activating T cells could be induced to undergo apoptosis by PD-1 when interacting with the specific ligand, PD-L1. In addition, the agent could enable graft imaging by both IVIS and MRI if the agent was equipped with a reporter, luciferase. In summary, these results concluded that the grafted islets labeled with the multi-functional nanoparticles facilitated immunosuppression and graft tracking for allotransplantation as well as autoimmune diabetes in NOD mice.

Th188. Transfer of CD8^{+} T Regulatory Cells GMP Manufacturing for Clinical Trial in Kidney Transplanted Patients

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CD8+ Treg cell therapy is effective in preclinical models but has never been evaluated in a clinical trial. We designed a phase 1/2a clinical trial of cell therapy using GMP manufactured autologous polyclonal CD8+ Tregs to treat kidney transplanted patients from living donors. First, we set up a method for the isolation of CD8+Tregs from peripheral blood of healthy individuals using positive magnetic selection and flow cytometry in a safe closed system. We determined optimal clinical-grade culture conditions to preserve high proliferation rate, baseline phenotypic profile, and suppressive function in vitro and in vivo in a model of acute GVHD in NSG mice. We verified that they were resistant to classic maintenance immunosuppressive drugs, persistent and efficient in vivo but not cytotoxic. The method was validated on cells from patients with renal insufficiency, then transferred in the GMP facility with 3 validation runs demonstrating the phenotypic and functional stability of CD8+ Treg, meeting quality controls and release criteria. The study will be a first-in-human, one-arm, open-label, prospective trial in patients with end-stage chronic renal failure requiring primary kidney transplantation, with 3 escalating doses of CD8+Tregs administered the day before the transplant without induction treatment and associated with classic maintenance immunosuppression. Patients will be monitored for long-term safety, immunosuppression burden and occurrences of infections. Transcriptomic and proteomic analyses on blood, biopsy, and urine samples will inform on persistence and migration of Tregs to the graft.

Tu180. Characterization of CD19 CAR T Cells Produced in Hypoxia

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Chimeric antigen receptor (CAR) T cell research has exploded over the last decade. Great strides have been made in optimizing CAR T therapy for several cancers, with much focus being placed on understanding the inhospitable tumor microenvironment, in the hopes of being able to produce CARs that maintain their functionality despite factors such as poor access to nutrients and low oxygen levels. Cell culture usually takes place at 21% O2. It has been reported that this is not optimal for culture and production of T cells, due in part to their overproduction of reactive oxygen species in response to high oxygen tension. In situ, T cells are never exposed to oxygen levels greater than 14% and often function at substantially lower levels. CAR T cells are often expanded for up to two weeks before being administered. Prolonged exposure to 21% O2 and the corresponding overproduction of reactive oxygen species may abrogate their function and survival in vivo. Using in vitro methods including repeated exposure to stimulus and assessment of mitochondrial fitness, we demonstrate that CD19-targeted CAR T cells can be produced in significantly lower oxygen tensions and retain their functionality, without appearing more exhausted than their counterparts produced at 21% O2. This project aims to highlight the impact of oxygen exposure during production as a possible reason for the lack of significant progress in treating solid tumors and other tumor diseases, such as chronic lymphocytic leukemia, that create inhospitable environmental pockets where CAR T cell function might be negatively impacted.

Tu181. Chimeric Autoantigen-t Cell Receptor (CATCR)-T Cell Therapies to Selectively Target Autoreactive B Cells

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All currently available drugs to treat autoimmune diseases are indiscriminate, suppressing both self-directed and protective immune responses. This lack of precision results in opportunistic infection, poor vaccine responses, and excess mortality. Precision immunotherapies that selectively eliminate autoreactive B cells, while sparing normal B cells, could treat autoimmunity without increasing the risk of infection, revolutionizing the management of patients with autoimmune diseases. Here, we developed such a precision therapy for the treatment of antiphospholipid syndrome (APS), an autoimmune disease caused by pathogenic antibodies against beta-2-glycoprotein I (β2GPI) that promote recurrent thrombosis and fetal loss. This was achieved by reprogramming primary human T cells to selectively eliminate anti-β2GPI B cells. To this end, we used CRISPR/Cas12a editing to express chimeric autoantigen-T-cell receptors (CATCRs), which incorporate the autoantigen β2GPI into different parts of the TCR-CD3 complex, on engineered T cells. By expressing β2GPI-CATCRs, T cells acquired the ability to selectively bind anti-β2GPI B cell receptors (BCRs) on the surface of autoreactive B cells in APS, inducing immune synapse formation and killing target B cells via the perforin–granzyme pathway. In co-culture experiments, β2GPI-CATCR-T cells selectively eliminated anti-β2GPI B cells and abolished anti-β2GPI autoantibodies —pathogenic drivers of thrombosis and fetal loss in APS— in a dose-dependent manner, providing an opportunity to treat and prevent APS. Complete target cell killing was observed at physiologically relevant effector:target cell ratios. Beyond APS, CATCR-T-cell therapies have the potential to treat autoimmunity without increasing the risk of infection, promising a future of antigen-specific immunotherapy for autoimmune and rheumatic diseases.

Tu182. Circular RNA AEBP2 Regulates Dendritic Cell Mediated Anti-tumor Immune Response

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Dendritic cells (DC), acting as a bridge between innate and adaptive immune responses, play an important role in anti-tumor immunity. Recently, circular RNA molecules have emerged as new regulators of DC development and show promise in optimizing DC-based immunotherapies; however, the effect of circRNA in DC modulation requires further investigation. Our study examines the role of circRNA AEBP2 (circAEBP2), where we have found circAEBP2 to be upregulated in mature DCs and downregulated in tolerogenic DCs. We hypothesize that circAEBP2 aids in DC maturation and enhances the anti-tumor immune response elicited by a DC-based vaccine in a murine melanoma model. Bone marrow derived DCs were cultured from C57BL/6 mice, followed by circAEBP2 silencing with siRNA or circAEBP2 overexpression through transfection with RNA from B16-F10 murine melanoma cells pre-transfected with circAEBP2 expressing plasmids. Anti-tumour effect of circAEBP2-modified DC vaccines were tested in a murine melanoma model. Silencing of circAEBP2 arrested DCs in an immature state and decreased their activation of allogenic T cells in favor of regulatory T cells (Treg) induction. Treatment with circAEBP2 over-expressed DC vaccine in vivo delayed tumor onset, reduced tumor growth, and enhanced activation of inflammatory T cells and reduced immunosuppressive Treg generation. We demonstrate that circAEBP2 regulates the development and function of DCs through activation of NF-κB via the hnRNP F/HMGB1/P-p65 signaling pathway. In conclusion, circAEBP2 regulates the development and function of DCs and enhances the anti-tumor effect of DC-vaccines in melanoma, highlighting the circularAEBP2-DC axis as a new promising target for development of effective DC vaccines.

Tu183. Clinical Characteristics Associated with Early Immune Reconstitution in Patients with Congenital Athymia After Treatment with Allogeneic Processed Thymus Tissue-agdc

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Congenital athymia (CA) is an ultra-rare disorder that results in severe immune deficiency and dysregulation due to lack of naïve T cells. Allogeneic processed thymus tissue-agdc is approved for immune reconstitution in pediatric

patients with CA (typical and atypical phenotypes), based on increased naïve T-cells after implantation through month 24. In the efficacy analysis population (n=95), mean age at implantation was 298 days, 52.6% had the typical phenotype, 65.3% received immunosuppressants, 93.7% experienced an infection-related adverse event (AE) in year 1, and 72.6% were alive at last follow-up. The analysis aims to identify clinical features associated with early immune reconstitution post-implantation, defined as >100 naïve CD4+ cells/mm3 at 6 months (M6). This was achieved by 26.9% (18 of 67) of patients vs 70.2% (40 of 57) who achieved immune reconstitution at 12 months (M12). Patients with early immune reconstitution had a lower mean age at implantation (M6=132.1 days; M12=232.4 days), the majority had a typical phenotype (M6=72.2%; M12 62.5%), and immunosuppressant medications decreased over time (M6=38.9%; M12=27.5%). The proportion of patients with infection-related AEs was lower in the early reconstitution group (M6=61.1%; M12=85.0%). All patients in both groups were alive at last follow-up. In summary, earlier age at implantation, typical phenotype, and decreased immunosuppressant medication use could contribute to earlier immune reconstitution in some patients receiving allogeneic processed thymus tissue-agdc. Although early immune reconstitution is associated with fewer infection-related AEs; patients carry a high risk from infections in the first-year post-implantation.

Tu184. Colon Cancer Immune Therapy and Prophylaxis
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Problem: Cancer resistance in colon cancer is linked to over expression of Factor H and its variants (Wilczek Ewa et al. in Int. J. Cancer: 122, 2030–2037 (2008)). The data suggests the inefficiency of immunotherapy with monoclonal antibodies is due to presence of complement immune regulators such as Factor H. Further this resistance can be over come by Factor H Antibody. Method: We have developed a balanced modulator of Factor H and D to overcome complement mediated immune resistance. Discussions: Overcoming immune resistance and inflammatory cascade of complement over activation will lead to improve immune therapy with current and evolving colon cancer therapies. Further it may also be used for prophylaxis for colon cancer in select subgroup of patients.

Tu185. Combining Human CD4$^+$ and CD8$^+$ Treg to Inhibit Experimental Acute GVHD
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Combining CD4+Tregs and CD8+Tregs for cell therapy would increase the number of starting Tregs, reducing the volume of blood drawn from patients; would simplify cell isolation, saving time and costing less; and could be more efficient by recognizing different antigens. Here, we explored the differences and benefits of combining them. Blood CD4+Tregs compared to CD8+Tregs displayed significantly lower methylation in the FOXP3-TSDR, 253 genes DE, and higher efficiency in suppressing CD4+ Tconv responses in vitro. In contrast, after overnight polyclonal activation, transcriptomic comparison showed no DE gene between subsets, similar efficacy in vitro, and their combination resulted in more efficient suppression than each individually. After separate expansion under pro-regulatory conditions, the combination of a half dose of CD4+Tregs and CD8+ Tregs in a model of humanized acute GVHD in NSG mice resulted in therapeutic effect similar to that of a full dose of CD4+ or CD8+ Tregs, and their addition improved long term mouse survival to 66%. To enrich for both FOXP3+ CD4+ and CD8+ Treg, we isolated T cells based on the CD45RClow/- expression marker and expanded them under pro-regulatory conditions. CD3+CD45RClow/- cells displayed similar suppressor efficacy to isolated CD4+ or CD8+Tregs both in vitro and in the GVHD NSG mouse model, without inducing any toxicity, when using a 1.5x doses, which should be largely offset by the production yield. CD4+ and CD8+Tregs show similarities and their complementarity for Tconv control and it is possible to isolate them from blood using only the CD45RClow/neg marker and appropriate culture conditions.

Tu186. Engineering Genetic Circuits to Promote Tumor Allorejection for Immunotherapy of Solid Cancers
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In cancer immunotherapy, there is a need for clinically relevant therapies that tackle solid cancers and minimize systemic toxicity experienced by patients. We utilize genetic circuits that force tumors to release therapeutics that promote localized immune responses, without harming healthy tissues. This project's aim was to promote tumor-specific allorejection in mouse strains. We developed a genetic circuit that selectively "hijacked" tumors of the C57BL/6 mouse strain, forcing them to express immunologically foreign alloantigens derived from a different strain, BALB/c. Consequently, C57BL/6 tumors would become "foreign" to the body, inducing tumor elimination via CD8 T-cell mediated allorejection. This circuit comprises 3 modules: Modules 1 and 2 (TF-sensor modules) harness tumor-specific activity of transcription factors and promoters; driven by TF-sensor modules, Module 3 (output module) enables tumors to express spleen-derived BALB/c alloantigens. Sensors of 20x and 6x tumor specificities were selected, following flow cytometry with ovarian cancer and melanoma cell lines. For complete circuit assembly, tumor cells (BPPNM, YUMM1.7) were lentivirally infected with all 3 modules and injected into C57BL/6 mice for in vivo validation. BPPNM mice treated with alloantigen-genetic circuits had 51.1% less tumor burden than the non-therapeutic negative controls' tumors 5 days post-tumor inoculation. YUMM1.7 alloantigen-treated mice had mean tumor burdens ~4x lower than negative control mice 6 days post-tumor inoculation. Alloantigen-treated tumors grew slowly, persisting 19 days post-tumor inoculation. Negative control tumors grew aggressively, prompting euthanization 10 days post-tumor inoculation. These in vivo findings suggest preliminary anti-tumor efficacy of alloantigen-genetic circuits via T-cell responses and lower systemic toxicity.

Tu187. Enhancing TCR Efficacy Through CD3ζ Modifications
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Modified T-cells are currently being rapidly developed for use as immunotherapies, with chimeric antigen receptor (CAR) T-cells currently being used to treat patients with B-cell malignancies, via CD19 targeting. However, despite their success CAR T-cells are limited by their exclusive recognition of proteins present on the cell surface. One way to expand the range of target antigens is to utilise T-cell receptor (TCR) mediated recognition. The TCR interacts with peptides presented by MHC molecules allowing the recognition of intracellular as well as cell surface proteins. For efficient expression in T cells, the TCRα/β chains form a complex with four CD3 chains, one of these being CD3ζ which is key in promoting signalling after TCR binding. Here we explore whether modifications to the CD3ζ chain can enhance TCR signalling and T cell function. These modifications include the addition of the co-stimulatory domains CD28 or 4-1BB, at the membrane proximal or the membrane distal position of the intracellular tail of CD3ζ. Our preliminary results show that the CD28 can improve TCR signalling and enhance T cell effector function. We will present a detailed analysis of how CD28 and 4-1BB signalling domains incorporated into two different positions of the CD3ζ chain affect TCR stimulation and T cell effector function. The modification of the CD3ζ chain provides an opportunity to improve TCR gene therapy by incorporating the conventional TCR signal 1 and co-stimulatory signal 2 in one molecular complex.

Tu189. Expansion of EBV Peptide-specific CD8 T Cells from Multiple Sclerosis Patients and Healthy Donors Reveals Dysregulation of Effector Responses that May Be Associated with Disease Pathogenesis
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Although 90% of adults are infected with EBV, recent studies have rekindled a role for this ubiquitous agent in the pathogenesis of MS. To assess whether Multiple Sclerosis (MS) is associated with defective T cell control of EBV-infected B cells, we employed a nanoparticle (np) based Artificial Immune Modulation (AIM) platform consisting of a cocktail of six EBV peptides presented on np decorated with HLA-A2 and anti-CD28 molecules to enrich, expand, and characterize EBV specific CD8+ T cells from healthy and MS HLA-A*02:01 donors. After 14 days, expanded cells were assessed for specificity and functionality as measured by intracellular cytokine staining and killing of EBV peptide loaded target cells. The expanded CD8+ T cells showed significant antigen specificity as shown by intracellular cytokine release and/or cytotoxic activity, which is consistent with previously published data. Interestingly, compared to heathy donors, such cells from MS patients exhibited functional defects in their responses to select EBV peptides. These results, analyzing the functional responses of EBV specific CD8 + T cells expanded from healthy donors and patients with MS, represent our initial effort to interrogate the hypothesis that MS may be associated with defective T cell control of EBV infected cells, which is consistent with a number of reports suggesting that a dysregulation of EBV specific immune responses is associated with the pathogenesis of MS creating potential opportunities for new therapeutic approaches in MS.

Tu190. T-cell Mediated Immune Responses Induced by Anti-cd19 Chimeric Antigen Receptor (ARI-0001) in Patients with CD19 Positive B-cell Malignancies

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Background: ARI-0001 cells express a second-generation CAR that combines the anti-CD19 single-chain variable fragment (scFv) originated from A3B1, a murine monoclonal antibody, conjugated with the transmembrane and co-stimulatory domains. Although the efficacy and safety of ARI-0001 cells has been demonstrated, the relevance of the cellular immune responses induced by ARI-0001 cells has not been fully investigated. Methods: Regarding cellular immune response, PBMCs from treated patients were stimulated with 35 HLA-II predicted peptides derived from CAR19 and CD4+ T cells were sorted through activation induced markers CD154 and CD69. After 30-35 days of expansion, CD4+ T-cell lines were screened with peptide pools and then individual peptides. Finally, it was blocked HLA-DQ, DP and DR during the individual peptide stimulation to explore which HLA present the peptide which triggers the T-cell activation. Results: In total 43 cell lines were obtained from 5 different patients who developed human anti-murine antibodies. Eight out of 43 cell lines were reactive against CAR19 peptides after 30-35 days of expansion. In relation to CAR19 peptides, immunogenic peptides for these eight cell lines were located at scFv (VL, linker and VH) and were presented by HLA-DR. Conclusions: These data suggest that ARI-0001 cells induce cellular immune responses. The most immunogenic domain of CAR19 is the extracellular part from murine monoclonal antibody. Even though scFv peptides induce CD4+ T cell activation, the detected immunogenic sequences do not include CDR regions. Finally, immunogenicity induced by ARI-0001 cells could be clinically important, specially before considering a second infusion.

W155. Cart-cell Therapy Directed Towards Novel CD84 Antigen for the Treatment of Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) is an heterogeneous malignancy with scarce treatment options in the relapsed or refractory (R/R) setting. CART cells have revolutionized the treatment of R/R multiple myeloma and B-cell malignancies; however, none has yet been approved for AML. CD84 (SLAMF5) is a member of the SLAM family of cell-surface immunoreceptors. Here, we validate the CD84 antigen as a novel target for AML and present a first-in-class CD84-directed CART cell (CART84) for AML. Patient primary AML blasts were analyzed by flow cytometry: while CD84 was overexpressed on their surface in the majority of AML cases, both de novo and at relapse, its expression was very low in CD34+ healthy hematopoietic stem/progenitor cells (HSPC). Moreover, healthy human tissues were analyzed by immunohistochemistry and CD84 expression was not found in the lung, liver, kidney, myocardium, skin, or brain, except in tissue monocytes/macrophages. We engineered second-generation CART cells with murine or fully human single chain variable fragments (scFv) and 4-1BB as a co-stimulatory domain (anti-CD84scFv-CD8αTM-4-1BB-CD3). CART84 exerted high cytotoxicity in vitro towards AML cells even at low effector:target ratios. In terms of safety, CART84 displayed a very low cytotoxic effect towards CD34+ HSPC, suggesting that CART84 may be less myelotoxic than other CART cells in AML. CART84 eliminated AML cells in vivo in a NSG mouse model using both AML cell lines and a patient-derived-xenograft (PDX). In summary, these results validate CD84 as a potential target for AML CART-cell therapy and support the therapeutic use of CART84 for R/R AML patients.

W156. Crispr/cas9-based Editing of KLRC1 Enhances the Efficacy of Primary cd33-targeting CAR-NK Cells Against Acute Myeloid Leukemia

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Chimeric antigen receptor (CAR) natural killer (NK) cells show strong antileukemic activity against acute myeloid leukemia (AML) in vivo. However, NK cell-mediated tumor killing is often impaired by tumor-mediated immune cell inactivation. Acute Myeloid Leukemia (AML) still represents a severe disease with limited therapeutic options, especially for elderly patients. Recently, we reported on the successful generation of primary CD33-targeting CAR(CAR33)-NK cells, which are highly effective against AML in vitro as well as in AML-xenograft mouse models (Albinger et al. BCJ 2022). Yet, CAR-NK cell function can be impaired by high levels of the inhibitory immune checkpoint receptor NKG2A (natural killer group 2A) expressed on NK cells (Bexte et al. Oncoimmunology 2022). Here, we describe a novel strategy to overcome CAR-NK cell inhibition mediated by the immune checkpoint NKG2A, which interacts with HLA-E expressed on AML blasts. We generated AML-targeted CD33-CAR (CAR33)-NK cells combined with CRISPR/Cas9-based gene editing of the NKG2A-encoding KLRC1 gene. Single-cell multi-omics analyses revealed a high proportion of activated cells in CAR33-NK and CAR33-KLRC1ko-NK pools, which was preserved following exposure to AML cells, and which correlated with improved antileukemic activity against AML cell lines and primary blasts in vitro and in vivo. We conclude that dual modified NK cells have the potential not only to bypass the suppressive effect of AML but also of a broad range of other cancer entities.

W157. Fine Tuning CD19-CAR Sensitivity by Modulating the CD28 Transmembrane and Intracellular Domains

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CD19-CAR T cells are emerging as promising therapies, yet exposed to on- and off-target toxicities possibly resulting from the formation of CAR-CD28 heterodimer. Modulations and functional implications of CAR-CD28 interactions have not been thoroughly studied. We engineered 2nd generation CD19-CARs with either a CD28ζ or 4-1BBζ intracellular-domain (ICD) with selected mutations in the CD28 transmembrane-domain (TMD) targeting: (A) the CD28 evolutionary conserved YxxxxT motif (M1a=Y166L, M1b=T171L, M2=LxxxxL), (B) the CD28-core polar amino acids (CYSxxxT->LLLxxxL=M4), (C) the GxxxA dimerization motif (to YxxxL=M5). Control wild-type CD28-TMD-CAR (WT-CAR) was used. CARs were linked to an EGFRt reporter and inserted into the TRAC locus of primary human T cells to control genomic integration. Single-cell sorted clones with increasing CD19 levels were generated. Except for the M5 construct, all four mutants significantly reduced CAR-CD28 formation independently of the ICD assessed by the division index to anti-CD28 mAb stimulation and confirmed using a split fluorescent protein system. Mutating the threonine (M1b) strongly reduced cell-surface CAR expression and sensitivity against CD19 -low but not -high targets. CAR expression was progressively restored in the M2, and M4 CAR construct, respectively, although it remained inferior to WT-CAR and was ICD dependent. Interestingly, the M4-CAR had lower on-target sensitivity than WT-CAR only in the context of a 4-1BBz ICD. Our data suggest that the TMD conformation dictates CAR expression level and its interaction with endogenous CD28. CAR T cell sensitivity can be modulated using selected TMD-ICD pairs, opening the perspectives to engineer safer CAR-T cell products for non-oncological applications.

W158. Generation of TIM-3 Inhibitor Single Domain Antibody by Phage Display Technique

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Background: T cell immunoglobulin and mucin domain 3 (TIM3) is one of the major inhibitory immune checkpoints expressed on T cells. Blocking the intraction between TIM3 and its inhibitory ligand galectin-9 may potentiate the effects of immunotherapy or overcome the adaptive resistance . Based on the unique features of nanobodies, we aimed to construct an anti-TIM-3 nanobody as an appropriate tool for manipulating immune responses for future therapeutic purposes. Methods: We immunized a camel with TIM-3 antigen and then, synthesized a VHH phage: prepared library from its B cell's transcriptome using nested PCR. Library. selection against TIM-3 antigen was performed in three rounds of panning. Using phage-ELISA, the most reactive colonies were selected for sub-cloning in soluble protein expression vectors. The Nanobody was purified and confirmed with a nickel-nitrilotriacetic acid (Ni-NTA) column, SDS-PAGE and Western blotting. A flowcytometric analysis was performed to analyze the binding and biologic activities of theTIM-3 specific nanobody with TIM-3 expressing HL-60 and HEK cell linesAn explanation of the study design and experimental method. Results: Specific 15kD band representing for nanobody was observed on the gel and confirmed with Western blotting. The nanobody showed significant specific immune-reactivity against TIM-3 with a relatively high binding affinity. The nanobody significantly suppressed the proliferation of TIM-3 expressing HL-60 cell line. Conclusion: we successfully prepared a functional anti-humanTIM-3 specific nanobody with a high affinity and an anti-proliferative activity on an AML cell line in vitro.

W159. Human Engineered Tregs Maintain Stability in Inflammatory Environment

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Regulatory T cells (Tregs) are essential for keeping the immune system in check, thus dysregulation of Tregs can lead to autoimmune disorders. Treg cell therapy has the potential to address this problem, however limitations of sorted and cultured Treg (cTregs) cell therapies include inherent plasticity and instability. Indeed, cTregs can lose FOXP3 expression and acquire the ability to express T effector (Teff) cytokines in non-favorable environments. To address these limitations, we have engineered human CD4 T cells into Tregs (EngTregs) endowed with stable FOXP3 expression and a rapamycin-activated, chemically induced IL-2 signaling complex (CISC). To address the stability and functionality of EngTregs, we compared them to cTregs at both steady state and in inflammatory conditions. At steady state, EngTregs demonstrate enrichment of core T regulatory cell gene signatures (CTLA-4, IL2RB, TNFRSF1B, TNFRSF18) compared to cTregs. EngTregs but not cTregs, maintain stability as measured by expression of FOXP3, CD25 and other Treg stability markers (e.g. EOS, CD27+CD70-). Stable FOXP3 expression in EngTregs is reflected in their suppressive activity against Teff cells. Importantly, EngTregs maintain FOXP3 expression in inflammatory environments and secrete IL-10 similarly to cTreg. However, unlike cTregs, EngTregs express little or no key Th2 cytokines (e.g. IL4 and IL13) in such environments. Engineering human Tregs has shown the potential to overcome key limitations of sorted Treg therapies by enhancing their stability and function and thus providing a novel therapeutic modality to restore immune tolerance.

W160. IL-10-producing Dendritic Cells Modulate B Cell Responses

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Tolerogenic dendritic cells (tolDC) play a critical role in promoting antigen(Ag)-specific tolerance. We developed a method to efficiently generate tolDC by genetic engineering of monocytes with lentiviral vectors encoding for human IL-10 (DCIL-10). DCIL-10 secrete supra-physiological levels of IL-10, modulate CD4 and CD8 T cell responses and promote allo-specific Type-1-regulatory T (Tr1) cells in vitro and in vivo. Since IL-10 has been shown to modulate B cell responses, we aimed at defining whether DCIL-10 modulate B cell responses in vitro in terms of activation and maturation in T-cell independent and dependent manner. Results revealed that proliferation, percentage of antibody-secreting cells and isotype switching were increased in naïve and memory B cells activated in the presence of DCIL-10. In memory B cells activation in the presence of DCIL-10 significantly decreased the IgG membrane expression, while increasing the IgG secretion. DCIL-10-mediated modulation of naïve B cell occurred in cell-to-cell contact, while modulation of memory B cells is IL-10 dependent. Memory B cells stimulated with DCIL-10 are less effective than those stimulated with control DC to promote allogeneic T cells activation. Finally, DCIL-10 promotes survival without modulating phenotype and isotype switching when naïve and memory B cells were activated in a T-cell dependent manner. In conclusion, for the first time we report that tolerogenic IL-10-producing DC directly modulate B cells in vitro. In vivo investigations are ongoing to better define the impact of DCIL-10 on B cell responses in vivo.

W161. Immune Reset in B Cell Mediated Autoimmune Diseases Using BCMA-CD19 Compound CAR (cCAR) in an Open Label Phase I Clinical Trial

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Vincent DeStefano[4], Masayuki Wada[4], Kevin Pinz[4], Greg Deener[4], Yu Ma[5], Min Wang[2], Fugui Li[2], Ming Hong[2], Lan Ting[2], Chanjuan Zou[2], Mingxia Wang[2], Ling Ding[2], Yingwen Liang[2], Yupo Ma[4] and Weijia Wang[2]
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Seventeen patients (Lupus-12, NMOSD-1, transplant DSA-4) dosed 7 Sept 2019 to 19 Dec 2022. Cessation of immunosuppressing medications prior to cCAR. Monthly IgG until B cells recovered. Pre/Post cCAR percent total B lymphocytes, BCR sequence measured in subset. Age range: 20-58, 15 females/2 males. Lupus (12 patients): mean baseline SLEDAI-2K=10.1, mean 4 elevated autoantibodies, 8/12 low C3. Results: cCAR well tolerated, no SAEs or cCAR related infection, no CRES, no CRS >Grade 1, mild fever only (< 40OC) resolved in a week. B cells depleted in 3-14 days. IgA, IgM undetectable at ~1 month. Autoantibodies negative 1-4 months (most undetectable 2 months). An immune reset confirmed via flow cytometry revealed predominantly naïve and non-class switched B cells Post-cCAR, as compared to Pre-cCAR (memory and class switched B cells). BCR deep sequencing (patients 3-4), showing IgG and IgA clonotypes are absent and non-class-switched BCR repertoires >95% IgM heavy chain. All patients treated >6 months in remission (SLEDAI-2K = 0, autoantibodies negative, normal complement including C3, no medications). Lupus symptoms improve quickly, 2.7 mean 1 month. Transient increase in cytokines Post cCAR returning at or below baseline in 3-6 months (no immunosuppression). All B cells recovered in 2-6 months with no relapses (7 of 1st 9 lupus pts SLEDAI-2K=0 by 3 months, no relapses). 1st patient at 38 months: no symptoms, no autoantibodies, no medication. Conclusion: Well tolerated immune reset achievable & homeostasis maintained without need for continuous treatment. Monitoring patients to ensure reconstituted their immunocompetent B cells & maintain long-term remission.

W162. Impact of Epichaperome Inhibitor PU-H71 on Anti-tumor T Cell Responses
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The epichaperome is a tightly integrated protein network with critical roles in tumor-associated microenvironment and cancer cell survival. The epichaperome inhibitor PU-H71 is under development in clinical trials. We tested the effects of PU-H71 on C57BL/6 mice responses to OVA. When administered at the time of priming, PU-H71 significantly increased the number of IFNγ-secreting T cells but had no effect once the OVA-specific T cell response was ongoing. PU-H71 improved in vivo anti-tumor immune responses in OVA-primed mice challenged with E.G7 (OVA+) but not EL4 parental cells (OVAneg). Lethally-irradiated B6C3F1 mice received bone marrow and splenocytes from C57BL/6 mice and were treated with PU-H71 at first signs of GvHD. We found that PU-H71 did not aggravate GvHD. We evidenced that epichaperome indeed forms in human CD19.4-1Bb.3z CAR-T cells upon activation. In vitro cytotoxicity of CAR-T cells toward NALM6 B-ALL cells was significantly improved by PU-H71. Finally, the therapeutic effect of the combination of PU-H71 and CAR-T cells was evaluated in NSG mice using NALM6-BLIV cells, with bioluminescence imaging and flow cytometry monitoring. PU-H71 administration increased CAR-T cell numbers in the bone marrow and improved their in vivo antileukemic efficacy against NALM6. These results show that PU-H71 augments antigen-specific T cell responses during priming, such as CAR-T cell cytotoxicity, with little effect on ongoing immune responses, including GvHD. PU-H71 also has an adjuvant effect on anti-tumor T cell responses while not aggravating GvHD, supporting its clinical evaluation in the post-transplant setting or in combination with CAR-T cells.

W163. Improved Immunogenicity and Stability of RBD of sars-cov-2 by Polymer Nanoparticles
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Translational Health Science and Technology Institute, NCR Biocluster, Faridabad, Haryana, New Delhi, Delhi, India

The pandemic caused by SARS-CoV-2 has led to 546 million cases and 6.3 million deaths worldwide. The receptor binding domain (RBD) of SARS-CoV-2 has emerged as the prime vaccine candidate. Purified RBD is, however,

weakly immunogenic and requires the use of multiple doses and adjuvants. Further, all the current SARS-CoV-2 vaccines require cold chain storage that incurs heavy cost. Biodegradable polymer particles as vaccine delivery system offer the advantages of improved immunogenicity, stability and modulation of immune response. In the current investigation, RBD of SARS-CoV-2 was delivered through polymer particles and evaluated in mice model. Results suggest that polymer particles are capable of mounting better and sustained antibody response without the use of alum. Further, the particles showed improved stability and could be safely stored outside cold chain. Thus, using biodegradable polymer particles for vaccine delivery offer improved immunogenicity and reduce reliance on cold chain. This can significantly reduce cost and increase the access of vaccines to resource limited settings given the inequitable distribution still looms the pandemic.

W164. In Situ Immuno-engineering of Host T-cells Using an Acellular Approach for Solid Tumors
Prakriti Tayalia, Hiren Dandia, Mamatha Pillai, Deepak Sharma, Arvind Ingle and Shubhada Chiplunkar
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T-cell based therapy is well-known for hematological malignancies, while solid tumors still pose a challenge as they are localized, protected from circulating T-cells, and heterogeneous. Additionally, ex vivo genetic modification of large numbers of tumor-specific T-cells is elaborate, expensive and time consuming. We have developed a porous, biocompatible scaffold for immobilization of lentiviruses carrying tumor-specific antigen receptor genes. These scaffolds, when implanted subcutaneously in mice, show no systemic inflammation and facilitate preferential recruitment of T-cells. Engineered T-cells when co-cultured with tumor cells in vitro show tumor cell killing accompanied with increase in anti-tumor cytokines. These scaffolds, when implanted in tumor bearing mice, enable recruitment and transduction of host T-cells with tumor antigen specific receptors. Tumor-specific T-cells home towards tumor as well as secondary lymphoid organs, thereby, leading to significant reduction in tumor size and a systemic increase in anti-tumor cytokines. Here, a material-based engineering of T-cells to provide a localized approach for solid tumor T-cell based treatment is presented. This could be developed as an alternative to ex vivo genetic programming of T-cells.

W165. *In Vitro* Expansion of Antigen-specific $CD8^+$ T Cells from Human Pbmcs Using Artificial T-cell Stimulating Microparticles
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While current T cell-based immunotherapies have shown moderate success, clinical translation of these methods has been hindered by biological variability, limited efficacy, and high expense. Here, we create a novel T cell stimulating platform for antigen-specific CD8+ T cell activation and expansion, eliminating the extensive ex vivo steps and thus making it accessible for many cancer patients. The scaffold is formed using thiol-modified hyaluronic acid cross-linked with PEGDA and adding DTT-reduced MHC-Ig dimer HLA-A*02 (A2) and anti-CD28. The gel is passed through a mesh device to form microparticles (MPs), which are then passively loaded with peptides for ease of multiple expansions. They were plated with human PBMCs thawed in culture media supplemented with human AB serum and a cocktail of cytokines (IL-1b, IL-2, IL-4, IL-6, and IFNγ). On days 7 and 14, cells were harvested and analyzed for proliferation of antigen-specific cells. After fourteen days of culturing PBMCs with A2 MPs, we could detect a significant expansion of antigen-specific T cells via flow cytometry. When stimulated with peptide-pulsed target cells for an intracellular cytokine assay, they expressed one or more of IFNγ, TNFα, and IL-2, demonstrating their polyfunctionality. We also observed a greater population of memory T cells, which indicates that these cells may persist longer in the body. We have developed a biomaterial-based T cell activation platform that provides critical signals, mimicking the lymph nodes. These MPs are effective in expanding antigen-specific T cells from human PBMCs, and these cells have robust killing of target splenocytes.

W166. Influence of the Proinflammatory Environment on Cell-based Tolerogenic Therapies in Multiple Sclerosis

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Introduction: Multiple Sclerosis (MS) is an autoimmune disease affecting the central nervous system. Whilst different immunomodulatory treatments are available, a cure for MS doesn't exist yet. Antigen-specific immunotherapies represent an approach to re-educate immunity toward homeostasis without general immunosuppression and could be curative. We developed an autologous monocyte-derived tolerogenic dendritic cell product (VitD3-TolDCs) which showed safety in a Phase I clinical trial in MS patients. In this study, we aim at profiling the influence of MS-intrinsic systemic inflammation on monocytes and VitD3-tolDCs derived from patients, in order to find pathways that could be modulated to increase the potency of the cell product. Methods: Monocytes (18vs18) and VitD3-TolDCs (7vs7) from naïve active MS patients and HD were profiled via flow cytometry and methylation microarrays. Next, gene expression was evaluated through qPCR and bulk RNAseq. Finally, we evaluated the capability of MS VitD3-TolDCs to induce allogeneic PBMCs proliferation compared to HD. Results: MS monocytes shift from classical to the intermediate subset and show increased expression of inflammation markers. Moreover, we identified gene expression and methylation changes and enrichment of specific transcription factors in MS monocytes. Finally, VitD3-tolDCs from MS patients, despite presenting less methylation changes, resulted less able to suppress allogenic proliferation. Conclusions: MS monocytes present an inflammatory phenotype and generate less powerful tolDCs in comparison to HD. Given the involvement of specific TFs in the gene signature of MS patients´ monocytes, we are currently exploring if these proteins could be targeted to increase the potency of tolDCs derived from MS patients.

W167. Mitigating Genomic Rearrangements in Multiplex Gene-edited CAR T Cells

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Multiple genetic modifications may be required to develop potent allogeneic chimeric antigen receptor (CAR) T cell therapies. Conventional CRISPR-Cas nucleases install sequence-specific DNA double-strand breaks (DSBs), enabling gene knockout or targeted transgene knock-in. However, simultaneous DSBs provoke a high rate of genomic rearrangements which may impede the safety of the edited cells. Here, we generate T cell receptor (TCR) replaced CAR T cells using CRISPR-Cas9 in combination with Cas9-derived base editing technology for additional DSB free knockouts within a single intervention. To enhance the persistence of allogeneic CAR-T cell products, B2M and CIITA can be knocked out to eliminate HLA-class 1 and 2, respectively. However, we observed guide RNA exchange between the editors by the presence of small insertions and deletions at the base editing target sites, as well as translocations between the edited loci. This is overcome by using CRISPR enzymes of distinct evolutionary origins. Combining Cas12a Ultra for CAR knock-in and a Cas9-derived base editor enables the efficient generation of triple-edited CAR T cells with a translocation frequency comparable to unedited T cells. The ability of resulting TCR- and HLA-negative CAR T cells to resist allogeneic T cell rejection in vitro represents a promising step towards the development of off-the-shelf CAR therapeutics. This single-step procedure for simultaneous non-viral CAR gene transfer and efficient gene silencing using different CRISPR enzymes for knock-in and base editing provides a safe and effective strategy for multiplex gene editing in CAR-T cells.

W168. Novel Chimeric Antigen Receptors (CAR) T Cells Deplete Specific CD8 T Cell Populations and Reduce Incidence of Type 1 Diabetes

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Pathologic T cells drive many diseases, including autoimmune diseases like type 1 diabetes (T1D). Currently approved treatments are limited in their ability to differentiate pathogenic from non-pathogenic T cells, causing significant risk of adverse effects including infection and malignancy. A potential solution are chimeric antigen receptors (CARs), synthetic constructs that repurpose T cell cytotoxicity specifically against target-bearing cells. We hypothesized this technology could be developed for targeted depletion of specific CD8 T cells populations. Working within the canonical ovalbumin system, we have developed two novel CAR designs which target CD8 T cells via their T cell receptor (TCR). The anti-Vβ5 CAR is a second-generation CAR with traditional extracellular scFv-domain that targets the β-chain(Vβ5) of the OT-I TCR. The OVA-MHC-I-Bait CAR replaces the scFv-domain with an extracellular MHC(H-2Kb) complex with cognate epitope. Using in vitro co-culture assays, both anti-Vβ5 and OVA-MHC-I-Bait CAR T cells demonstrate activation by and robust killing of OT-I T cells despite expected activation of OT-I T cells. Both CARs demonstrate in vivo killing of OVA-reactive CD8 T cells generated via adjuvanted-ovalbumin vaccination, resulting in decreased protection against ovalbumin-expressing Listeria monocytogenes. We found that adoptive transfer of OVA-reactive T cells from OVA-vaccinated mice will induce T1D in RIP-mOVA mice. Subsequent administration of both CARs was able to prevent T1D. In conclusion, we have developed two TCR-targeting CARs which precisely kill specific CD8 T cells with resultant functional immune changes including altered microbial immunity and prevention of T1D. Durable, specific depletion of CD8 T cells has broad therapeutic applications.

W169. Precision Biomaterials for Investigating the Activation Requirements of Human Regulatory T Cells
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Regulatory T cells (Tregs) have been investigated as cellular therapeutics for the treatment of autoimmune diseases and transplant rejection. An essential step for Treg therapy is their manufacturing via ex vivo activation and expansion. High variability of patient Treg expansion poses a major challenge to the development of this therapy. Commonly used activation reagents—such as commercially available αCD3 and αCD28 antibody-coated beads—lack tunability of stimulation cues and may not reflect the activation requirements needed for Treg expansion. Thus, we developed a DNA-scaffolded biomaterial platform which permits the precision control over the surface density and stoichiometry of complementary-DNA-conjugated biomolecules via surface-hybridization. Here, we investigated the role of αCD3 and αCD28 surface ratio and density in Treg activation. Tregs displayed distinct αCD3 and αCD28 signaling thresholds for expansion, requiring significantly more αCD28 costimulation for maximal proliferation while a wide-range of the amount of αCD3 minimally impacted expansion. Increased αCD28 signaling and higher particle to cell ratio resulted in increased expansion and higher percentage of central-memory phenotype (CCR7+ CD45RO+). Increasing the particle to cell ratio for suboptimal formulations minimally improved proliferation, highlighting the dependence of signaling moieties acting in cis on particle surfaces. Ongoing work will investigate the impact of donor Treg characteristics on expansion requirements and validate the therapeutic efficacy of Tregs expanded using a wide-range of αCD3 and αCD28 stimulation conditions in a xenogenic GVHD model. We aim to identify activation conditions that reliably expand highly functional Tregs from most donors.

W170. Selective Depletion of Follicular Helper T Cells Using Chimeric Antigen Receptor Natural Killer Cells
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Hyperactivity of follicular helper T (Tfh) cells is a core feature of autoimmune disease and chronic infection. To date, no FDA-approved therapy exists for the selective depletion of Tfh cells, in part due to the lack of a specific cell-surface marker of these cells. While many immune cells express low levels of programmed cell death protein 1 (PD-1), Tfh cells express uniquely high levels of PD-1. To selectively deplete Tfh cells, our lab developed a chimeric

antigen receptor (CAR) that endows effector cells with a potent capacity to selectively kill PD-1-high target cells. Conventional CAR ectodomains recognize target cells via high affinity single-chain variable fragments of antigen-specific antibodies, putatively killing targets that expresses any antigen. Our CAR uses the extracellular domain of programmed death-ligand 1 (PD-L1) that recognizes PD-1 with micromolar affinity, facilitating selective killing of targets with high but not those with intermediate or low surface expression of PD-1. Accordingly, we demonstrate that our PD-L1 CAR natural killer (NK) cells specifically kill PD-1-high Tfh cells while sparing other subsets of naïve, effector, and regulatory T cells with lower expression levels of PD-1. We validated CAR-mediated killing of PD-1-high target cells in multiple model systems. Mouse PD-L1 CAR T cells selectively killed PD-1-high splenic CD4 T cells in vitro and in vivo. PD-L1 CAR NK cells offer diverse applications for selectively depleting Tfh cells in contexts such as transplant rejection, allergy, lymphoma, autoimmune disease, and latent viral infection.

W171. Serum- and Feeder-Free Generation of CD4 Single Positive T Cells and Regulatory T Cells from Human Induced Pluripotent Stem Cells

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Increased interest in developing regulatory T cell (Treg) therapies is driven by the medical need to treat autoimmune and inflammatory diseases caused by loss or dysfunction of Tregs. However, primary Treg cell products are limited in cell numbers, subject to donor variability and difficult to multiplex edit. Human induced pluripotent stem cell (iPSC)-derived Tregs (iPSC-Tregs) could solve these issues by serving as a renewable source of a consistent edited cell product. While generation of iPSC-derived CD4 single-positive (CD4sp) T cells and CD4sp FOXP3+ Tregs using organoids was reported, here we show, for the first time, a serum- and cell feeder-free differentiation process suitable for largescale production. Using PMA and ionomycin, we generated CD4sp T cells from iPSCs at high efficiency (~3700 CD4sp/iPSC) and converted them to Tregs using TGFb and ATRA. We also demonstrated 80% non-viral, targeted integration of an HLA-A2 CAR in iPSCs by electroporating zinc finger nuclease mRNA and donor plasmid. The iPSC-Tregs +/- HLA-A2 CAR phenotypically resemble primary Tregs, are demethylated (>85%) at FOXP3 TSDR and secrete IL-10 but no proinflammatory cytokines (IL-2, IFN-g, TNFa). Comparing a validated Treg gene signature among iPSC-Tregs, primary Tregs, induced Tregs, and conventional CD4 T cells by single-cell RNA sequencing, iPSC-Tregs and primary Tregs clustered more closely together. Importantly, the iPSC-Tregs suppress T cell proliferation via HLA-A2 CAR and TCR stimulation in vitro. Our work is the first to demonstrate an iPSC-based platform for generating functional Tregs under serum- and feeder-free conditions amenable to manufacturing for delivering Treg therapies at scale.

Immunogenetics

Th190. Chromatin Conformation During CD4+ T Cell Activation Implicates Autoimmune Disease Candidate Genes

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Genome-wide association studies (GWAS) have identified hundreds of genetic signals associated with autoimmune disease. The majority of these genetic signals are located in non-coding regions and likely impact cis-regulatory elements (cRE). As cRE function is dynamic across tissue types and cellular states, profiling the epigenetic status of cRE across physiological processes is necessary to characterize the molecular mechanisms by which autoimmune variants act to contribute to disease risk. Here, we localized risk variants from 15 autoimmune GWAS to cRE active during TCR-CD28 costimulation of naïve human CD4+ T cells. To characterize how dynamic changes in gene expression correlate with cRE activity, we measured gene expression, chromatin accessibility, and promoter-cRE contacts across three phases of naive CD4+ T cell activation using RNA-seq, ATAC-seq, and HiC. We identified 1,200 protein-coding genes physically connected to accessible disease-associated variants for 423 GWAS signals, at least one-third of which are dynamically regulated upon activation. From these maps, we functionally validated a novel stretch of intergenic enhancers whose activity is required for activation-induced IL2 gene expression and is influenced by autoimmune-associated genetic variation. The set of genes implicated by this approach are enriched for genes shown by high-throughput CRISPR screens to control CD4+ T cell proliferation and function, and we also pharmacologically validated 8 novel implicated genes as potent regulators of T cell activation. These studies directly show how autoimmune variants and the genes they regulate influence processes involved in CD4+ T cell proliferation and activation.

Th191. Genome-wide Crispr-interference Screens Connect Non-coding Autoimmune Risk Variants to T Cell Functions

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Autoimmune diseases have complex genetic inheritance patterns making their genetic origins difficult to determine. Genome-wide association studies have identified thousands of variants associated with autoimmune diseases, but the causal variants for >99% of these trait associations have yet to be identified and functionally validated. Thus, there continues to be a lack of understanding of the mechanisms that promote genetic risk for these complex traits. As part of our efforts to connect non-coding variants to an effect on cellular function, we targeted >1000 variants in T cell cis-regulatory regions that associate with multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, psoriasis, and type 1 diabetes using genome-wide CRISPR-interference screens in primary human T cells. We defined >50 variants that were in regulatory regions that control T cell proliferation in activated Th0 cells and >30 variant regulatory regions that control IFNg secretion in differentiated Th1 cells. We connected each regulatory region to putative target genes, identifying 124 gene candidates, which significantly enrich for T cell proliferation and immune dysfunction pathways. Many gene candidates are previously unknown to affect T cell proliferation or cytokine secretion. Variant regulatory regions acted at an average distance of 106 kb from their putative target genes with a range of 1 bp to 1 Mbp, suggesting that enhancers play a major role in regulating T cell phenotypes and that variants perturb T cell functions from distal parts of the genome. This work represents a crucial step to define the effects of risk variants on disease-relevant cellular functions.

Th192. Overactive STAT3 Drives Dysregulated Accumulation of Self-reactive and Autoimmune-associated cd21low cd23low B Cells

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Signal transducer and activator of transcription 3 (STAT3) plays pleiotropic roles in hematopoietic and non-hematopoietic cells, by regulating gene expression downstream of cytokine and hormone receptors. STAT3 constitutive activation and somatic gain-of-function (GOF) mutations recur in B-lymphomas and germline heterozygous STAT3 GOF mutations cause early-onset multi-organ autoimmune disease. Affected individuals variably present with type-1 diabetes, autoimmune thyroid disease (AITD), rheumatoid arthritis, gut enteropathies and autoimmune cytopenias. The AITD, cytopenias, hypogammaglobulinemia and B-memory lymphopenia observed in STAT3 GOF syndrome point to B cell tolerance defects. However, little is known of the B cell-intrinsic effects of overactive STAT3. Here, we address this question in human patients and mice engineered to carry the most common mutation causing STAT3 GOF syndrome, STAT3T716M, or STAT3K658N found in malignancy and STAT3 GOF syndrome. We demonstrate that GOF STAT3 causes aberrant accumulation of polyclonal CD21low B cells resembling those that accumulate in humans and mice with age, chronic infections, immunodeficiency and autoimmune disease. STAT3 GOF allowed aberrant accumulation of self-reactive SWHEL B cells recognising a blood cell-surface autoantigen, and in humans of VH4-34+ B cells recognising the I/i self-antigen. We use ex vivo cultures, BCR deep-sequencing, flow cytometry, single-cell RNA sequencing and chromatin immunoprecipitation sequencing to reveal cell-intrinsic effects of overactive STAT3 in CD21low B cells. Our findings reveal the landscape of genes and proteins dysregulated by overactive STAT3 in B cells. We propose a novel mechanism to help explain the over-accumulation of autoantibody-enriched CD21low B cells in autoimmune diseases associated with IL-6 and IL-21 over-abundance.

Th193. Thymus Tissue Regeneration in 22q11.2ds Mouse Models (DiGeorge) Using Mesenchymal Cell Replacement and Selected Vasodilative Drugs

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Thymus hypoplasia occurs in several clinical conditions including 22q11.2 deletion syndrome (22q11.2DS). 22q11.2DS is the most common human microdeletion disorder, affecting ~1/2100. Patients born with this deletion can have congenital malformations including thymus hypoplasia, cardiac defects, hypoparathyroidism and/or dysmorphic facial features. Thymuses from 60-70% 22q11.2DS patients are smaller and produce fewer T cells than normal. For individuals with a severe hypoplasia, an allogenic thymus tissue graft is needed to restore thymopoiesis. To determine the molecular mechanisms contributing to a small thymus, we compared the subsets of cells needed for the development of the thymus using embryos from various 22q11.DS mouse models and controls. Reaggregate fetal thymic organ culture assays revealed that replacing mesenchymal cells from 22q11.2del hypoplastic lobes with normal ones restored tissue expansion and thymopoiesis. Thymic epithelial cells and endothelial cells used as substitutes were unable to restore the growth. Single cell RNA sequencing of normal and hypoplastic thymus lobes revealed differential expression of transcripts that primarily impacted 5 distinct mesenchymal cell subsets and the one endothelial cell group in 22q11.2DS. These transcripts are involved in extracellular matrix modeling, collagen deposition, cell-cell interactions, and growth. Thymus growth in 22q11.2DS tissues was restored in RTOC assays in the presence of vasodilators, minoxidil or PGE2. We now report that minoxidil injections in timed pregnant 22q11.2DS mice restore thymus tissue expansion in affected embryos. Such findings suggest novel strategies aimed at preventing thymic hypoplasia in utero that may have clinical value.

Tu191. A Human ITPR3 Variant Causes Immunodeficiency and Growth Delay by Negatively Impacting Calcium Responses

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The inositol 1,4,5 triphosphate receptors (ITPRs) span the endoplasmic reticulum, regulating intracellular calcium levels upon diverse receptor-ligand interactions. Three isoforms, ITPR1, ITPR2 and IPTR3 form homo- or hetero-tetramers, with autosomal recessive mutations in ITPR3 causing Charcot Marie Tooth disease. We identified 2 unrelated children with immunodeficiency and peripheral neuropathy who have the same de novo ITPR3 mutation. This ITPR3 variant (c.7570C>T) results in a p.Arg2524Cys substitution within the calcium pore. Patient 1 is a 5-year-old African American female who had abnormal newborn screens for SCID. She has T cell deficiency, a predominance of memory T cells, low mitogen responses and depressed B cells. Small in stature, she has conical deciduous teeth, anhidrosis, gait abnormalities and bilateral vocal fold paralysis. Nerve conduction studies (NCS) demonstrated sensorimotor polyneuropathy most prominent in lower extremities. Patient 2 is a 12-year-old boy born to parents of Pakistani descent with similar clinical presentations. The ITPR3 mutation was genocopied in mice using CRISPR/Cas technologies. The Itpr3Wt/R2524C mice are significantly smaller than littermate controls with severe B cell immunodeficiency, reduced peripheral T cell numbers and impaired antigen-receptor induced calcium responses. While T cell development appears normal based on cell numbers, early B cell development is severely attenuated. The data indicate that the single allelic Itpr3 mutation acts as a dominant negative protein. Notably, the targeting of ITPR3 could enable the two related ITPR family members to function normally, potentially improving immune, neuronal, and endocrine functions in the patients.

Tu192. Alterations in Circular RNA Landscape in Septic Peripheral Blood Mononuclear Cells Before and After Intensive Care

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Sepsis, the leading cause of death in intensive care units (ICU), is associated with alterations in genomic expression in leukocytes. Understanding of molecular mechanisms and current treatments for sepsis remain to be improved. Circular RNAs (circRNAs) are a novel class of non-coding RNA that is increasing shown to participate in complex pathologies. We hypothesized that circular RNAs in peripheral blood mononuclear cells (PBMCs) actively mediate the pathogenesis of sepsis. PBMCs were isolated from three sepsis patients before and after intensive care, and RNA sequencing was conducted with total RNA. CircRNAs were identified using four annotation pipelines and their expression was compared. Expression of circular RNA ASPH (circASPH), the most differentially expressed circRNA, was validated through quantitative PCR in a larger sample size. In silico analysis was conducted to predict potential interactions with mRNAs and RNA binding proteins (RBPs). Overall circRNA expression in septic PBMCs were found to be more abundant and less diverse at ICU admission (ICU-AD) compared with ICU discharge (ICU-DC). We identified 938 circRNA species, with 34 differentially expressed between ICU-AD and ICU-DC. CircASPH was found to be highly expressed at ICU-AD, its level positively correlated with the length of stay in ICU. CircASPH was predicted to bind four microRNAs including miR-670, miR-7975, miR-6818 and miR-384 and interact with proteins TLR2 and ZC3H12D. This study characterized differential expression of circRNA in septic PBMCs between ICU admission and discharge and explored their potential role as novel markers or targets for treatment of sepsis.

Tu193. Decoding Regulatory T Cell Identity and Stability to Inform Better Regulatory T Cell Therapies

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Regulatory T cells (Tregs) are a specialized subset of CD4+ T cells that maintain self-tolerance and immune homeostasis. Due to their natural capacity to suppress excessive inflammatory immune responses without causing global immune deficiency, Tregs are a promising candidate for cellular therapeutics to treat autoimmune disease, prevent transplant rejection, and perhaps more broadly, heal inflamed tissues. However, Tregs can destabilize when exposed to chronic inflammation, a phenomenon marked by loss of suppressive capacity and the lineage-defining transcription factor FOXP3. Understanding the genetic factors controlling FOXP3 expression may nominate regulators important for Treg stability and reveal manipulable targets to improve Treg cellular therapies. Advances in CRISPR technologies have enabled rapid and scalable identification of genetic elements controlling gene expression in primary human cell types. Using CRISPRi and CRISPRn-based genetic screens, we identified novel cis- and trans-regulators controlling FOXP3 maintenance and suppression in primary human Tregs. Notably, these regulators included nine FOXP3-suppressive trans-regulators and a suppressive cis-element associated with lncRNA expression. Ablation of these elements under destabilizing conditions revealed a subset that prevents destabilization and enhances FOXP3 expression. Overall, this work reveals a network of novel cis- and trans- regulators governing FOXP3 expression and Treg stability and informs the rational design of next-generation Treg cellular immunotherapies.

Tu194. Delineating Target Genes for Autoimmune Disease-associated Enhancer Variants Using 3D Genomics

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To demonstrate that autoimmune-associated enhancer variants have a functional impact, we first prioritized GWAS and proxy variants for rheumatoid arthritis (RA), type 1 diabetes (T1D), allergy, and asthma by overlapping them with disease-relevant cell type enhancer marks H3K4me1 (Th0, Th1, Th2, and Th17), resulting in approximately 8150 enhancer variants. Next, we generated Hi-C maps for the Th1, Th2, and Th17 cell types and captured enhancer regions of interest, recovering approximately 80% of the targeted region and allowing us to assigned target genes for 70% targeted T1D enhancers, 78% of targeted RA enhancers, 67% targeted asthma enhancers and 70% of targeted allergy enhancers. Our results validate known eQTL target genes, and also identify cell type-specific regulatory interactions, including interactions with genes important for maintaining cellular specificity. In order to validate regulatory loops, we have performed CRISPRi for a subset of enhancers in different T cell types. To determine functional enhancer variants, we performed massively parallel reporter assays (MPRA) for a subset of the prioritized variants. Here we tested 1435 Th1 enhancer variants and 2375 Th2 enhancer variants in their respective cell types. At 5% FDR, 53 enhancer variants show significant allelic activity in Th1 cells, and 91 variants show significant allelic activity in Th2 cells. By combining capture Hi-C target genes with MPRA activity and CRISPRi validations, we have identified putative causal variants and novel target genes for autoimmune disease-associated enhancer variants.

Tu195. Long-term Evaluation of Good's Syndrome Reveals a Unique Adult-onset Progressive Immune Deficiency

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Good's syndrome (GS) is a rare immune deficiency of thymoma, hypogammaglobulinemia and recurrent infections. A lack of premorbid and longitudinal data has limited our understanding of a unique human model of adult-onset B-cell lymphopenia. To characterize the clinical evolution and natural progression of disease, we prospectively followed 8 GS patients (6 males:2 females) continually at a single center from 1984 to 2022. Clinical events, complete blood counts, immune phenotyping, and serum immunoglobulin quantification, were captured at semi-annual outpatient visits and all hospitalizations. The mean age at GS diagnosis was 56±12 years with mean follow up of 11.8±10 years. Two individuals died during follow up due to opportunistic infections. At diagnosis, 7/8 patients had

significantly low levels of IgG and undetectable IgM. 4/8 had undetectable IgA. Remaining IgG, IgA and IgM continued to decline during follow up. All patients had an absence of peripheral B-cells at diagnosis and in one patient, this absence preceded the IgG decline. Progressively, patients developed lymphopenia[< 1x109-lymphocytes/L] (n=5), neutropenia[< 1.9x109/L] (n=3), anemia[< 13g/L-hemoglobin] (n=4), thrombocytopenia[< 150x109-platelets/L] (n=3), and CD4 lymphopenia[< 500-cells/ul] (n=4). A mean decline of 30% in lymphocytes and 25% in platelets was observed 10 years post thymoma diagnosis. Cytopenias progressed with age and correlated with significant clinical outcomes including opportunistic infections. While cytopenias have been noted in previous case studies, this study is the first to show the progressive nature and the temporal association with opportunistic infections. Based on these findings, an evaluation of the bone marrow should be included in the clinical follow-up and studies on the pathophysiology.

W173. Integrative Functional Genomics Points to Natural Killer Cells as Key Drivers in Pathogenesis of Ankylosing Spondylitis

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Multiple lines of evidence indicate that ankylosing spondylitis (AS) is a lymphocyte-driven disease. However, which lymphocyte populations are critical in AS pathogenesis is not known. AS is a highly heritable disease, with estimates of the genetic contribution to AS ranging from 69-90%. Genome wide associations studies (GWAS) have identified >100 loci associated with AS, with the vast majority of likely causal variants being non-coding. Here we integrate GWAS data with epigenomic and transcriptomic datasets in human lymphocytes to identify key cell subsets mediating genetic susceptibility to AS. We use public ATAC-seq on sorted peripheral immune cell subsets of healthy subjects, single-cell RNA-seq on lymphocytes of AS patients and healthy controls, and low-input RNA-seq on 7 sorted lymphocyte populations of healthy subjects. To link cell type-specific open chromatin regions or gene expression with GWAS we use 3 methods: Linkage Disequilibrium Score-regression in Specifically Expressed Genes (LDSC-seg), single-cell disease-relevance score (scDRS) and SNPsea. We validated that our methods could identify T-cells as the main drivers of Rheumatoid Arthritis. We discovered that open chromatin regions and gene expression that is specific to Natural Killer (NK) cells compared to other immune cell-types, are enriched in genetic risk loci for AS. These results are consistent between two independent GWAS, and using 3 independent methods in 3 independent functional genomics datasets. Our data points to putative causal genes for AS that may play important roles in NK cell biology. Unexpectedly, these results suggest that NK cells may be key mediators of the genetic susceptibility to AS.

W174. Multiomics Atlas of Human B Cell Activation Helps Decipher Lupus Pathogenesis

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Systemic lupus erythematosus (SLE) is an autoimmune disease that is highly heterogeneous and remains among the most difficult to control. Its heritability is high (up to 66%), and more than 100 genetic susceptibility loci have been identified. Most of these loci implicate common non-coding variants that likely affect gene regulation, especially in activated B cells. However, for most risk loci the regulatory effects and target genes remain unknown. Despite B cells being a main driver of SLE, there is a lack of functional genomics data in human B cells during activation. In this study, we first transcriptomically characterized B cell activation by targeting the B cell receptor (BCR), Toll-like

receptor (TLR) 7, TLR9, and CD40 activation pathways at multiple time points, totalling 29 in vitro conditions in five healthy subjects. We then focused on B cells activated via BCR, TLR7, and a condition to differentiate B cells into a subset (IgD- CD27- CXCR5- CD21- CD11c+, DN2) expanded in SLE patients. Utilizing bulk RNA-seq on our cohort of 23 healthy individuals with enriched heterozygosity for SLE risk variants, we identify pathway-specific allele-specific expression of SLE genes. In order to pinpoint the specific regulatory elements likely affected by risk variants, we performed ATAC-seq. Finally, we single-cell profiled mRNA and 137 surface proteins (CITE-seq) to evaluate SLE gene regulation in specific B cell states. In this work, we generated a unique resource of human B cell activation and provide an in-depth analysis of the contributions of SLE risk loci to specific activation pathways.

W175. Role of Killer Immunoglobulin Like Receptors (KIR) and Their Ligands HLA-C in Recurrent Miscarriage. a Case Control Study from Saudi Arabia

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Unknown etiology of Recurrent Miscarriages (RM) causes discrepancies in the diagnosis and treatment at a global level. Therefore, it is necessary to investigate the possible and unexpected causes and related risks like Killer Immunoglobulin like Receptors (KIRs) of the NK cells, its ligand HLA class-1. This is a case control study aimed to investigate the role of highly polymorphic NK cell KIRs, and their ligands, HLA C. There were 68 women with proven RM patients and 91 controls who had successful pregnancies and shared the same ethnic background. Five (5) ml of blood was collected in EDTA, the KIR and HLA-class 1 genes were genotyped in patients and healthy controls. Data were analyzed and reported that, the frequency distribution of the inhibitory KIR2DL1/L2/L3 and 2DL5 and activating KIR2DS2/S3/S4 were lower in patients than in controls, but did not achieve statistical significance About 37 and 25 distinct KIR haplotypes in patients and controls respectively were documented. No significant distribution differences reported of haplotypes between cases and controls. In this study we observed that the activating centromeric BB haplotypes were significantly more common in RM patients, suggesting that it may be a risk factor for RM, particularly when the HLA C2C2 is a ligand, whereas centromeric AB haplotype, HLAC1C2, is more prevalent in the control group and is linked to successful pregnancies.

W176. Sexual-dimorphism in Human Immune System Aging and Responses to Bacterial Pneumonia Vaccines

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Differences in immune function and responses contribute to health- and life-span disparities between sexes. However, the role of sex in immune system aging and immune responses is not well understood. By profiling peripheral blood mononuclear cells from 172 healthy adults (22–93 years old) using ATAC-seq, RNA-seq and flow cytometry we uncovered an accelerated aging phenotype in men; where T and B cell inactivation and monocyte activation with age was more pronounced in men compared to women. To study whether there are sex-differences in vaccine responses of older adults, we recruited 40 older adults (60 years and older) and studied their responses to two available pneumococcal vaccines: T-dependent Prevnar and T-independent Pneumovax. Using flow cytometry, bulk and single cell RNA-seq we uncovered an activated baseline immune phenotype which was negatively associated with Prevnar vaccine responses. Individuals with this activated phenotype had more circulating pro-inflammatory Th17 cells and more cytotoxic CD16+ NK cells, and less Th1 cells. This immune phenotype was associated with age and sex of donors, where older men were more likely to have this phenotype and did not mount strong responses to Prevnar. Overall, our study uncovered how older adults respond to different pneumococcal vaccines and demonstrated the significance of considering biological sex and the baseline immune state while administering these vaccines.

W177. Single-cell CRISPR Screens in Primary Human T Cells Identify Regulators of th2 Cell Skewing

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CRISPR screens have become the primary discovery engine in modern biology. At Myllia, we combine CRISPR screening with single-cell RNA sequencing in primary T cells, leveraging two transformative technologies to enable genetic screening for complex phenotypes. We utilize pooled CRISPR screens to map the impact of thousands of genetic perturbations at single-cell resolution which allows to identify novel drug targets or to elucidate unknown mechanisms of actions of drugs. Primary human T cells are key players in autoimmunity and other inflammatory diseases, but also represent attractive targets for immunotherapy of cancer. To enable the discovery of novel targets, we built a workflow that utilizes CD4+ T cells from peripheral blood and allows functional genomic screens. Upon activation, naïve CD4+ T cells proliferate and differentiate into specific Th1, Th2, or Th17 cell subsets. Here, we present data of a pooled CRISPR screen for regulators of T cell fates in which we aimed to identify genes whose knockout boosts or attenuates the ability of primary naïve CD4+ T cells to become Th2 cells. In our screen, the different T cell subsets could be captured using curated transcriptomic signatures. Importantly, several gene KOs introduced in a pooled fashion using CRISPR/Cas9 accumulated in distinct subpopulations, suggesting that these genes regulate the differentiation of naïve T cells into the various T helper cell subsets. Overall, our pooled CRISPR screening platform enables to decipher primary T cell plasticity and identifies genes that could serve as drug targets in autoimmunity, inflammation and immuno-oncology.

W178. Single-cell Transcriptomic Characterization Reveals Changes in MAIT and Regulatory T Cells with Advancing Age in a Diverse American Population

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Immunosenescence (ISC) is the natural aging of the immune system that consists in gradual age-related changes within immune-cell composition and function. Age-associated cellular phenotypic changes are well-established, yet few studies extensively characterized the transcriptomic profile of ethnically diverse populations. Here, we assessed transcriptomic and proteomic profiles in a large-scale manner (n=200, age: 25-88 years), using single cell-RNA-sequencing and CITE-Seq. We identified 31 distinct PBMCs cell-clusters associated with age after adjusting for sex and technical covariates; including established age-related populations such as Naïve CD8+ (p=6.35x10-23) and CD4+ (p=1.0x10-9) and NK-cell subsets (p=0.0007). However, unbiased clustering revealed new age-associated immune-cell subset changes, namely cytotoxic CD4+ CTLT-cells (p=0.009), mucosal associated invariant T-cells (MAIT) (p=0.0013), regulatory T-cells (p=0.006) and □-T-cells (p=9.51x10-5). The MAIT cluster is subdivided into three subtypes, all of which decrease with age. We report reciprocal ISC trajectory changes within the MAIT and regulatory T-cell compartments, supporting a favorable tumor environment. These data are then used to calculate an immunologic age that is enhanced compared to our cytometry-derived estimates. Our data offer a new level of resolution of peripheral blood immunosenescence across diverse populations. While certain associations with genetic ancestry are seen, they're not related to those cellular populations influenced by advancing age. These data enabled

us to identify more cell-subtypes that inform the immunologic age calculation, and further produced accurate estimates improving personalized therapeutic strategies that harness the immune system. The reciprocal MAIT and regulatory T-cell trajectories also shint at dysfunction that may contribute to higher cancer incidence in older individuals.

W179. The Impact of Forkhead Box N1 Transcription Factor Variants Determined with Functional Assays and Reaggregate Thymus Organ Cultures

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Thymus hypoplasia is evident in individuals with autosomal recessive, compound heterozygous and single allelic Forkhead Box N1 (FOXN1) mutations. FOXN1 regulates thymic epithelial cell (TEC) development and function, which is essential for T cell development. The impact of diverse human FOXN1 mutations on TECs and thymopoiesis remains unclear for most variants. We determined the consequence of different FOXN1 variants on protein activity (luciferase reporter assays), localization (cell imaging), and dominant negative capabilities (co-expression studies). With these assays, we mapped the nuclear localization signal in FOXN1, identified de novo sequences in human FOXN1 that mediate dominant negative functions and uncovered domains modulating transcriptional activities. Both loss-, partial loss- and gain- of function FOXN1 variants were uncovered. To determine their impact on thymopoiesis, we modified reaggregate thymus organ cultures (RTOC) by incorporating flow sorting to isolate and recombine different cell subsets. In this system, only FOXN1 sufficient TECs will support thymopoiesis when combined with hematopoietic, mesenchymal, and endothelial cell subsets. Murine TECs from “nude” embryos (lacking FOXN1) are unable to support this thymus growth. The flow sorted “nude” TECs were reconstituted with purified TAT-Foxn1 fusion proteins prior to reaggregation. The transduction of wildtype TAT-Foxn1 re-established RTOC growth, thymopoiesis and T cell development within a 10-day culture period. This RTOC strategy can enable one to define the FOXN1 variants of unknown significance (VUS) as benign, partially, or fully attenuated in their capacity to support thymopoiesis. Taken together, our findings establish FOXN1 genotype-phenotype relationships and reveal a novel hymopoietic screening strategy.

W180. Therapy of Aging

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The Problem: Complement-mediated inflammation is a contributor to the dysfunction in lipid metabolism. This is associated with the pathogenesis of aging involving different diseases such as Age related macular degeneration (AMD); Alzheimer's disease; Atherosclerosis and C3 glomerulopathy (Meri S and Haapasalo K (2020)"Function and Dysfunction of Complement Factor H During Formation of Lipid-Rich Deposits. Front. Immunol. 11:611830. doi: 10.3389 /fimmu.2020.611830) Method: One solution to aging problem is to strengthen the physiological working of alternate complement system by inhibiting its dysfunction. Results: Sulfonic nano polymers in right dose and formulation strategy can be used for inhibition of alternate complement system dysfunction and can increase its potential for the therapy for aging as well as prophylaxis for age related illnesses.

Immuno-oncology

Th194. Precise Spatial Multiplexing of Protein Biomarkers for Immune Profiling in Tissue Samples with Chipcytometry

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Immunohistochemistry (IHC) is the most widely used diagnostic technique in tissue pathology. However, IHC is associated with several limitations including the labeling of just a few markers per tissue section and limited quantification of cell populations. As a result of plex limitations, key insights about tumor biology are missed, which could be important for advancing our understanding of tumor biology and ultimately improving patient outcomes. ChipCytometry is a novel image-based platform for precise spatial multiplexing that addresses these challenges by combining iterative immuno-fluorescent staining with high-dynamic range imaging to facilitate quantitative phenotyping with single-cell resolution. The platform enables simultaneous detection of dozens of markers on a single tissue section and enables accurate quantification of protein expression levels necessary to deeply profile single cells, understand interactions between key immune cells, and identify topographic biomarkers. Here we demonstrate how standard FCS files are generated from multichannel OME-TIFF images, enabling identification of cellular phenotypes via flow cytometry-like hierarchical gating while maintaining spatial information about each cell. ChipCytometry has the potential to advance precision medicine in immuno-oncology and inform the discovery of novel biomarkers by enabling quantitative analysis of cellular phenotypes in the spatial context. The ChipCytometry platform enables simultaneous detection of multiple protein markers on a single tissue section for deep immune cell profiling in the tumor microenvironment. Combined with the single-cell spatial information, such data sets provide an opportunity for the discovery of new complex multiplexed biomarker signatures to inform therapeutic development.

Th195. Simplifying High-parameter Phenotypic and Functional Characterization of Cancer Immune Cells

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Interrogating immune cell composition and function is critical for disease prognoses, monitoring immunotherapies, and identifying novel biomarkers and therapeutic targets. CyTOF® is high-plex flow cytometry that exploits metal-isotope-tagged antibodies. In contrast to fluorescence-based cytometers, CyTOF is not limited by fluorescence overlap, enabling rapid design of 40-plus-marker panels. To expand the clinical and preclinical utility of a 30-marker Maxpar® Direct™ Immune Profiling Assay™ (Maxpar Direct Assay), nine Expansion Panels were developed for deeper phenotyping of cell types and activation states, including markers to characterize ex vivo and activated myeloid cells, T cells, and NK cells. Peripheral blood mononuclear cells from healthy donors and donors with multiple myeloma were stimulated in vitro, then stained in the 30-antibody Maxpar Direct Assay tube with the NK Cell Expansion Panel (NKp30, NKp46, CD181, PD-1, NKG2A, ICOS, and TIGIT) or the T Cell Expansion Panel 3 (OX40, TIGIT, CD69, PD-1, Tim-3, ICOS, and 4-1BB). Surface staining was followed by intracellular staining with the Basic Activation Expansion Panel antibodies (IL-2, TNFα, IFNγ, perforin, granzyme B) after cell fixation and permeabilization. Anti-CD107a was added during stimulation to measure degranulation. Samples were acquired on a CyTOF XT™ instrument in automated batch acquisition mode. Automated analysis was performed with Maxpar Pathsetter™ software to quantify immune cell activation, cytokine responsiveness and marker expression. Maxpar reagents and analysis tools thus provide an end-to-end workflow to characterize immune cells in health and disease. For Research Use Only. Not for use in diagnostic procedures.

Th196. Tcf-1 Promotes Genomic Instability and T Cell Transformation in Response to Aberrant β-catenin Activation

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Understanding the mechanisms promoting chromosomal translocations of the rearranging receptor loci in leukemia and lymphoma remains incomplete. Here we show that leukemias induced by aberrant activation of β-catenin in thymocytes, which bear recurrent Tcra/Myc-Pvt1 translocations, depend on Tcf-1. The DNA double-strand breaks (DSBs) in the Tcra site of the translocation are Rag-generated whereas the Myc-Pvt1 DSBs are not. Aberrantly activated β-catenin redirects Tcf-1-binding to novel DNA-sites to alter chromatin accessibility and downregulate genome-stability pathways. Impaired homologous recombination DNA-repair and replication-checkpoints lead to retention of DSBs that promote translocations and transformation of DP thymocytes. The resulting lymphomas, which resemble human T-ALL, are sensitive to PARP inhibitors (PARPi). Our findings indicate that aberrant β-catenin signaling contributes to translocations in thymocytes by guiding Tcf-1 to promote the generation and retention of replication induced DSBs allowing their coexistence with Rag-generated DSBs. Thus, PARPi could offer therapeutic options in hematologic malignancies with active Wnt/β-catenin signaling.

Th197. TDG Mediated Tregs Inhibition in Oral Squamous Cell Carcinoma

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Thiodigalactoside (TDG), a synthetic inhibitor of β-Galactoside Binding Protein (β-GBP), an immuno-modulatory protein, which regulates regulatory T cells (Tregs) which are responsible for tumor immuno-suppression. We observed the skewed immunological balance between Treg (CD4+CD25+FOXP3+) and Th17 (CD4+IL17A+) cells in peripheral circulation of oral squamous cell carcinoma (OSCC) patients by multicolor flowcytometry. The anti-tumour effects were studied by performing MTT, propidium iodide (PI) staining, annexin-V binding assay and ELISA respectively. The Tregs used in all co-culture assays were separated by MACS. An increased Th17/Tregs ratio was observed in oral cancer patients leading to immuno-suppressive tumor microenvironment. Treatment of oral cancer cells with β-GBP showed growth promoting effects i.e. significant increase in growth and angiogenesis of oral cancer cells. It also showed proliferative effect on Tregs. However, the treatment with its inhibitor TDG showed cytotoxic effects on both the Treg cell subsets and also oral cancer cells. TDG treatment resulted in Treg cell growth inhibition and also decreased frequency of IL10+ and IL35+ Treg cells indicating less immuno-suppressiveness. Subsequently, TDG treatment significantly ($p< 0.001$) inhibited the growth of OSCC cells with a concomitant induction of apoptosis, cell cycle arrest and anti-angiogenesis. It appears that TDG concurrently prevents many tumor promoting effects by Treg inhibition. Therefore, therapeutic targeting of β-GBP by TDG can overcome Treg mediated immunosuppression in oral cancer patients. Hence, TDG may be developed as chemopreventative/chemotherapeutic drug for treatment of patients with OSCC.

Th199. The FAM72 Gene Family Promotes Cancer Development by Disabling the Base Excision Repair System

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Genetic lesions are central contributors to cancer development and progression, and understanding the sources of these lesions will provide a better understanding of the mechanisms of carcinogenesis. Our previous work showed that the uncharacterized murine Fam72a gene promotes mutagenic DNA repair during antibody maturation by causing the degradation of Uracil DNA glycosylase 2 (UNG2), a component of the base excision repair (BER) system. Humans encode four almost identical paralogues of FAM72 called FAM72A-D, which are absent in all other mammalian genomes. The role of these four paralogues in human biology is unknown. A previous report showed that FAM72A is over-expressed in many cancers. Since FAM72A promotes mutagenesis by degradation of UNG2 in B

cells, we hypothesized that overexpression of the FAM72 gene family might promote cancer. Here we show that FAM72A-D is overexpressed in many human cancers and inversely correlates with UNG2 protein levels in tumorigenic tissue. However, FAM72A but not FAM72B-D causes degradation of UNG2. Consistent with this effect, FAM72 expression correlates with a higher mutation load in many tumor types, and with poorer survival in several cancers. To directly test if Fam72a is sufficient to promote cancer, we generated transgenic mice that overexpress Fam72a in multiple tissues. We observed that Fam72a overexpression promotes increased colonic polyps in the Apcmin background compared to controls. These data show that the novel FAM72 gene family are drivers of cancer development in both mice and humans and advances our understanding of the underlying molecular mechanisms that precipitate cancer development and progression.

Th200. Tissue Spatial Analysis by Imaging Mass Cytometry and Longitudinal Peripheral Blood Flow Cytometry in Checkpoint Inhibitor Toxicity

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Immune checkpoint inhibitor (ICI) colitis is among the most prevalent immune-related adverse events (irAEs). Deeper pathophysiological understanding of irAEs can contribute to evidence-based irAE management. We used imaging mass cytometry (IMC) along with in-house developed cell-segmentation, data-normalization and lineage-annotation pipelines to study ICI colitis (N=18) spatially in high immunological detail. Tissue analyses were complemented with longitudinal immunophenotyping and serum proteomics in ICI-treated patients that developed irAEs or remained irAE-free (N=44). CD8+ T cells (CD8s) dominated the infiltrate of both αPD-1 and combined ICI (CICI) colitis. CICI-colitis showed highest CD8 granzyme B (GzmB) production across groups and serum GzmB was also higher in CICI than αPD-1-colitis. Activated CD8s with CD69hiCD103hi tissue resident (TRM)-like phenotype, more abundant in epithelium than lamina propria, mainly explained high GzmB in CICI-colitis. We found elevated Th1/Th17 balance in CICI vs αCTLA-4-colitis and, accordingly, in peripheral blood CICI irAEs (not limited to colitis) were mainly characterized by significantly enhanced proliferation of CD4+ Th1-associated effector memory T (TEM) cells. IrAEs following αPD-1 were associated with modestly enhanced Th1 response reflected by increasing CXCL9/CXCL10, but without Th1/Tc1 subset changes. Our data demonstrate that epithelial cytotoxic CD8+ TRM cells and local Th1 skewing are associated with the highest cytotoxicity in CICI-colitis. In peripheral blood, we demonstrate marked increases in proliferation of CD4EM T cells and mainly Th1-associated cytokines. These findings indicate that tissue TRM cells play an important role in colitis development. They may be directly activated by ICI, or dependent on CD4EM T cells, which drive especially CICI irAEs.

Th202. Transcription Factor Cooperation in T-cell Development and Leukemogenesis

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T-cell Acute lymphoblastic leukemia (T-ALL) is an aggressive disease with only a 10% 5-year survival rate for patients over 60, due to genomic instability. We have shown that β-catenin and TCF-1 cooperate in DP thymocytes to promote genomic instability leading to lymphomagenesis. We have also established that TCF-1 and HEB cooperate in the DP stage. Now, we aim to determine the molecular underpinnings of T-ALL and thymic development, by elucidating the interplay of all three factors, β-catenin, TCF-1, and HEB. Aberrant activation of β-catenin promotes the development of Lymphoma/Leukemia equivalent to human T-ALL and blocks thymocyte development in the DP stage. Ablation of TCF-1 in thymocytes with activated β-catenin rescues from lymphomagenesis, but the developmental block persists. Antithetically, developmental progression is achieved only when HEB is conditionally

knocked out. In an effort to explain this phenomenon, we found several genes associated with DP maturation that were downregulated by the activation of β-catenin (e.g. Klf2, Ccr-7, etc.) and reverted upon deletion of HEB. This is associated with changes in the binding of TCF-1 to DNA. Genomic sites that acquire TCF-1 binding upon β-catenin activation forfeit this binding when HEB is deleted, while simultaneously, novel TCF-1 binding sites emerge. These new binding sites are significantly more enriched for the conserved TCF-1 motif than TCF-1-bound sites in WT thymocytes. This observation promotes the notion that HEB guides TCF-1 towards alternative binding sites, thus altering gene expression. These findings highlight the complex interactive relationship of TCF-1 and HEB in both normal development and disease.

Th203. Transcriptional Programs of Primary AML Cells Define Their Susceptibility to T Cell Killing and Correlate with Patient Survival

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Less than 30% of patients with acute myeloid leukemia (AML) survive, and new therapeutic approaches are urgently needed. We are developing cytotoxic CD4 T cells, engineered by lentiviral gene transfer of IL10, as a therapy for AML. These CD4 T cells kill AML cells in vitro through a TCR-independent mechanism, and impair AML progression in vivo. However, some primary AML samples are resistant to killing by our cytotoxic CD4 T cells. To understand this resistance, we analyzed the single-cell transcriptome of primary AML cells and CD4 T cells before and after their in vitro interaction. This analysis uncovered transcriptional programs of AML cells that correlate with sensitivity or resistance to CD4 T cell killing. Using a large public AML RNA-seq dataset, we found the resistance-associated AML programs significantly enriched in AML patients with poor survival. Moreover, interaction with AML cells perturbed the CD4 T cell transcriptome. While killing-resistant AML cells only had a modest effect, interaction with killing-sensitive AML cells led to a strong CD4 T cell activation and AML killing. Further, we observed that killing-sensitive AML cells upregulated a gene encoding one of the key proteins required for the immune synapse formation with T cells. The knock-out of that gene from a killing-sensitive AML cell line dramatically increased its resistance to CD4 T cell killing. Altogether, we found that AML sensitivity to T cell killing depends on their ability to form an immune synapse, and AML resistance to T cell killing may contribute to poor survival of AML patients.

Th204. Tumor Exosomal mir-15a Downregulates CD25 and Induces γδT-17 Cells to Promote Radioresistance in Nasopharyngeal Carcinoma

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Nasopharyngeal carcinoma (NPC) is one of the most prevalent and aggressive tumors in Southeast Asia. Radiotherapy is the first-line treatment for NPC, but its therapeutic efficacy is poor in some patients due to radioresistance. Chronic inflammation in tumor microenvironment is thought to be associated with radioresistance. γδT-17 cells are important cellular source of inflammatory cytokine and associated with tumor progression. However, whether and how γδT-17 cells affect radiotherapy in NPC remains elusive. In this study, we identified abundant γδT-17 cells in NPC tissues. Exosomes-derived from NPC cells (NPC-Exos) could induce γδT-17 cells. NPC-Exos-induced γδT-17 cells could further promote NPC radioresistance both in vitro and in vivo. Blockage of IL-17 secreted from NPC-Exos-induced γδT-17 cells enhanced the radiosensitivity of NPC cells and increased radiation-induced cell death. Mechanistic study revealed that both IL-17-driving cytokines from DCs and down-regulated CD25/IL-2 signaling of γδ-T cells induced by NPC-Exos contributed to the induction of γδT-17 cells. Furthermore, down-regulated CD25/IL-2 signaling was mediated by the delivery of tumor exosomal miR-15a to γδ-T cells. miR-15a inhibitor could successfully suppress the induction of γδT-17 cells by NPC-Exos. Our study demonstrates a novel immunoregulatory effects of NPC-Exos and provides implications to overcome NPC radioresistance.

Th205. Unraveling the Relationship of Periodontal Dysbiosis in Oral Squamous Cell Carcinoma
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The microbiota of the oral cavity is composed of bacteria, fungi, viruses, archaea, and protozoa. Imbalances in the periodontal microbiota in a susceptible host and the proliferation of keystone pathogens are responsible for causing oral dysbiosis. When dysbiosis occurs, the subgingival periodontal bacteria trigger a chronic inflammatory process, destroying periodontal support tissues and systemic low-grade inflammation. This systemic can contribute to oral cancer development. Oral squamous cell carcinoma (OSSC) represents 95% of cancers in the oral cavity. Despite advances in diagnosis and treatment, OSCC is diagnosed late, starting with a pre-malignant lesion known as leukoplakia. Recently, periodontitis has been related to the development of chronic diseases. We evaluated oncogenic transformation in a cohort of patients with leukoplakia or OSCC the different signatures in long non-coding RNAs and the immunomodulatory molecules involved in malignant transformation, these studies were complemented with histopathology analysis. Also, we corroborate these markers in paraffin samples from secondary tumors to evaluate the metastatic niche. We obtained a difference in non-coding RNA signature samples from patients with leukoplakia and OSCC, which correlates with the different immunomodulatory molecules, and the histopathology studies in leukoplakia and OSCC. In addition, we determined the presence of keystone periodontal pathogens in the tissue samples of leukoplakia and OSCC and we study the small extracellular vehicles (EVs) from saliva to evaluate biomarkers for diagnosis of these pathologies. This study provides a new perspective on the OSCC since it includes new components to the view: the inflammatory effect of periopathogens, EVs, and the non-coding RNAs.

Tu197. Adipose-derived, her2/neu Tumor-Targeted, Human Mast Cells have Antitumor Effects *in vivo* After I.V. Injection
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The use of one's own cells to treat tumors is typified by chimeric antigen receptor T cells (CAR T) therapy yet cells with anti-tumor properties being investigated continues to grow. We have previously proposed a new strategy using tumor-targeted mast cells (MC) obtained from autologous sources and demonstrated proof-of concept previously in vitro and in vivo. We sought to exploit the anti-tumor mediators in MC granules to selectively target them to tumor cells using tumor specific immunoglobin E (IgE) and controllably trigger release of anti-tumor mediators upon tumor cell engagement. We used a human HER2/neu-specific IgE to arm human MCs through the high affinity IgE receptor (FcepsilonRI). The ability of intravenously (i.v.) injected HER2/neu-targeted MCs to effect HER2/neu-positive human tumors was assessed using a immunocompromised xenograft mouse model. It is shown for the first time that MC injected i.v. shrink tumors. These studies provide further proof of concept that MC have anti-tumor properties and could possibly provide another strategy for developing adoptive cell transfer therapeutics for patients.

Tu198. Association of Treatment Outcomes with Fc-dependent Antibody Effector Functions Using Systems Serology
Lenny Moise[1], Amanda Gross[2], Thomas Broge[2], Thomas Linnekin[2], William Graham[2], Kimberly Piper[2], Henry Buda[2], Lev Brown[2], Tom Shneer[2] and Jishnu Das[3]
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Growing evidence for associations between humoral immune responses and development of cancer and autoimmunity suggests that antibodies may be useful biomarkers for prediction of disease progression and therapeutic efficacy. The fragment crystallizable (Fc) region in antibodies carries structural determinants of Fc receptor and complement component 1q binding that triggers effector innate immune cell and complement functions

involved in homeostasis, pathogenesis, and therapeutic efficacy. A systems strategy that integrates the Fc-dependent biophysical and functional properties of diverse antibody repertoires and correlates corresponding multivariate profiles with specific outcomes or endotypes would be beneficial for biomarker identification. We have developed the Systems Serology platform that consists of a suite of robust, high-throughput, rapid, and highly reproducible bead- and plate-based assays, adaptable to analyze antigen-specific antibody responses. The platform has provided an unprecedented depth of resolution of qualitative differences in the humoral immune response to many bacterial, viral, fungal, and parasitic pathogens and has identified correlates of protection in vaccine trials. In a pilot cancer study, we investigated Fc-dependent effector functions in baseline and post-treatment serum samples from melanoma patients who received a novel toll-like receptor immunotherapy. Antibody effector functions such as antibody-dependent complement deposition and antibody-dependent NK cell activation were observed to be more robust earlier in treatment in the responder group compared to the non-responder group. The Systems Serology platform holds promise to uncover Fc-dependent effector functions associated with clinical outcome in areas of health concern beyond infectious disease.

Tu199. Automated Gating of Pharmacodynamic Biomarker Expedites Data Analysis and Enables Novel Biomarker Exploration in Preclinical Studies

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Introduction: Flow cytometric-based pharmacodynamic (PD) assessments are increasingly incorporated into dose optimization efforts as they provide evidence of direct target engagement and downstream bioactivity. With the expansion in parameters evaluated by conventional flow cytometry there is a concomitant increase in data volume, leading to an unmet need to streamline data analysis. Objective: To overcome data volume burdens, a bioinformatics approach was used to expedite data analysis generated from preclinical cynomolgus monkey studies. Methods: Target engagement of a T cell agonist, PD-1-GITRL bispecific monoclonal antibody (bsAb), on CD4+ central memory T cells (Tcm) and downstream immune cell activation were evaluated in cynomolgus whole blood using a 16-color 4-Tube flow cytometric assay during two in vivo preclinical studies. flowDensity, flowType and a statistical filter were encoded into a bioinformatics pipeline to capture immune cell populations and identify novel pharmacodynamic effects observed post-dosing. Results: Automated gating accurately identified immune populations despite biological variation with similar precision to manually gated data. Computational analysis quickly recapitulated pharmacodynamic effects, such as target engagement of PD-1 and GITR by PD-1-GITRL bsAb and TIGIT upregulation on CD4+ central memory T cells, observed by manual gating. The addition of flowType and a statistical filter elucidated over 600 additional PD biomarkers that demonstrated significant change compared to baseline data points and between dosing groups. Conclusion: Inclusion of bioinformatic scripts not only reduced analysis time, but also revealed novel pharmacodynamic biomarkers. The efficiency of automated gating to streamline data enables improved refinement of dosing decisions for optimal drug development.

Tu200. Characterising the Tumour-associated Regulatory T Cell (Treg) Niche

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The molecular mechanisms underlying regulatory T cell (Treg)-mediated modulation of early cancer development are underexplored. The Notch ligand Jagged1 (Jag1), highly expressed in skin Tregs, is critical for facilitating hair follicle (HF) regeneration by mediating HF stem cell activation. Here, we aim to elucidate the role of Jag1pos Tregs in mediating tumour infiltrating (TI)-immune cell and progenitor cell activity during early melanoma development. We mined public single cell RNA-sequencing (scRNA-seq) dataset ofTI-immune cells in D5, D8 and D11 B16F10 melanoma to asess Jag1pos Treg abundance during tumour development. B16F10 cells were subcutaneously

implanted into the right flank of Foxp3eGFP-Cre/ERT2Jag1fl/fl (homozygous flox) mice at day 14 after tamoxifen (0.1 mg/g of body weight/ day) i.p. induction for 5 consecutive days (days 1–5). Flow cytometric immunophenotyping was performed to compare the activation status of Tregs and other TI-immune effector cells between Jag1pos Treg-sufficient and -deficient melanoma tumours in vivo. scRNA-seq analysis revealed highest levels of Jag1pos Tregs in early tumours, in line withour flow cytometric data. Intriguingly, these intratumoral Jag1pos Tregs also showed higher Ki67 expression than their Jag1neg counterparts. Conditional deletion of Jag1 on Tregs attenuated tumour growth by 3.5-fold in tamoxifen-treated homozygous flox mice relative to Foxp3eGFP-Cre/ERT2Jag1fl/wt (heterozygous flox) controls at day 15 post tumour-implantation. Analysis of tumour cell digests revealed a decrease of Ki67 expression in total Tregs in homozygous flox mice compared to heterozygous flox controls. Our results suggest that Jag1 may be necessary for the initial expansion of Tregs in early TME driving tumour progression.

Tu201. Characterization of Myeloid-derived Suppressor Cells in Response to Restraint Stress in Preclinical Models of Ovarian Cancer

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Ovarian cancer (OC) is the fifth-leading cause of cancer death among females in the United States. Myeloid-Derived Suppressor Cells (MDSCs) are immature and immunosuppressive cells that play a key role in the tumor microenvironment (TME). Tumor-associated MDSCs aid immune evasion and are associated with poor prognosis in cancer patients. Chronic stress has been shown to increase tumor-associated inflammation and promote immune escape. However, the role of chronic stress on MDSCs infiltration and function in OC is poorly understood. This study aims to determine the role of chronic restraint stress on MDSCs infiltration and biology in the TME. We hypothesize that restraint stress results in the upregulation of MDSCs infiltration in TME. To address this, we inoculated 3 to 4-month-old C57BL/6 female mice with ID8 or IG10 ovarian cancer cells and subjected them to restraint stress (2 hours daily) for 6-8 weeks. Unstressed mice were used as controls. Upon sacrifice, tumors were collected for immunofluorescence (IF) and flow cytometry (FC) analyses. IF and FC were used to characterize MDSCs by the expression of cell surface markers (CD11b+ and Gr-1+ (Ly-6G/Ly-6C)). Our results suggest that chronic restraint stress led to increased infiltration of MDSCs in the TME in ID8 ($p=0.0018$) and IG10 ($p=0.0018$) mouse models. Also, FC results show an increased infiltration of MDSCs (CD11b+/Ly-6G) ($p=0.04$) in IG10 tumors. These data suggest that chronic stress may modulate the OC TME, specifically the regulation and function of MDSCs, which impact the OC progression and TME immunosuppression.

Tu202. CHRM4 Promotes Immunosuppressive Response in the Tumor Microenvironment to Promote Neuroendocrine Differentiation of Prostate Cancer

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Androgen deprivation therapy (ADT) is typically applied to decrease the tumor burden in advanced prostate cancer cases. However, emerging evidence of prolonged androgen receptor (AR) pathway blockage can change the archetypal course of the disease, which results in histological dedifferentiation and alterations in the lineage of prostate cancer cells. Our previous study showed that stimulation of the muscarinic acetylcholine receptor 4 (CHRM4) leads to the development of neuroendocrine differentiation (NED) in prostate cancer after ADT. Here, we aimed to investigate the role of CHRM4 in the tumor microenvironment (TME) to demonstrate its potential as a clinical predictive diagnostic for NED of prostate cancer. We identified the mechanism of how AR inhibition increases CHRM4 expression in prostate cancer after ADT. Our findings also suggest that overexpression of CHRM4 may stimulate immunosuppressive cytokine secretion from immune cells in the TME that trigger an immunosuppressive response of prostate cancer cells, leading to NED and metastasis. Immunosuppressive cytokines mediate feedback mechanisms in the TME by activating CHRM4-driven immune checkpoint pathways, promoting the progression of

NED, migration, invasion, and proliferation of prostate cancer cells. We explored the therapeutic efficacy of targeting CHRM4 as a potential treatment for NED prostate cancer and evaluated the level of immunosuppressive cytokines in the TME as a prognostic biomarker for predicting NED progression in prostate cancer.

Tu203. Circulating Tumor DNA and T Cells Expressing PD-1 and CD39 Enable Neoantigen-targeted T-cell Therapies from Liquid Biopsies

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Lymphocytes targeting neoantigens play an important role in the antitumor efficacy of cancer immunotherapies. However, the strict need of a tumor biopsy both to identify candidate neoantigens and reactive T cells or TCRs can underestimate tumor heterogeneity in the advanced metastatic setting and limits the broad applicability of personalized T-cell therapies. Here, we explored the concomitant detection of non-synonymous mutations via WES and isolation of neoantigen-specific T cells and TCRs using liquid biopsies from patients with metastatic breast, gynecological, colorectal or head and neck cancers of epithelial origin and compared to those detected in the tumor. Biomarker-based isolation of circulating CD8+ and CD4+ T cells allowed the identification of neoantigen-specific T cells in five of six patients, being PD-1hiCD39+ the combination that most consistently captured CD8+ and CD4+ neoantigen reactivities. WES of cfDNA from six patients preferentially identified clonal somatic mutations from tumor biopsies and enabled the detection of seven of 13 neoantigen-specific T-cell responses in four of five patients harboring neoantigen reactivities. Our results underscore peripheral blood as an alternative source to identify cancer-specific neoantigens and CD8+ and CD4+ neoantigen-specific T lymphocytes and TCR from patients with epithelial cancers, bearing important implications for monitoring and exploiting personalized T-cell responses in cancer patients.

Tu204. Clonally Expanded cd38hi Cytotoxic CD8 T Cells Define the T Cell Infiltrate in Checkpoint Inhibitor-associated Arthritis

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Immune checkpoint inhibitor (ICI) therapies used to treat cancer, such as anti-PD1 and anti-CTLA-4 antibodies, can induce a range of immune-related adverse events (irAEs) including ICI-arthritis. To assess the T cell response of ICI-arthritis and how it overlaps with spontaneous autoimmune diseases, we compared T cells from joints of twenty patients with ICI-arthritis to two archetypal autoimmune arthritides, rheumatoid arthritis (RA) and psoriatic arthritis (PsA). Single cell transcriptomic and TCR repertoire analyses highlighted clonal expansion of an activated effector CD8 T cell population in joints and blood of ICI-arthritis patients. These CD8 T cells, identified as CD38hiCD127-, were uniquely enriched in ICI-arthritis joints compared to RA and PsA and displayed an elevated interferon signature. In vitro, type I interferon induced CD8 T cells to acquire the ICI-associated CD38hi phenotype and boosted cytotoxic

function in a novel cytotoxicity assay. In a cohort of patients with advanced melanoma (n=31 pairs), ICI therapy dramatically expanded circulating CD38hiCD127- CD8 T cells, more so than any other T cell population. These CD38hiCD127- CD8 T cells were Ki67+ and frequently bound by the therapeutic anti-PD-1 drug. TCR sequencing of paired blood and synovial fluid T cells from ICI arthritis patients revealed anti-PD-1 drug-bound CD8 T cells in synovial fluid had marked clonal overlap with drug-bound CD8 T cells in circulation but almost no overlap with non-drug-bound CD8 T cells in circulation. These results suggest ICI therapy directly targets CD8 T cells in patients with ICI-arthritis to induce a novel autoimmune pathology distinct from spontaneous autoimmune arthritides.

Tu205. Cytokines Surge in the Blood Years Before a Cancer Diagnosis in Elderly Individuals

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How the human immune system interacts with a tumor before diagnosis is almost entirely unknown. Here we analyzed a longitudinal cohort of 133 healthy individuals (from 18 to over 90 years old), 28 of whom developed cancers over nine years. We found that a large group of cytokines surges 2-3 years before a cancer diagnosis, but not with other conditions (such as inflammatory or cardiovascular diseases). And these cytokine surges precede a cancer diagnosis only in those 80 years or older. In the The Cancer Genome Atlas (TCGA) we found that from 1,741 tissues across 8 types of cancers, elderly individuals elevate the transcription of a broad set of cytokines (including IFNG, IL1B, IL15 and others). Defects in cellular senescence (TP53 or CDKN2A/p16 mutations) sensitize the cells for the inflammation associated with advanced aging. Advanced aging elevates IRF1, a p53 and p16-independent transcription factor for apoptosis and inflammation. The rise of cytokine transcription in early-stage cancer tissues thresholds at the age of 80 years, similar to our serum findings.

Tu206. Development of a Macrophage-based Prognostic Scoring System and Evaluation of Bufalin as a Macrophage Phenotype Modulator in Head and Neck Cancer Using 2D and 3D Models

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Tumor-associated macrophages are key components of the tumor microenvironment (TME) and have been shown to play important roles in the progression of head and neck cancer. As a result, novel treatment approaches are focused on reprogramming M2 macrophages to adopt the M1 phenotype. First, a scoring system based on the high or low density of M1 CD80+ and M2 CD163+ macrophages and on the tumor-infiltrated phenotype was developed in a clinical series of 54 head and neck squamous cell carcinoma patients. Interestingly, this macroscore was found to be more powerful than TNM criteria and p16 status and also significantly associated with poor prognosis for these patients. Additionally, a 3D coculture model was established to analyze the influence of cancer cells on monocyte recruitment and their polarization. This model demonstrated that cancer cells are responsible for monocyte recruitment and M2 polarization, resulting in an immunosuppressive microenvironment with an increased production of IL8 and IL10 cytokines. Finally, we focused on a new compound found in toad venom. Bufalin is an endogenous cardiotonic steroid with reported anti-cancer and immunomodulatory properties. Our data indicated that bufalin reprogram M2 macrophages towards the M1 phenotype underlining its potential as an antitumor immune modulator. Overall, this research highlights the power of the macroscore as a new valuable prognostic biomarker and sheds light on the immunosuppressive tumor microenvironment. Moreover, it indicates that modulating macrophages in the tumor microenvironment using bufalin could be a promising immunotherapeutic strategy for the treatment of cancer.

Tu207. Differential Benefit and Safety of Neoadjuvant Chemotherapy in Patients with Breast Cancer According to Pretreatment Serum Prealbumin Levels

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Background: Prealbumin is a sensitive and easy-to-access laboratory marker that reflects nutrition state, a crucial determinant of immune response that plays a vital role in the host defense against cancer. However, little evidence is reported on prealbumin and neoadjuvant chemotherapy (NAC) for breast cancer. We aimed to evaluate the relationships between prealbumin and treatment efficacy, survival outcomes, and safety in breast cancer receiving NAC. Methods: Patients receiving cisplatin-paclitaxel-based NAC were included. Predictive value of prealbumin for pathological complete response (pCR) was evaluated by logistic regression analysis. Kaplan-Meier estimates were used to calculate survival rates, examined by log-rank test and Cox proportional hazards regression. Chi-squared test was performed to evaluate the association between adverse events and prealbumin. Results: A total of 262 patients were included. Higher prealbumin levels were related to postmenopausal status (p=0.015) and higher body mass index (p=0.002). The pretreatment prealbumin levels could serve as an independent poor predictive factor for pCR (OR=0.487, 95% CI 0.243-0.974, p=0.042), with an interaction between prealbumin and hormone receptor in the postmenopausal patients (p=0.042). Besides, pretreatment prealbumin levels were also an independent poor prognostic factor for disease-free survival (adjusted HR=2.097, 95% CI 1.064-4.131, p=0.032). Furthermore, more hand-foot syndrome (p=0.003), rash (p=0.007), and insomnia (p=0.018) occurred in patients with lower prealbumin levels. Conclusions: This study revealed that higher pretreatment serum prealbumin levels were a risk factor for chemosensitivity and prognosis but a protective factor for side effects in breast cancer receiving NAC. Prospective studies and basic research are warranted to elucidate its biomarker potential.

Tu208. Vitamin B5 Enhances the Antitumor Activity of Human Vγ9Vδ2-T Cells

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γδ-T cell-based cellular tumor immunotherapy has achieved success in some cancer patients, but the rapid exhaustion of proliferation and effector responses in γδ-T cells caused by repetitive phosphoantigens stimulation limit their clinical application. In this study, using a novel formula by treating the peripheral γδ-T cells with vitamin B5 (VB5), we found that VB5 significantly increased the proliferation of Vγ9Vδ2-T cells with enhanced effector functions against different cancer cells. Exogenous administration of VB5 reprogrammed and increased the oxidative phosphorylation (OXPHOS), which further promoted the proliferation of phosphoantigen-expanded Vγ9Vδ2-T cells and increased the productions of lytic granules like granzyme A/B and perforin, and effector cytokine IFN-γ for enhancing their anti-tumor activities against tumor cells. In murine tumor model, treatment of Vγ9Vδ2-T cells with VB5 controlled tumor growth and prolonged mice survival. Our study offers a novel approach by treated Vγ9Vδ2-T cells with VB5, which reprogram their bioenergetic profile toward OXPHOS pathway and then enhance their proliferation and the efficacy of γδ-T cell-based tumor therapy.

W181. Antibodies to PODXL as an Approach to Targeting Tumor Stem Cells

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Podocalyxin (PODXL) is a CD34-related stem cell sialomucin normally expressed by kidney podocytes, vascular endothelia and early hematopoietic progenitors, ESCs and iPSCs. Intriguingly, PODXL is frequently upregulated on a wide range of human cancers and is consistently linked to poor prognosis. Furthermore, we find that inactivation of PODXL in human tumor cell lines cripples their ability to form tumors in xenografted mice, suggesting a functional role in tumor growth and metastasis. Accordingly, we developed several hundred monoclonal antibodies (mAb) to human PODXL as potential immunotherapeutics. One mAb, PODO83, binds to the juxtamembrane domain of PODXL and exhibits a potent ability to block primary tumor growth and development of distal metastases in mice. A second mAb, PODO447, shows exquisite specificity for a tumor glycoform of PODXL but no reactivity with PODXL expressed by any normal human tissue. Intriguingly, evaluation of PODO447 expression on ovarian cancer tumor microarrays suggest that this epitope is selectively expressed by tumors with a "cold" immunophenotype (those lacking infiltrating T and B cells) and thus could be a promising therapeutic for this poor outcome subset of tumors. Although PODO447 lacks an intrinsic ability to block tumor growth, when coupled to toxins as an antibody drug conjugate (ADC) it exhibits dose-dependent tumor cell killing in vitro and in vivo. In summary, we have identified PODXL as a functionally significant molecule expressed by a wide range of poor outcome human tumors and developed two novel PODXL-targeting immunotherapeutic reagents that show promise in preclinical models.

W182. Evaluation of Hhla2's Dual Function on Innate and Adaptative Immunity

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HHLA2 protein, a newly emerging B7 family's member naturally expressed by epithelial cells of the gastrointestinal tract, has immunostimulatory and immunosuppressive functions on T and NK cells depending on the receptor engaged. Its overexpression, observed in many human tumours notably in PD-L1neg, suggests that HHLA2's inhibitory function might result in tumour escape. HHLA2's activating receptor CD28H is express on 70% of circulating NK cells and on all naïve T cells. CD28H engagement increases T cell proliferation, cytokine production and NK cell cytotoxicity. The inhibitory effect of HHLA2 mediated by the KIR3DL3 receptor remains poorly understood. Importantly, KIR3DL3 and CD28H can simultaneously bind to HHLA2 at non-identical sites, with CD28H recognizing HHLA2-IgV1 domain while KIR3DL3 epitope remains undetermined. To elucidate the consequences of HHLA2's engagement on T and NK cells, we developed K562 cells expressing HHLA2. HHLA2 expression inhibits T and NK cell responses to K562 stimulation. Interestingly, we found that HHLA2 expression results in a rapid loss of membranous CD28H on T and NK cells. We then developed K562 cells expressing only HHLA2's IgV1 portion. These cells only bind the activating receptor CD28H but not KIR3DL3. T or NK cells cultured with K562-V1 cells are then no longer inhibited and do not lose their CD28H expression. Whether we are describing a novel tumour escape mechanism tuned by HHLA2+ tumour cells through CD28H down-modulation on T and NK cells needs to be further clarified with tumour cells naturally expressing HHLA2.

W183. Flow Cytometry-based High Throughput Assay Uncovers a TNFα-JAK1-ICAM-1 Cytotoxic Axis Utilized by Double Negative T Cells Against Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) remains a challenging disease with high relapse rates and treatment-associated toxicities following induction chemotherapy and allogeneic hematopoietic stem cell transplantation (allo-HSCT). Ex vivo expanded allogeneic CD3+CD4-CD8- double negative T cells (DNTs) from healthy donors effectively target primary and chemotherapy-resistant AML cells, without developing graft-vs-host disease, in preclinical models. Further, the feasibility, safety, and potential efficacy of allogeneic DNT therapy were demonstrated among AML patients who relapsed after allo-HSCT (ChiCTR1900022795). However, some AML cells resist DNT-mediated cytotoxicity and the mechanisms remain largely unclear. To dissect the underlying mechanisms, a flow cytometry-

based high throughput screening (HTS) was conducted to identify surface molecules that were differentially expressed during effector-tumour cell interactions. By comparing cell surface protein expression levels between DNTs co-cultured with DNT-susceptible versus -resistant AML cells, we identified a novel TNFα-dependent DNT anti-leukemic pathway against AML. DNTs secreted TNFα upon interacting with susceptible AML targets, which sensitized AML cells, including those previously resistant to DNTs, to greater DNT-mediated cytotoxicity without inducing direct apoptosis. Mechanistically, TNFα unexpectedly signalled through JAK1 in AML cells to upregulate ICAM-1 (CD54). This allowed DNTs to engage AML cells more effectively through an ICAM-1 receptor, LFA-1 (CD18/CD11a), for increased killing. Altogether, this study highlights the utility of the flow cytometry-based HTS to discover important immune-cancer cell interactions, and the potential to augment the TNFα-JAK1-ICAM-1 axis for enhanced DNT therapy against AML, including DNT-resistant AML populations.

W184. Foxp3$^+$ Regulatory T Cells Co-localize with CD8$^+$ T Cells in Tumors Responding to PD-1 Blockade

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PD-1 blockade has been a breakthrough in cancer therapy, inducing durable remission in patients. Yet, 2/3 of patients do not respond to treatment in melanoma, the solid tumor type with maximal efficacy. Objective responses are associated with higher degrees of pre-existing inflammation in the tumor, while regulatory T cells (Treg) play a dominant role in suppressing anti-tumor responses. However, it is still unclear how PD-1 blockade impacts Treg cell localization and how Treg cells themselves impact treatment outcome. To dissect this, we used the highly immunogenic YUMMER1.7 melanoma model which, following anti-PD-1 treatment, yields a bimodal distribution of tumor volumes indicating high and low responders. By flow cytometry, we identified that responsiveness to anti-PD1 was correlated with the presence of IFNγ+ CD8+ cells. Immunofluorescence studies established that in both high and low responder tumors, Treg cells preferentially localize in zones of dense CD8 infiltration. Furthermore, tumor-infiltrating Treg cells from both groups displayed potent suppressive capacity in vitro, suggesting that CD8+ cells are evading Treg cell suppression locally in high responders. Further characterisation revealed that, exclusively in high responders, tumor-infiltrating Treg cells progressively acquire Th1-like characteristics, namely T-bet and IFNγ expression, which has been hypothesized to dampen their suppressive function within tissues. To assess Treg cell suppressive capacity in situ, we are using spatial proteomics to compare their distribution and phenotypic heterogeneity across regions of high and low tumor cytotoxicity. Taken together, these data suggest a potential mechanism through which PD-1 blockade relieves Treg cell suppression locally in hot tumors.

W185. High Complexity Immunophenotyping Spectral Flow Cytometry Demonstrates Long-term Stability in Proteomic Stabilized Banked Human Whole Blood Samples

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Introduction: Immunophenotyping human blood in clinical trials often requires real-time assessment and/or isolation of mononuclear cells from large blood volumes, limiting the ability to evaluate cellular biomarkers of development stage candidate therapeutics. Coupling spectral flow cytometry with convenient methods to bank human whole blood enables evaluation of broad ranges of cellular biomarkers. Objective: To overcome operational challenges such as blood volume limitations, batching errors, and sample stability, we evaluated a high-parameter spectral cytometry panel using Proteomic™ stabilized human whole blood samples, stored at -80deg. Methods: A 33-color Cytek Aurora full spectrum cytometry panel characterized common and rare immune cell populations from 1mL of heparinized Proteomic Stabilizer (Smart Tube Inc) preserved human whole blood over 9 months. Banked samples were thawed,

stained, and analyzed in triplicate monthly. Immune cell percentages were monitored for precision and stability and a minimum cell event determination was evaluated using down-sampling in FCS express on 100K CD45+CD15- cell events. Results: Common immune cell populations were stable (< 25% CV) up to 9 months. A minimum of 25,000 CD45+CD15- collected cellular events demonstrated precision (< 25% CV) for rare populations with minimal change associated with storage at 9 months. Conclusion: Broad immunophenotyping using Proteomic stabilized samples reduces blood volume needs and increases the number of conveniently evaluable cellular biomarkers with a high degree of precision and stability. Used in combination these tools enable broad coverage cellular biomarker monitoring in the clinical setting.

W186. Immune Cell Function in Murine Model of Immune-mediated Adverse Events

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Within the last few years there has been increased awareness of immune-mediated adverse events associated with cancer immunotherapies. Basic research into how the immune system is responding to these treatments and promoting new autoimmune-like diseases has been limited. We explored immune-mediated adverse events during immunotherapy in autoimmune-prone mice by assessing autoantibody production, kidney function, and immune cell phenotypes. Changes in activation and exhaustion via flow cytometry were also assessed within CXCR5+ and CXCR5- T cell subsets. Our previous research showed that CXCR5+CD8+ T cells drive autoimmune disease by promoting antibody class-switching and plasma cell differentiation. We observed elevated inhibitory marker expression in autoimmune CXCR5+CD4 and CXCR5+CD8 T cells that could be reactivated during therapy, potentially driving immune-mediated adverse events. CXCR5+CD8+ T cells also upregulate markers of exhaustion during chronic infection and cancer; the reactivation of these cells is a good cancer prognosis during immunotherapy. We tested whether autoimmune CXCR5+CD8+ T cells induce immune-mediated adverse events following checkpoint blockade treatments, and whether these events mimic autoimmune pathology. These studies suggest a deeper understanding of the relationship between immune-mediated adverse events and specific immune cell populations. This research may increase our future ability to better modulate immune-mediated adverse events within patients being treated for cancer.

W187. Immuno-STAT Platform: TCR-selective Engagers for Selective Targeting of IL-2 to Tumor-Specific T Cells

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Cue Biopharma

IL-2 immunotherapy is severely hampered by safety and tolerability concerns. Selective targeting of IL-2 to tumor-specific T cells provides an opportunity to create a therapeutic index, hence maximizing potential benefit while limiting safety risks. The CUE-100 series of Immuno-STATs (ISTs) are rationally engineered Fc fusion proteins containing bivalent peptide-HLA complexes and four attenuated IL-2 molecules to preferentially activate tumor-specific T cells. This scaffold bypasses the need for antigen-presenting cells and stimulates T cells directly, including in the tumor microenvironment. CUE-101, the lead clinical candidate, incorporates an immunodominant peptide from the HPV E7 oncoprotein to activate T cells targeting HPV+ cancers. As monotherapy, CUE-101 has been safely dosed up to 8 mg/kg in 2L+ recurrent/metastatic head and neck cancer with favorable pharmacodynamics and evidence of anti-tumor activity. Parallel dose escalation of CUE-101 in combination with pembrolizumab supports further enhancement of anti-cancer efficacy. These clinical data provide proof of concept for selective modulation of the tumor-specific T cell compartment.

Harnessing modularity of the IST platform has enabled rapid generation of additional clinical candidates targeting other tumor antigens. CUE-102 is 99% sequence identical to CUE-101 but targets a Wilms' Tumor 1 (WT1) peptide

for WT1-positive malignances. Preclinically, CUE-102 selectively expands polyfunctional and cytotoxic WT1-specific CD8+ T cells in vitro and in vivo. Future ISTs targeting shared T cell cancer epitopes, such as mutated KRAS, are also under development and will be discussed. Taken together, the clinical de-risking achieved with CUE-101 supports broad applications of this platform to target diverse cancers.

W188. Improving NK Cell Immunotherapy Against Rhabdomyosarcoma

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Natural killer (NK) cells are innate effector cells known for their high intrinsic cytotoxic capacity and the possibility to be safely applied as 'off-the-shelf' third party donor cell therapy. CD19-specific chimeric antigen receptor (CAR)-expressing NK cells showed promise for the treatment of B-cell malignancies, while so far only limited data exist on the potential of CAR-NK cells as a therapy for solid tumors. Here, we investigated peripheral blood derived NK cells for the treatment of rhabdomyosarcoma (RMS), the most common soft tissue cancer in pediatric patients. Relevant pathways in the NK-RMS interaction were studied by RNA sequencing analysis and RT-qPCR. The cytotoxic potential of NK cells was analyzed in 2D-cultures and 3D spheroids. Interestingly, we observed a decrease in NK cell cytotoxicity in rechallenge killing assays following pre-cultivation of NK with RMS cells (E:T=5:1) for 2 hours in comparison to non-challenged NK cells, indicating that the NK-RMS interaction impaired NK antitumor activity. Blocking the inhibitory NKG2A-HLA-E and the PD-1/PD-L1 axis by CRISPR/Cas9-mediated KO of the respective receptor in NK cells did not improve NK-cell mediated cytotoxicity. To enhance tumor cell recognition, CAR-NK cells were engineered by lentiviral transduction to express a cetuximab-derived CAR (225.28.z) that targets the epidermal growth factor receptor (EGFR). EGFR-CAR NK cells showed stable CAR expression (35-60%), a similar surface marker profile as non-transduced NK cells, and significantly increased cytotoxicity against both, the alveolar RMS cell line RH30 and the embryonal RMS cell line RD. Efficacy of EGFR-CAR NK cells against chemotherapy-resistant RMS are currently investigated.

W189. LAIR-1 Signal Transduction Determines Leukemic Cell Fate

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In acute myeloid leukemia (AML), a significant unmet need remains for therapeutics that selectively eradicate leukemic stem cells (LSCs) and blasts while sparing normal hematopoietic stem cells (HSCs) and healthy immune cells. LAIR-1 is an immune cell-surface expressed inhibitory receptor that has been identified as a therapeutic target in AML. LAIR-1 receptor inhibitory signaling is induced on immune cells when it binds to collagen domain-containing proteins in the extracellular matrix (ECM). However, the function of LAIR-1 on leukemic cells remains unclear. In this work, we utilized a LAIR-1 agonist monoclonal antibody (mAb) to delineate LAIR-1 signaling in leukemic cells compared to signaling in healthy cells. We show that augmentation of LAIR-1 signal transduction in AML cells by an agonist LAIR-1 mAb and ECM collagens induces cell death through mTOR and caspase-7-mediated apoptosis. Conversely, LAIR-1 signaling in healthy cells does not induce cell death, and instead regulates TNFα, IL-6, and IL-17 cytokine production in response to LPS and interferon danger stimuli. Importantly, multiple in vivo and ex vivo models demonstrate that targeting LAIR-1 with an agonist mAb kills LSCs and blasts without adversely affecting HSCs or healthy leukocytes. This novel mechanism of LAIR-1 function provides a unique therapeutic opportunity in myeloid leukemias via agonism of the LAIR-1 receptor.

W190. Local and Systemic Immune Response Against Tumor Specific and Tumor-associated Antigens in Patients with Localized Uveal Melanoma (UM)

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Mutations in the splicing factor SF3B1 generate immunogenic public neoantigens (neoAgs) allowing tracking tumor-Ag specific T cell response. SF3B1Mut related neoAg specific T-cells have been found in the blood of metastatic UM (mUM) patients. Using tetramers specific for SF3B1mut related neoAgs or for a melanocyte differentiation (Melan-A) Ag, we evidenced the presence of memory CD8 T cells for these tumor Ags in the blood of patients bearing SF3B1mut UM tumors indicating that the immune system has been in contact with these tumor Ag. As the eye is an immune privileged site without draining lymph nodes, where would happen the priming step is not obvious. To assess the immune response towards tumor-specific Ag in the primary eye tumor, we studied 20 enucleated patients whose tumor was SF3B1wt. In tumors, we observed a large proportion of CD8+ T cells expressing CD39 and PD-1 suggesting local neoAg reactivity. Among them, 1-10% were specific for Melan-A. Using single cell RNAseq, we are currently characterizing the expanded clonotypes and their associate transcriptome in three patients. Notably, only 1 out of 8 patients with Melan-A infiltrates in the eye harbored memory Melan-A specific CD8 T cells in the blood while in the 7 other patients, the Melan-A were still naïve (CCR7+CD45RA+) suggesting an absence of systemic response or a failure to detect it in the blood. Conclusion: An anti-tumor immune response takes place in the eye since the early stage of tumor development without clear systemic response in many cases.

W191. Microenvironments of Malignant Versus Non-malignant Metastases to the Gut

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Metastasis is the development of secondary malignant growths at a site distant from its origin. We used multiplex immunofluorescent staining and spatial analysis to compare metastatic endometrial cancer with endometriosis, a non-malignant metastasis. Using colon and small bowel disseminated specimens from 3 patients per disease, 90 fields of views (FOVs) per patient, and 37 fluorescence antibodies. Spatial analysis was performed to quantify the expression of cells and the composition of communication between leukocytes in endometriotic lesions versus endometrial carcinoma. Endometrial lesions are well-demarcated, consisting of glandular epithelial structures with strong expression of HLA-I in late endometriosis and expression of HLA-II in the initial stages. These lesions are active sites of antigen presentation between the early ectopic epithelium and T-cells and between professional CD206+HLA-II+ cells. Tertiary lymphoid follicles in endometriosis present themselves more frequently with distinct T-cell and B-cell zones more than twice as frequent and have a consistently higher incidence of contacts between T-cells, antigen-presenting cells, and B-cells versus endometrial carcinoma. By contrast, expression of HLA-I is sporadic in endometrial cancer metastases and has lower densities of CD206+HLA-II+ cells and contact with T-cells, indicative of lower immune activity. Surprisingly, T-cells have more frequent contact with tumor epithelial than ectopic endometrial cells. Our observations indicate differences in lesion morphology, expression of HLA by disseminated cells, infiltration by immune cells, formation of lymphoid follicles, and antigen presentation. These studies provide the rationale for the investigation of the epigenetic and gene expression programs by which disseminated cells contribute to shaping their immediate environment.

W192. Multi-omic Analysis Data from Phase 1 Clinical Trial of CAN-2409 + Valacyclovir in Combination with Nivolumab in High-grade Glioma Demonstrates Systemic Activity After Locally Delivered CAN-2409 Viral Immunotherapy

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Introduction CAN-2409/valacyclovir is a locally delivered viral immunotherapy. Preclinical data suggested potential synergy of CAN-2409 with anti-PD1 in an anti-PD1 resistant high-grade glioma (HGG) model. We evaluated the effects of combination CAN-2409/valacyclovir with nivolumab and standard of care (SoC) in HGG patients. Methods Forty-one HGG patients were treated in a phase 1 clinical trial (February 2019 - March 2021) with CAN-2409/valacyclovir and SoC plus nivolumab (administered biweekly from week 3 for up to 52 weeks). Comprehensive biomarker analysis on tumor and longitudinal peripheral blood samples was performed by the CIMAC-CIDC Network to characterize local and systemic immune response to treatment. Results Survival data were previously reported (SITC 2022). Baseline tumor tissue WES demonstrated frequent in-frame mutations in ARID1B, PAXIP1 and missense mutations in MUC16. Selected combinations of mutations showed statistically significant associations with overall survival. Plasma proteomics and CyTOF revealed activation of a systemic immune response after CAN-2409 monotherapy (week 3) and after co-treatment with nivolumab (week 5): we observed an increase in CD161+CD4+ naïve (p=0.0037, w3), activated CD4+ (p=0.0379, w3; p=0.0011, w5), and CD8+ T cells (p=0.0189, w5). There was also a decrease in immuno-suppressive TIM3+NK cells (p=0.0337, w3; p=0.0324, w5). Combination therapy resulted in expansion of TCR clone density, diversity and clonality in tumors and PBMCs. Imaging analysis of baseline and post-treatment tumor samples by MIBI is ongoing. Conclusion Combination treatment with CAN-2409/valacyclovir plus nivolumab and SoC induces immunological changes in both the injected tumor and peripheral blood indicative of activation of an adaptive T cell immune response.

W193. Peripheral Blood Immunophenotyping for Identification of Pre- and Post-treatment Immune Correlates of Checkpoint Inhibitor Response in Melanoma

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While checkpoint inhibitor therapies have revolutionized cancer treatment over the last decade, it has also become clear that efficacy varies greatly, with some patients showing complete responses whereas others are refractory to therapy. Robust biomarkers of response are currently lacking, thus we aimed to identify correlates of clinical response to the PD-1 blocking antibody pembrolizumab by immunophenotyping peripheral blood of patients with advanced malignant melanoma before and after treatment. We can demonstrate that response to PD-1 blockade was associated with increased expression of the proliferation marker Ki67, particularly in CD28+CD8 T cells. Meanwhile, individuals with progressive disease showed a transient increase in CTLA-4 expressing Treg and we can use ratios of activated and proliferating Treg to CD8 T cells to distinguish response following PD-1 blockade. Remarkably, using unsupervised clustering analysis of pre-treatment T cell subsets we can identify differences in individuals that

responded to PD-1 blockade compared to individuals that did not. These differences mapped to expression of the proliferation marker Ki67 and the costimulatory receptor CD28 as well as the inhibitory molecules 2B4 and KLRG1. While these results require validation in larger patient cohorts, they suggest that flow cytometric analysis of a relatively small number of T cell markers in peripheral blood could provide a valuable tool for stratification of PD-1 blockade treatment response prior to therapy initiation. We are now expanding this approach to individuals treated with combination therapy of PD-1 and CTLA-4 blocking antibodies to understand how T cell populations relate to clinical response in this setting.

W194. Precise Integration of Truncated Cars into the cd3-zeta Gene Conveys Potent Cytotoxicity in T and NK Cells

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The use of chimeric antigen receptor (CAR)-reprogrammed immune cells through adoptive transfer has the potential to revolutionize oncology, autoimmune diseases, and transplant medicine. However, the current reliance on personalized cell manufacturing using viruses as vectors for gene transfer makes these therapies difficult to access due to logistical challenges and high costs. One promising way to overcome these limitations is virus-free gene editing, which allows for precise engineering of safe CAR immune cells without alloreactivity, enabling large-scale production of allogeneic CAR products. In this study, we transfer truncated CAR-transgenes not encoding a main activation domain into the human CD3ζ (CD247) gene using CRISPR-Cas. This creates functional CAR fusion-genes that utilize the endogenous CD3ζ gene as the CAR's activation domain. This procedure allows CAR-reprogramming of conventional T cells, γ/δ T cells, regulatory T cells and natural killer (NK) cells. In T cells, this strategy prevents T cell receptor (TCR) surface expression, thereby eliminating the risk of alloreactivity. CD3ζ-CD19-CAR T cells show better tumor control than lentivirus-transduced CAR T cells in vivo. Furthermore, CD3ζ-integration of a HER2-CAR conveys similar tumor control but with reduced susceptibility to activation-induced cell death in vitro compared to TCRα constant (TRAC)-edited T cells. In contrast to the TRAC approach, CD3ζ editing also enables reprogramming of NK cells to enhance their cytotoxicity in a CAR-dependent fashion and without affecting missing-self recognition or antibody-dependent cytotoxicity. Thus, our findings suggest that CD3ζ gene editing can serve as a universal platform technology to redirect killer lymphocytes for allogeneic off-the-shelf cell therapies.

W195. Removal of Endogenous TCR Chains for Tcr-based Immunotherapies

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The aim of any cancer therapy is to target and kill cancer cells without affecting normal cells. Tumor neoantigens provide targets for therapies based on adoptive transfer of T cell expressing specific TCRs. However, a major issue in transducing T cells with a transgenic TCR is the preexisting expression of TCRs in the recipient lymphocytes. These endogenous TCRs compete with the transgenic TCR for surface expression and allow mixed dimer formation, potentially causing autoreactivity. To circumvent these problems, we developed a novel CRISPR-based method that successfully removes both endogenous TCR α and β chains without affecting the transgenic TCRs. We show that the combination of our guide RNAs successfully removes the endogenous TCRαβ chains with an efficiency of more than 80% in primary T cells. We also demonstrate that this CRISPR strategy results in markedly increased surface expression of the correct transgenic TCR, which positively correlates with a robust killing activity in vitro. We validated the beneficial effect of endogenous TCRαβ knockout in a human immune system mouse model using a clinically relevant TCR targeting a melanoma antigen recognized by T cells (MART-1). In preliminary studies, removal of the endogenous TCR limited the graft-versus-host response without compromising effector function. Ongoing

studies will determine whether GvHD inhibition is complete. Together, our studies provide evidence that the simultaneous removal of both endogenous TCRαβ chains improves anti-tumor performance in a preclinical model for human metastatic melanoma. If confirmed, the reduced risk of GvHD will be highly relevant for “off-the-shelf” allogeneic cell therapy.

W233. Oral Immunization with Highly Attenuated Listeria-based Cancer Vaccines Elicits Protective Gastrointestinal Focused Immunity in Colorectal Cancer

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Gastrointestinal (GI) cancers, including colorectal, small intestine, and pancreatic cancer are a major public health issue worldwide. Commonly used treatment strategies in the clinic include surgery, radiotherapy, and chemotherapy that may provide short-term control of tumors. Cancer vaccines have recently emerged as a promising immunotherapeutic strategy to eliminate tumors while providing long-lasting protection. Listeria monocytogenes (Lm) is an intracellular bacterium that stimulates potent innate and adaptive immune responses that are critical for tumor control. Indeed, Lm-based intravenous (i.v.) vaccines have emerged as a promising strategy to elicit a robust tumor-reactive CD8 T cell response. Despite some reported therapeutic efficacy in clinical trials for various solid tumors, this approach requires improvements. Our prior research demonstrated that foodborne Lm infection of mice induced qualitatively and quantitatively superior CD8 T cell responses that were focused in GI tissues when compared to i.v. infection. Based on this finding, we hypothesized that oral Lm immunization would provide better protection against GI tumors. In this study, we used a modified Lm strain that contains a mutation in the epithelial invasion protein InlA, allowing efficient invasion of murine intestinal epithelium to mimic oral immunization in humans. our findings indicate that highly attenuated Lm-based vaccines elicit robust CD8 T cell responses and demonstrate an excellent safety profile when administered by oral immunization of mice. More importantly, oral Lm immunization provided rapid antigen-specific protection in an orthotopic transplant model of colorectal cancer with a tumor rejection rate of 92%.

Infectious Diseases

Th206. Broadening T Cell Help by Antigen Cross-Linking as a Universal Vaccine Platform Against Rapidly Evolving Pathogenic Viruses

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The effectiveness of influenza vaccine is limited by the heterogeneous immune response it elicits. Factors such as age, sex, and pre-existing antibody titers only partially explain this heterogeneity. Using a novel deconvolution analysis we find subtype-specific bias as a major source of this inter-individual variation. Since the 1940's it has been proposed that this phenomenon is due to prior strain exposure, referred to as "Original Antigenic Sin". But our analysis of flu vaccine responses in a monozygotic twin cohort showed that antigenic imprinting from past exposures can only partially explain strain preference (r2=0.25, p=4.7x10-5). Thus, we hypothesized that host genetics may play a major role in subtype-bias. Consistent with this is our finding that strain-preferences are evident after vaccination in an influenza naïve infant cohort indicating that prior exposure is not essential. We determined the source of this genetic component to the wide variation in influenza hemagglutinin peptide presentation by different HLA class-II alleles resulting in an imbalanced, subtype-specific CD4+ T follicular help after vaccination. To address this, we developed a tunable, plug-and-play vaccine platform to better calibrate T cell help. In a human tonsil organoid system, we demonstrated that engineered, cross-linked HA induces a significantly higher response against multiple influenza subtypes in comparison to the inactivated influenza vaccine. In summary, we have developed an effective strategy to overcome response bias in a population with diverse genetic backgrounds and pre-existing immunity. Furthermore, we successfully adapted this platform to generate a pan-coronavirus vaccine.

Th207. Heme Oxygenase 1 (HO-1) Expression in Dendritic Cells Promotes Antiviral Immunity Against Herpes Simplex Virus Skin Infection

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Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) infections are highly prevalent in the human population, producing a wide range of diseases that go from mild to life-threatening. These viruses infect epithelial cells and neurons where they establish latency, but also dendritic cells, key immune cells involved in the initiation and regulation of antiviral immunity. HSVs block DC maturation and function and ultimately leads to their apoptosis. Heme oxygenase-1 (HO-1) is an inducible host enzyme with increasingly reported antiviral activity. Thus, we sought to assess whether HO-1 modulates the function and viability of DCs upon infection with HSVs. Importantly, we found that HO-1 expression in HSV-inoculated DCs significantly recovers DC function, noteworthy cell viability and hampers viral egress. The stimulation of HO-1 expression in HSV-infected DCs promoted the expression of anti-inflammatory molecules in Dos and elicited the activation of virus-specific CD4+ T cells with regulatory (Treg) and Th17 phenotypes. Furthermore, stimulation of HO-1 expression in HSV-infected DCs transferred into mice favored their migration to the draining lymph nodes, the activation of virus-specific T cells and improved the outcome of HSV-1 skin infection. These findings suggest that HO-1 expression in DCs limits the deleterious effects of HSVs over these cells and induces a favorable virus-specific immune response in the skin against HSV-1.

Th208. P66 is a Bacterial "Don't Eat Me" Checkpoint That Mimics Mammalian CD47 and Facilitates Macrophage Evasion by Borrelia Burgdorferi

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Innate immunity, the first line of defense against pathogens, relies on efficient elimination of the invading agents by phagocytes. Thus in the co-evolution of host and pathogen, pathogens developed mechanisms to dampen and evade phagocytic clearance. Here, we report that bacterial pathogens can evade clearance by macrophages through molecular mimicry of a mammalian anti-phagocytic “don’t eat me” signal. Using a high affinity structural probe for human CD47, a dominant “don’t eat me” signal, we discovered a bacterial protein that mimics CD47’s structure on the surface of Borrelia burgdorferi (Bb), a bacterial spirochete that can cause Lyme Disease (LD) in mammals. Blockade of the mimic delays the infection in vivo. We identified P66, a known virulence factor, as the bacterial mimic of CD47, although P66 lacks sequence homology with CD47. We find that both the initial rate of phagocytosis and total number of phagocytic events are higher for p66 knockout Bb compared to wildtype upon incubation with human-serum derived macrophages. Finally, we determined that patients who return to health following treatment of LD are more likely to generate antibodies to P66 compared to patients who do not. This study demonstrates molecular mimicry as a means used by Bb to inhibit macrophages and evade phagocytic clearance. This is the first report of a bacterial protein signaling through a mammalian ‘don’t eat me’ receptor and this mechanism may have broad implications for understanding host-pathogen interactions and developing therapeutic strategies to combat bacterial infection.

Th209. Presence of Japanese Encephalitis Virus-specific Igm and Igg Antibodies in Suspected Pediatric Febrile Illness Cases in Aseer Region, Southwestern Saudi Arabia: A Transitory Experience

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The presence of Japanese Encephalitis (JE) has not been documented in Saudi Arabia, and no systematic screening for JE in vectors (mosquito) or vulnerable populations has been conducted. In the current study, for the first time in southern Saudi Arabia, the presence of JE virus-specific IgM and IgG antibodies was evaluated in blood samples from children visiting the pediatric clinic at the University Hospital of King Khalid University. The results showed 20% seropositivity for JE with 19 (17.7%) subjects testing positive for IgM and two (1.8%) for IgG. No double positivity for IgM and IgG was observed. The mean age of JE-seropositive subjects was 3 and 2 years of age for boys and girls, respectively. The occurrence of the clinical presentation of convulsion was statistically significant in JE-seropositive cases compared to the unknown viral etiology group. Paralysis and altered consciousness were only observed in JE-seropositive individuals, with the majority of cases showing JE seropositivity at date of illness (DOI) from 1 to 3 days, compared to DOIs of 1 day to 4 weeks for cases of unknown viral etiology. From the results, it is evident that JE is present in the community and causes febrile illness; screening for JE can be expedited in suspected viral meningitis and encephalitis cases. Further investigations including cerebrospinal fluid and larger seroprevalence studies can shed more light on the incidence and prevalence of JE in this region of Saudi Arabia

Th210. Regulation of Chemokines Expression Modulated by E6/E7 Oncogenes of HPV Frequently Detected in Cervical Cancer in the Mexican Population

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In Mexico, Cervical Cancer (CC) is ranked second and third in mortality and incidence among pathologies associated with cancer in women. The Human Papillomavirus (HPV) is considered the main risk factor. The E6 and E7 HPV oncogenes have an immunomodulatory effect related to the induction of carcinogenic processes through the uncontrolled expression of different molecules. This study aims to evaluate the expression of chemokines modulated by the E6/E7 oncogenes of frequent HPVs circulating in the Mexican population, to analyze their expression and

prognostic value in the development of CC. The expression of a chemokines panel was analyzed in cell models expressing E6/E7 oncogenes of HPV -16, -18, - 38b, -107, and -122; and in CC-derived cell lines (SiHa, CasKi, HeLa, SW756, and C33a) by next generation sequencing (NGS) and validated by qPCR. Additionally, chemokines expression was determined using the OncoDB tool and a database of 324 cervical squamous cell carcinoma (CESC) biopsies. Finally, survival analyses (Kaplan-Meier) were performed using databases from the repository "The Cancer Genome Atlas Program" (TGCA). Our results show that CXCL2 and CXCL8 expression increases in the presence of E6/E7 of various HPV genotypes and is overexpressed in CC-derived cell lines and CESC biopsies. This behavior has an unfavorable impact on the survival index in patients with CC. Thus, measuring the expression levels of CXCL2 and CXCL8 could improve the screening techniques for diagnosing/prognosis of cervical lesions.

Th211. SIMON, a Novel Tool for Pattern Recognition and Knowledge Extraction from Multi-dimensional Clinical Data

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To understand the immune system, we need to embrace and welcome its complexity. The immune system comprises multiple cell types that work together to develop an effective response. Which of myriad cell types are important in a particular response, however, is not well understood. In recent years, new technological breakthroughs allowed to obtain enormous amounts of information from a single blood draw by measuring multiple cell types simultaneously. This led to the explosion of data and we are nowadays in need of tools to help us understand data. One solution is to apply machine learning algorithms to extract meaningful patterns from such high-dimensional data. To accomplish that, we developed an approach to automate a machine learning process, named Sequential Iterative Modeling “OverNight” (SIMON). SIMON is specifically suited for clinical data collected across multiple cohorts containing inconsistent features with many missing values. Our approach runs 180 machine learning algorithms to find the ones which fit any given data distribution. Such process maximizes predictive accuracy of the generated models. SIMON was applied to data from five clinical studies across eight consecutive influenza seasons and in COVID-19 infection. Over 3,000 parameters were considered, including serological responses , serum cytokines, cell subset phenotypes, and cytokine stimulations. SIMON identified several immune cell subsets, that correlated with an effective antibody response to influenza vaccination and that can predict COVID-19 severity. Overall, SIMON is a powerful tool for data-driven research that facilitates pattern recognition and knowledge extraction from high-dimensional clinical data collected across multiple cohorts.

Th212. T Cell Transcriptional Signatures of Influenza A/H3N2 Humoral Response to High Dose Influenza and Adjuvanted Influenza Vaccine in Older Adults

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Influenza poses a major health risk for the elderly, with increases in morbidity/mortality with age. >90% of influenza-associated deaths occur in individuals ≥65 yrs of age and are predominantly associated with influenza A/H3N2. The objective of our study was to identify CD4+Th cell transcriptomic genes/signatures associated with antibody (Ab) response, age, sex, BMI, and CMV IgG-serostatus to high-dose (HDFlu) and adjuvanted (MF59Flu) influenza vaccines in older adults. A cohort of 234 healthy adults (median age 71.5: IQR 67.9-76.0 yrs), immunized with HDFlu or MF59Flu was enrolled. Day 0 median influenza A/H3N2-specific HAI titers (1/40; IQR 1/40-1/80) demonstrated the presence of pre-existing Abs. Overall CMV seroprevalence was 56%, and higher (p=0.01) in women (58.2%) than in men (52.3%). There were marginally significantly higher HAI titers observed to HDFlu compared to MF59Flu at Day 28-Day 0 (p=0.06). We found a negative correlation between CD4+Th cell gene expression and CMV-serostatus for several immune-related genes: TRAIP/TRIM27/ILK/CTSS, r<-0.22. Age-dependent correlations were found between HAI and expression of immunologically-relevant genes (KPNA5/ZFP90/ICAM2). We also discovered vaccine type-

specific expression profiles in genes with known immune function: CaMKIV that encodes the calcium/calmodulin-dependent serine/threonine kinase IV (p=0.0008, q=0.1575); its regulator the TMEM38B/transmembrane protein 38B; and the transcriptional coactivator CBP/CREB binding protein, associated with HAI response. The most significant gene associated with Ab titer change to both HDFlu (p=2.12E-13) and MF59Flu (p=1.79E-09) was the TXNIP/thioredoxin interacting antiviral protein. In conclusion, our findings provide evidence that differential Ab response to influenza vaccination is associated with gene expression profiles that differ between vaccines.

Th213. Therapeutic Potential of Monoclonal Antibodies Against Lymphatic Filariasis

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Our laboratory developed a multivalent recombinant fusion protein vaccine, rBmHAXT, for prophylaxis against human lymphatic filariasis (LF). Seed stock of the vaccine is ready for cGMP manufacturing for phase 1 clinical trial. A consistent quality control process is essential for manufacturing the vaccine during the cGMP engineering runs to ensure that there are no batch to batch variations. We contracted with the Fred Hutchinson Cancer Center to prepare mouse B cell hybridomas against rBmHAXT. Several clones were generated. We did extensive screening on the clones and narrowed down 10 clones that produced IgG mAbs that bound to various component peptides of the rBmHAXT and showed significant binding to the native rBmHAXT. In this project, we further characterized these 10 clones for their ability to kill the infective larvae of the LF parasite (Brugia malayi) in vitro and in vivo. Our in vitro antibody-dependent cell mediated cytotoxicity assay showed that four mAbs have significant larval killing abilities in vitro ($p < 0.0001$). We also demonstrated the binding of these four mAbs to the larval surface and the Fc region of these mAb to the macrophages suggesting a role for macrophages in the killing of the infective larvae. These findings also confirmed that the mAbs can bind to the surface of the infective larvae. Challenge studies will demonstrate the ability of these four mAbs as therapeutic monoclonal antibodies against LF.

Th214. Unlocking Serology's Secrets: Harnessing Novel Immune Biomarkers to Predict Lyme Disease Progression and Recovery

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There are currently an estimated 476,000 cases of Lyme disease annually in the US, caused by the bacteria Borrelia burgdorferi (Bb). At least 10% of people with Lyme disease experience persistent symptoms after antibiotic treatment. Currently, there are no tools to predict Lyme disease illness trajectories; a significant barrier to research progress. While there historically has been a significant focus in the field on using bulk IgG and IgM antibodies to diagnose Lyme, we quantified all the different IgG subtypes, IgE, and IgA isotypes of antibodies. We found that the plasma of acute Lyme patients who went on to fully recover after antibiotics contained opposite levels of subtypes and isotypes than patients who developed persistent symptoms. We identified a novel protective immune profiling ratio of the different antibody types in patients who went on to recover. To further explore this, we developed a new FLow-based Immune Profiling technology (FLIP) to better profile Bb-specific antibody isotypes and subtypes. The FLIP innovatively uses live Bb as bait to precipitate out pathogen specific antibodies. We found sex differences in the levels and kinetics of certain Bb-specific antibody isotypes, including IgE. This is important because we found concerningly high levels of IgE that binds to Bb in a third of people experiencing persistent symptoms. In mice this IgE triggers mast cell degranulation. This could indicate the development of an allergy type response to Bb or parts of Bb that are also found in other bacteria, and point to treatment options used for allergies.

Tu209. Characterization of Neuroimmunological Alterations Induced by Infection with the Human Respiratory Syncytial Virus

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Respiratory viruses are the leading cause of acute lower tract respiratory infection, mainly in infants, elderly and immunocompromised individuals, causing high morbidity and mortality rates. The most important of this kind of virus is the respiratory syncytial virus (hRSV), which can cause severe clinical pathologies, including bronchiolitis and pneumonia. However, increasing evidence shows this virus's ability to cause neurological alterations, such as seizures, encephalitis, and encephalopathy. Reports have been shown to detect hRSV RNA and pro-inflammatory molecules in cerebrospinal fluid from patients with neurological signs, supporting the notion of neuroinvasion and neuroinflammation caused by hRSV. Previous data has shown the detection of viral RNA and proteins in the brain of infected mice. In this research, we show that the viral infection alters the blood-brain barrier permeability, altering the tight junctions' expression and allowing the immune cells to infiltrate and increase pro-inflammatory molecules during and after the infection. Moreover, hRSV can infect astrocytes, microglia, neurons, and endothelial cells. Also, the alteration in the glutamate receptors' expression could cause behavioral impairment observed in mice after the hRSV clearance. The brain hRSV-infected cells such as astrocytes, neurons, and endothelial cells can secrete pro-inflammatory cytokines that could alter the neurological synapse's normal establishment and contribute to the long sequels observed in the mice model. Our work provides new insight into the effect of hRSV on the central nervous system and underscores the need to further understand how respiratory virus can damage brain function in humans.

Tu210. Characterization of T Cell-mediated Immunity Against Oral Keratinocytes Infected with Intracellular Bacteria

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Oral lichen planus (OLP) is a chronic inflammatory mucosal disease that involves band-like infiltration of activated T cells and liquefactive degeneration of basal epithelial cells. Our previous study has shown abundant bacterial infection within the epithelium and lamina propria of OLP lesions. This study aimed to investigate the hypothesis that bacterial antigens can be presented to CD4+ T cells by oral keratinocytes, leading to liquefactive degeneration. To demonstrate this, we used immortalized mouse oral keratinocytes (IMOK), OTII peptide-expressing E. coli 7.2 (a strain isolated from an OLP tissue) and OTII-specific CD4+ T cells. First, we confirmed that E. coli stained with pHRodo invades keratinocytes. We examined the expression of the molecules required for interaction with CD4+ T cells, including MHC class II, CD40 and ICAM-1, on IMOK cells. While ICAM-1 was upregulated both by IFNγ and E. coli infection, MHC class II was only induced by IFNγ, and CD40 expression required synergistic effect of IFNγ and E. coli infection. The OTII CD4+ T cells obtained from transgenic mice were differentiated into Th1/Th17 cells using polarizing cytokines. The polarized OTII Th1/Th17 cells were activated by IMOK cells pulsed with OTII peptide. When OTII Th1/Th17 cells and IMOK infected with OTII-expressing E. coli were cocultured, Th1 cells effectively cleared intracellular E. coli, while Th17 cells were able to rescue IMOK from cell death induced by IFNγ and E. coli. This study will help understand the interplay of infected keratinocytes and Th1/Th17 cells in the pathogenesis of OLP.

Tu211. Comparative Single Cell Analysis Reveals Distinct Transcriptional and Epigenetic Immune Signatures of Sars-cov-2 and Respiratory Syncytial Virus Infections in Infants

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Both SARS-CoV-2 and respiratory syncytial virus (RSV) can cause severe disease in infants, leading to hospitalization or even death. However, how these viruses affect the immune systems of infants at the molecular and

cellular level remains unknown. To fill this gap, we profiled peripheral blood mononuclear cells (PBMCs) from 68 infants: 31 SARS-CoV-2 infected (10 convalescent, 12 moderate, 9 severe), 19 RSV infected (5 mild, 7 moderate, 7 severe), and 18 healthy controls, using single cell RNA-sequencing (scRNA-seq) and single nucleus assay for transposase-accessible chromatin with sequencing (snATAC-seq). scRNA-seq data revealed common infectious disease signatures including virus-induced interferon-stimulated transcriptional (ISGhi) states observed across T, B, and Myeloid lineages and depletion of dendritic cells. In contrast, we observed i) a SARS-CoV-2 infection specific depletion of γδ T cells, ii) RSV infection specific expansion of cytotoxic (GZMKhi) memory CD4+ T cells, iii) RSV specific depletion of CD56bright NK cells, and iv) depletion of CD16+ NK cells in severe RSV infection. Epigenetic analyses revealed that binding activity for NK maturation transcription factors T-BET (TBX21) and EOMES was lower specifically in CD56bright NK cells from RSV-infected infants. Moreover, in CD14+ Monocytes, the binding activity for interferon regulatory factors IRF7 and IRF9 and interferon mediator STAT1:STAT2 heterodimer was increased upon RSV-infections yet decreased upon severe SARS-CoV-2 infection. Altogether, we uncovered shared and distinct immune responses to SARS-CoV-2 and RSV infections in infants, highlighting RSV specific changes in circulating NK cells, and transcriptionally shared interferon signatures that are epigenetically distinct between SARS-CoV-2 and RSV infections.

Tu212. Deciphering Human Immune Responses in Infection Diseases Using Machine Learning
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In recent years, there has been a significant increase in the amount of data being generated in the biomedical field. This data is crucial for advancing our understanding of health and disease mechanisms, as well as predicting clinical outcomes. However, the lack of powerful analytical tools has slowed the translation of this knowledge. To address this issue, a software called SIMON has been developed. This software uses over 180 state-of-the-art machine learning algorithms to aid in pattern recognition and knowledge extraction from multiple types of biomedical data. SIMON has a user-friendly interface, standardized pipelines, and automated machine learning methods to help identify optimal algorithms. This allows both technical and non-technical researchers to easily identify important patterns in biomedical data. The software has been tested on various types of biomedical datasets and has been shown to be accurate, easy to use, and powerful. It has been used to identify patterns associated with favorable outcomes for patients with SARS-CoV-2 and influenza, which can help in the development of more effective vaccines and understanding the long-term impact of these viruses on the immune system. SIMON has the potential to accelerate the development of vaccines that provide long-term protection against pandemic viruses.

Tu213. Developing a Vaccine for Dracunculus Medinensis (Guinea Worm)
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One of the major focus of our laboratory is to develop vaccines for neglected tropical helminth parasitic infection. Dracunculiasis, popularly known as Guinea Worm Disease (GWD), is a tropical parasitic infection of humans caused by the nematode, Dracunculus medinensis and the infection is transmitted through drinking water. Migration and emergence of female worms through the skin results in blisters complicated with secondary infections. There are no effective treatment options for GWD. However, eradication of human dracunculiasis is nearly achieved by providing safe drinking water, prompt symptomatic treatment of infected individuals and preventing contamination of water sources. However, several reports demonstrated that dracunculiasis is a zoonotic infection with mainly dogs as carrier hosts. There is no treatment or vaccine to control GWD in dogs. Thus, the major focus of this study was to develop a vaccine for dog dracunculiasis. Genome of D. medinensis is available, but only very few genes are annotated. Based on previous reports from other helminths that are closely related to D. medinensis, we identified, cloned and expressed three antigens (DmHSP, DmTPX and DmCol-4) of D. medinensis. Homolog of all three antigens were previously shown to be excellent vaccine candidates against other closely related parasites. We also

prepared a trivalent fusion protein of all three antigens as DmHTC. Immunogenicity studies in a mouse model showed that all three antigens and the fusion protein are highly immunogenic with IgG titers (>1:20,000) and antigen specific memory T cells. Challenge studies will determine the vaccine efficacy of the identified candidates.

Tu214. Therapy of Multi Drug Resistant Neisseria Gonorrhoeae
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The Problem: Novel therapies are urgently needed to combat the global threat of multi drug-resistant pathogens such as Neisseria gonorrhoeae. Method: Neisseria gonorrhoeae, express glycans, including N-acetylneuraminic acid (Neu5Ac), that mimic host structures to evade host immunity. Neu5Ac is a negatively charged 9-cabon sugar that inhibits complement, in part by enhancing binding of the complement inhibitor factor H (FH) through C-terminal domains (19 and 20) on FH. (Ram S. et al. "Utilizing complement evasion strategies to design complement based antibacterial immunotherapeutics: lessons from the pathogenic Neisseriae" Immunobiology. 2016 October ; 221(10): 1110–1123. doi:10.1016/j.imbio.2016.05.016) Result: Sulfonic nano polymers by targeting complement evasion strategies of Factor H can be designed as complement based antibacterial for Multi Drug Resistant Neisseria gonorrhoeae as immunotherapeutics. Such strategy will have added advantage to target immune evasion, immune virulence, immune resistant and drug resistant microbes. Discussions: Sulfonic nano polymers through formulation methods can be enhanced for therapeutic application of drug resistant Neisseria Gonorrhoeae.

W196. Distinct M. Tuberculosis Antigens Induce Human CD4 Responsive T Cells That Differ in Phenotypes and Maturation States
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Overreliance on studies of a few immunodominant M. tuberculosis (Mtb) antigens (out of > 4,000 proteins) is a bottleneck to identification of protective antigens/epitopes against tuberculosis. We stimulated PBMC from recently exposed, QFT+ HIV negative human adults with distinct Mtb antigens; four immunodominant antigens (classical); ESAT-6, PPE18, PPE46, and EspI, and four novel antigens (antigens with T cell epitope sequence variation demonstrating evidence of evolutionary diversifying selection); RimJ, Rv0012, Rv0010c and LldD2, and assessed CD4 T cell differentiation and functionality by intracellular cytokine staining for IFN-g, TNF-α, IL17, and GM-CSF. We discovered that CD4 T cell responses to distinct Mtb antigens are highly variable but, unexpectedly, classical antigens induce predominant Th1 while novel antigens elicit predominant Th17 cell responses. Specifically, the novel antigens responses are characterized by IL17 production, expression of RORgT, and CCR6, while classical antigens exhibit IFN-g production, expression of T-bet, and CXCR3. Whereas IL17 and IFN-g responses are enriched in novel and classical antigens respectively, TNF-a and GMCSF responses are prevalent in both classes of antigens. Additional analysis revealed that CD4 T cells that respond to Mtb antigens by producing IFN-g are predominantly central memory, while cells that produce IL17 in response to the same antigens are evenly distributed in effector and central memory phenotypes. In contrast, Staphylococcus Enterotoxin B (SEB)-responsive IFN-g and IL17 cells are predominantly effector memory. Studies to determine whether these differences in CD4 T cell responses determine the outcome of Mtb infection, progression to active TB disease or non-progression are ongoing.

W197. High-dimensional Spectral Cytometry Panels for Whole Blood Immune Phenotyping in the Milieu Interieur 10-year Longitudinal Study
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The Milieu Intérieur (MI) project aims to establish the boundaries of a healthy immune response and define the determinants of immune response variation, integrating the impact of hereditary and non-hereditary factors. Immunophenotyping of the 1000 healthy donor cohort by flow cytometry in the initial study in 2011 revealed seropositivity to cytomegalovirus, age, and sex as major determinants for the variability of immune cell counts and showed that genetic factors influence the expression levels of surface immune cell markers. Ten years later, 415 participants from the original cohort were recruited for a longitudinal assessment to compare individual ageing effects with population-level effects (MIv3). One of the main objectives is to define the changes in the initially measured immune phenotypes of peripheral blood cells and to study the contribution of genetic, epigenetic and environmental determinants on these modifications. To this end, we have 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 permits the reproducible quantification of roughly 200 immune cell phenotypes through standardized immunophenotyping at a single site. Repeatability testing of the two panels shows coefficients of variation (CVs) between 1.09% and 9.13% (cell proportions) and between 4.33% and 14.82% (absolute cell numbers). The reproducible results were with CVs in the range of 3.55 –13.30%. This highly standardized protocol was applied to MIv3 cohort and samples from patients with diverse autoimmune diseases.

W198. IRF5 Mediates Persistent Inflammatory Response in HIV-1 Infected Macrophages

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People living with HIV (PLWH) experience chronic inflammation, which contributes to co-morbidities in virally-suppressed HIV+ individuals. HIV-1-infected macrophages constitute important mediators of chronic innate immune activation. Additive effects of aging and persistent HIV-1 infection can promote macrophage dysfunction, contributing to chronic inflammation, referred to as “inflammaging”. We had previously reported that nuclear export and cytoplasmic expression of HIV-1 intron-containing RNA (icRNA) activates MAVS-dependent innate immune sensing and persistent type I IFN responses in macrophages. However, signaling pathways downstream of icRNA sensing have not been fully elucidated. In this study, we investigate whether selective dysregulation of HIV-1 icRNA sensing pathways in aged macrophages can lead to chronic innate immune activation. We utilized patient derived monocyte-derived macrophages (MDMs) from young (< 35 years old) and aged (>50 years old) donors, and observed significantly higher induction of inflammatory responses upon HIV-1 infection and icRNA-expression in older (>50 yo) MDMs (IP-10 expression was enhanced 4-fold). Interestingly, knock-down of IRF5 expression, but not IRF3 or IRF7, all of which are phosphorylated and translocated to the nucleus upon MAVS activation, in THP1/PMA macrophages and MDMs, robustly attenuated icRNA-induced IP-10 secretion. Furthermore, knockdown of IRF5-associated ubiquitin ligase TRAF6 decreased IRF5 nuclear localization and icRNA-induced IP-10 production. In contrast, establishment of HIV-1 infection in macrophages induced nuclear localization of IRF5 that further perpetuated IRF5-mediated inflammatory cytokine expression. Studies in progress will address whether IRF5 expression or activation is dysregulated in HIV-infected aged macrophages and contributes to the “inflammaging” phenotype observed in aging HIV+ individuals.

W199. Leveraging Dried Blood Spot-based Sampling and Room Temperature Preservation for Flow Cytometry Analysis and Sorting

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Blood cell analysis is a major pillar of biomedical research and healthcare. As these analyses are performed in central laboratories, a rapid shipment from collection site is needed because cells and biomarkers may degrade rapidly. Dried blood spots allow the preservation of nucleic acid, protein or metabolite molecules, but entire cells have never been recovered for downstream analysis. Herein, we demonstrate that leucocyte drying, preservation and subsequent elution from a specific polyester membrane is compatible with flow cytometry analysis and sorting. While red blood cells are lysed upon drying, most leucocytes are permeabilized upon the considered drying process, which allowed for an easy staining of all cellular compartments. Providing recovery above 50%, elution from the polyester membrane of previously dried leucocytes could be performed by its simple but yet vigorous shaking while immersed in buffer. As leucocytes eluted from dried blood spots were initially found to have an altered structure upon analysis, it could be improved by adding fixative in the elution buffer. While ongoing studies are performed, more than 100 common immunophenotyping markers were already tested and found to be compatible with the proposed approach. Specimen stability for more than three months as well as RNAseq after sorting have also been demonstrated. Leucocytes from blood can thus be dried, shipped and/or stored for up to several months, then recovered for a wide variety of analyses. We believe this approach may facilitate not only biomedical applications worldwide, but also multicentric clinical research studies which often face implementation challenges.

W200. MIT Mucosal and Systemic Signatures Triggered by Responses to Infectious Organisms (MAESTRO) Clinical Study

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Borrelia burgdorferi is a bacterial spirochete that causes Lyme disease (LD). Patients with LD exhibit a high degree of variability in clinical presentation and disease severity, suggesting individual immune responses may drive this heterogeneity. Unfortunately, even after receiving a correct diagnosis and standard of care antibiotic treatment, approximately 10% of people will experience persistent symptoms. This is similar to observations with Long COVID, which has significant overlap in symptom presentation as chronic LD. Currently, there are no known biomarkers that can distinguish between acute and chronic LD, or predict which acute LD patients will return to health. We have found an antibody signature that can distinguish acute LD patients who continued to have chronic symptoms after treatment from those who returned to health. With that, we have designed a clinical study, MAESTRO, to test the accuracy of our ratiometric antibody score and to create an extensive biobank to investigate differences between acute and chronic LD, compared to Long COVID and healthy controls. Through the MIT Center for Clinical and Translational Research, we will collect blood, saliva, and urine samples, as well as optional sweat, throat swabs, and vaginal/menstrual samples. Biological specimen sampling will be paired with cognitive, eye, and hypermobility testing. We will also follow the acute LD participants who experience symptoms post-treatment, as well as matched participants from the other cohorts, longitudinally with follow-up visits at 6 and 12 months. These data will allow us to test our antibody ratio as a biomarker for predicting recovery from LD.

Inflammatory Diseases

Th215. Dynamic Circulating T Cell Activation States in Extremely Premature Infants

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Extremely premature infants (EPI) are susceptible to infections and inflammatory diseases. To define peripheral T cell ontogeny in EPI serial blood samples obtained in the first 2 months of life were compared to cord blood from term and preterm infants and healthy adults. We analyzed phosphoprotein expression (CyTOF) and transcriptome(scRNAseq). Differences in T cell abundance, phosphoprotein expression, transcriptome, and clonality were noted. Naïve CD4 and regulatory T cells peaked at 1 month, while naïve CD8 T cells continuously increased. Central memory (CM) CD4 T cells were detected in cord blood and decreased from birth to 1 month. Meanwhile, effector memory (EM) CD4 and CD8 T cells were low at birth and increased by two months of age. scRNAseq demonstrated an expansion of cycling T cells at 1 and 2 months of age and clonal expansion in memory CD8 T cells. PI3K/mTOR pathway (pS6) was suppressed at 1 month and increased at 2 months of age in memory T cell populations. TCR activation (pZAP70) was high in naïve and CM CD4 and CD8 populations concomitant with high dendritic cell activation as measured by HLA-DR, pCREB, and pMAPK, suggesting ongoing antigen presentation and memory T cell generation. pSTAT5 levels increased at 1 and 2 months of age consistent with more cycling T cells. In EPI, there is a dynamic progression of T cell signaling, transcriptome and clonality demonstrating activation, proliferation, antigen presentation, and memory formation. Thus, even very preterm infants can mount antigen-specific adaptive immune responses early in life.

Th216. Establishing Leptomeninges Immune Dynamics and Regulatory Control of T Cell Infiltration in the Degenerating Human Brain

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Although several studies have highlighted peripheral lymphocyte infiltration in the degenerating human brain and accumulation of T cells in the meninges in mouse models of Alzheimer's disease (AD), mechanistic studies into the relationship between immune cells in the brain and its borders are lacking. Here, we used single cell RNA/TCR sequencing of fresh postmortem leptomeninges and paired brain from patients across multiple neurodegenerative diseases to define the relationship between meningeal immunity and immune changes in the degenerating brain. Clonally expanded T cells were detected across both the leptomeninges and brain with significant TCR overlap between the two compartments, and differential expression analysis suggested that infiltrated T cells in the degenerating brain may be activated in the meninges. CellChat analysis between leptomeningeal immune cells further revealed activated T cells as potential targets of HLA-E mediated NK cell recognition. Interestingly, meningeal and brain NK cells were characterized by elevated transcription of the novel neurotrophic c-KIT ligand meteorin-like (METRNL). In vitro analyses are currently underway to assess whether NK cells can regulate activated T cells through NK receptor mediated targeting of HLA-E and whether METRNL plays a role in sensitizing activated T cells to NK cell regulation. In parallel, recombinant METRNL dosing studies in human monocyte-derived microglia-like cells (MDMI) are underway to assess whether NK cell secreted factors can modulate human microglial inflammation and amyloid phagocytosis. This study therefore establishes the relationship between the degenerating human brain and leptomeninges immunological niches and introduces a potential mechanism of NK cell mediated control of neuroimmunity.

Th217. Interleukin-4 Activates Olfactory Sensory Neurons and Induces Loss of Smell in Mice Through Distinct Molecular Signatures

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Background: Dupilumab, which binds to interleukin-4 receptor alpha (IL-4Rα) and inhibits signaling of both IL-4 and IL-13, rapidly improves sense of smell in patients with chronic rhinosinusitis with nasal polyps (CRSwNP) via unknown mechanisms. The current study aimed to investigate the effects of IL-4 and IL-13 in smell function in mice. Methods: Calcium uptake was measured in primary murine olfactory sensory neurons (OSN) after acute challenge with IL-4 and/or IL-13. Sense of smell in mice was assessed at baseline and after 5 consecutive days of intranasal administration of IL-4 (10 μg) and/or IL-13 (10 μg) by time to discover hidden food. IL-4Rα antibody (dupilumab surrogate) was injected intraperitoneally. After treatments, the olfactory epithelium was dissected and subjected to RNA extraction for transcriptomic profiling and protein extraction for proteomic analysis. Results: IL-4 and IL-13 increased calcium uptake in OSN. Interestingly, intranasal administration of IL-4, but not IL-13, induced anosmia. In transcriptome analysis, only IL-4 upregulated genes involved in olfactory/calcium signaling, neuronal regeneration, and immune response, and downregulated genes encoding olfactory receptors. Proteomic analysis demonstrated an effect of IL-4, but not IL-13, on inflammatory cell recruitment to the olfactory epithelium and showed activation of neuroinflammation pathways. Finally, IL-4Rα blockade restored the basal level of gene and protein expression, and the sense of smell. Conclusions: Our data show IL-4, but not IL-13, elicits loss of smell in mice and support the hypothesis that the therapeutic effects of dupilumab in restoring smell function in patients with CRSwNP may be mediated predominantly through IL-4 signaling.

Th218. Longitudinal Single-cell RNA Sequencing and T Cell Receptor Analysis of Paired Central and Peripheral Immune Cells in Familial and Sporadic ALS Patients

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Amyotrophic Lateral Sclerosis (ALS) is a multisystem neurodegenerative disorder characterized by the loss of motor neurons in the motor cortex and spinal cord. In the US over 5,000 people are diagnosed with ALS each year. Mutations in the FUS gene can lead to a more aggressive, heritable ALS (fusALS) when compared to other sporadic ALS (sALS). While microglial activation and Treg dysfunction have been observed in both disease states, we lack a comprehensive understanding of how the central and peripheral immune system change throughout disease progression and differ between disease classification. We attempt to address this knowledge gap by following fusALS (n = 15) and sALS (n = 8) patients over their disease course, collecting blood and cerebrospinal fluid (CSF) at multiple timepoints and cortex samples posthumously. Biofluids were subjected to 5' single cell RNA and T cell receptor sequencing. Notably, we observed different proportions of CD4 Th17 and gamma-delta cells between fusALS blood and CSF samples while sALS patients displayed an enriched TEMRA-like population in the CSF. These cell type proportional differences were sustained throughout disease progression. High levels of clonal expansion were captured in all samples. In sALS patients, hyperexpanded clonotypes were localized to MAIT and TEMRA-like populations in the blood and CSF. Additionally, flow cytometry analysis of sALS autopsy samples revealed T cells infiltrating into the motor cortex and spinal cord. This work adds to the growing body of evidence implicating immune involvement in ALS and reveals several specific immune system states unique to fusALS and sALS.

Th219. Measuring Natural Killer Cell Cytotoxicity of Human K562 Cells by Fetal Hemoglobin Release Is Comparable to the Traditional Chromium51 Method

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Measurement of Natural Killer (NK) cell function is a valuable tool in the evaluation of Hemophagocytic Lymphohistiocytosis and reduced function forms part of the established diagnostic criteria. Typically, NK cell cytotoxicity is assessed via measurement of the lysis of human K562 (leucoerythroblastic leukemia) cells. Here, K562 cells are incubated with chromium51 to allow uptake into the cells. Following an incubation period with patient Peripheral Blood Mononuclear Cells (PBMCs), lysis of K562 cells is assessed by measuring the supernatant radioactivity. This assay is laborious, requiring the use and storage of radioactive material in the laboratory which is onerous and a potential hazard. Fetal Hemoglobin (HbF) is the dominant hemoglobin expressed in erythrocytes in-utero and in neonates, but expression is typically < 1% after 12 months of age. Human K562 cells are known to express HbF. We hypothesised that NK cell cytotoxicity of K562 cells could be measured by quantifying HbF release into the supernatant, therefore abrogating the need for radioactive material. We performed a series of NK cell function testing by isolating PBMCs through Ficoll-Paque separation and incubating these with K562 cells spiked with chromium and unmarked K562 cells in reducing effector to target ratios (50:1, 25:1, 10:1, 5:1 and 1:1). The traditional chromium51 assay was done in parallel with HbF measurement. HbF in the supernatant was measured by NanoBit® (Promega) bioluminescent detecting antibodies. We found excellent correlation between the two methods, suggesting that bioluminescent measurement of HbF is a suitable surrogate method for measurement of NK cell cytotoxicity.

Th220. Neuroimmune Correlates of Suicidal and Self-harming Behavior Among Patients with Personality Disorders: A Pilot Study

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The results of a pilot study of immune-related biomarkers among patients (N=235) with personality disorders in association with suicidal behavior will be presented. The study has examined the associations of a broad range of biomarkers reflecting neuroinflammation, compromised blood brain barrier function and altered cytokine and chemokine signaling with self-harm and suicidal behavior among individuals with personality disorders. Biomarkers include plasma protein levels of several mediators of neuroinflammation, chemokines, blood-brain barrier biomarkers, CAMS, IL-18 system, and defensins. Levels of the neuroimmune factors are compared between patients with personality disorders who have and those we do not report a self-harm/suicidal behavior. Comparison include patients with personality disorder and healthy controls that are gender and age-matched. Samples are obtained from a large database of healthy controls and psychiatric population with personality disorders as part of a Norwegian network of personality disorder.

Th221. Peripheral Immunome Dysregulations Reflect Osteoarthritis (OA) Joint Disease Severity

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Osteoarthritis (OA) is a prevalent joint inflammatory condition that is especially common among older people. It is recognized that OA pathogenesis involves a complex interplay of joint damage, poor cartilage regeneration, and inflammation. However, how the peripheral immunome is perturbed by the local joint inflammation is not well understood. To investigate the peripheral immunome dysregulation in OA, we employed mass cytometry time-of-flight (CyTOF) to investigate peripheral blood mononuclear cells (PBMCs) obtained from patients with OA of varying severity (n = 64) and age, sex, and race-matched healthy controls (HCs) (n = 37). We observed peripheral immunome dysregulation that evolves with the disease severity of the OA joint. In early OA, inflammatory immune subsets such as natural killer (NK) and gamma-delta T cells were identified to be dysregulated suggesting the possibility of these subsets infiltrating the joint to initiate and perpetuate inflammation. As the disease progresses to late OA, we discovered further involvement of CD45RO+ and CTLA-4+/PD-1+ T cell subsets suggesting memory formation and immune exhaustion. Furthermore, we also observed an involvement of regulatory subsets in late OA

suggesting the homeostatic attempts to temper the chronic inflammation. In silico deconvolution of RNA-sequencing data obtained from synovial samples revealed joint level immune dysregulations that mirror our peripheral immunome findings. Our study has revealed profound peripheral immunome changes that provide insights to the immunopathogenesis of OA. By uncovering the underlying peripheral immunome changes in OA, we provide further evidence that there is a significant immune component in the disease process of OA.

Th222. Relapsing Myocarditis Following Initial Recovery of Post COVID-19 Vaccination in Two Adolescent Males

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Despite the undeniable public health benefits of COVID-19 spike-protein vaccines, acute myocarditis have been reported. We describe two cases of recurrent myocarditis following mRNA-Covid-19-vaccine occurring in adolescent males (15 years-old) with no previous history of COVID-19. During the first episode both patients presented with fever and chest pain few days after the second dose of BNT162b2-mRNA-Covid-19 Vaccine. The blood exams showed increased cardiac enzymes, both hsTnT (610 and 450 ng/mL respectively) and NT-proBNP (1000 and 1170 pg/mL respectively). The left ventricular ejection fraction (LVEF) was normal at echocardiogram but cardiac magnetic resonance scanning (CMR) were consistent with myocarditis in both patients (regional hyperintensity on T2-weighted imaging and late gadolinium enhancement). They received supportive treatment with full recovery. The 6-months follow-up evaluation demonstrated good clinical conditions with normal cardiological findings and the CMR showed persistent lesions in left ventricle‘s wall. Few months after the 1st episode (9 and 12 months respectively), both patients presented at ED with fever, chest pain and increased cardiac enzymes. Whereas no decreased LVEF was observed, the CMR showed new focal areas of oedema and stable lesions respectively. HHV7 infection was found in both and supportive treatment along with IGIV was started. Both patients reached full recovery after few days. These case reports warn clinicians on performing a strict follow-up in patients with CMR-confirmed myocarditis following mRNA-based-COVID19 vaccine. More efforts are necessary to depict the underlying mechanisms of myocarditis after SARS-CoV2 vaccination to understand the relapse risk and host-related factors favoring such condition and the long-term sequelae.

Th223. Role of Microrna-mediated Macrophage Polarization in Immune Response During Cholestatic Liver Injury

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Macrophages are important components of innate and adaptive immunity that are highly heterogeneous and plastic. Primary sclerosing cholangitis (PSC) is chronic liver diseases characterized with immune responses, inflammation and fibrotic scar formation which would ultimately lead to choleostasis. The current study aimed to clarify the role of liver specific microRNA in choleostasis associated macrophage polarization, immune response and liver fibrosis. A liver specific miR-34a knockout mouse was established by crossing floxed miR-34a mice with the albumin cre mice, and subjected to bile duct ligation surgery relative to controls. Bile duct ligation surgery induced liver injuries which include necrosis, fibrosis, and immune cell infiltration. Interestingly, liver specific miR-34a knockout significantly alleviates liver damages which may be due to decreased biliary ductular reactions associated with immune response and liver fibrosis. It was found that miR-34a-mediated ductular reactions are possibly through Sirt-1-mediated immune response and fibrosis. Furthermore, hepatocyte-derived miR-34a regulates LPS-induced immune response and fibrotic response likely through paracrine effects. Enhanced expression of macrophage M1 markers, CD86 and TNFα was observed in BDL WT mice, which was significantly reduced in BDL miR-34a liver specific KO mice. On the other hand, M2 markers including arginase1 and FIZZ 1, was decreased in BDL WT mice, but recovered after the

depletion of miR-34a. These data provide strong evidence that macrophages in the context of immune response and hepatic fibrosis exhibit miR-34a regulated M1/M2 polarization. The current study demonstrates that liver specific miR-34a plays a deleterious role in macrophage polarization, immune response and liver fibrosis during cholestasis.

Th224. Sex-specific Unconventional Neutrophils Determine Immunological Outcome in Autoinflammatory Disease

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Neutrophils are the most abundant immune cell type that first responds to immune insults in circulation. Although associative evidence suggests that sex differences in neutrophil may be linked to the sex-specific incidence of inflammatory diseases, mechanistic links remain elusive. Here, we identified extensive sex-specific heterogeneity in neutrophil composition under normal and inflammatory conditions at single-cell resolution. Using a combination of single-cell RNA sequencing analysis, neutrophil-specific genetic knockouts and transfer experiments, we found that dysregulated unconventional (interferon-responsive/T cell regulatory) neutrophil subsets led to higher incidence and severity of autoinflammatory Behçet's disease (BD) in males. In addition, male-specific negative effects of both genetic factors and circulating exosomes on interferon-α responsive neutrophil subset that also contributed to vulnerability to disease in males. IFN-α2a, an FDA-approved immune- regulatory agent, also exerts its immunomodulatory effects by promoting interferon-α responsive neutrophil subset more immune-regulatory phenotype. These findings identify sex-specifically distinct neutrophil subsets with different functionalities, and highlight unconventional neutrophil subsets as therapeutic targets for effective prevention and treatment of inflammatory diseases.

Th225. Single-cell Multiomics Approach for Early Drug Discovery

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Drug discovery pipelines aim to deliver candidate molecules to human clinical trials. Each potential molecule requires strong evidence of biodistribution, biological activity, and lack of toxicity prior to testing in humans. This process is long, expensive, and has a high attrition rate requiring many biological targets to be considered in the early stages of validation in order to ensure a single entry into clinical trial. Integrative omics approaches provide biological and clinical insights for characterizing drug candidates and exploring their functions and connections. However, there is significant heterogeneity in the biological activity of drugs on cells within tissues due to specific differences in the proteome, transcriptome, and metabolome of individual cells. Therefore, single-cell technologies are crucial in providing the necessary resolution to reach a comprehensive understanding of the biological properties underlying human health and disease. Systematic single-cell analyses enable the exploration of heterogeneity within cell populations greatly advancing our understanding of less-defined cell subsets. We present our systematic approach combining high-resolution single cell profiling technologies, state-of-the-art computation and machine learning methodologies, and the latest biological and pharmaceutical findings, to accelerate drug discovery through single-cell forward- and back-translation.

Th226. Successful Anti-integrin Alpha4/Beta7 Therapy Reduces Intracolonic Dendritic Cells, Not Effector T Cells in Inflammatory Bowel Disease

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Vedolizumab controls intestinal inflammation in Crohn’s disease and ulcerative colitis by preventing integrin α4β7 on circulating immune cells from binding the addressin MaDCAM-1 on vascular endothelial cells in inflamed intestinal

lamina propria. This was believed to achieve remission by preventing migration of effector T cells into tissue. We tested this hypothesis by evaluating the blood and colonic mucosal immune cells of 13 vedolizumab recipients before and during therapy. We found no changes in effector T cell frequency, mRNA transcriptome, or T cell receptor repertoire overlap between circulating and mucosal T cells to follow vedolizumab initiation, regardless of clinical efficacy. Instead we found a precipitous drop in mucosal CD1c+ dendritic cells (DCs), particularly in treatment-responsive patients. To determine if this was a unique effect of α4β7 blockade rather than simply a nonspecific effect of resolving inflammation, we evaluated 71 biopsies from vedolizumab recipients and 71 control biopsies from inflammatory bowel disease patients not on vedolizumab, matched 1:1 in terms of anatomic location, disease type, inflammation, and concomitant IBD therapy (other than vedolizumab). Again, we found no differences in effector T cell frequency or mRNA transcriptome profile associated with vedolizumab use. Instead, we found significantly fewer CD1c+ DCs exclusively in the biopsies of treatment-responsive patients. Indeed, circulating DCs express more α4β7 than any T cell and more dramatically down-regulate it upon exposure to vedolizumab in vitro. We thus strongly suggest that vedolizumab controls Crohn's disease and ulcerative colitis by blocking the migration of DCs, not effector T cells, to the inflamed mucosa.

Th227. The Glycolytic Pathway Modulates the Astrocytic Function During the Development of Experimental Autoimmune Encephalomyelitis

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Astrocytes contribute to the maintenance of the health of the central nervous system (CNS) and have also been implicated in the onset and progression of neurodegenerative diseases. Recently, it has become evident that metabolism regulates the pathological responses of microglia and astrocytes. Glucose uptake by astrocytes provides substrates that can be processed by the hexosamine pathway (HBP). UDP-GlcNAc is the end product of HBP, which is used as a substrate for O-GlcNAcylation, a post-translational modification controlled by the enzymes O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA). O-GlcNAcylation is associated with the triggering inflammatory responses by immune cells, and the development of neurodegenerative diseases. Here, we report that culture-purified astrocytes responded to the inflammatory stimulus by TNF and LPS through the production of IL-6, CCL2, CXCL1 and CXCL10. The production of these mediators correlates with an increase in the expression of enzymes in the HBP. Additionally, we noticed that increased O-GlcNAcylation by the suppression of OGA activity using Thiamet-G reduced the production of IL-6 and CXCL1. However, in vivo we observed a reduction in the expression of OGT and OGA in the spinal cord of animals submitted to the experimental model of autoimmune encephalomyelitis (EAE). We also generated and validated astrocyte-specific OGT conditional knockout mice. Aldh1creER/OGTfl/fl mice were immunized with MOG35–55 peptide. Notably, the loss of OGT in astrocytes significantly reduced the clinical severity. These data, although preliminary, demonstrate that the hexosamine pathway regulates the inflammatory response of astrocytes and may be a future target for the treatment of neurodegenerative diseases.

Th228. To Quantify the Kinetics of the Degree of Inflammation by a Novel Quantitative *in vivo* Imaging Technique: PET Imaging Study in the CIA Mouse Model

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Hypothesis of this study was that as in the PET-CIA mouse model the degree of inflammation can be quantified so we should be able to determine the kinetics of the degree of changes in inflammation in longitudinal studies. Arthritis was induced using bovine type II collagen in DBA/1J mice (n=30), out of which 20 mice developed arthritis on Day 28. The mice had PET imaging on the week 1st, 2nd, 3rd and 5th; that is imaging on the days 0, 28, 35, 42 and 56. The maximum 18F-FDG uptake (SUV score) was determined for the most severe joint in each mouse to generate a comprehensive PET score (PS). The median clinical score (CS) of the most affected limb increased with time to 2, 2, 3, 3.5 and 3 respectively on day 28, 32, 42 and 56. The PS SUV score on day 0 was 1.02. PS median values

increased to 1.52 on day 28 ($p < 0.05$), 3.5 on day 35 ($p < 0.05$), 4.5 on day 42 ($p < 0.05$) and 3.5 on day 56 ($p < 0.05$). Our results confirm that the kinetics of the total inflammatory load can be quantified. Further on treating these mice with tofacitinib we could see significant therapeutic efficacy within the 1st week of therapy. Thus, this will be a novel tool for monitoring the inflammatory load and a unique tool to measure efficacy or resistance to therapies at an early stage. With time we are transferring these observations to quantify the degree of inflammation in human with RA and PsA.

Th229. ZFYVE21 is a Mediator of Inflammatory Liver Disease
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Non-alcoholic steatohepatitis (NASH) is characterized by lipotoxicity and hepatic inflammation and predisposes patients to hepatocellular carcinoma. The immune mechanisms underlying NASH are undefined, hindering the development of therapeutics. We have identified an early endosome effector, ZFYVE21, that, when knocked out in endothelial cells in mice, elicits NASH by 3 months of age. Livers from these mice show signs of endothelial cell dysfunction and upregulate a broad panel of pro-inflammatory mediators including CCL5, a known mediator of NASH. Endothelial cell-specific ZFYVE21 regulates inflammatory processes associated with NASH.

Tu215. A Deep Dive into a Wide Spectrum: Redefining Blood Cell States in Health and Disease
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Blood is a window into human health and often used for disease diagnostics. It has been profiled by single-cell multi-omics technologies at greater depth than any other human tissue, but to-date these findings have not translated to clinical utility, possibly due to the focus on a different disease in every study. With the Human Cell Atlas moving towards providing a healthy reference map for all cell states in the human body, inevitable questions will arise about how this is altered in disease. To this end, we are building a blood single-cell multi-omics atlas encompassing over 5 million cells, incorporating healthy and 16 distinct immune-related disease states. Studying gene programs across diseases allowed us to understand how each cell state functions across different conditions and go beyond the current cell state definition that includes a set of unique markers it expresses. Trajectory analysis enables setting new boundaries to how we define a 'healthy' condition and studying in a new resolution how circulating immune cells change during disease. We identify shared dynamics of different cell states across disease conditions to better understand how the immune system works as a whole in the changing environment. This blood cell atlas provides a catalogue of the full spectrum of circulating PBMCs, enhancing healthy data atlases and fundamentally supporting future annotation of blood data in health and disease. Utilizing this compendium allows to answer a range of important questions in immunology with unprecedented resolution.

Tu217. A Humanized Mouse Model of VEO-IBD by Crispr/Cas9 Editing of the IL10 Gene in Human Hspcs
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Very early onset inflammatory bowel disease (VEO-IBD) is a severe form of IBD that affects children younger than 6-years-old. In VEO-IBD, uncontrollable immune responses to gut microbiome-derived antigens result in excessive and chronic inflammation of the intestines. The most common and severe form of VEO-IBD is caused by loss-of-function

(LOF) mutations of the IL10, IL10RA, and IL10RB genes, which encode the immunoregulatory cytokine IL-10 or its receptor subunits. Although Il10-deficient mice provided critical insights in IBD, differences in IL-10 biology and IBD pathophysiology between mouse and human warrant the need for a humanized mouse model of VEO-IBD. Therefore, we designed a multi-guide CRISPR/Cas9 gene editing strategy in which the IL10 locus of human CD34+ hematopoietic stem and progenitor cells (HSPCs) is disrupted with high efficiency and negligible off-target events. As a control, rAAV-mediated knock-in (KI) of wildtype IL10 cDNA at the endogenous IL10 locus of these IL10-knock-out (KO) HSPCs was achieved with high integration efficiency. To create a humanized VEO-IBD mouse model, we transplanted specific-pathogen-free (SPF) NSG-SGM3 mice with wild-type and IL10-KO HSPCs and observed high reconstitution of the human immune system in multiple tissues including spleen, bone marrow, and mesenteric lymph nodes. However, we did not observe colitis in mice transplanted with IL10-KO HSPCs which was comparable to germ-free Il10-deficient mice. Therefore, we aim to challenge these mice with IBD-triggering bacteria in follow-up cohorts. In conclusion, the successful reconstitution of mice with human IL10-deficient HSPCs will, upon further optimization, allow the development of a humanized mouse model of VEO-IBD.

Tu219. ACE2 Expression in the Liver of Obese People Is Associated with Anthropometric, Biochemical, and Proinflammatory Cytokine Variables

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Obesity is characterized by chronic low-grade inflammation associated with developing and progressing metabolic dysfunction-associated fatty liver disease (MAFLD). The pathogenesis of MAFLD depends on multiple factors, such as the disruption of the renin-angiotensin system (RAS) balance. There is evidence that increased activation of the hepatic classical RAS axis or inhibition of the alternative axis is associated with increased expression of lipid oxidation, oxidative stress, and inflammation genes. Moreover, it has been proposed that obesity can trigger a loss of balance in this system. To understand in greater depth the characteristics of the hepatic RAS in obesity, this work aimed to determine in liver samples from patients with obesity the association of ACE2 with cytokine protein levels, as well as with different biochemical, clinical, and anthropometric parameters. Thus, seventy liver tissue samples from patients with obesity were included. To assess the expression of ACE2 and proinflammatory cytokines, RT-qPCR was performed. The concentration of cytokines was evaluated using an immunoassay with magnetic beads. We found that the expression of ACE2 is higher in men than in women, in people with comorbidities vs. without comorbidities, and in subjects with systemic arterial hypertension (SAH) vs. no HAS. Likewise, an inverse correlation was found between the expression levels of ACE2 and the serum concentration of GGT. Finally, we found that TNF-α positively influences ACE2 mRNA levels. Based on these results, it is likely that with increasing TNF-α, the susceptibility to develop MAFLD increases, and as TNF-α levels in the liver increase and consequently, ACE2.

Tu220. Agonist Anti-chemr23 Mab Inhibits Netosis and Neutrophil-mediated Inflammation

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Neutrophils are the most abundant immune cells in humans playing essential roles in invading pathogens and debris elimination. However, neutrophil-associated chronic inflammation has now emerged as key pathogenic mechanism in many autoimmune and chronic inflammatory diseases. Neutrophil extracellular traps (NETs) process, initially released to limit pathogen dissemination, is now recognized as a key driver of tissue fibrosis. Resolution of inflammation is elicited by pro-resolving lipids (e.g. Resolvin E1) which target GPCRs, such as ChemR23, inducing neutrophil apoptosis, and blunting neutrophil tissue recruitment. We previously reported that ChemR23 mRNA is overexpressed in human chronically inflamed tissues and demonstrated ChemR23 is expressed at the surface of neutrophils only upon inflammatory settings. We generated a novel agonist anti-ChemR23 mAb, cross-reacting with

human and cynomolgus monkey, and found it induces selective inflammatory neutrophil apoptosis and potent inhibition of NETosis by both human and cynomolgus neutrophils in vitro. In vivo, we observed that the agonist anti-ChemR23 mAb accelerated the resolution of skin inflammation induced by intradermal LPS or UV-killed bacteria injection in cynomolgus monkeys, reducing erythema, limiting neutrophil skin infiltrates and decreasing neutrophil IL-8 chemokine expression in the inflamed tissue. No decrease of neutrophil count has been observed in the periphery, in accordance with the translocation of ChemR23 at the surface only upon inflammatory stimuli. Altogether, our findings show that agonist ChemR23 mAb could control neutrophil recruitment, induces local inflammatory neutrophil apoptosis, and extinguishes inflammatory NETosis. This new class of pro-resolutive mAb constitutes an innovative therapeutic approach to fill unmet needs in chronic inflammatory diseases.

Tu221. ALTB-168, an Immune Checkpoint Enhancer (ICE) Targeting P-selectin glycoprotein-1 (PSGL-1), is Effective in Treating T Cell Mediated Inflammatory Diseases

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P-selectin glycoprotein ligand 1 (PSGL-1), which has been described as an adhesion molecule, was recently discovered to be a novel immune checkpoint regulator that modulates T cell homeostasis. Here we show that PSGL-1 is upregulated in activated T cells compared to naïve T cells. Cross-linking of ALTB-168 (neihulizumab, an anti-PSGL-1 agonistic antibody) down regulates early T-cell receptor mediated signaling, T cell proliferation, and cytokine secretion. This inhibitory function is independent of the migration function of PSGL-1 as the binding of P-selectin to PSGL-1 is not interfered by ALTB-168. In a pre-clinical GVHD model, ALTB-168 treatment resulted in increased population of apoptotic CD3 T cells, reduced severity of GVHD and prolonged survival of the mice. In a trans-vivo delayed type hypersensitivity (DTH) study where human PBMC, previously sensitized with tetanus toxoid (TT), was implanted in the mouse footpad together with TT, the induration of the mouse footpad was reduced by ALTB-168 in a dose-dependent manner. DTH studies performed in non-human primate (NHP) also showed that ALTB-168 inhibited skin induration as well as the inflammation of the lesion site. Intriguingly, ALTB-168 treatment does not impact the development of T-cell dependent antibody production, suggesting a marginal impact in primary T cells - a significant safety advantage in clinical development. Indeed, no significant adverse events have been reported for ALTB-168, in which nearly 200 subjects were treated. These data support the continued development of ALTB-168, the first anti-PSGL-1 agonistic antibody that down regulates T cell effector function, for the treatment of human inflammatory diseases.

Tu222. Alterations in the Gut Microbiota in Advanced Age-related Macular Degeneration may be Involved in its Pathogenesis

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Investigating the relationship between alterations in the gut microbiota in age-related macular degeneration (AMD) can uncover a completely novel mechanism of this blinding eye disease. A cross-sectional case-control study of advanced AMD patients and age-similar healthy control subjects from a university practice was performed. We used 16S rRNA gene sequencing to characterize gut bacterial differences and flow-cytometry-based bacterial cell sorting taxa-specific coating of the intestinal microbiota with immunoglobulin A (IgA-SEQ) to show that high IgA coating uniquely identifies colitogenic intestinal bacteria. We analyzed 85 advanced AMD and 49 healthy control subjects. An intestinal dysbiosis in advanced AMD was demonstrated at the phyla and genus levels (e.g. increased differential abundance of Proteobacteria-Gammaproteobacteria and reduction in Firmicutes-Clostridia [$p < 0.05$]). Increased genetic risk score in AMD subjects was associated with decreased gut bacterial alpha diversity (Spearman's $r=-0.3$, $p=0.0086$ value), whereas AREDS (2) supplementation significantly increased alpha diversity. The percentage of IgA-bound gut bacteria was significantly increased in ARMS2 homozygous risk allele patients (23.7%, $p=0.008$)

compared to non-risk allele AMD subjects. AMD and control subjects showed differences in IgA coating indices for Prevotella and Ruminococceae genera. Metabolite pathways represented by the gut bacteria that were differentially abundant in AMD vs. control subjects included lipid metabolism and carotenoid biosynthesis pathways that are known to be important in AMD pathogenesis. AREDS supplementation and genetic risk are crucial determinants of intestinal microbial alterations in advanced AMD and may point to novel therapeutic targets to treat this blinding condition.

Tu223. Anti-tnf Treatment Response Classifier for Inflammatory Bowel Disease Patients

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Background: Inflammatory bowel disease (IBD) is a chronic inflammatory autoimmune disease which includes ulcerative colitis (UC) and Crohn's disease (CD). It is characterized by chronic inflammation of the gastrointestinal tract and rectal bleeding. Anti-TNF-α Ab therapy is a frontline targeted therapy for IBD but it is not clear why some patients fail to respond or develop resistance after a short time. Clinical biomarkers capable of predicting anti-TNF-α Ab response at baseline can help guide therapy decisions, reduce treatment costs and lead to better treatment outcomes. Here we developed a robust predictive model, characterized by a gene signature, capable of discriminating anti-TNF-α response in IBD patients at baseline. Methods: We collected public IBD transcriptomic datasets containing Mayo score-based response labels. Following normalization, baseline colonic UC samples were used to train and evaluate an Elastic Net classification model employing a leave-one-out, nested cross-validation strategy. The model was evaluated in independent cohorts consisting of UC or CD baseline colonic samples from different platforms. Finally, the resulting gene classifier was compared to a previously published classifier. Results: Based on our gene signature, cross-validated predictive model accuracy on the UC training set was 84%. Our model also classified independent colonic baseline samples, interrogated on different platforms, with an accuracy of 86% (UC) and 83% (CD). Lastly, we used principal component analysis to compare our gene-signature with a published signature to evaluate their ability to separate responders from non-responders. In various testing datasets, our signature separated samples as well, or better, than the previously published signature.

Tu224. Asthmagraph – Knowledge Graph Framework for Drug Target Prioritization in Asthma

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Knowledge graphs (KGs) use a graph-structured data model to store interlinked descriptions of entities – objects, events, situations or abstract concepts – with free-form semantics (unstructured data). They could be thought as of a collection of statements (e.g. "A activates B") that are gathered into a domain knowledge and organized in a graph structure. KGs are a powerful way to efficiently store and retrieve domain findings, as well as interrogate their relationships through graph "traversing". Importantly, KGs also allow for the inference of new relationships not present in the initial collection of statements used as data input, as well as the effect of perturbations in the graph. Starting from a large database of pathway and network interactions (Qiagen Biological Knowledge Base), we extracted the subnetwork of entities related to asthma (AsthmaGraph), based on pathogenic cell-type, asthma related pathways and genes. Using the KG framework as a foundation, we analyzed the causative connections between genes and immune conditions related to asthma. Furthermore, using a graph embedding, we applied deep learning and infer properties of weakly characterized entities in the network. Our results demonstrate a significant enrichment in clinically validated therapeutic targets amongst the causative connections, supporting the effectiveness of this approach in the target identification process for drug discovery and clinical development. By expanding the same approach to other immune conditions, we will also be able to reposition known drugs on other medical indications.

Tu225. Clinical Outcomes of Adalimumab in Real-world Treatment of Non-infectious Uveitis from a Single Tertiary Center in Tokyo

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Purpose: Tumor necrosis factor-alpha inhibitor, adalimumab (ADA), has been widely used in the treatment for non-infectious uveitis (NIU). The present study investigated the long-term clinical outcomes of adalimumab for non-infectious uveitis in the real world practice. Methods: Records of 32 patients (women: 18, men: 14, 62 eyes) with NIU followed for at least 6 months after starting ADA , were retrospectively reviewed. The rate of treatment failure after starting ADA therapy was evaluated in both active NIU patients and inactive NIU patients using the criteria defined in VISUAL I and VISUAL II clinical trials Results: The mean age was 50.2 years (14~79). One fourth had unclassified NIU and Vogt-Koyanagi-Harada disease (n=8) and sympathetic ophthalmia (n=4) were included. The most common type of uveitis was panuveitis (87%). The most common indication of ADA was failure to oral immunosuppressive drugs. The rate of active NIU at initiation of ADA was 43% (n=14). The median follow-up period after initiation of ADA was 24 months. The rate of treatment failure was 22% (n=7) at 6 months after initiation of ADA and 44% (n=14) over follow-up. The dose of oral corticosteroid and cyclosporine at 12 and 24 months was significantly reduced compared to that prior to ADA therapy. The ADA treatment was discontinued at 5 patients (16%) and no serious adverse events were observed over follow-up. Conclusions: ADA showed the efficacy and safety for NIU and corticosteroid/cyclosporine sparing effect. The rate of treatment failure increased during long-term follow-up period.

Tu226. Constitutive IL-1RA Production by Modified Immune Cells Protects Against Tissue and Systemic il-1-mediated Inflammatory Disorders

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IL-1 is a proinflammatory cytokine with multiple functions. While it is important for fighting infections and promoting healing, overproduction of IL-1 leads to severe inflammatory conditions. Anti-IL-1 blockade with anakinra, a recombinant form of the naturally occurring human IL-1-receptor antagonist (IL-1RA), is the standard of care for patients with autoinflammatory disorders. However, anakinra has several potential drawbacks, like a short half-life, poor tissue penetration, and side effects. To circumvent these limitations, we empowered immune cells with a stable IL-1RA production by adoptively transferring in mice hematopoietic stem-progenitor cells (HSPCs) transduced with a lentiviral vector encoding human IL-1RA. IL-1RA-expressing HSPCs engrafted, self-renewed and differentiated into all immune cell types with amplitude and kinetic similar to control mice. No microscopic abnormalities were observed in the immune and non-immune organs of chimeric mice, indicating that stable IL-1RA expression was well tolerated. Cell-mediated IL-1RA delivery significantly reduced acute neutrophil recruitment in response to crystal-mediated peritonitis to a similar extent as anakinra. In contrast, immune cell-derived IL-1RA was superior to anakinra in ameliorating the clinical phenotype of CAPS at both systemic and tissue levels. In addition, our approach significantly improved the clinical score and survival, and reduced immune cell infiltrates in the spinal cord of mice with EAE. Our results demonstrate that stable IL-1RA production by immune cells protects mice against IL-1-mediated inflammation in three experimental models, opening new therapeutic avenues for treating autoinflammatory diseases caused by IL-

1 dysregulation.

Tu227. Drug Induced Interstitial Lung Disease and Inhibitors of Interleukin 1 and Interleukin 6
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Background: Previously we reported an emerging highly fatal, diffuse lung disease (DLD) in Still's disease patients, associated with exposure to anakinra, canakinumab, tocilizumab or rilonacept. We discovered that patients met diagnostic criteria for definite or probable drug reaction with eosinophilia and systemic symptoms (DRESS) and that 80% carried DRB1*15 alleles (OR>15) with or without DLD. Here, we compared those stopping to those not stopping the implicated drug for survival probability, resolution of DLD, and treatment intensity (TI) required to subsequently manage Still's. Method: Retrospective multicenter Still's-DRESS cases were collected during Still's treatment with any interleukin 1 or 6 inhibitor (IL-1i/IL-6i). Resolution of DLD after drug withdrawal was defined as normal oxygen saturation without supplementation, absence of respiratory symptoms, and normal chest HRCT. TI was assessed by the number of immunosuppressive medications (ISM) required to manage Still's. Results: 74.4%(58/78) of Still's-DRESS developed DLD; 48.3%(28/58) stopping IL-1i/IL-6i were followed ≥1 year after drug stop. To date, 61%(17/28) show lung disease resolution vs 0%(0/58) during IL-1i/IL-6i. Additionally, in Still's cases withdrawing drugs, followed ≥1 year, regardless of DLD, 48.0%(36/75) decreased TI versus 52.0%(39/75) continuing IL-1i/IL-6i. At one year after drug stop, fewer ISMs were needed for disease control: ≤ 1 ISM in 69%(24/36) vs 2%(8/39), p=7x 10-5 stopping versus continuing IL-1i/IL-6i after initial DRESS features, respectively. Survival favored stopping vs continuing any IL-1i/IL-6i [HR=0.24(95% CI: 0.08,0.74)p=0.0127]. Conclusions: Stopping all IL-1i/IL-6i in this condition improves survival, may resolve DLD and simplify subsequent Still's disease management implicating these drugs in pathophysiology. Specific mechanism remains unknown.

Tu228. Therapy of C3 Glomerulopathy
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In the pathogenesis of Kidney disease such as C3 Glomerulopathy, the human CFHR–Factor H gene cluster encodes are emerging as novel complement and immune modulators. (Peter F. Zipfel, Thorsten Wiech, [...], and Christine Skerka " CFHR Gene Variations Provide Insights in the Pathogenesis of the Kidney Diseases Atypical Hemolytic Uremic Syndrome and C3 Glomerulopathy" J Am Soc Nephrol. 2020 Feb; 31(2): 241–256. Published online 2020 Jan 24. doi: 10.1681/ASN.2019050515) Method: The underling cause of inflammation is due to hyper activation of complement pathways. Sulfonic nano polymers are balanced modulator of Factor H and Factor D and offer an attractive therapeutic target for C3 Glomerulopathy. Discussions: Sulfonic polymers are backed with extensive human safety record and can be advanced readily with additional formulation changes.

W201. ALTB-268, a Tetravalent anti-psgl-1 Antibody Derived from ALTB-168, Shows Enhanced Potency in Treating T Cell Mediated Inflammatory Diseases
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We have discovered a novel anti-PSGL-1 monoclonal antibody, ALTB-168 (neihulizumab), that acts as an immune checkpoint enhancer (ICE) by down-regulating T effector function. With this unique mechanism of action, ALTB-168 has been advanced clinically for the treatment of T-cell mediated inflammatory diseases. ALTB-168 was found to induce inhibitory signaling upon binding to PSGL-1, which is enhanced by cross-linking with anti-human antibody in

vitro. An Fv engineered tetravalent antibody, with four PSGL-1 binding sites, can potentially facilitate the clustering of cell surface PSGL-1 and the downstream signaling compared to a conventional bivalent antibody. Here we show that a tetravalent version of ALTB-168, named ALTB-268, demonstrated greater than 10-fold higher potency in in vitro T cell activation inhibition assays compared to ALTB-168. When compared in a human-mouse trans-vivo DTH study as well as in a non-human primate (NHP) DTH study, greater than 3-fold higher potency was observed for ALTB-268. The increased potency is likely related to differences in stoichiometry and increased avidity rather than increased affinity, as a single 268 molecule can bind to more PSGL-1 compared to a single 168 molecule, while similar affinity for both ALTB-168 and ALTB-268 was measured by SPR or ELISA. Most importantly, a similar safety profile as ALTB-168 was observed for ALTB-268 in NHP toxicology assessments, with a NOAEL of 120 mg/kg in a definitive 28-day weekly repeat-dose toxicity study, and a bioavailability of 70% by subcutaneous (sc) route. These data support the clinical development of ALTB-268, sc, for the treatment of T-cell mediated inflammatory diseases.

W202. Clonally Expanded T Cells Reveal a Pathophysiologic Distinction Between Disease Subtypes in Human Uveitis

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Uveitis is a heterogeneous group of inflammatory eye diseases leading to blindness. Lack of pathophysiologic understanding underlies the failure of empiric treatment for 30-60% of patients. We used single cell RNA and V(D)J sequencing to study ocular immune cells in aqueous biopsies from 23 uveitis patients. We found evidence of antigen-driven immune responses in uveitis including clonal expansion of ocular lymphocytes. The most highly expanded T cells shared a core gene signature that included CXCR6, HOPX, LGALS1/3 and IFNG and lacked SELL, TCF7 and CCR7, consistent with antigen-driven T cell activation. Highly expanded CD4 T cells were specifically enriched in RORA and IFNG with lower levels of RORC and IL17F, corroborating murine studies that suggest Th1 responses drives uveitis. Highly expanded CD8 T cells expressed comparatively low levels of granzyme K, perforin and granulysin, suggesting these cells have reduced cytotoxic potential within the immune privileged eye. We found a wide variation between patients in the degree of clonal expansion, leading us to hypothesized that the quality of immune responses may vary between disease types. We therefore used principal component analysis to determine whether the aggregate composition of immune cells could differentiate clinical disease subtypes. We then performed bootstrapping to generate confidence intervals for each variable and identified 7 which contributed robustly to discriminating patients with distinct clinical presentations. These notably included the degree of clonal expansion of CD4 and CD8 T cells, as well as B cells, suggesting that antigen-driven inflammation is a key pathophysiologic feature differentiating patients with uveitis.

W203. Drug Repurposing in Pulmonary Fibrosis Based on the Immune Mechanism of the Disease

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Idiopathic pulmonary fibrosis (IPF) is a chronic and refractory interstitial lung disease with no effective treatment other than lung transplantation. Although there are two approved drugs for IPF, pirfenidone and nintedanib, they were not able to completely cure the disease. Therefore, there is an urgent need to develop of a new therapeutic agent for IPF. To find effective drug for IPF, we chose a drug repositioning strategy. In this study, we investigated the effect of a xanthine derivative, theophylline that has long been used for the treatment of asthma, on pulmonary fibrosis. Administration of theophylline attenuated the fibrotic changes of lung tissues and improved mechanical pulmonary functions in a bleomycin (BLM)-induced pulmonary fibrosis. A variety of studies have reported the importance of Th17 cells and IL-17 in IPF. IL-17 was detected in the sera of BLM-treated mice as well as patients with IPF, and lung fibrosis was inhibited by IL-17A antibody. Theophylline treatment suppressed IL-17 production through inhibiting cytokines controlling Th17 differentiation; TGF-β, IL-6, IL-1β and IL-23. Inhibition of IL-6 and IL-1β by theophylline is

mediated by suppressing BLM-induced ROS production and NF-κB activation in epithelial cells. We further demonstrated that theophylline inhibited TGF-β-induced epithelial-to-mesenchymal transition in epithelial cells through suppressing phosphorylation of Smad2/3 and AKT. Taken together, these results demonstrated that theophylline exhibits the potent anti-fibrotic effect on pulmonary fibrosis by inhibiting Th17 differentiation and TGF-β signaling, and thus suggest that theophylline may be a potential new therapeutic agent for IPF.

W204. Elevated Levels of IL-35 in Different Stages of Rheumatoid Arthritis and its Association with the Presence of Autoantibodies

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Rheumatoid arthritis can cause pain, inflammation and destruction of joints. IL-35 is a recently described immunosuppressive and anti-inflammatory cytokine. Several roles of IL-35 in the immune and inflammatory regulation of autoimmune diseases have been described. In this work we determined the relationship among the serum concentrations of IL-35 expressed in different stages of RA pathology and its association with autoantibodies. Donor samples were collected (n=64): 1) Healthy, 2) CCP+, 3) Patients with early Arthritis, 4) Patients with established rheumatoid arthritis according to ACR-AULAR classification. Determinations of IL-35 were made by ELISA according to manufacturer´s instructions. Statistical analysis was carried out in Graph Pad Prism software. No significant differences were identified on the proportions of clinical variables and demographics among groups. A statistically significant difference ($P< 0.05$) was identified when analyzing the concentrations of IL-35 in the CCP+ group compared to controls. This was more pronounced than those on early and established RA. Additionally associations with autoantibodies were identified for both CCP and CarP autoantibodies and other clinically relevant variables. Our data suggest an important clinically relevant link among IL-35 and the presence of autoantibodies, particularly CCP. The clinical implications of such results await further experimentation.

W205. Evaluation of Highly Sensitive Immunoassay Technologies for Measurement of Low Abundant Human Cytokines

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Traditional immunoassay methodologies such as ELISAs and multiplex arrays demonstrate limitations in sensitivity. These factors reduce the utility of traditional immunoassays for the detection of low-abundant plasma cytokine and chemokine levels in healthy subjects and patients, thus hampering statistical analysis among study groups. To identify the best platform for use in low abundant biomarkers, we conducted a comprehensive cross-platform and cross-assay evaluation across two leading platform technologies. The goal of this study is to evaluate the technical performance in plasma samples from kidney transplant patients with/without CMV viremia and healthy controls receiving seasonal influenza vaccination (cohort from the University of Georgia - Center for Vaccine Research for High-Risk Populations). We measured IL-2 and IL-15 using the Single molecule ultrasensitive assay (Simoa) compared to the Luminex array in plasma samples from 24 kidney transplant patients with/without CMV viremia. All 24 samples have detectable IL-2 by Simoa, 21/24 (87%) were not detectable by Luminex. 22/24 (92%) of samples have detectable IL-15 by Simoa whereas 7/22 (32%) were not detectable by Luminex. In a cohort of healthy individuals receiving a seasonal influenza vaccine, we were able to detect IL-1a in 189/212 (89%) by Simoa while it was not detected in 125/189 (66%) by Luminex. Our data suggest that ultrasensitive immune assays represent a highly accurate approach for studies that include healthy subjects or populations with a potentially heightened inflammatory state.

W206. Evaluation of Potential Protective Effects of the Immunomodulatory Strains Lacticaseibacillus Rhamnosus UCO-25A and Limosilactobacillus Fermentum UCO-979C upon an Improved Model of Ulcerative Colitis

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Ulcerative colitis has abruptly increased its incidence and prevalence, affecting millions of people. Current therapies aim to achieve long-term remission but are expensive, invasive and carry side effects as they are mainly based on the immunosuppression of the patient. Alternative therapies such as the use of probiotics modulating chronic inflammatory response or promoting the restoration of the intestinal epithelial barrier, have acquired great worldwide relevance as a strategy to prevent, treat, and achieve remission of the disease. There are various studies about the role of probiotics in the course and prevention of ulcerative colitis, whose promising results vary based on, among other factors: the selected probiotic strains or their combination, the study model used, the stage of the disease, and the form of administration. Therefore, this study evaluates the role of two probiotic strains Lacticaseibacillus rhamnosus UCO-25A and Limosilactobacillus fermentum UCO 979C, that have previously demonstrated an immunomodulatory capacity upon the innate immune response; for their application as a possible strategy to improve the management and evolution of ulcerative colitis. For this, an in vitro model of ulcerative colitis induced with sodium dextran sulfate in Caco-2 cells, grown on an extracellular matrix membrane from the small intestine submucosa, was used to achieve an improved approximation of the disease. After co-cultures with the bacterial strains, a modulation of the immune response was observed through the variation of different inflammatory markers in the cells, indicating a clear effect of the probiotic strains upon the improved disease model.

W207. EVO101 is a Novel, Topically Applied IRAK4 Inhibitor That Can Suppress Inflammation in Human Skin Tissue Models

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Interleukin-1 receptor associated kinase 4 (IRAK4) is a key component of MyD88 dependent pathways, including signaling downstream of Toll-like receptors (TLRs) and IL-1 family cytokine receptors. EVO101 is a potent IRAK4 inhibitor currently in clinical development (NCT05579899) as a 0.1% topical cream formulation for the treatment of atopic dermatitis (AD). Leveraging our ex vivo human skin tissue model, we show that EVO101 can potently and dose-dependently inhibit inflammation induced by TLR and IL-1 family cytokine stimulation. Additionally, topically applied 0.1% cream formulation of EVO101 demonstrates robust anti-inflammatory activity in TLR and IL-1 stimulated human skin, with performance on par with high potency topical corticosteroids. As AD is known to have significant contributions from Th2 inflammation, we also evaluated EVO101 in Th2 stimulated human skin. 0.1% EVO101 topical cream performed on par, or superior to, other topical therapeutics, such as topical ruxolitinib (JAK inhibitor), topical tapinarof (AhR modulator), and topical calcineurin inhibitors. To interrogate this finding further, we evaluated if IL-1 family cytokines could drive Th2 inflammatory markers, and if Th2 inflammation resulted in upregulation of IL-1 family cytokines, thus providing a positive feedback loop. Indeed, individual IL-1 family cytokine stimulation of human skin resulted in upregulation of Th2-associated cytokines/chemokines. Additionally, Th2 stimulation of human skin resulted in upregulation of IL-1 family cytokines. These findings suggest that IL-1 family cytokines can drive and perpetuate Th2 inflammation, and that inhibition with an IRAK4 inhibitor (such as 0.1% EVO101 topical cream) may have positive impact in a heterogenous Th2 disease, such as AD.

W208. Genetic Variants in IBD Chilean Patients are Related to Clinical Outcomes

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Latin population has been underrepresented in IBD GWAS. We investigate the association of IBD risk variants reported in previous GWAS studies with clinical outcomes in Chilean patients. Methods. Chilean individuals with IBD (145 UC and 47 CD) were genotyped using Illumina GSA Arrays. From IBD GWAS (Jostin et al. and Liu et al.), we selected gene variants and looked for them in our Chilean IBD group. Then, we built a Chilean dataset. Using this dataset, we performed a Spearman correlation matrix to correlate clinical outcomes with IBD variants. Further, we built regression models to predict the clinical outcomes using the variants obtained from the correlation matrix ($p < 0.05$). Then, we selected the best models using significance testing (P values) or likelihood-based information criterion, such as the Akaike Information Criterion (AIC) and plotted the models using a Receiver Operating Characteristic Curve (ROC). Finally, to evaluate the association among variants in each model, we perform a Gene Ontology biological process enrichment analysis using PANTHER (Fisher, FDR). Results. The best predictive regression models (more than 80% AUC >80) for the clinical outcomes were surgery, clinical/endoscopy remission for more than five years, and naïve anti-TNF. Association with genes related to genetic variants was observed significantly ($p< 0.05$) in the enrichment analysis for the model Clinical/endoscopy remission of more than five years (rs6837335,rs11742570,rs6871626,rs38904,rs7133914,rs7134599,rs1708507, and rs6142618). Conclusion. Candidates' genes related to clinical outcomes in our Chilean IBD cohort were related to epithelial, innate, and adaptative immune responses and host-microbial interactions.

W209. Genome-wide Association Study Analysis of Disease Severity in Acne Reveals Novel Biological Insights

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Acne vulgaris is a common skin disease that affects >85% of teenage young adults where >8% develop severe lesions that leaves permanent scars. Genetic heritability studies of acne in twin cohorts have shown that the heritability for acne is 80%. Numerous genome-wide association studies (GWAS) have shown that 14-29 loci are associated with increased risk of developing acne when compared to healthy individuals. Here, we performed a multi-ethnic GWAS analysis to capture disease severity in acne patients by using individuals with normal acne as a control. Our cohort size consists of 1279 control acne and 538 severe cases with a total of 1817 individuals from Nashville Biosciences. We replicated this analysis using FinnGen cohorts. We therefore identified a novel locus that crossed the 5x10-8 threshold and several loci with suggestive association level. We also performed mendelian randomization and colocalization using eQTLs to identify causal genes. Lastly, we performed gene-set enrichment analysis to implicate biological pathways that drive disease severity in Acne.

W210. Hereditary Angioedema and Pregnancy

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The management of pregnancy and childbirth in women with hereditary angioedema (HAE) has important practical implications. We present a clinical case – patient A., 26 years old. The diagnosis of HAE was established at the age of 25 years. The disease made its debut at the age of 10 years. The patient became pregnant one year after the diagnosis was made. During pregnancy noted frequent attacks of angioedema. The most severe course of HAE was in the second trimester, when attacks occurred every 3 days and were manifested by swelling of the hands, forearms, abdominal and lumbar areas, buttocks, thighs and feet, which caused severe physical discomfort. The doctor found during the interview that the patient did not comply with the prescribed treatment regimen, which probably caused the worsening of the disease during pregnancy. Doctor recommended compliance with the pre-assigned regimen of drug administration - 1000 IU every 3 days. Physicians were of great concern regarding the choice of method of delivery for a pregnant woman with HAE. As a result, a healthy child was born by caesarean

section. In this case, in addition to the long path to the diagnosis, we have the problem of compliance by patients with the frequency of prophylactic administration of the drug for the treatment of HAE. It is important to focus attention on this problem with conversation with the patient, because only the introduction of an adequate amount of the drug can give the expected therapeutic effect.

W211. High-dimensional Immunophenotyping Reveals Unique Immune Cell Aberrations in Patients with Undiagnosed Inflammatory and Autoimmune Diseases

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An estimated 25 to 30 million Americans and hundreds of millions of patients worldwide suffer from rare or undiagnosed diseases. While genomic sequencing has proven to be a valuable tool in uncovering disease etiology arising from a monogenic defect, limited diagnostic strategies are available to interrogate immune dysregulation in patients with autoimmune or inflammatory conditions that do fit into traditional disease categories. We employed high-dimensional immunophenotyping through mass cytometry to study patients with severe, undiagnosed immunologic conditions from the Undiagnosed Diseases Network (UDN). Our outlier analyses demonstrated significant enrichment of outlier features in UDN patients, with 5/16 UDN patients (31.3%) exhibiting an unusual expansion of defined or novel cell populations compared with 0/141 (0.0%) control patients. One patient with debilitating global erythroderma, anhidrosis, and alopecia had a markedly expanded population of CD25hiCD127- regulatory T cells (Tregs) comprising 53.9% of all T cells with prominent cutaneous infiltration. T cell receptor clonality studies revealed the Treg population to be polyclonal, and single-cell RNA sequencing (scRNA-seq) analyses showed an activated interferon signature in the Treg and memory CD4+ T cell populations. T cell-directed therapies were trialed, and longitudinal scRNA-seq analyses revealed a reduction in Treg frequency with abatacept and subsequent clinical improvement with Janus kinase inhibitors. Our study demonstrates that high-dimensional immunoprofiling offers a promising approach to defining pathogenic immune dysregulation and guiding therapy in individual patients with unusual immune-mediated diseases.

W212. htlv-1-specific CD4+ T Cell Contribution to Neuroinflammation in htlv-1-associated Mylopathy

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Human T cell leukaemia virus type 1 (HTLV-1) is a retrovirus that infects 10-20 million people. Most infected people remain asymptomatic carriers (ACs), while 2-5% of HTLV-1 carriers develop adult T-cell leukaemia and another 2-5% develop inflammatory diseases including HTLV-1-associated myelopathy (HAM). HAM is an inflammatory disease of the CNS characterised by chronic inflammatory demyelination that resembles progressive spinal forms of multiple sclerosis, and for which no curative treatments are available. Individuals with higher HTLV-1 proviral loads (PVL) are at an increased risk for HAM. CD4+ T cells carry 95% of the HTLV-1 PVL and predominate in the early CNS lesions, suggesting the involvement of CD4+ T cells in HAM pathogenesis. However, the contribution of HTLV-1-specific CD4+ T cells to disease pathogenesis is unknown. By performing RNA-seq analysis of HTLV-1-specific CD4+ T cells from HAM patients and ACs, we identified over 500 differentially expressed genes in HAM patients when compared to high PVL ACs including upregulation of EOMES, SEMA4A, IL17RC, and the downregulation of IL1RN, which we have confirmed by flow cytometry. Pathway enrichment analysis showed that HAM patients upregulate signalling through Rho GTPases, have increased NRAGE death signalling through JNK, and increased signalling by p75NTR. These pathways modulate CNS resident cells including microglia and astrocytes, which we have confirmed in ex vivo co-culture experiments with HTLV-1-specific CD4+ T cells. Our results on the contribution of CD4+ T cells to HAM

pathogenesis has identified novel potential targets for HAM treatment.

W213. Identification of Atopic Dermatitis (AD) Endotypes Based on Lesional Skin Transcriptomic Data
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Background: Atopic dermatitis (AD) is a highly heterogenous inflammatory skin disease with a diverse clinical manifestations and treatment responses. Therefore, patient stratification based on the underlying molecular mechanisms/endotypes is critical for the development of targeted therapeutic approaches. Aim: We sought to identify robust and reproducible AD endotypes using lesional skin transcriptomic data. Methods: We carried out an integrated unsupervised clustering analysis using both K-means and NMF (non-negative matrix factorization) algorithms with lesional skin transcriptomic data from a collection of 8 independent cohorts respectively (total AD patients=427). The subtypes from each of the 8 cohorts were systematically compared with each other by looking at the correlation of cluster-specifically expressed genes. The endotypes that are consistently identified from all cohorts were further characterized by clinical information and disease-relevant cell and pathway signatures. Results: Two main disease subtypes were consistently identified across all the 8 AD cohorts. The first subtype was characterized as inflammatory with relative higher disease severity (e.g. EASI/SCORAD/POEM), and higher activity of immune cells and pathways (such as T, B, Th1 and Th2). The second subtype was characterized as metabolic with relative lower disease severity, but higher activity for melanocyte and fatty acid metabolic pathways. The integrated results of K-means and NMF clustering indicate distinct molecular mechanisms underlying these two subtypes. Conclusion: Two distinct and reproducible AD endotypes were identified and further characterization of these endotypes would help generate hypotheses of patient stratification strategy for targeted therapies.

W214. Immune-related Biomarkers and Suicidal Behaviors: A Meta-analysis
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Biomarkers that can differentiate between psychiatric disorders with and without a history of suicidal behavior from each other and from healthy volunteers may explain part of the pathogenesis of suicidal behavior and aid its prevention. We conducted the hitherto largest meta-analysis comparing immune biomarkers between subjects with and without suicidal behaviors. The study protocol was registered with PROSPERO, CRD42020212841. Two independent reviewers screened the publications, extracted data and evaluated study quality. Standardized mean differences (SMD) were calculated for all comparisons and pooled with random-effects models. Heterogeneity between studies was assessed with the I2 statistic and publication bias was evaluated by funnel plots. Data were based on twenty-four studies including 2042 persons with suicidal behaviors and 4471 comparison subjects, and four immune-related biomarkers (CRP, IL-6, TNF-α and IL-1β). Suicidal behavior was associated with higher CRP blood levels compared with: healthy controls (SMD [95%CI] = 1.33[0.67-2.00]); patients with depression alone (SMD [95%CI] = 0.67[0.08-1.26]); and patients with any psychiatric disorders (SMD [95%CI] = 0.49[0.25-0.73]). IL-6 blood level was higher in patients with suicidal behaviors compared with healthy controls (SMD [95%CI] = 1.43[0.49-2.38]). IL-1ß blood level was lower in depression with suicidal behaviors compared with depression without suicidal behaviors (SMD [95%CI] = -0.34[-0.67 to -0.01]). These results implicate the immune system and inflammatory response in suicidal behavior independent of a relationship to major psychiatric disorders. Future studies are needed to determine the cause-and-effect relationship of these immune system biomarkers with risk of suicidal

W215. Increased Intrathecal Interferon-gamma Is Associated with Paramagnetic Rim Lesions in Multiple Sclerosis

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Background: Paramagnetic Rim Lesions (PRL) are a subset of chronic demyelinating lesions in Multiple Sclerosis (MS), characterized by iron-rich, pro-inflammatory microglia encircling the periphery. These lesions have recently been shown to predict poorer overall prognosis. They have also been associated with elevated levels of cerebrospinal fluid (CSF) total protein, neurofilament light, and chitinase-3-like protein suggestive of a perpetuating or causative role in intrathecal inflammation. Objectives: We sought to explore inflammatory CSF correlates of PRL to better elucidate their underlying associated pathophysiology. We hypothesized that patients with PRL would exhibit greater CSF inflammation associated with dysregulated microglial activity. Methods: This is a retrospective, cross-sectional cohort study. We assessed cellular and soluble mediators of inflammation in CSF by employing 1) spectral flow cytometry and 2) oligonucleotide-linked-antibody proteomics (Olink Proteomics) of 96 cytokines/chemokines. PRL were identified on standardized clinical 3T MRI collected in close proximity to CSF sample acquisition. Results: 27 Patients with diagnosed MS were analyzed via Olink and assessed for PRL status. Of this cohort, 6 were additionally assessed by flow cytometry. We found that patients with PRL had higher Interferon-Gamma (IFN-g) and STAM Binding Protein (STAMBP) in CSF. We also observed increased Oligoclonal Bands and an over-representation of CD138+ CD19+ plasma cells within CSF. Conclusions: PRL are associated with increased intrathecal IFN-g, STAMBP, and plasma cells in MS. Whether these inflammatory mediators drive the formation of PRL, are a consequence of PRL formation, or are the result of a parallel inflammatory process is a promising subject for future study.

W216. Investigation of T Cell-induced Neuroinflammation in Alzheimer's Disease

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Previous studies have implicated T cells in the brains and CSF of Alzheimer's disease (AD) patients. However, most studies are small and focus primarily on the hippocampus at later Braak stages, and little is known about their role over the course of the disease.

Informed by scRNAseq of immune cells isolated from fresh autopsies, we have investigated T cell frequencies and subtypes in the aging and AD brains. Namely, we compared T cell frequency in four brain regions affected by AD—entorhinal, prefrontal and posterior cortices, and hippocampus—across all Braak stages (from Braak 0 – minimal AD pathology - to Braak 6 - extensive pathology mixed with other neuropathologies) by automated segmentation and quantitative analysis of immunofluorescence (IF)-stained post-mortem brain tissue sections. We therefore characterize the phenotype and topology of T cells in their perivascular, parenchymal, and meningeal niches.

In the first 23 individuals (out of 100 being profiled), we found that T cell number is higher in the hippocampus compared to the other three regions but only at Braak stages 4 and 5 (reduced in Braak 6). A small number of CD8+ T cells in the AD brains exhibit tissue-resident traits, while some in the perivascular space are activated. Most interestingly, our data suggest that local inflammation might cause accumulation of granzyme K-secreting CD8+ T cells and C3 complement activation in AD brains. This study clarifies the timing and location of T cells in the course of AD: they do not anticipate but rather follow the spread of pathology.

Learning Objectives:

- Upon completion, participant will be able to further understand the roles that T cells play in Alzheimer's disease brain.

- Upon completion, participant will be able to learn more about T cell-microglia interaction in Alzheimer's disease brain.
- Upon completion, participant will be able to learn the differences of T cell quantity and subtypes in different brain regions across all Alzheimer's disease stages.

W217. Systems Immunology Approaches Identify Potential Drug Targets to Prevent Brain Inflammation in Refractory Epilepsy

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Epileptogenesis is a pathological process that causes spontaneous recurrent seizures in Epilepsy. Patients non-responsive to antiepileptic drugs are categorized as drug-refractory epilepsy (DRE). Epileptogenic triggers are multifactorial and not well understood. We aimed to address here the hypothesis that inappropriate, immunologically driven pro-inflammatory mechanisms contribute to the pathogenesis of refractory epilepsy in humans We used single cell Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-seq) to dissect the architecture of the immuno-transcriptome of the diseased epileptic human brain. Systems and Network biology methods were used to analyze the cellular network facilitating immune cell infiltration into the brain. Ligand Receptor network analysis was performed to show intercellular communication and to identify potential drug targets. Our approach uncovered a surprisingly well organized pro-inflammatory microenvironment pivoting on activated, pro-inflammatory microglia in a closely-knit network where resident cells attract and manipulate in a pro-inflammatory fashion infiltrating innate and adaptive immune cells. Furthermore, for the first time we showed physically interacting microglia with infiltrating immune cells that co-enhances proinflammatory capacity of interacting cells. Moreover, we discovered potential Ligand receptor interaction that could be blocked to prevent brain inflammation by available approved therapeutics Altogether, our study characterizes the DRE focus in the human brain as an immunologically competent and pro-inflammatory micro-environment and unravels the potential mechanism of immune cell infiltration in the epileptic brain lesions.

W218. Therapy of High Risk Pregnancy and Its Complications

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Problem: Dysregulated Complement Activation as a Common Pathway of Injury in complications of high risk pregnancy such as Pre eclampsia, miscarriage and anti phospholipid syndrome (Lynch A. M. and Salmon J.E. "Dysregulated Complement Activation as a Common Pathway of Injury in Preeclampsia and Other Pregnancy Complications" in Placenta. 2010 July ; 31(7): 561–567). Method: That appropriate complement inhibition is an absolute requirement for normal pregnancy. Defective placental development was associated with pregnancy loss and solely dependent on alternative pathway activation. Results: Sulfonic nano polymers offer balanced modulation of dysfunction of alternate complement system. Discussions: Nano formulation methods of sulfonic polymers with human safety record offer an extremely attractive therapeutic option to improve pregnancy outcome in high risk pregnancy and should be an integral part of therapy in pregnancy.

Mucosal Immunology

Th230. Transforming Growth Factor-β3 Maintains Intestinal Tolerance

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Development of inflammatory immune responses towards antigens derived from dietary proteins underlies food allergy and celiac disease. This situation is avoided by oral tolerance, a mechanism through which ingested proteins elicit the differentiation of FoxP3+ regulatory T cells (Tregs) in a TGF-β-dependent manner. In this work, we analyzed the expression of the three TGF-β isoforms and found that TGF-β3 (Tgfb3) was the isoform most abundantly expressed in the small intestine and other barrier tissues. Using isoform-specific neutralizing antibodies and conditional knock-out mice, we provide evidence that supports a fundamental and non-redundant role for TGF-β3 in the establishment of oral tolerance. We demonstrate that, though FoxP3 induction was equivalent when naïve CD4 T cells were activated in the presence of TGF-β1 or TGF-β3, the genetic expression profile of Tregs generated with TGF-β3 differed substantially from that of Tregs induced by TGF-β1. TGF-β3 promoted the expression of gut-homing molecules and a metabolic profile that allowed for rapid functionality and survival. These characteristics were associated with a better capacity to limit intestinal inflammation in an adoptive T cell transfer colitis model. Finally, tamoxifen-induced deletion of Tgfb3 caused the development of severe colitis associated to numerical and functional defects in lamina propria Tregs. Collectively, our results indicate that TGF-β3 is essential for the maintenance of the intestinal immune homeostasis. Its local production is necessary for the establishment of tolerance to oral proteins and its presence is required for the preservation of intestinal health.

W219. Alveolar Macrophage Subsets Defined by Single Cell RNA Sequencing Associate with Mortality in Acute Respiratory Failure

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Alveolar macrophages are highly plastic and are the most abundant cell in the homeostatic human lung. The transcriptional and phenotypic characteristics of alveolar monocyte and macrophage (mono-AM) subsets in acute respiratory failure are poorly understood. In this study, we wanted to identify novel mono-AM subsets in patients with acute respiratory failure, examine changes over time, and test for associations between subsets and mortality. From a cohort of patients with non-infectious lung injury on mechanical ventilation (n=8), we performed bronchoalveolar lavage (BAL) and collected peripheral blood within 96 hours of hospital admission and 72 hours later. We utilized CITE-seq (scRNA-seq and cell surface proteins) to establish mono-AM subsets. Six AM subsets and 2 monocyte subsets were identified in BAL. In paired peripheral blood, we identified monocyte subpopulations with similar gene expression programs to those in BAL. The proportion of AM subsets with cholesterol processing and complement gene expression profiles increased over time. Cell-surface proteins including CD71, CD163, and CD86 distinguished AM transcriptional subsets. In a second cohort of mechanically ventilated patients (n=51), we used flow cytometry to identify mono-AM subsets defined by our CITE-seq. We found a higher proportion of the alveolar CD163/LGMN subset (CD71-CD163+) was associated with mortality ($p < 0.01$). In this study of patients with non-infectious lung injury, we identified novel alveolar mono-AM populations as well as previously described subsets in COVID-19. The proportion of mono-AM subsets rapidly changes and can be identified by cell surface proteins. This facilitated our

identification of associations between CD163/LGMN AMs and mortality.

W220. Gamma-delta T Cells Suppress Microbial Metabolites that Activate Striatal Neurons and Induce Repetitive/compulsive Behavior

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Intestinal gamma-delta (gd) T cells play an important role in shaping the gut microbiota, which is critical not only for maintaining intestinal homeostasis but also for controlling brain function and behavior. Here, we found that mice deficient for gd T cells (gd-/-) developed an abnormal pattern of repetitive/compulsive behavior, which was dependent on the gut microbiota. Colonization of WT mice with gd-/- microbiota induced repetitive/compulsive behavior whereas colonization of gd-/- mice with WT microbiota abolished the repetitive/compulsive behavior. Moreover, gd-/- mice had elevated levels of the microbial metabolite 3-phenylpropanoic acid in their cecum, which is a precursor to hippurate (HIP). HIP reaches the striatum and abnormally activates dopamine type 1 (D1R)-expressing medium spiny neurons, leading to repetitive/compulsive behavior. Altogether, these data suggest that intestinal gd T cells shape the gut microbiota and their metabolites and prevent dysfunctions of the striatum associated with behavior modulation.

W221. Harnessing the Microbiome to Regulate Systemic Innate Immunity

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While many studies have correlated changes in the gastrointestinal microbiome with systemic immune-based diseases, the mechanisms underlying the crosstalk between the microbiome and the systemic immune system are poorly understood. We have now identified a microbiome Bacteroidota-derived lipodipeptide—L654—a TLR2/TLR6 ligand that accesses the systemic circulation, as a microbiome-derived regulator of systemic innate immunity. We previously documented that Multiple Sclerosis patients have significantly lower serum L654 levels than healthy controls, leading us to postulate that: 1) L654 regulates systemic innate immunity by entering the systemic circulation and mediating weak TLR2-signaling that maintains "normal" levels of innate immune signaling feedback inhibitors (e.g., A20); and 2) inadequate systemic L654 levels result in poorly regulated, enhanced innate immune responses. In prior proof-of-concept studies, we reported that increasing systemic L654 levels by administering exogenous L654 intravenously significantly diminishes systemic innate immune pro-inflammatory responses and attenuates murine autoimmunity. In the present study, our goal is to directly study the role of the microbiome in this mode of immunoregulation. Using wild-type C57BL/6 mice, we now report that decreasing microbiome Bacteroidota using a specific protocol of oral antibiotics reduces fecal and serum L654 levels and significantly enhances innate immune pro-inflammatory responses. Moreover, replenishing levels of L654 in antibiotic-treated mice reverses this innate immune enhancement. Overall, our results suggest that: 1) L654 is a microbiome-derived mediator of systemic innate immune regulation and; 2) our approach now allows the microbiome to be harnessed therapeutically for enhancing or diminishing immunity in the context of infectious, malignant, or autoimmune disease.

W222. Pro-inflammatory and Exhausted T Cell Signatures Characterize Severe Male Infertility

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Severe male infertility has been associated with low health status and earlier onset of age-associated comorbidities compared to fertile men. Besides, men with idiopathic non-obstructive azoospermia (iNOA) display a proinflammatory signature in the testis. In the hypothesis that a deregulation of the immune system could associate to infertility and impact the healthiness of severe infertile men, for the first time, we comprehensively analyzed by multiparametric flow cytometry and scRNAseq analysis the immune status of infertile men in comparison age-matched fertile men. Severe infertile men showed a higher frequency of granulocytes and proinflammatory dendritic cells (DC) in sperm fluid and peripheral blood compared to fertile men and an overall proinflammatory signature in both tissues. Despite the lower frequency of CD4+ and CD8+ T cells in peripheral blood of infertile men, these cells co-expressed exhaustion markers (PD-1, TIM-3, KLRG-1, Eomes, and T-Bet) at higher frequency compared to cells from fertile men. Functional studies indicated that CD8+ T cells from severe infertile men were slightly hypo-proliferating and produced lower levels of GM-CSF and IL-2 compared to T cells from fertile men. Our results reported a previously undescribed association between severe male infertility and a systemic and local proinflammatory immune status, with circulating T cells phenotypically and functionally resembling exhausted T cell precursors. Overall, our study could explain the low health status of infertile men and will be instrumental to improve the clinical management of male infertile subjects.

W223. Short-chain Fatty Acids Foster Bile Acid-metabolizing Microbiome which Ameliorate Allergic Inflammation of the Airways via fxr/tgr5 Signaling

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We previously found that short-chain fatty acids (SCFAs) significantly reduced asthma severity through sequential induction of the immune-regulatory cells, Mac1+Ly6G+Ly6Clo granulocytic myeloid-derived suppressor cells (PMN-MDSCs) and Tregs. Additionally, mice supplemented with SCFAs harbored increased Clostridiales and decreased Bacteroidales. The altered gut microbiome was associated with a distinct bile acid metabolomic profile which can be attributed to the unique function of a SCFA-enriched microbial taxon, Clostridium scindens. C. scindens is unique for its expression of the 7 alpha-dehydroxylase, an indispensable enzyme biotransforming unconjugated primary to secondary bile acids. We found pharmacological activation of either of the FxR or TGR5 bile acid receptor induced signals that expanded PMN-MDSCs and Tregs and mitigated the severity of allergic airway diseases. We hypothesize that C. scindens protects against allergic inflammation of the airways through FXR and TGR5 signaling and forms a feed-forward loop in SCFA-elicited immune regulation. To investigate the effect of C. scindens in prevention and treatment of allergic airway disease, we generated the GN12 mice, the BALB/c counterparts of the sDMDMm2 mice harboring OligoMM12, a consortium of 12 representative gut microbial species which specifically lacks 7α-dehydroxylase activity. We found GN12 mice colonized with C. scindens (GN12-CS) had more PMN-MDSCs and Tregs and developed significantly less severe allergic disease than GN12 mice. The present study provides a proof of principle that distinct microbes (C. scindens) exploit their metabolites (bile acids) to form a feed-forward loop within the mucosal microenvironment that shapes host's response towards mucosal allergen sensitization and modulates inflammatory processes of the airways.

W224. Single Cell Sequencing Shows Specific Adaptation of Human Breast Milk T Cells

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Breastfeeding provides important benefits to the neonate, including protection against infections and support in immune development. Breastmilk contains a range of immunoactive components, but how and where these contribute to immunity remains poorly understood. Here, we characterized breastmilk T cells using single cell RNA sequencing and flow cytometry. Breastmilk CD4+ and CD8+ T cells exhibited an effector/memory profile, with immune response signaling, proliferation and a Th1/cytotoxic profile with high cytokine production capacities.

Activation and regulation appeared well balanced in breastmilk, with a prominent Treg population and co-expression of proliferation and immune regulatory markers in conventional memory T cells. Tissue-related gene expression and surface expression of tissue-resident memory T cell (T-RM) markers indicated that breastmilk T cells represent tissue-adapted rather than circulatory T cells. We showed that an activated effector profile was enriched in the breastmilk CD8+ T-RM population. We hypothesize that these cells played a role in local breast tissue defense against damage and infections introduced by the suckling infant. On the other hand, high expression of a diverse range of homing receptors leaves room for a role of breastmilk T cells in neonatal immune defense, as is supported by previous animal research. We observed a broad homing profile, with (co-)expression of skin-, gut- and inflammatory homing receptors and an enrichment of proliferation, activation markers and cytokine production in these homing cells. Together, our data suggest that breastmilk contains an adapted T cell population with an activated effector profile and potential functionality in maternal and/or neonatal tissues.

W225. Tissue Remodeling in Human Nasal Epithelium Induced by S100A8 and S100A9

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Background: Chronic inflammation triggers tissue remodeling of the human nasal epithelium (HNE). In inflammatory conditions, S100A8 and S100A9, damage-related molecular patterns, have potent pro-inflammatory activity, but their effect on tissue remodeling such as squamous metaplasia is not known. Therefore, we aimed to investigate the roles and regulatory mechanisms of S100A8 and S100A9 on tissue remodeling in chronic rhinosinusitis (CRS). Methods: The expression of S100A8 and S100A9 were confirmed after type 1 and/or 2 cytokines were treated on HNE cells. In addition, the expression of S100A8, S100A9, and their complex calprotectin was confirmed by immunohistochemistry staining and immunofluorescence in the tissues of CRS patients. After treatment with S100A8, S100A9, and calprotectin in cultured HNE cells, RT-PCR, and Western blot were performed. In addition, gene ontology analysis was performed through bulk RNA sequencing. Results: TNF-α and IL-1β increased the expression of S100A8 and S100A9 in HNE cells. The expression of S100A8 and S100A9 was increased in the squamous epithelium of CRS tissue. Treatment with S100A8 and S100A9 in HNE cells increased the expression of Involucrin and MMP-9. Bulk RNA sequencing analysis showed that S100A9 had an effect on keratinization and inducing the cornified envelope of HNE cells. Conclusion: S100A8 and S100A9 were associated with tissue remodeling such as squamous metaplasia. However, the S100A9 showed a more pronounced association. Increased expression of S100A8 and S100A9 in CRS tissue, especially squamous epithelium, correlated with disease severity of CRS. Therefore, we suggest that S100A8 and S100A9 are biomarkers reflecting the severity of inflammation in CRS.

W226. Type 2 Cytokines Arrest ccr2-expressing Monocyte-derived Cells in a Pathogenic State in Asthmatic Airways

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Mononuclear phagocytes (MNP) regulate tissue homeostasis and inflammation but are not well-characterized in human airways during allergic inflammation. We leveraged segmental allergen challenge (SAC) in allergic asthmatics (AA) and allergic non-asthmatic controls (AC) to distinguish transcriptional profiles specific to asthma from those associated with allergy alone. Single-cell RNA-sequencing was performed on endobronchial brushings collected at baseline and 24 hours after SAC (AC=4; AA=4), and on co-cultures of blood CD14+ monocytes and primary airway epithelial cells (AEC) at air-liquid interface. Logistic regression identified cells associated with AC (OR< 1) or AA (OR>1). Differential gene expression analysis was performed between groups (FDR< 0.1). Bronchoalveolar lavage protein was quantified via multiplex assay (AC=14; AA=13). Clustering of 8,510 airway mucosal MNP identified 14 subsets including macrophages, dendritic cells, and monocyte-derived cells (MC). MC2 (SPP1) associated with AC

at baseline (OR 0.52, [0.31-0.89]) and after SAC (OR 0.26, [0.18-0.36]). MC4 (CCR2) associated with AA after SAC (OR 2.77 [1.29-5.92]) and upregulated IL-13-induced genes involved in eicosanoid synthesis and matrix remodeling, including MMP12. MMP-12 protein levels were higher in AA compared to AC after SAC (745.7 [176.7-1957.7] vs. 228.6 [69.7-493.5] pg/mL, P=0.02). RNA velocity predicted a differentiation continuum from MC4 to MC2, with acquisition of genes directing macrophage identity, phagocytosis, and lipid metabolism. Consistent with this, blood monocytes transcriptionally aligned with MC4 but acquired an MC2 profile after co-culture with AEC. Thus, type 2 cytokine signaling in asthmatic airways may prevent macrophage differentiation and instead arrest MC in a pathogenic state that drives airway inflammation and pathologic remodeling.

Stem Cell and Organ Transplantation

Th231. Dynamic Establishment of Recipient Resident Memory T Cell Repertoire After Human Intestinal Transplantation

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Understanding formation of the human tissue resident memory T cell (TRM) repertoire requires access to longitudinal human non-lymphoid tissues. We longitudinally assessed the origin, distribution, and specificity of human TRMs at phenotypic and clonal levels in patients who have undergone intestinal transplantation (ITx). Applying next generation sequencing to blood, lymphoid tissue, and gut samples from 16 ITx patients, we found that donor age ≥ 1 year and blood T cell macrochimerism (peak level ≥ 4%) were associated with delayed establishment of stable recipient TRM repertoires in the transplanted ileum. T cell receptor (TCR) overlap between paired gut and blood repertoires from ITx patients was significantly greater than that between paired repertoires from healthy controls, demonstrating increased gut-blood crosstalk after ITx. Recipient T cells rapidly populated grafts from donors < 1 year old and crosstalk with the circulating pool remained high for years of follow-up. TCR sequences identifiable from pre-Tx recipient gut but not lymphoid tissues were overrepresented in post-Tx allograft ileum, demonstrating a circulating counterpart of pre-existing intestinal TRMs. Recipient T cells were distributed widely throughout the gut, including allograft and native colon, which had substantial repertoire overlap. Both alloreactive and microbe-reactive recipient T cells persisted in transplanted ileum, contributing to the TRM repertoire. Together, our study provides novel spatial, temporal, and functional insights into human TRM repertoire establishment.

Th232. Single Cell Transcriptomic Analysis of Renal Allograft Rejection Reveals Novel Insights into Intra-graft TCR Clonality and Rejection Mechanisms

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Alloreactive expansion and transcriptomic heterogeneity of intragraft T-cells is poorly understood. Here, we characterize clonally expanded CD8+ T-cell populations (CD8EXP) during acute renal allograft rejection (AR) under various immunosuppression (IS) regimens. Biopsy samples were obtained at the time of initial AR diagnosis from 10 patients on either iscalimab (anti-CD40 mAb), belatacept (CTLA4-Ig), or tacrolimus maintenance IS and subjected to 5' single-cell RNA + TCRα/β sequencing. Seurat and Loupe V(D)J analysis identified differentially expressed genes (DEGs) between individual CD8EXP clonotypes. Expanded clonotypes were defined as >2 cells with identical TCRα/β. Initial AR samples revealed a stunning degree of TCR restriction with original CD8EXP observed at initial AR remaining present in ongoing rejection biopsies as well as paired urine samples. In contrast, CD8EXP were largely eliminated upon rejection resolution. Transcriptomes of CD8EXP demonstrated a variety of effector states, and co-culture of Jurkat76 cell lines expressing CD8EXP-specific TCRs with donor PBMCs revealed alloreactivity of CD8EXP. Intriguingly, a resident memory phenotype was found in patients with persistent rejection and eventual graft loss. Finally, DEGs suggested that the type of IS significantly affected gene expression of CD8EXP, which was further analyzed through immunostaining. In conclusion, AR is characterized by a remarkably limited number of distinct CD8EXP that persist in ongoing rejection and disappear with rejection resolution, which is also reflected in paired urine samples. Persistent CD8EXP develop a resident memory phenotype that may be associated with allograft loss, and transcriptional profiles of the CD8EXP are distinct and dependent on the type of IS therapy.

Th233. TCL1A Involvement in B Cells in Long-term Kidney Transplantation

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Operational tolerance is defined as allograft acceptance without immunosuppression. TCL1A, a gene involved in the prevention of apoptosis in B cells, is overexpressed in the blood of operational tolerant patients (TOL). We also observed an increase level of apoptosis in B cells from TOL compared to non-TOL. The goal of our study is to decipher TCL1A role in tolerance and long-term transplantation. We performed a phenotype analysis of peripheral blood cells by spectral cytometry of a cohort composed of TOL (n=8) and patients under immunosuppression with stable function transplanted for one year (STA, n=8) or for more than 5 years (long-term transplanted: LTT, n=6) and healthy volunteers (HV, n=10). Supervised analysis shows an increase of total B cells and GZMB+ B cells frequencies in TOL, in accordance with our previous reports. Regarding TCL1A expression, unsupervised analysis highlighted that naïve B cells (IgD+CD27-) are divided in two populations, with an increase frequency of the TCL1Alow subset in TOL (median= 37,50%) compared to non TOL (STA, p= 0,0016 and LTT, p= 0,0079). This naïve TCL1Alow B cell population in TOL is also characterized by a lower expression of IgD and CD24. The emergence of such a naive TCL1Alow B cell population, with the pro-apoptotic behavior of TOL B cells is in favor of a tiny control of blood B cells, likely contributing to maintain the B cell response in operationally tolerant patients. Ongoing characterization of this naive TCL1Alow B cell population will provide important insight in their role in immune tolerance.

Th234. The Blood and Bone Marrow Have Distinct Patterns of Innate and Adaptive Lymphoid Immune Reconstitution in Humans Following Allogeneic Hematopoietic Cell Transplantation

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In myeloid hematologic malignancies, the bone marrow (BM) represents the tumor microenvironment, a potentially key site of anti-leukemic immunity following allogeneic hematopoietic cell transplantation (allo-HCT). Despite the importance of immune reconstitution (IR) after allo-HCT, IR data in BM are limited and much of our understanding arises from peripheral blood (PB) studies. We hypothesized that lymphocyte reconstitution in BM may be distinct from that of PB, and that characterization of BM IR may elucidate populations associated with outcomes. To test this, we prospectively compared IR via multicolor flow cytometry on fresh serial BM and PB samples (n=110 BM, n=115 PB) from pre-transplant (recipient) to one-year post-transplant in 33 adults with hematologic malignancies undergoing allo-HCT at our institution. We found that NK cells were the first lymphocytes to emerge in BM and PB, but NK cells continued to outnumber T cells in BM after their frequencies became similar in PB by day 100. Additionally, while T cells outnumbered NK cells as a percentage of lymphocytes in PB thereafter, NK and T cell frequencies in BM were similar for the duration of year-one. Furthermore, CD69 expression was significantly greater in BM compared to PB for T and NK cells by day 100, suggesting potential acquisition of a tissue-resident profile. Though rare, ILC1/2/3s had distinct distributions and phenotypes (increased CD69 in BM, increased CD25 in PB) in blood and marrow. Our work reveals tissue-specific features of lymphocytes in BM following allo-HCT, underscoring the importance of tissue-level studies of clinically relevant immune subsets in humans.

Th235. The Cure of Type 1 Diabetes

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Problem: The current progress in The Cure of Type 1 Diabetes is limited due to the instant blood mediated inflammatory reaction (IBMIR) occurring in the liver hours and days after islet infusion. This is due to hyper activation of complement and coagulation pathway (Gamble A.C. Anissa et al: Review: REVIEW "The journey of islet cell transplantation and future development" ISLETS 2018, VOL. 10, NO. 2, 80–94 https://doi.org/10.1080/19382014.2018.1428511 Method: Early inhibition of Complement and coagulation pathway can be achieved safely by balanced inhibition of Factor D. This can be done by sulfonic nano polymers. Result: It is possible to improve islet cell transplantation results by including sulfonic polymers as inhibitor of complement and coagulation cascade along with islet cell infusion. This will allow improve islet cell tolerance, viability and functionality.

Th236. The Predicted Indirectly Recognizable HLA Epitopes (PIRCHE) Scores for HLA Class I and II Graft-versus-host Disparity are Associated with Better Outcome in Haploidentical Transplantation of Relapsed Neuroblastoma Patients

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The clinical significance of Predicted Indirectly Recognizable HLA Epitopes (PIRCHE) scores for HLA Class I and II Graft-versus-Host Disparity was evaluated to predict outcome in 40 patients with relapsed neuroblastoma who underwent haploidentical stem cell transplantation (haplo-SCT) from 2012 to 2022. The outcomes according to PIRCHE -I and -II scores, and significance of PIRCHE score levels according to clinical and biological characteristics before and after haplo-SCT were evaluated. The 5-year probabilities of progression-free survival (PFS) and overall survival (OS) from haplo-SCT in 40 patients were 20.5±8.9% and 30.2±8.3%, respectively. A higher PIRCHE-II score (≥39) was associated with better survival (5-yr PFS 46.4±16.3% vs. 0.0%, P< 0.001; 5-yr OS 49.1±14.4% vs. 18.2±9.0%, P=0.047) and was an independent good prognostic factor in multivariate analysis (PFS: hazard ratio 0.025, 95% confidence interval 0.09–0.72, P=0.010; OS: hazard ratio 0.038, 95% confidence interval 0.16–0.93, P=0.035). A higher PIRCHE-I score was associated with faster hematologic recovery (neutrophil p=0.046; platelet p=0.021) and shorter fever (≥38.0) duration (P=0.04). The PIRCHE-I score was also higher in patients who did not experience CMV reactivation compared to those who did (median 27 vs. 19, P=0.027). Acute and chronic GVHD were not associated with PIRCHE scores in our study. In conclusion, higher PIRCHE scores are associated with better survival, faster hematologic recovery and less infection in haplo-SCT for children with recurrent neuroblastoma.

Th237. The Reverse Transcriptase Multiplex Ligation Dependent Probe Amplification (RT-MLPA) Assay as a Simple Molecular Tool for Diagnosis and Classification of Rejection in Formalin-fixed Paraffin-embedded Kidney Transplant Biopsies

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Background The Banff recommendations for the diagnosis of kidney transplant (KT) rejection includes molecular assessment of the transplant biopsy. However, implementation of molecular tools in clinical practice is still limited, partly due to the required expertise and the high cost. The reverse transcriptase multiplex ligation-dependent probe amplification (RT-MLPA) assay is a rapid and affordable assay that permits simultaneous evaluation of a restricted gene panel using paraffin-embedded tissue blocks. We aimed to develop and validate a gene panel and to propose an open-access tool for the diagnosis and classification of KT rejection. Methods A bicentric cohort of 220 KT biopsies, with 52 antibody-mediated rejection (AMR), 51 T cell-mediated rejection (TCMR) and 117 no-rejection controls was assessed. A 17-gene panel was identified, including genes significantly associated with at least one of these diagnoses. A support vector machine classifier (SVM) was developed. A subset of 109 biopsies was also

assessed using the Nanostring B-HOT panel to compare the two assays. Results The SVM classifier train and test accuracy scores were 0.84 and 0.83, respectively. In the test cohort, the F1-score for AMR, control and TCMR were 0.88, 0.86 and 0.69, respectively. Gene expression levels assessed by RT-MLPA or Nanostring correlated: r=0.68, p< 0.0001, with a concordant molecular profile in 81% of the samples. Conclusions The 17-gene panel RT-MLPA assay, developed here for FFPE tissue blocks, is a simple molecular tool that can be used to diagnose kidney transplant rejection and classify it between humoral or cellular rejection, without the need for additional biopsy.

Th238. T-reg Mediated Split Immunological Tolerance After Intestinal Transplant in a Porcine Model
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Background. Intestinal transplantation is the only remaining treatment for patients with intestinal failure who failed parenteral nutrition. Unfortunately, it carries the highest rate among solid organ grafts of immunologic complications, including rejection and graft-vs-host disease. We developed a porcine intestinal transplant model across a full-MHC-mismatch to evaluate chimerism and cellular immune responses associated with rejection. Methods. The recipient was T-cell depleted using CD3-immunotoxin and anti-CD8-antibody. The recipient's intestine was removed and replaced by a full-MHC-mismatched graft. Immunosuppression, mimicking our clinical protocol, consisted of tacrolimus (goal: 16-18ng/ml) and 1mg/kg prednisone for 90 days and was subsequently weaned over 30 days to assess immune responses. Peripheral and mucosal chimerism were tracked weekly and biweekly, respectively. Mixed lymphocyte assays (MLR) with and without CD25-depletion were done monthly using peripheral and mucosal lymphoid cells. Findings. The recipient was successfully weaned from immunosuppression without clinical or pathological signs of rejection. Peripheral blood chimerism was lost 10 days after transplantation. Recipient chimerism in the graft mucosa peaked at 80% and stabilized at 60%. Peripheral blood lymphocytes in MLRs maintained a robust response against the donor, while the mucosal lymphocytes showed donor-specific unresponsiveness that was abrogated when T-regs were depleted. Conclusion. We unexpectedly achieved tolerance to a full-MHC-mismatched intestinal graft in our pig model, which appears to have been locally mediated by T-regs within the graft but not the peripheral blood. We intend to repeat this experiment to see if the findings are reproducible and if the mechanisms favoring tolerance can be applied to our clinical regimen.

Th239. New HLA Alleles Discovered by Next Generation Sequencing in Clinical Laboratory
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The area of immunogenetics is undergoing a revolution because to next-generation sequencing (NGS), which makes it possible to accurately and precisely identify HLA for exons and introns that had not previously been typed. Our laboratory began using NGS for routine testing in October 2021. Genotyping was performed by NGS on the Illumina MiSeq next generation sequencer. Sequences were determined using locus-specific primers supplied by GenDx. Sequences were analyzed using NGengine. The 16 new alleles were discovered during routine HLA typing (1 HLA-B, 1 DRB1, 3 DRB3, 7 DQA1, 2 DPA1, 2 DPB1). Among them, there were two cases on mismatch in core exon. In one case, DQA1*01:01:08 is the most similar known allele. This allele differs from DQA1*01:01:08 at codon 19 in exon 2. One nucleotide change from C to T (TAC to TAT) results in a synonymous mutation coding for tyrosine. The other was identified in siblings being typed at two different times. DQA1*01:01:01:01 is the most similar known allele and differs from at codon 45 in exon 2. Three nucleotide (AGG) insertion from AAG GAG ACT to AAG GAG GAG ACT results in glutamic acid added to the amino acid sequence. We submitted data to the IPD-IMGT/HLA Database for naming (GenBank, OP487692; IMGT HWS10065829). Historically, many laboratories had not routinely typed the HLA-DQA1 and -DPA1 alleles, which made up the majority of newly identified alleles. All laboratories, vendors, and database administrators will need to make the designation of novel alleles as automatic if the numbers continue to rise.

Tu229. A Role for Iga in GZMB$^+$ Regulatory B Cells in Tolerance

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Transplanted patients tolerating a kidney graft without immunosuppression (TOL) display a higher number of circulating regulatory B cells, acting through a granzyme B (GZMB)-dependent manner. IgA+ plasmablasts/plasma cells have been associated with regulatory B cells secreting IL-10, but nothing is known regarding GZMB+ Bregs. The objective was to explore a potential link between IgA and GZMB+ B cells in non-transplanted (HV) and kidney-transplanted patients (KTP). Patients with: stable kidney graft function under immunosuppression, progressive degradation of kidney function, stable kidney graft function under one immunosuppressive drug, TOL patients and HV volunteers were included. The quantification, subclasses and glycosylation levels of IgA were determined by ELISA, IgA forms by Western blot; scRNAseq was performed on GZMB+ Bregs, multiparametric flow cytometry analysis on patient PBMC. The suppressive properties of IgA+ B cells were analyzed in B-T cell co-cultures. We showed that IgA blood levels were increased in TOL patients, with a significant increase of IgA1. There was no change in the forms or glycosylation level of IgA between KTP. Transcriptomic analysis revealed higher IgA expression in GZMB+ Bregs and, conversely, higher GZMB expression in IgA+ B cells. Flow cytometry analysis showed a higher expression of GZMB in IgA+ B cells from TOL compared with other KTP. Finally, IgA+ B cells demonstrated higher T-cell suppressive properties than IgA- B cells and total B cells. These findings suggest a strong link between IgA+ and GZMB+ B cells in TOL patients with increased IgA1 frequency, higher GZMB and IgA expression and higher suppressive properties.

Tu230. Adoptive Transfer of Group 2 and Group 3 Innate Lymphoid Cells Prevents Rejection in Humanized Models of Islet Transplant

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Allogeneic islet transplantation is a potentially curative therapy for individuals with type-1 diabetes (T1D). However, it does not eliminate underlying autoimmunity, and individuals require life-long immunosuppression to prevent rejection. In murine studies, group 2 innate lymphoid cells (ILC2s) prevented islet rejection and group 3 ILCs (ILC3s) supported tolerance within islets, respectively. Here, we assess whether human ILC2s or ILC3s improve islet transplant in humanized models. In vitro and in vivo studies demonstrate ILC2s and ILC3s are not cytotoxic and do not negatively impact engraftment of allogeneic human islets transplanted in NOD-scidIL2Rgammanull(NSG) mice. Using an antigen-specific model of islet transplant rejection, where HLA-A2+ human islets are transplanted into NSG mice and following engraftment of islets, HLA-A2-specific CAR T cells with or without ILCs are transferred intravenously to induce rejection, we demonstrate the ability of ILCs to prevent CAR T-cell-mediated islet rejection. Both ILC2s and ILC3s suppress antigen-specific rejection by CAR T cells, resulting in sustained normal blood glucose levels when ILCs are present. In vitro studies revealed ILC2s suppress both autologous and allogeneic CD4+ and CD8+ T cell IFN-γ production and decrease expression of apoptosis-inducing molecule FasL on CD8+ T cells. ILC3s also display a moderate ability to inhibit allogeneic T cells in vitro, although not as efficiently as ILC2s, suggesting ILC3 effects in vivo may be via indirect T cell interactions. Collectively, these findings support that adoptive cell therapies with human ILC2s and ILC3s have potential in promoting tolerance in transplantation.

Tu231. Assessment of Hematological Parameters of Petrol Filling Workers at Petrol Stations in Gondar Town, Northwest Ethiopia: A Comparative Cross-sectional Study

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Background: Petrol filling workers are highly vulnerable to these harmful substances which lead to blood disorders such as leukemia and aplastic anemia, and bone marrow failure. Thus, this study aimed to assess the hematological parameters of petrol-filling workers. Methods: A comparative cross-sectional study was conducted from January to March 2019 in Gondar town, Ethiopia. A total of 110 study participants comprising 55 study groups and 55 controls group were recruited by a convenient sampling technique. 3 ml of venous blood was collected for the determination of hematological parameters. Independent t-test and Mann-Whitney U test were used to compare the mean or median difference between parametric and non-parametric parameters, respectively. Pearson product-moment and Spearman's rank-order bi-variable correlations analyses were used to describe the correlation between hematological parameters with duration of exposure. P value ≤ 0.05 was considered statistically significant. Results: The study revealed that mean RBC and Hb level as well as the median HCT, MCHC, platelet count, absolute lymphocytes count, and RDW values of petrol filling workers showed a significant increment compared with the controls. On the other hand, the MCH value of petrol filling workers showed a significant decrement compared with controls. Moreover, the duration of exposure showed a significant positive correlation with RBC and MCHC. However, a significant negative correlation was observed with MCV. Conclusion: This study showed that the majority of hematological parameters of petrol filling workers showed an increment compared with controls. However, the further longitudinal studies should be conducted to explore the impacts on hematopoiesis.

Tu233. Comparison of the Occurrences of Long-term Toxicities in Patients Who Received Allogeneic Hematopoietic Stem-cell Transplantation With or Without Total Body Irradiation

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Objectives: The purpose of this study was to compare long-term toxicity incidences, including secondary malignancies with or without total body irradiation (TBI), in patients receiving allogeneic hematopoietic stem cell transplantation (HSCT) using data from a national population-based database. Methods: We performed this work using the Healthcare Bigdata system of the Republic of Korea. We identified 4,554 analyzable patients from 2009 to 2016. Incidence rate ratios (IRRs) for secondary cancers (Sec.Ca), cataracts, hypothyroidism, chronic kidney disease (CKD), myocardial infarctions (MIs), or strokes were compared, and propensity-score matching was used. Standardized incidence ratios were also estimated. Results: TBI was conducted on 1,409 patients (30.9%). In the propensity-score matched cohort, no overall survival differences based on TBI were observed. After the median follow-up time of 58.2 months, 143 patients were diagnosed with Sec.Ca (crude ratio 3.4%). The most commonly observed Sec.Ca were gastrointestinal cancers followed by lymphomas. Incidence rates per 1000 PY were 6.56 and 7.23 in the TBI and no-TBI groups, respectively (p=0.594). The incidence rates of cataracts (IRR, 1.60), CKD (IRR, 1.85), and hypothyroidism (IRR, 1.50) were increased after TBI. However, there were no significant differences in the occurrence of MI and stroke according to TBI. Conclusions: Our results suggest that modern TBI may not additionally increase the risk of secondary malignancies after allogeneic HSCT, although increased risks of other conditions were noted. Physicians should carefully consider individualized risks and benefits of TBI, with a particular focus on age group. Further studies with long-term follow-up are needed to confirm these findings.

W227. Immune Profiling of γδ T Cells After Human Intestinal Transplantation Reveals Their Roles in Lymphohematopoietic Graft-vs-host Responses and Graft Rejection

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We performed phenotypic and clonal tracking of donor- and recipient-derived γδ T cells after human intestinal transplantation (ITx) in blood, intestinal graft and bone marrow (BM). We previously demonstrated that donor T cell macrochimerism (peak level ≥4% in blood) is associated with less rejection. Here we found that increased γδ blood chimerism was present in patients with higher levels of total T cell chimerism. Remarkably, donor γδ T cells were detected in recipient BM 105–357 days post-Tx. Single-cell profiling of BM-infiltrating donor γδ T cells from 3 pediatric donors revealed both Vδ1- and Vδ2-dominant clonotypes with cytotoxic effector phenotypes that might contribute to graft-vs-host responses. BM-infiltrating donor δ2+ T cells are dominated by sequences with zero N-additions that likely originate during fetal life and are shared across pediatric, but not adult, donors, suggesting an age-related distribution and migration pattern. In contrast to αβ T cells, the turnover dynamics of γδ T cells in the graft showed a stronger association with donor age than with the status of macrochimerism. Graft-repopulating recipient γδ T cells showed effector phenotypes early post-Tx and gradually developed into cytotoxic resident-memory T cells with "private" non-Vγ9δ2 clonotypes. In one patient, the top dominant Vδ2 sequence (mainly Vγ5δ2) in blood during quiescence was also the top dominant clone in later rejecting ileal graft samples, but with much lower frequencies in earlier quiescent grafts, indicating active crosstalk of γδ T cells between blood and intestinal grafts during rejection. γδ T cells may influence chimerism and rejection after ITx.

W228. Impact of Pre-transplant Conditionings on Bone Marrow Stromal Cells (MSCs) in Immune Dysregulation Disorders

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Mesenchymal stromal cells (MSCs) regulate hematopoietic stem and progenitor cells (HSPC) homeostasis within the bone marrow (BM) niche and result functionally impaired after common preparative conditionings, reducing the BM ability to receive transplanted cells and increasing the graft versus host disease risk after transplants. The BM niche maintenance is fundamental to improve the HSCT success in immune dysregulation disorders. Thus, we isolated and characterized MSCs from patients and mouse models of these diseases, and we evaluated the standard preparative conditionings effects over non-genotoxic approaches on MSCs. Preliminary results showed that wild-type (WT) mice-derived MSCs have a fibroblast-like morphology, express canonical surface markers, can mesodermal differentiate and support HSPCs. On the contrary, we observed a reduced clonogenic capacity and impaired proliferation, and a trend in altered gene expression and phenotype in MSCs derived from recombination-activating genes (RAG)-deficient mice. Moreover, irradiation-based conditioning reduced MSCs number and increased expression of supportive genes in WT and affected murine MSCs, with impairment in their clonogenic capacity. Human MSCs from patients affected by Mevalonate Kinase Deficiency (MKD) and Wiskott Aldrich syndrome (WAS) showed a stable growth in culture. However, we found an altered expression of supportive genes and a reduced CD146 expression in WAS-MSCs. Patient-derived MSCs appeared impaired in terms of anti-inflammatory and ROS-involved factors in basal conditions and/or after in vitro stimuli. In vitro irradiated human MSCs showed a growth drop compared to the untreated counterpart. MSCs interaction within the niche after preparative conditionings will be further investigated using in vitro and in vivo models.

W229. Modulation of Antibody (Ab) Secretion from Human Bone Marrow (BM) Plasma Cells as a Mechanism of Rapid Serum Hla-specific Ab Rebound After Proteasome Inhibitors

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In transplantation, proteasome inhibitors (PI) have been used to target plasma cells (PC). However, after an initial decline of Ab titers, with PI removal, serum Ab levels tend to rebound weeks later. Whether old PC die and are

replenished by new plasmablasts or newly-minted Ab secreting cells (ASC), or if the existing PC merely modulate Ab secretion without undergoing apoptosis, is unknown. Furthermore, it is unknown if all BM ASC subsets regulate Ab secretion equally. Here, we tested whether human early and late (long-lived) BM ASC subsets modulate Ab secretion with a second-generation PI, carfilzomib (CFZ). Using serum and BM ASC from a pilot trial (ITN089ST) of CFZ in combination with belatacept in five highly-sensitized patients, we measured serum HLA-specific antibodies and BM ASC subsets before and after treatment. We also measured in vitro modulation of IgG secretion by ELISpots and by single cell analysis ± CFZ. In the serum, a reduction of immunodominant serum HLA titers were observed after the first cycle; however, a rapid rebound was observed as early as 10 days. BM ASC were also reduced by, on average, 54% and 23% in early and late subsets, respectively. Interestingly, CFZ initially decreased Ab secretion in all BM ASC, but only late subsets recovered Ab secretion at the single cell level. In conclusions, CFZ selectively depleted early BM ASC and the rapid serum HLA Ab rebound in CFZ treated patients may be due to resilience or modulation of Ab secretion rather than apoptosis of late BM ASC subsets.

W230. On the Clonal Evolution of Pathogenic and Non-pathogenic Donor-specific B Cell Responses to Kidney Transplantation in Human recipients

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Donor-specific (DS) B cell responses generate antibodies that sometimes cause rejection of organ transplants but at other times not. We ought to determine how donor-specific B cells weigh on the outcomes of clinical transplants. We used intact cells to isolate avidly donor-specific, self-specific and third party-specific B cells from clinical kidney transplant recipients, and sequenced their Ig V regions. Nearly all recipients had appreciable numbers of B cells that avidly bound donor cells at 37oC during the first several months after transplantation. DS IgVH sequences revealed several clonal evolution patterns. In some recipients, DS B cells exhibited promiscuous specificity (avid binding both to donor cells and to autologous cells) before or shortly after transplantation evolving later to high specificity (absence of dual recognition). In others, donor-specific B cell clones were not promiscuous initially, but promiscuous recognition emerged at 1 year. DS B cells encoded predominantly germline IgVH predominantly germline and polyreactive antibodies during periods of stable function. New highly mutated clones encoding Ig of high affinity, high specificity and pathogenicity expanded before or at the time of rejection. We concluded that DS B cell responses that avidly bind autologous cells are not pathogenic and may confer benefit since recipients with these responses exhibit stable graft function. Our results also suggest that expansion of non-promiscuous DS B cells with highly mutated IgV regions (likely reflecting T cell-dependent responses) is associated with and may cause rejection. Whether outcomes reflect antibodies or other functions of the B cells remains unknown.

W231. Single Cell Analysis Reveals Distinct CD8 T Cell Phenotypes and Functions in CMV Primary Infection and Reactivation in Kidney Transplant Recipients

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Cytomegalovirus (CMV) infection is a risk factor for graft loss and patient mortality in solid-organ transplantation. Single-cell transcriptomics of peripheral blood CD8 T cells from kidney transplant (Tx) recipients (KTRs) pre and 1wk post-CMV viremia and 1yr post-Tx identified terminal effector populations (CD28-, TCF7-, IL7R-, KLRG1+, GZMB+) enriched in KTRs with reactivation of latent CMV infection (n=2) compared to KTRs (n=2) after primary CMV infection. Highly cytotoxic FCGRIIIA+ (CD16) clusters (GZMB+, PRF1+, GNLY+) and “NK cell-mediated cytotoxicity” and “FcγR-mediated phagocytosis” pathways (KEGG database) were enriched in KTRs at 1wk and 1yr post-reactivation. We evaluated peripheral blood CD8 T cell response of KTRs with CMV primary infection (n=4) and reactivation (n=4), with paired CMV- KTRs, pre- and ~2yrs post-Tx against CMV peptides and anti-CD16 antibody. KTRs with CMV reactivation and KTRs with poorly-controlled primary infection (peak CMV viral load >10,000 IU/mL;

multiple episodes of viremia) had high CD8 T cell activation (CD69, CD137), effector cytokine secretion (IFN-γ and TNF-α) and degranulation (CD107a) to anti-CD16 stimulation, post-viremia, but primary infection resulted in poor CMV recall response 2yrs post-viremia compared to KTRs with reactivation both pre- and post-viremia. CMV-responsive CD8 T cells also lack CD16 expression. Our results suggest that CMV memory response can be difficult to establish and maintain after primary infection under immunosuppression. While latent CMV infection can be better controlled under immunosuppression, the presence of CMV-induced CD16+ CD8 T cells suggests an NK-like potential in mediating allograft injury, such as via ADCC in KTRs producing donor-specific anti-HLA antibodies.

W232. T Cell Receptor Sequencing Used to Monitor Changes Associated with Two Immunosuppressive Regimens in Pediatric and Adult Recipients of Allogeneic Hematopoietic Stem Cell Transplant

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Assessment of immune dysregulation after allogeneic hematopoietic stem cell transplantation (allo-HCT) is essential to efforts in reducing morbidity and mortality associated with complications from disease relapse, infectious disease, and graft versus host disease. Immunosuppressive regimens are routinely administered to allo-HCT patients in an effort to suppress alloreactive T-cells. To better understand differences in the effectiveness of new immunosuppressive regimens, it is helpful to evaluate restoration of immune competence, and to longitudinally monitor expansions of donor T cells and the dynamics of T-cell receptor (TCR) repertoire reconstitution in transplant recipients. We investigated these dynamics with the immunoSEQ® Assay (Adaptive Biotechnologies, Seattle, WA) in both the donor stem cell product and peripheral TCRß repertoires of pediatric and adult patients enrolled on the ABA2 multicenter randomized trial of abatacept for GVHD prophylaxis (NCT# NCT01743131, PMID: 33449816). All patients received a standard immunosuppressive regimen of a calcineurin inhibitor (tacrolimus or cyclosporine) and methotrexate (MTX). The CNI/MTX group (n=288 samples analyzed) was compared to a subset of patients that additionally received abatacept (ABA, n=526 samples analyzed). In both groups, there wasn't a significant difference in the peripheral repertoire skewness at day 180 and 365. Furthermore, we found that clones from the donor transplant were present at a moderate frequency as far out as one-year post-infusion. These findings shed light on post–allo-HCT adaptive immune reconstitution and demonstrate that immunoSEQ can be used to quantifiably assess immune responses, track clonal specificities, and may help in evaluating alloreactive responses following allo-HCT.

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