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Contents

Allergy

Th7. Vitamin D Modulates Th2 Response by Mediating T Regulatory Cells in Allergic Conjunctivitis

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Allergic conjunctivitis (AC) is one of the most common diseases encountered in eye clinics. Recently, Vitamin D deficiency has been shown to be associated with allergic AC. However, the therapeutic potential of vitamin D for AC and the underlying mechanisms of its actions remain still unknown. This present study was designed to evaluate the efficacy of vitamin D to suppress ovalbumin (OVA)-sensitized AC in a murine model. BALB/c mice were sensitized with OVA for two weeks, and mice were challenged by OVA eyedrops for 12 days with intraperitoneal injection of vehicle or 1α, 25-dihydroxyvitamin D3 (1,25(OH)2D3). We evaluated clinical signs, the infiltration of inflammatory cells into conjunctiva, regulatory T cells (Treg) in drainage LN, serum OVA-specific IgE , and Th2 cytokines in vitro T cells. AC development and conjunctival infiltration of eosinophils and mast cells were significantly impaired with 1,25(OH)2D3 treatment. In addition, 1,25(OH)2D3 suppressed production of OVA-specific IgE in serum and Th2 cytokines in vitro T cell assays, compared to vehicle group. Interestingly, 1,25(OH)2D3 led to increase Treg cells population in draining LNs and suppressive levels of clinical signs and inflammatory cells infiltration into conjunctivae were reversed by depleting Treg cells. Our results suggest that vitamin D supplement alleviate allergic conjunctivitis, indicated by suppressing Th2 response in draining LNs and inflammatory cells infiltration into conjunctiva through increasing population of Treg cells in draining LNs. Therefore our results demonstrated the therapeutic potential of vitamin D for allergic diseases including asthma or atopic dermatitis.

Th10. Effect of Cigarette Smoke on Immune Response to Pollen Allergen in the Lung

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Introduction: Cigarette smoke (CS) is a major risk factor for pulmonary diseases. Alveolar macrophages (AM) are known to play an important role in the lung immune system. Cryptomeria Japonica pollen (CJp) is known as pollen allergen. However, it is not fully understood the relationship of CS and CJp. Therefore, we investigated the effects of CS and CJp on immune cells response in the lung. Materials & Methods: Mice were exposed to CS during 10 days. The next day, mice were inhaled 600μg of CJp. After CJp inhalation, bronchoalveolar lavage (BAL) cells were obtained. Expression of cytokines mRNA were measured by RT-PCR. Chemotactic active ity were measured by EZ-TAXIScan. Surface antigens measured by FACS. Histopathological findings was analyzed by H&E stain. Results: The number of neutrophils was significantly increased in mice treated with CJp. The number of CJp-induced neutrophils was decreased by CS. Although IL-1β and CXCL2 mRNA expressions of AM were increased by CJp, the expressions were decreased by CS. Chemotactic activity of neutrophils for CJp were decreased by CS. Percentage of CD11b in AM was increased by CJp, Increased CD11b positive cells by CJp were decreased by CS. Neutrophils were accumulated and kept in the lung interstitial tissue by CS. Conclusions: CJp induced neutrophil and lung inflammation. CS inhibited neutrophils influx to alveolar space and increased accumulation of neutrophils in the lung interstitial tissue by the suppressions of IL-1β and CXCL2 mRNA expressions in AM. These results suggest that CS may exacerbate immunological allergic response against pollen allergen.

Th51. High Compliance of Oral Immunotherapy and Improvement of Health-related Quality of Life in a Long-term Follow-up for Food Allergy Study

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Oral immunotherapy (OIT) has been investigated extensively in clinical studies as a promising allergen-specific approach in managing food allergies. Data on long term compliance with dose maintenance regimens is necessary to understand the durability of OIT. Participants and caregivers who participated in OIT studies at our clinical translational research unit were contacted to respond to IRB-approved surveys: food allergy long-term follow up questionnaire (FALTFU), food allergy quality of life questionnaires (FAQOL), and burden of treatment (BOT). BOT was measured with a 1 (extremely positive) to 7 (extremely negative) scale to assess OIT treatment. Among 148 participants responded to the FALTFU questionnaire, 108 (73%) were 12 years or young at enrollment, 70% participants incorporated at least one of their OIT foods in their daily diet. More younger participants (0-12 years) continued the food compared to older participants (>12 years) (80% vs 40%, p< 0.001). Reasons to discontinue the food included frequent adverse reactions (n=16) and Epinephrine auto-injector (n=2). The most common OIT foods were peanut (n=124), cashew (n=38) and walnut (n=36) with 68%, 82%, and 75% participants still ingesting these foods, respectively. FAQOL improved post-study among 36 children (p< 0.001) and 39 caregivers (p< 0.001). More younger participants (0-12 years) rated slightly to extremely positive on BOT compared to older participants (87% vs 73%, p=0.048). This is the largest OIT follow-up study showing continued dosing with food, positive perceptions of treatment with significant improvement in FAQOL. These findings were most prominent in those who started OIT at a younger age.

Th59. Investigating the Immune Basis of Green Tea Induced Liver Injury in Healthy Donors Expressing HLA-B*35:01

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Introduction: Green Tea, derived from the Camellia sinensis plant, is a common constituent of herbal supplements. Green Tea extracts (GTE) have been linked with immune-mediated liver injury and associated with the expression of HLA-B*35:01. Epigallocathecin-3-O-gallate (EGCG) is the most abundant catechin found in GTE and is implicated in liver injury. The aim of this study was to investigate the immunogenicity of EGCG in healthy donors positive and negative for the HLA-B*35:01 risk allele. Methods: PBMC and T-cell priming assays using cells from HLA-B*35:01 positive and negative healthy donors (n=4) were undertaken to assess EGCG's ability to prime naïve individuals, with and without immune checkpoint blockade. EGCG-responsive T-cell clones (TCC) were generated via repetitive mitogen stimulation examined for specificity, phenotype, cytokine secretion and pathways of T-cell activation. Results: Significant proliferative responses were identified in PBMC from 2/4 allele positive and 2/4 negative donors. EGCG-specific priming of T-cells was observed in the presence of PD-1 and PD-L1 blockade. A number of EGCG-specific TCCs were identified from healthy donors expressing HLA-B*35:01. TCCs proliferated in a dose-dependent manner and secreted IFNγ, IL-5, IL-13 and Granzyme B. TCC had a CD4+ phenotype, with activation restricted to MHC Class II. Conclusion: These findings suggest EGCG has an intrinsic ability to be immunogenic and can effectively prime naïve T-cells from HLA-B*35:01 positive and negative donors. Blockade of co-inhibitory signaling pathways potentiates the priming seen. EGCG is able to activate CD4+ TCC, stimulating the release of pro-inflammatory and cytolytic cytokines, driven through MHC Class II interactions.

Th84. Leveraging the OASIS Resource to Query LEAP GWAS Results in an Unbiased or Targeted Fashion

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Intro: Genome wide association studies of the Learning Early about Peanut (LEAP) study participants has resulted in multiple publications and a vast amount of data remains to be interrogated. Given the quantity of data, we determined that there was a need to make this information publicly available to query in a way that is "scientist friendly". Methods: A new online platform, the Omics Analysis, Search, and Information System (OASIS), was developed to provide a user-friendly platform for interrogating LEAP GWAS data. OASIS integrates this data with functional annotations from GTEx and a wealth of bioinformatics resources. On demand analyses including visualization of linkage disequilibrium and Manhattan plots can easily be generated. Results: OASIS can be queried in an unbiased fashion or for genes or single nucleotide polymorphisms (SNP) of interest. To illustrate targeted use of the database, a query for CMA1, a gene previously associated with eczema and IgE levels in eczema, was performed. The peak association for CMA1 was SCORAD at 12 months study duration (OR=15 and $p< 0.001$). CMA1 was also associated with peanut-specific IgG4 at multiple timepoints suggesting that CMA1 may be associated with development of blocking antibodies. CMA1 appeared to have a relationship to peanut allergy status at 60 months in those avoiding peanut (OR 9.2, $p=0.099$). CMA1 may not only be associated with total IgE levels in patients with eczema, but also in clinical food allergy status. Conclusions: OASIS is a "scientist friendly" web-based system for mining genetic associations in the LEAP GWAS dataset.

Fel D 1 Monoclonal Antibody Combination Modulates Asthma and Allergic Molecular Pathways in Nasal Mucosa Upon Cat Allergen Challenge in a Phase 2, Randomized, Double-blind, Placebo-controlled Study

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A single dose of Fel d 1 monoclonal antibodies (REGN1908/1909) was evaluated for the prevention of early asthma responses (EAR) by cat allergen in cat-allergic mild asthmatic subjects (NCT03838731). Subjects were randomized to single-dose subcutaneous REGN1908/1909 600 mg (n=29) or placebo (n=27) prior to cat-allergen exposure in a controlled environmental exposure unit. Forced expiratory volume in 1 second (FEV1) was measured up to 4 hours during cat-allergen exposures at baseline and on days 8, 29, 57 and 85. EAR was defined as ≥20% reduction from baseline FEV1. REGN1908/1909 significantly prevented EAR incidence versus placebo on days 8 (48.3% vs 81.5%), 29 (44.8% vs 88.0%), 57 (55.6% vs 76.9%) and 85 (50.0% vs 87.5%) ($P < 0.05$ for all except day 57). Nasal brushings were performed on day 29 to investigate nasal mucosal changes in gene expression with RNAseq. Using placebo-controlled gene set enrichment analysis, Fel d 1 treatment significantly downregulated nasal mucosal derived type 2 asthma signatures (normalized enrichment score [NES] = –1.67, $q=0.003$, GSE152004), in addition to an epithelial asthma gene signature (NES = –2.23, $q < 0.001$, GSE85567).

Th104. GPCR19 Agonist (HY209) Regulates P2X7 Ion Channel-mediated NLRP3 Inflammasomal Activation and Ameliorates Atopic Dermatitis in Mice

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Atopic dermatitis (AD) is the most common chronic inflammatory skin disorder, characterized by intense itch and recurrent eczematous lesions. Although T helper 2 cell-mediated immune responses are dominant, there is growing evidence showing that AD disorder involves multiple immune pathways. Due to lack of proper therapy, the strategies of T cell modulators in combination with targeting keratinocytes and/or myeloid cells might hold promise for achieving disease control in AD patients better than T cell modulators alone. In this regards, targeting NLRP3 inflammasome (N3I) of keratinocytes and skin-infiltrating/resident myeloid cells might open new insight for AD patient's therapy. Because of the presence of polymorphisms, subtypes, ubiquitous expression, and redundant pathways, inhibition of N3I components is ineffective for inflammatory disorders. We show that HY209 regulates both priming and activation phases of N3I of keratinocytes. HY209 receptor, GPCR19, colocalizes with P2X7R which enhance Ca^{++} mobilization that leads to N3I activation of keratinocytes. HY209 desensitizes P2X7R, inhibits BzATP-mediated Ca^{++} mobilization and suppresses P2X7R expression in addition to inhibiting NF-kB activation of keratinocytes. Transcription of N3I components, and processing of immature IL-1β and IL-18 were suppressed by HY209 treatment *in vitro* and In vivo. Topical and oral treatment with HY209 ameliorates pro-inflammatory features of atopic dermatitis in Balb/c mice induced by DNCB and further confirmation using MC903 and oxazolone mice AD models. Considering the effects of HY209 alone on atopic dermatitis, HY209 might improve clinical symptoms of AD patients synergistically when combined with therapeutics targeting pathogenic Th2 cells together.

AO. Inhibition of IgE and IgG Production by FICZ and Tofacitinib in Low-dose Allergy Model

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Signaling pathways regulating IgE production are not well known. Several small molecule inhibitors are used to decrease allergic symptoms. Among them aryl hydrocarbon receptor (AHR) agonist 6-formylindolo(3, 2-b)carbazole (FICZ) and JAK pathways inhibitor Tofacitinib (TOFA) are shown to ameliorate atopic dermatitis. The aim of this work was to estimate the role of FICZ and TOFA in IgE and IgG production in mouse model of allergy to a mixed pool of allergens. BALB/c mice were immunized 20 times into mouse withers with 100 ng/injection/mouse of Gal d 1, Bos d 5, Der p 3, and Amb a1 equimolar mixture in saline (PAs) without of with FICZ (20 ug/injection) or TOFA (20 ug/injection). IgE and IgG production was estimated by ELISA. Inflammation in the withers and axillary lymph node was analyzed by histology. Immunization of mice with PAs resulted in allergen-specific IgE titers increasing in a row Amb a1 (20±3) = Der p3 (22±8)< Bos d5 (480±29)< Gal d1 (1144±220). FICZ decreased 100, 84, 65, and 20 % of IgE response accordingly the row while TOFA was more efficient and decreased 100, 72, 62, and 69 % ones. Both FICZ and TOFA also decreased IgG production. IgE and IgG levels correlated significantly ($p< 0.05$) both in control and experimental groups. These results show that there is a dominance of some allergens in the mixture; AHR and JAK pathways are both involved in IgE and IgG production however TOFA was more efficient.The work was supported by the RFBR grant 20-015-00360.

Reduced mTORC1 Signal in Autosomal Dominant Hyper-IgE Syndrome Can Be Partially Rescued with Addition of Exogenous Glutamine

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Loss-of-function (LOF) mutations in the IL-6/STAT3 signaling pathway can lead to early life allergic inflammation, elevated IgE, and infection, though the mechanism for the allergic inflammation remains elusive, especially since IL-6 receptor blockade later in life does not lead to atopy. LOF mutations in the antigen receptor induced CARD11-BCL10-MALT1 complex can also lead to atopy, as well as impaired nuclear factor-kB and the mammalian target of rapamycin complex 1 (mTORC1) signaling. While NFkB component LOF does not lead to allergic disease in humans, mouse models strongly suggest that CARD11 and MALT1 are required for adequate mTORC1 activation via facilitating import of glutamine—leading to increased Treg function, increased Th1 and decreased Th2 differentiation. We therefore sought to determine if impaired mTORC1 signaling might also be observed and potentially underlie the atopy in a STAT3 dominant negative (DN) model. Here, we show that in mouse and in human T-cells, chemical inhibition or dominant negative mutations in STAT3 result in reduced TCR-induced mTORC1 signaling. The addition of exogenous glutamine could partially restore mTORC1 signaling *in vitro.* Allergic-inflammation associated RORgT-/T-bet+ Tregs—which can also result from dysbiosis—predominated in STAT3DN patients and mice, a phenotype which could also be reversed with glutamine supplementation. Finally, oral glutamine supplementation reduced total serum IgE levels in the STAT3DN mice. These data suggest that the STAT3 pathway is required for normal TCR-mediated mTORC1 signaling and prevention of allergic phenotypes early in life, and that exogenous glutamine can reverse some of the consequences of impaired STAT3 function.

Autoimmune Diseases

Tu6. Identification of Novel DRB1*01:01-restricted T Cell Epitopes in Rheumatoid Arthritis

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The shared epitope amino acid sequence motif in the beta chain of HLA-DR is the primary genetic risk factor for anti-citrullinated protein antibody (ACPA)-positive rheumatoid arthritis (RA). Among common HLA-DRB1 alleles, the shared epitope is found in DRB1*04:01, DRB1*04:04, and DRB1*01:01 proteins. DRB1*04:01-restricted T cell epitopes have been previously found in RA autoantigens such as vimentin, α-enolase, and aggrecan. However, less is known about DRB1*01:01-restricted T cell epitopes in RA. To this end, 244 citrullinated peptides covering all possible citrullination sites in vimentin, α-enolase, aggrecan, fibrinogen-α, and fibrinogen-β were synthesized and tested for binding to DRA-DRB1*01:01 protein in a competition binding assay. Thirty-nine peptides were identified that bound DRA-DRB1*01:01 with an IC50 < 50 μM. The immunogenicity of these peptides was evaluated with 12 blood samples from ACPA-positive RA participants. PBMCs or CD8-depleted PBMCs were stimulated with peptides for 15-18 days and stained with the corresponding DRA-DRB1*01:01 tetramers. CD8-depletion enhanced expansion of tetramer-positive T cells. Two T-cell epitopes from aggrecan and one from fibrinogen-β were detected in 50% or more of the samples. The citrullinated-Agg 553 epitope was most often observed and typically had the most robust expansion. All three predominant T cell epitopes were further verified by isolating tetramer-positive T cell clones. Citrullinated-Agg 553 had previously been shown to be a predominant DRB1*04:01-restricted T cell epitope, thus finding an overlap in predominant T cell epitopes between shared epitope alleles. These results provide a panel of tetramers spanning multiple citrullinated antigens for studying DRB1*01:01-restricted T cell responses in ACPA-positive RA subjects.

Tu9. IL-6 Targeted Therapies Directed to Cytokine or Cytokine Receptor Blockade Drive Distinct Alterations in T Cell Function

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Multiple therapeutics that inhibit IL-6 at different points in the signaling pathway are in clinical use yet whether the immunologic effects of these interventions differ based on their molecular target is unknown. We performed short term interventions in individuals with type 1 diabetes using anti-IL-6 (siltuximab) or anti-IL-6 receptor (IL-6R; tocilizumab) and investigated the impact of this *in vivo* blockade on T cell fate and function. Immune outcomes were influenced by the target of the therapeutic intervention (IL-6 versus IL-6R) and by peak drug concentration. Tocilizumab but not siltuximab reduced IL-6 driven STAT3 phosphorylation, ICOS expression on T follicular helper cell populations and TCR driven STAT3 phosphorylation (TCR/pSTAT3). Siltuximab on the other hand, uniquely reversed resistance to Treg mediated suppression and increased TCR/pSTAT3, and IL-10, 21 and 27 production in T effectors. Together these findings indicate that the context of IL-6 blockade drives distinct T cell intrinsic changes that alter function.

ORAL. Antigen Specific T Cell Phenotypes Distinguishes Type 1 Diabetes Patients with High or Low Residual C-peptide

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Type 1 Diabetes is caused by immune mediated destruction of beta cells, leading to dependence on exogenous insulin. After disease onset, subjects with diabetes exhibit diverse levels of residual beta cell function. Persistence of residual c-peptide correlates with improved glycemic control and its preservation has been used as a clinical endpoint. However, the immunologic factors that differentiate subjects who maintain measurable c-peptide (slow progressors) from those do not (rapid progressors) are not well understood. To define immunologic factors that differentiate slow and rapid progressors, we recruited a cohort of subjects with diabetes that included slow progressors (c-peptide > 0.1ng/ml) and rapid progressors (undetectable c-peptide) who had known HLA class I genotypes. We then utilized HLA class I tetramers to enumerate and characterize beta cell specific CD8+ T cells and sorted these cells to perform single cell transcript analysis and to assemble T cell receptor (TCR) sequences. Notably, we observed differences in epitope diversity between slow and rapid progressors. Single cell RNA-Seq revealed clear patterns of differential gene expression between slow and rapid progressors indicating differences in effector, exhaustion, and regulatory pathways. Analysis of autoantigen specific CD8 TCRs suggested differences in clonal expansion between rapid and slow progressors and revealed identical TCRs within different cell states. Cumulatively, this work revealed specific immunologic factors that differentiate subjects who maintain measurable levels of c-peptide from those whose levels continue to decline. These aspects of T cell function and repertoire that correlate with disease progression provide important insights that will drive further studies.

Th37. Clonally Expanded CD8 T Cells Are Recruited to the CSF in Neurosarcoidosis Through the Chemokine Receptors CXCR3 and CXCR4

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Sarcoidosis is an idiopathic granulomatous disease that damages multiple vital organs, including the central nervous system (CNS), i.e., Neurosarcoidosis. How antigen-specific lymphocytes are recruited to the CNS in Neurosarcoidosis remains poorly defined. To identify cerebrospinal fluid (CSF) specific, antigen-driven T and B cell responses, we performed single-cell RNA sequencing of CSF and blood cells from Neurosarcoid participants coupled to T and B cell receptor sequencing. In contrast to pulmonary sarcoidosis, which is driven by CD4 T cells, we found consistent CD8 T cell clonal expansion enriched in the CSF. These CSF-enriched CD8 T cells were composed of two subsets based on chemokine expression: $EBI2^{+}CXCR3^{+}$ dual expressors and $CXCR4^{+}$ single expressors. Correspondingly, the ligands for CXCR3 (CXCL9 & CXCL10) and CXCR4 (CXCL12) were elevated in the CSF compared to the blood. Finally, we found a specific reduction of $EBI2^{+}CXCR3^{+}$ dual expressors and $CXCR4^{+}$ single expressors in blood CD8 T cells in Neurosarcoidosis, but not healthy controls, consistent with recruitment out of the blood into the CNS. Thus, anti-specific CD8 T cells appear to be recruited to the CNS in Neurosarcoidosis via two parallel chemokine axes.

Th45. Autoreactive CD8+ T Cells are Restrained by an Exhaustion-like Program that is Maintained by LAG3

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CD8$^+$ T cell exhaustion is a differentiation state initiated by chronic high antigen stimulation, in which T cells become unresponsive to stimulation and lose effector function. Impaired chronic viral and tumor clearance has been attributed to CD8$^+$ T cell exhaustion and can be partially reversed upon blockade of inhibitory receptors (IR) such as programmed cell death protein 1 (PD1). Despite detailed analysis of CD8$^+$ T cell these models, a role an exhaustion program in autoimmunity is still not fully appreciated. The pancreatic islets are a site of chronic high antigen stimulation in Autoimmune Diabetes (AD). We therefore hypothesized that intra-islet CD8$^+$ T cells are restrained by an exhaustion program that can be partially reversed by IR deletion. Using the Non-obese Diabetic mouse model of spontaneous AD, we show that intra-islet CD8$^+$ T cells phenotypically, transcriptionally, epigenetically, and metabolically possess features of exhausted T cells, yet maintain key differences that may contribute to their persistent pathogenicity in AD. This “restrained” phenotype can be perturbed, and disease can be remarkably accelerated in the absence IR Lymphocyte Activating Gene 3 (LAG3). CD8$^+$ T cell-restricted deletion of LAG3 promotes enhanced effector-like function and trafficking into the islets, while diminishing enrichment of an exhausted phenotype, revealing a previously underappreciated role for an exhaustion program in limiting autoimmunity. Single deletion of LAG3 from the surface of CD8$^+$ T cells, strikingly alters the developmental state and pathogenicity of intra-islet CD8$^+$ T cells, implicating LAG3 as a potential target for future autoimmune therapeutics.

Th49. Dysregulated Transferrin Receptor Disrupts T Cell Iron Homeostasis to Drive Inflammation in Systemic Lupus Erythematosus

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T cells in systemic lupus erythematosus (SLE) exhibit mitochondrial abnormalities including elevated oxidative stress. Because excess iron can promote these phenotypes, we tested iron regulation of SLE T cells. A CRISPR/Cas9 screen identified the Transferrin Receptor (CD71) as important for Th1 cells but detrimental for induced regulatory T cells (iTreg). Activated T cells induce CD71 to increase iron uptake, but this was exaggerated and prolonged in T cells from SLE-prone mice which accumulated iron. Treatment of T cells from SLE-prone mice with a CD71 blocking antibody reduced intracellular iron and mTORC1 signaling and restored mitochondrial physiology. While Th1 cells were inhibited, CD71 blockade enhanced iTreg and their FoxP3 expression. *In vivo* this treatment reduced kidney and liver pathology, reduced CD4 T cells, and increased IL-10+ T cells in SLE-prone mice. Importantly, disease severity in patients with SLE correlated with CD71 expression on T cells and blocking CD71 enhanced IL-10 secretion. Excess iron uptake via CD71 dysregulation thus contributes to T cell dysfunction and can be targeted to correct SLE-associated pathology.

Th52. Childhood Idiopathic Nephrotic Syndrome is Characterized by Antigen-Experienced B-Cell Responses and Diminished Immunoregulation that are Differentially Targeted by Distinct Immunosuppressive Treatments

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Idiopathic nephrotic syndrome (INS) is the most common chronic glomerular disorder in children featuring frequent relapses of heavy proteinuria. Effacement of podocyte foot processes – the major determinants of glomerular filtration – is the definitive and often sole histopathological manifestation of INS. Nevertheless, a T-cell-dependent etiology is suspected given the efficacy of broadly immunosuppressive drugs (glucocorticoids, GC) and T-cell-targeting agents at inducing remission, albeit with significant toxicity. Recently, the search for GC-sparing treatment alternatives identified the B-cell-depleting biologic rituximab (RTX) as a potent inducer of long-term remission and thereby positioned B-cells as the probable culprits of INS. Since the immune processes leading to podocyte effacement and the mechanisms by which GC and RTX mediate remission are unknown, we sought to characterize T- and B-cells in pediatric INS using single cell RNA-sequencing (scRNAseq) and high-parameter flow cytometry. Comparisons of patient and healthy PBMC by scRNAseq (N=4/group) and flow (N=10/group) revealed a T-cell-dependent antigen-experienced B-cell phenotype and a broadly activated T-cell phenotype with diminished regulatory T- (T_{REG}) cell frequencies during INS onset/relapse. GC treatments specifically reduced plasma cell proliferation and rescued T_{REG}-cell abundance, whereas RTX treatment provided long-term ablation of most antigen-experienced memory B-cells in both longitudinal (N=7) and cross-sectional (N >10/group) flow-based investigations. Accordingly, the eventual resurgence of memory B-cells following RTX treatment correlated with proteinuric relapses. Overall, we demonstrate that pediatric INS features a T-cell-dependent antigen-driven B-cell response and a T_{REG}-cell defect that are differentially targeted by GC and RTX, thus emphasizing the importance of adaptive immunity in INS.

Th69. Oral Mucosal Breaks Trigger Unique Systemic Immune Responses that Mediate Rheumatoid Arthritis

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Periodontitis is a risk factor for developing rheumatoid arthritis (RA) with anti-citrullinated protein antibodies (ACPA), implicating oral mucosal inflammation in RA pathogenesis. Here, we performed paired analysis of human and bacterial transcriptomics in longitudinal blood samples from RA patients. We discovered that patients with RA and periodontitis experienced repeated oral bacteremias, which triggered innate inflammatory pathways that were associated with clinical arthritis flares. These inflammatory pathways included activation of an ISG15+HLADRhi monocyte subset that is expanded in the inflamed RA synovium. Further, the oral bacteria observed transiently in blood were broadly citrullinated in the mouth, and their in situ citrullinated epitopes were targeted by extensively somatically hypermutated ACPA encoded by RA blood plasmablasts. Together, these results suggest (i) periodontitis results in repeated breaches of the oral mucosa releasing citrullinated oral bacteria into circulation, which (ii) activate innate immune pathways that are observed in inflamed RA synovium and during clinical arthritis flare, and (iii) stimulate recurrent rounds of ACPA expressing B cell activation and somatic hypermutation associated with progression to and persistence of clinical RA.

Th74. *Ex vivo* Fold Expansion of Human Regulatory T Cells Negatively Correlates with Preservation of Beta Cell Function in Adolescent T1D subjects

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Tregs are critical for peripheral immune tolerance, and defects in Tregs have been described in patients with T1D. Adoptively transferred autologous polyclonal expanded Tregs (exTregs) were safe and well-tolerated in prior phase 1 clinical trials. The Sanford/Caladrius Trex trial, a double-blinded, placebo-controlled, multi-center exploratory Phase 2 study, assessed a single treatment with exTregs (1-24x106cells/kg) in adolescents with recent-onset T1D (NCT02691247). Overall, exTreg therapy did not preserve the residual β-cell function, quantified as change over two years in C-peptide AUC-mean during a mixed meal test (p=0.39). However, the dose of exTregs and outcome varied greatly across subjects, providing a dataset that can be queried for correlates to outcome. *Ex vivo* fold expansion of exTregs (246 ± 149.9-fold, range 24.3-643-fold) negatively correlated with C-peptide change (r=-0.33, p=0.01), suggesting that infused Tregs may influence the outcome. Indeed, exTregs expressed more CD25, CD38, HLA-DR, and CXCR3 than endogenous Tregs. Preliminary analysis of a subset of subjects (n=32) shows a transient increase of total Tregs in treated subjects. Additionally, no significant changes in conventional T cell differentiation and activation after exTreg therapy compared to placebo were observed to date. By contrast, the dose of exTregs did not correlate with the preservation of beta cell function (r=-0.15, p=0.25), nor did peak frequency (day 7 post-infusion) of Tregs following treatment, suggesting that Treg number does not predict outcome. Instead, the activation state of exTregs may impact outcome. Studies are ongoing to characterize the endogenous and exTregs from treated (n=63) and placebo (n=44) subjects.

Th75. Reduced CD8 T Cell Exhaustion in DR4 Risk Subjects is Selectively Augmented with Abatacept in RA

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Exhausted CD8 T cells (T_{EX}) are associated with worse outcome in cancer yet better outcome in autoimmunity. However, factors contributing to reduced T_{EX} in autoimmunity are not well understood. Here, we identify co-expression of TIGIT and KLRG1 as identifiers of human T_{EX}. TIGIT+KLRG1+ CD8 memory T cells share EOMES signature genes in healthy controls (HC), type 1 diabetes, and rheumatoid arthritis (RA) subjects; are increased with age and in chronic viral-specific cells; and are hyporesponsive (reduced proliferation and cytokine production) in vitro. We applied this inclusive measure of T_{EX} to larger longitudinal and autoimmune cohorts. Consistent with a genetic determinant, T_{EX} are stable within HC (within-individual mean range in frequency 6.5% (95% CI: 5.6-7.4%)) but vary between individuals (inter-individual mean frequency 4.2-59.8%) resulting in a high intraclass correlation coefficient of 92.0%. In a large cohort of HC and RA subjects (n >90/cohort), lower levels of EOMES transcriptional modules and T_{EX} cells were associated with RA-associated HLA risk alleles (primarily DR4), regardless of disease status. In a treatment cohort, an increase in T_{EX} following treatment was more prevalent in RA HLA risk subjects (11/16, 69%, p=0.0043) than non-risk subjects (4/10, 40%,p= ns) treated with abatacept; no change in T_{EX} was observed with adalimumab (anti-TNF). The HLA association and selective modulation with abatacept suggests that T_{EX} may be indirectly influenced by APC or T cell help, that is blocked by abatacept. Moreover, targeting factors that increase T_{EX} may be more effective in HLA risk subjects, a step towards personalized medicine.

Th89. Increased Intrathecal Activity of Follicular Helper T Cells in Patients with Relapsing-Remitting Multiple Sclerosis

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Follicular helper T (Tfh) cells play a critical role in protective immunity helping B cells produce antibodies and are likely implicated in the pathogenesis of various autoimmune diseases. This study investigated a possible role of Tfh cells in the pathogenesis of multiple sclerosis (MS). We investigated phenotype, prevalence, and function of Tfh cells in blood and cerebrospinal fluid (CSF) from controls and patients with relapsing-remitting MS (RRMS) and primary progressive MS (PPMS). Additionally, we assessed the migratory capacity of Tfh cells in patients with MS in an *in vitro* blood-brain-barrier coculture assay of primary human astrocytes and brain microvascular endothelial cells. This study identified two phenotypical and functionally distinct Tfh cell populations; $CD25^-$ (Tfh1-like) and $CD25^{int}$ (Tfh17-like) Tfh cells. Whereas only minor differences in Tfh cells were found in blood between patients with MS and controls, we observed an enrichment of $CD25^-$ Tfh cells intrathecally in patients with RRMS and PPMS and $CD25^{int}$ Tfh cells in patients with RRMS, compared to controls. Frequencies of intrathecal $CD25^-$ Tfh cells and $CD25^-$ Tfh:Tfr cell ratio correlated positively with IgG-index in patients with RRMS. Despite enrichment of intrathecal Tfh cells in patients with MS, no difference in the migratory capacity of peripheral Tfh cells was observed when compared to controls. Our study indicates substantial changes in intrathecal Tfh dynamics in MS, particularly in patients with RRMS suggesting that the intrathecal inflammatory environment of patients with RRMS enables increased recruitment of peripheral Tfh cells rather than Tfh cells having an increased CNS migratory capacity.

Th97. Utilization of a Clinical Data Research Network to Improve Identification of Systemic Lupus International Collaborating Clinics Classification Criteria Attributes in Patients with Systemic Lupus Erythematosus

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Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by a heterogenous clinical phenotype that may present differently over time and between patients, which can create challenges for identification and treatment of individuals visiting multiple care centers. Clinical data research networks (CDRN) aim to pool electronic health records (EHR) to provide more complete clinical information for patients shared across care centers. We sought to identify whether differences existed in algorithms Systemic Lupus International Coordinating Clinics (SLICC) classification criteria with the inclusion of data from multiple sites. Previously published algorithms for the identification of the SLICC attributes were adapted to the PCORnet Common Data Model used by the Chicago Area Patient Centered Outcomes Research Network (CAPriCORN), initially identifying 1,231,130 persons having at least one SLICC attribute. A subset of 1,251 individuals with data from ≥1 CAPriCORN sites and ≥ 3 instances of a SLE ICD code was retained for the primary analysis. The number of non-redundant attributes satisfied when data was included from multiple sites was quantified and compared to attributes met at the primary (most-visited) site. The average number of SLICC attributes was 3.6 per person when data was included from only one site. This increased to 4.5 when data from multiple sites were included ($p < 0.0001$). Rates of individual SLICC attributes were also compared, all of which were identified at significantly higher rates with multisite data inclusion. The results suggest CDRNs can be used to increase the amount of non-redundant data for increased identification of SLE attributes.

Th99. Multi-omic Profiling in Celiac Disease Reveals Somatic Driver Mutations in Rogue T Cell Clones

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It has long been proposed that the pathogenesis of human autoimmune diseases may share common origins with lymphoid cancer. However, a major hurdle in understanding the immune pathogenesis of autoimmune diseases is distinguishing self-reactive “rogue” lymphocytes from normal immune cells required for host defence. In Celiac disease, both the environmental trigger (gluten) and major autoantigen (transglutaminase 2) are well characterised, but the underlying mechanisms by which rogue lymphocytes initiate and drive disease are unknown. Here, we apply multi-omic technologies that enable detailed DNA, RNA and protein measurements at the single-cell level to profile tens of thousands of immune cells isolated from small intestine duodenum biopsies from individuals with Celiac disease. We utilise the T-cell receptor as a natural barcode across multiple single-cell experiments to identify expanded rogue T cell clones and their gene and cell-surface protein expression profiles, along with any somatic DNA driver mutations. Strikingly, we identify in multiple patients expanded T cell clones with mRNA and cell-surface protein expression profiles of cytotoxic effector cells, that harboured missense somatic mutations in T cell lymphoma driver genes *STAT3*, *STAT5B* and *DDX3X.* These mutations have been described as strong gain-of-function mutations in multiple T cell lymphomas, highlighting a novel mechanism by which T cell clones escape immune tolerance and adopt a rogue phenotype in Celiac disease. Overall, our results highlight the power of single-cell multi-omics in identifying and characterising rare pathogenic clones in a common human autoimmune disease and provide direct evidence for a shared pathogenesis of autoimmunity and lymphoid malignancy.

Tu11. High-Dimensional Immunophenotyping Reveals Altered Regulatory T Cell Fitness Between Inactive and Active Disease in Juvenile Idiopathic Arthritis

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Juvenile Idiopathic Arthritis (JIA), a prevalent autoimmune condition in children, is characterized by unpredictable, T-cell rich inflammatory flares of joints. Despite increased CD4+FoxP3+ regulatory T cell (Treg) numbers within the synovial fluid (SF) of active joints, these fail to suppress autoimmunity. Here, we utilize in-depth phenotyping by 5-laser full spectrum flow cytometry and unbiased high dimensional analysis to identify and distinguish unfit Treg sub-populations present in JIA SF (n=18), clinically active peripheral blood (PB, n=29) compared to inactive disease (n=17) and healthy control PBMCs (n=18). We designed and verified a 33-parameter panel to assess cellular composition (monocyte, B, NK, dendritic and T cell subsets) and comprehensive Treg phenotype (functional markers, activation, memory, co-receptors). SF saw most changes with 12/18 cell composition and 8/10 Treg clusters changed in frequency. SF CD4-, CD4+ T cells and Tregs showed increased expression of GITR, HLA-DR, CD71 and CD69, with SF Tregs expressing high CTLA-4 and PD-1 levels. However, despite a more active state, SF Tregs were not more proliferative than blood Tregs. The co-stimulatory receptor CD226 was increased on SF Tregs, mostly co-expressed with TIGIT while blood Tregs rarely co-expressed both co-receptors. Moreover, we identified a sub-population of CD137low ID2intermediate Tregs in PB of inactive individuals, with reversed expression pattern in PB Tregs from active disease. High dimensional flow cytometry revealed subsets of Tregs within the inflammatory joint of JIA and in blood of active compared to clinically inactive patients. These may have biomarker potential for predicting flare-ups and offer potential new mechanistic targets.

Tu12. An In Silico Analysis to Predict the Function of Genes Associated with Type 1 Diabetes, Systemic Lupus Erythematosus, and Juvenile Idiopathic Arthritis in Colombian Patients

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Background: Autoimmune diseases are complex-heterogeneous. There are clinical and genetic grounds for assuming similar genetic mechanisms in these diseases Aim: Predict the function and interactions of genes associated (MGAT5, RUNX1, PSD3, GRB2, GABRA2, CD86, KSR2, and EDEM3) with T1D, SLE, and JIA. Methods: We analysed whole-exome-sequences of 75 patient diagnosed with T1D (n=25), SLE (n=25) and JIA (n=25) recruited in Barranquilla-Colombia. Single and multi-locus linear mixed-effects-models were used to identify genomic-variants associated with SLE, JIA, and/or T1D. We prioritize the analysis to the following genes MGAT5, RUNX1, PSD3, GRB2, GABRA2, CD86, KSR2, and EDEM3. We use biological network predictor server such as GeneMANIA, STRING, and Phenolyzer to predict Genetic and Protein-interactions. Results: GenMANIA reported a genetic-interaction between CD86 and MGAT5; Co-expression of CD86, MGAT5, PSD3, GRB2 and EDEM3; and the physical-interaction between CD86 and GRB2 in the T cell activation pathway. Phenolyzer displayed three seed-genes (GRB2, RUNX1, EDEM3) that interact with other genes; GRB2 associate to SLE and interact with KSR2 and CD86; CD86 is a bridge with RUNX1 that relate to JIA and interact with MGAT5, EDEM3 and GABRA2. k-means-clustering of STRING showed 3 cluster of protein-protein interactions (C1-GABRA2, PSD3, RUNX1; C2-EDEM3, KSR2, MGAT5; C3-CD86, GRB2). Conclusions: Taking together these in silico results, we found that these eight genes are involved in different pathways; however, the role of these genes should be analyzed particularly in the pathophysiology of each disease. Some genes confirm their association with SLE, and JIA but other are reported for the first-time associated with T1D.

Tu14. Functional and Molecular Characterization of Th2 Cell Subsets in Type 1 Diabetes

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Type 1 diabetes (T1D) is an inflammatory autoimmune disease resulting in destruction of insulin secreting beta-cells by T cells. Soon after diagnosis, most T1D patients experience a period of partial remission, characterized by reduced insulin requirement, that can last from several weeks to over a year. The mechanism for partial remission is not known. We have identified and validated a novel CD25+CD127+ CD4 T cell population that is present at a higher frequency in T1D patients with long remission compared to those with short or no remission, and in those who maintain beta-cell function. CD25+CD127+ cells are 90-95% memory cells, the majority of which have an anti-inflammatory Th2-type profile. CD25+CD127+ memory Th2 cells are different from CD25- memory Th2 cells (CD25-neg) - they secrete significantly higher levels of Th2 cytokines, and differential gene expression analysis (DGE) of scRNAseq and CITEseq data shows that Th2-associated genes PTGDR2, IL9R, IL2R, IL17RB, IL4R, GATA3, CCR4, IL10RA, as well as PRDM1 (transcriptional repressor of T-bet and IFN-) and GATA3-AS1 (regulator of GATA3 transcription) are significantly upregulated in CD25+CD127+ memory Th2 cells compared to CD25- memory Th2 cells supporting their anti-inflammatory profile. In addition, genes GZMK, IL6ST, IL12RB and IFNG-AS1, (regulates IFN-g expression) are significantly downregulated. Pathway analysis and differences in CD25+CD127+ Th2 cells in T1D compared to healthy will be discussed. These data support the idea that CD25+CD127+ memory Th2 is a unique cell subset mechanistically linked to protecting from T1D progression.

Tu18. Targeted PD-1 Agonists as localized Immune Suppressants against Skin Autoimmune and Inflammatory Diseases

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Tissue-restricted immune modulation is a promising approach to overcome many issues associated with current immunosuppressants and provide improved therapies against autoimmune diseases. We recently described targeted PD-1 agonist bispecifics that consist of a high affinity TCR targeting domain fused to a PD-1 agonist moiety to inhibit autoreactive T cells. These molecules, once bound to target cells, activate the PD-1 pathway on interacting T cells and potently inhibit inflammatory cytokine production and cytotoxic activity. Importantly, in the absence of target cell binding, these targeted bispecifics are unable to inhibit T cells. To advance this targeted immune suppressive approach towards the clinic, skin-directed PD-1 agonists are now being engineered to treat T cell driven diseases such as vitiligo and atopic dermatitis. For this, two separate skin targets have been identified and characterized: a novel peptide HLA melanocyte antigen and a highly abundant, HLA-unrestricted target found on skin antigen presenting cells. PD-1 agonist bispecifics engineered against these targets inhibit TCR signaling and inflammatory cytokine release in Jurkat and primary T cells with picomolar potency. Again, the immune suppressive activity of these novel molecules is dependent on target cell binding. Overall, the immune modulating bispecifics described here have the potential to deliver potent, skin-restricted T cell inhibitors that avoid systemic immunosuppression and could benefit patients with dermatological autoimmune or inflammatory diseases.

Tu19. Multi-parametric Interrogation of the Systemic Lupus Erythematosus (SLE) Immunome Reveals Multiple Derangements

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Systemic lupus erythematosus (SLE) is an autoimmune disease with unpredictable disease course due to alternating remissions and flares. Mechanistic insights are required for better assessment. To achieve this, SLE is best interrogated with a multi-parametric, holistic approach such as mass cytometry (CyTOF). Peripheral blood mononuclear cells (PBMCs) from 26 SLE subjects (41 samples, median age 39.5years) and 27 age-matched subjects underwent CyTOF. Data was analysed and visualised using our laboratory-designed machine learning tool. An activated CD8+CXCR3+CLA+ T-cell subset (CD8+CD45RA+CD38+CLA+CXCR3+) was enriched in SLE (median: 0.19%,interquartile range: 0.13-0.35% of CD45+ PBMCs) versus healthy (0.08%,0.05-0.09%; p< 0.0001). CXCR3 and cutaneous lymphocyte-associated antigen (CLA) are involved in skin-homing, with CLA function enhanced in inflammatory dermatoses. This suggests the importance of skin involvement in SLE immunopathogenesis even if skin manifestations are absent. Further analysis of the CD3+ cell compartment revealed unchanged natural T-regulatory cells (TREGS) between healthy and disease while some TREG-like populations (FoxP3+CD25-) are significantly increased in disease, highlighting a deranged TREG-driven immunoregulatory response. Thirdly, an activated CD8+BAFF+ T-cell subset (CD8+CD45RA+iCOS+ BAFF+) is enriched in SLE (0.975%,0.433-1.53%) versus healthy (0.28%,0.1-0.49%; p< 0.0001). B-cell activating factor (BAFF) supports autoreactive B cell survival in autoimmune disease; an anti-BAFF drug (belimumab) is FDA-approved for SLE. Despite increasing use of belimumab, not all patients respond equally, so BAFF inhibition alone may not adequately alter disease activity. Studying these cell subset interactions in detail to identify pathological pathways may facilitate improvements in current SLE theragnostics. With a multi-parametric unbiased approach, we identified immune subsets of immunopathogenic importance for further studies.

Tu41. KIR+CD8+ T Cells Target Pathogenic T Cells and are Active in Autoimmune Diseases and COVID-19

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Previous reports show a small subset of $CD8^+$ T cells expressing Ly49 proteins in mice can suppress autoimmunity in a model of demyelinating disease. Here we find a markedly increased frequency of $CD8^+$ T cells expressing inhibitory Killer cell Immunoglobulin like Receptors (KIR), the human equivalent of the Ly49 family, in the blood and inflamed tissues of various human autoimmune diseases. Increased KIR^+CD8^+ T cells in the gut also correlate with disease activity in Celiac disease (CeD) patients. Moreover, KIR^+CD8^+ T cells can efficiently eliminate pathogenic gliadin-specific $CD4^+$ T cells from CeD patients' leukocytes in vitro. Together with gene expression data, this shows that these cells are the likely equivalent of the mouse $Ly49^+CD8^+$ T cells. Furthermore, we observe elevated levels of KIR^+CD8^+ T cells, but not $CD4^+$ regulatory T cells, in COVID-19 and influenza-infected patients, and this correlates with disease severity and vasculitis in COVID-19. Single-cell RNA and parallelized TCR sequencing reveals that expanded KIR^+CD8^+ T cells from these different diseases and healthy subjects display shared phenotypes and similar T cell receptor sequences. Selective ablation of the murine counterpart in virus-infected mice leads to exacerbated autoimmunity developed after infection. These results characterize a regulatory $CD8^+$ T cell subset in humans which we hypothesize functions to control pathogenic cells in autoimmune and infectious diseases, with important implications for the cellular dynamics and possible therapeutic approaches to suppress unwanted autoimmunity.

Tu42. JAK Inhibitor Ruxolitinib Prevents Autoimmune Diabetes in Non-Obese Diabetes Mice through Inhibition of the JAK-STAT Pathway in Multiple Cell Types

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In Type 1 diabetes, pancreatic islet β cells are destroyed by diabetogenic CD8 T cells. Interferons (IFNs) play pathogenic roles in diabetogenic CD8 T cell activation. Ruxolitinib, a Janus Kinase (JAK) 1/2 inhibitor, inhibits receptor signaling of IFNs. The aim of the study was to investigate effects of ruxolitinib on diabetogenic immune responses and diabetes development in non-obese diabetic (NOD) mice. Female NOD mice were treated with ruxolitinib or vehicle for 10 days prior to disease onset, and mice were monitored for diabetes development. Further, the mechanism of ruxolitinib action was explored. Insulitis scores were decreased in ruxolitinib-treated NOD pancreas. Ruxolitinib prevented proliferation of adoptively transferred diabetogenic 8.3 CD8 T cells. Bone marrow dendritic cell (BMDC) maturation was affected by ruxolitinib, with evidence of impaired DC function. The proportion of $IFN\gamma^+CD4^+$, $IFN\gamma^+CD8^+$ and $NKG2D^+CD8^+$ T cells were decreased in NOD pancreas after ruxolitinib treatment. In vivo killing of islet peptide–pulsed splenocytes was reduced in ruxolitinib-treated NOD PLNs. After ruxolitinib treatment, diabetes development was inhibited in NOD mice and NOD SCID mice that were adoptively transferred with diabetic splenocytes. Further, ruxolitinib inhibited induction of pSTAT1 in BM and peritoneal macrophages as well as lymphocytes stimulated by IFNα, and the upregulation of pSTAT1 in response to IFNα was lower in ruxolitinib-treated PLN cells than in control mice. Our data demonstrate that ruxolitinib inhibits diabetogenic immune responses and diabetes development through inhibiting the JAK-STAT pathway in multiple cell types. Thus, early targeted therapy may be used to delay or prevent disease onset.

Tu43. NaCl Exposure Results in Increased Expression and Processing of IL-1β in Meniere's Disease Patients

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Meniere's disease (MD) causes fluctuating hearing loss, aural fullness and episodic vertigo. Initial management of MD is a low sodium diet and use of diuretics. A subset of MD patients have an autoimmune etiology. Recent autoimmune disease literature suggested that high sodium salt intake causes inflammation resulting in a higher risk and clinical worsening. Our previous studies have demonstrated elevated interleukin-1β (IL-1β) levels in corticosteroid-unresponsive autoimmune inner ear disease (AIED) patients and hearing could be improved with use of an interleukin-1 receptor antagonist anakinra. We recently identified that patients with AIED uniquely process IL-1β to a 28kDa form that is biologically active and proinflammatory. In this study, we characterized the production, processing and release of the pro-inflammatory cytokines IL-1 and IL-6 from PBMC of MD patients in response to sodium chloride (NaCl). We also queried whether diuretics, corticosteroids or anakinra could alter pro-inflammatory cytokine expression in patients with MD. We observed that MD patients showed processing of IL-1β to the 28kDa product in peripheral blood mononuclear cells (PBMC) when cultured in high sodium salt (NaCl) culture environment, and that this product is abrogated with diuretic triamterene-hydrochlorothiazide (T-HCTZ). Studies are underway to characterize which cell type in PBMC is majorly responsible for processing of IL-1β to the 28kDa.

Tu48. Naïve Arthritogenic SKG T Cells have a Defect in Anergy and a Repertoire Pruned by Superantigen

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How autoreactive CD4 T cells subvert tolerance to cause rheumatoid arthritis remains unknown. The SKG autoimmune arthritis model can be used to investigate these questions. Due to a mutation in ZAP70—a tyrosine kinase critical for T cell antigen-receptor (TCR) signaling—SKG T cells have severely impaired TCR signaling capacity resulting in a known defect in central tolerance. It is less clear whether they have an independent defect in peripheral tolerance. We used a reporter for antigen-receptor signaling in SKG mice to profile a T cell subpopulation that we previously identified is enriched for arthritogenic naïve CD4 T cells before arthritis onset by bulk and single cell RNA- and TCR-sequencing. Our analyses reveal that despite their impaired proximal TCR signaling, a subset of SKG naïve CD4 T cells that have recently encountered endogenous antigen upregulate gene programs associated with positive regulation of T cell activation and cytokine signaling at higher levels than wild type cells in the pre-disease state. These arthritogenic cells also induce genes associated with negative regulation of T cell activation but do so less efficiently than wild type cells. Furthermore, their TCR sequences exhibit a previously unrecognized biased peripheral TCR Vβ repertoire likely driven by endogenous viral superantigens. These particular Vβs, known to recognize endogenous mouse mammary tumor virus (MMTV) superantigen, are further expanded in arthritic joints. Our results demonstrate that autoreactive naïve CD4 T cells which recognize endogenous viral superantigens are poised to contribute to arthritis pathogenesis, influenced by their altered transcriptome.

Tu62. Impaired TIGIT Expression on B Cells Drives Circulating Tfh Cell Expansion in Multiple Sclerosis

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B cell depletion in patients with relapsing remitting multiple sclerosis (RRMS) markedly prevents new MRI lesions and disease activity, suggesting the hypothesis that altered B cell function leads to the activation of T cells driving disease pathogenesis. Here, we performed comprehensive analyses of memory B cells from patients with MS and healthy age-matched controls stimulated with CD40L and IL-21, modeling the help of follicular helper T cells (Tfh cells), and found a differential gene expression signature in multiple B cell pathways. Most striking was impaired TIGIT expression on MS-derived B cells mediated by dysregulation of the transcription factor TCF4. Activated circulating Tfh cells (cTfh cells) expressed CD155, the ligand of TIGIT, and investigation of TIGIT+ B cells revealed their capacity to suppress the proliferation of IL-17-producing cTfh cells via TIGIT/CD155 axis. Finally, CCR6+ cTfh cells were significantly increased in MS and their frequency was inversely correlated with that of TIGIT+ B cells. Together, these data suggest that the dysregulation of negative feedback loops between TIGIT+ memory B cells and cTfh cells in MS drive the activated immune system in the disease.

Tu77. Selective CD226 Knockout on Regulatory T Cells Reduces Diabetes in Female Non-obese Diabetic Mice

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T cell co-stimulation serves as a critical checkpoint for T cell development and activation, and several genetic variants affecting co-stimulatory pathways confer risk for autoimmune diseases. A single nucleotide polymorphism in *CD226* (*rs763361*; G307S) has been shown to increase susceptibility to type 1 diabetes (T1D), multiple sclerosis, and rheumatoid arthritis. Our group previously found that knockout of *Cd226* protected non-obese diabetic (NOD) mice from disease and that CD226 activity on $CD4^+$ T cells was required to confer disease through adoptive transfers into immunodeficient NOD.*scid* recipients. However, the impact of CD226 signaling on individual immune subsets remained unclear. Our efforts here focus on $CD4^+$ regulatory T cells (Tregs) based on our work showing that human $CD226^+$ Tregs exhibited reduced $FOXP3^+Helios^+$ purity and suppression following expansion. Crossing the NOD.$Cd226^{-/-}$ and Treg-fate tracking strains (NOD.*Foxp3*-GFP-Cre.*R26*-YFP) resulted in increased "early" Tregs ($p< 0.0001$) and decreased ex-Tregs (GFP^-YFP^+; $p< 0.0001$) in the pancreatic lymph nodes. We next generated a Treg-conditional KO (cKO) strain by crossing NOD.$Cd226^{fl/fl}$ and NOD.*Foxp3-Cre* strains and observed selective CD226 knockout on Tregs. Treg cKO mice had decreased insulitis ($p< 0.0001$) and diabetes incidence ($p=0.044$) compared to control mice (NOD.$Cd226^{+/+}$.$Foxp3^{Cre/+}$). cKO mice had increased naïve ($CD44^-CD62L^+$) and decreased effector/memory ($CD44^+CD62L^-$) subset frequencies of Tregs ($p=0.0149$ and 0.0362) and conventional $CD4^+$ T cells ($p=0.0016$ and 0.0040) compared to controls. These studies highlight the role for CD226 in controlling Treg lineage stability in the NOD mouse model of T1D and the potential for therapeutic targeting of this pathway to restore immunoregulation in autoimmune disorders.

Tu79. Searching for Environmental Triggers of Juvenile Dermatomyositis Using Antibody Profiling Technology

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Background: Idiopathic inflammatory myopathies (IIMs) are a group of autoimmune muscle diseases characterized by chronic progressive muscle weakness. The causes of these autoimmune myopathies are unknown. The prevailing hypothesis is that environmental triggers can precipitate disease in individuals with genetic susceptibility. Antibody profiling can serve as an unbiased method of characterizing environmental exposures. Methods: We used Phage ImmunoPrecipitation Sequencing (PhIP-Seq) with a library that covers all known protein toxin and virulence factors ("ToxScan"), to identify environmental IgG antibody reactivities in IIM patients. Candidate antigens were identified via comparison of IIM groups (cases) to controls using a case-control analysis software. The most significant reactivity in cases compared to controls was validated using Mesoscale Diagnostics (MSD). Results: PhIP-Seq with the ToxScan library identified increased anti-flagellin antibodies in juvenile dermatomyositis (JDM). The anti-flagellin antibodies displayed reactivity to several flagellin peptides from various bacterial species with conserved epitopes. Increased flagellin reactivity in JDM patients, compared to age-matched controls (including sibling and twin controls), was validated by MSD. Additionally, flagellin reactivity in JDM patients is associated with an age of 10 years or younger. Conclusions and Next Steps: Profiling antibody reactivities targeting environmental antigens can be used to identify triggers of autoimmune diseases, such as IIMs. Ongoing studies will use PhIP-Seq to determine IgA reactivities in JDM flagellin-positive patients. Ultimately, this work will deepen our understanding of the complex interplay between the microbiota, host immunity, and the development of JDM and other IIMs.

Tu91. Evaluation of Indirect Immunofluorescent Assays for LGI1 Antibody Detection in Patients with Anti-LGI1 Encephalitis

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LGI1 antibodies (LGI1-abs) are probably the most common cause of autoimmune limbic encephalitis and the second most common cause of autoimmune encephalitis. Therefore, detection of these antibodies is important to establish a definitive diagnosis of anti-LGI1 encephalitis. Herein, we assess the clinical performance of two antigen-specific indirect immunofluorescent assays (IIFA) for LGI1-abs in serum and CSF: 1) commercial IIFA with cells transfected with a GPI-LGI1 construct, and 2) in-house IIFA with HEK293 cells co-transfected with LGI1 and ADAM23 constructs. We also evaluated if cerebrospinal fluid (CSF) or serum is the most suitable sample to detect LGI1 antibodies by Indirect Immuno Histochemistry (IIHC) as screening assay. We retrospectively examined paired serum/CSF of 70 patients known to be LGI1-abs positive by IICH in at least one of the samples. Among the 70 sera, 69 (98.5%) were positive by IIHQ, 66/70 (94.3%) by commercial IIFA, and 58/70 (82.8%) by in-house IIFA. Regarding the CSF samples, 68/70 (97.1%) were positive by IIHC, 58/70 (82.8%) by commercial IIFA, and 70/70 (100%) by in-house IIFA. The performance of brain tissue IIHC as a diagnostic screening method is independent of the sample source (serum or CSF). A negative test in CSF by commercial IIFA does not exclude the presence of LGI1-abs in patients with suspected anti-LGI1 encephalitis. Based in our experience, LGI1-ADAM23 IIFA using CSF is the most sensitive method to study patients with suspected LGI-1 encephalitis.

Th171. Gain of Function Mutation in SKAP2 Leading to Autoimmune Diabetes

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The study of the genetic basis for type 1 diabetes (T1D) has benefited tremendously from examination of rare individuals with likely monogenic forms of the disease. Using whole exome sequencing of individuals with T1D enrolled in a monogenic diabetes registry, we identified an individual with a gain-of-function mutation (G153R) in the *SKAP2* gene. This individual also has multiple other autoimmune manifestations. Multiple GWAS studies have identified a strong genetic linkage between *SKAP2* polymorphisms and T1D, however the mechanisms by which alteration of *SKAP2* could lead to autoimmune T1D are unknown. SKAP2 is expressed primarily in myeloid cells, where it functions as an adapter protein in the integrin signaling pathway, linking cell surface integrins to actin rearrangements that occur following leukocyte adhesion. The *SKAP2 G153R* mutation resulted in a hyper adhesive phenotype in macrophages cultured from the patient. Knock-in (KI) mice containing the G153R substitution in murine *Skap2,* on the NOD genetic background, developed accelerated diabetes with histologic evidence of increased myeloid cell invasion of pancreatic islets. Female NOD.SKAP2 KI mice also have high levels of autoantibodies to a wide range of tissue antigens, consistent with increased systemic autoimmunity. Neutrophils from G153R KI mice had increased integrin signaling while dendritic cells from these animals showed increased antigen presentation capacity to islet-specific transgenic T-cells. This study suggests that hyperactive integrin signaling in myeloid cells can drive autoimmunity, including type 1 diabetes.

Preclinical Evaluation of a Precision Medicine Approach to DNA Vaccination in Type 1 Diabetes

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Antigen-specific immunotherapy involves the delivery of self-antigens as proteins or peptides (or using nucleic acids encoding them) to reestablish tolerance. The Endotope platform supports the optimal presentation of endogenously expressed epitopes on appropriate MHC class I and II molecules. Using specific epitopes that are disease-relevant (including neoepitopes and mimotopes) and restricted to the subject's MHC haplotypes provides a more focused and tailored way of targeting autoreactive T cells. We evaluated the efficacy of an Endotope DNA vaccine tailored to the non-obese diabetic (NOD) mouse in parallel to one expressing the Proinsulin protein, a central autoantigen in NOD mice, and assessed the influence of several parameters (e.g. route, dosing frequency, disease stage) on diabetes prevention. Secretion of encoded peptides and intradermal delivery of DNA offered more effective disease prevention. Long-term weekly treatments were needed to achieve protection that can persist after discontinuation, likely mediated by regulatory T cells induced by at least one epitope. Although epitopes were presented for at least two weeks, weekly treatments were needed, at least initially, to achieve significant protection. While Endotope and Proinsulin DNA vaccines were effective at both the prediabetic normoglycemic and dysglycemic stages of disease, Proinsulin provided better protection in the latter stage, particularly in animals with slower progression of disease, and Endotope limited insulitis the most in the earlier stage. Thus, our data support the possibility of applying a precision medicine approach based on tailored epitopes for the treatment of tissue-specific autoimmune diseases with DNA vaccines.

Th177. Understanding the T Cell Repertoire in Autoimmune Diseases Using High-dimensional Single-cell Analysis of Antigen-specific CD4+ and CD8+ T Cells

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Despite approvals of new medicines to treat patients with autoimmune diseases, the development of durable new disease modifying therapies that address the underlying causes of disease remain elusive.

To advance the development of new diagnosis and treatment strategies for antigen-specific T cell-driven autoimmune diseases, new insights into disease-associated T cell specificities will be required. We have developed the three integrated technologies of our DECODE™ platform to discover disease-associated TCR-pMHC specificities and further elaborate the functionality of associated T cell clonotypes. Using DECODE Antigen, we experimentally determine peptides that are well-presented by class II MHC. DECODE Synapse profiles the reactivities and phenotypes of primary T cells using hundreds of DNA barcoded multimers. With DECODE TCR, we de-orphan or assess the cross-reactivity of high-value TCRs isolated from disease tissue using thousands to millions of genetically encoded epitopes. To develop our Autoimmune Synapse Maps, we apply the DECODE platform to analyze T cells derived from the peripheral blood, spleen, lymph nodes, and disease-associated tissue of carefully selected cohorts of patients and healthy donors. Our synapse map of HLA-A*02:01 in Type 1 Diabetes includes dozens of novel epitopes enriched in T1D patients. Multiple clonotypes activated by these shared epitopes are TNF / IFN producing, effector memory cells. We have also begun to develop synapse maps on multiple class I and class II alleles in T1D, Multiple Sclerosis, and vitiligo. Our discovery platform has the potential to provide an approach to identify the antigens that matter for antigen-specific tolerizing therapies.

Th183. Two Sources of Autoreactive T Cells in Patients with FOXP3 Mutations

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T-cell ontogeny in thymus gives rise to immense amount of T cells with different TCR specificities, ensuring protection against wide variety of pathogens. Although the self-reactive T cell clones are largely deleted during the development in a process of negative selection, autoreactive T cells could be readily detected in healthy individuals. The autoreactive clones are being suppressed by various mechanisms among which CD4+FOXP3+ regulatory T cells (Treg) are the best recognized. However, to which extend Treg suppress autoreactive T cells expansion in human In vivo is unclear. Here, we analyzed TCR repertoire of patients with *FOXP3* mutations, a key transcription factor of Treg, and found increased TCR autoreactivity in bona fide T effector cells. Interestingly, the increased TCR autoreactivity positively correlates with increased number of cells with Treg epigenetic signature, which loss Treg phenotypic markers including FOXP3 expression. Since Treg cells are physiologically self-reactive, we speculate that the loss of identity Treg might represent a new pool of autoreactive T cells exacerbating the autoimmunity and thus promoting expansion of autoreactive T cells. In line with the hypothesis of pathological potential of loss of identity Treg cells, we observed increased production of proinflammatory cytokines by CRISPR/Cas9 mediated FOXP3 knock out Treg and increased proliferation in response to TCR-mediated stimulation. Collectively, these data suggest that mutation in *FOXP3* causes loss of Treg suppressive function leading to of autoreactive T cells in periphery and simultaneously increased number of Treg which lose their identity and are capable of exacerbating the autoimmune reaction.

Tu100. A Pathogenic Th17/CD38+ Macrophage Feedback Loop Drives Inflammatory Arthritis Through TNFa

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Rheumatoid arthritis (RA) and psoriatic arthritis pathobiology involves interplay between innate and adaptive immune components and resident synoviocytes. Single-cell analyses of patient samples and relevant mouse models have characterized many cellular subsets in RA. However, the impact of interactions between cell types is not fully understood. Here, we temporally profiled murine arthritic synovial isolates at the single-cell level to identify perturbations conserved in human RA. Notably, macrophage subtypes like those in RA patients were expanded in arthritis and linked to promoting the function of Th17 cells in the joint. *In vitro* experiments identified a capacity for macrophages to maintain the functionality and expansion of Th17 cells. Reciprocally, Th17-derived TNFa induced CD38+ macrophages that increased Th17 functionality. Synovial CD38+ macrophages were expanded during arthritis and their depletion or blockade via TNFa-neutralization alleviated disease while reducing IL-17A-producing cells. These findings identify a cellular feedback loop that promotes Th17 pathogenicity through TNFa to drive inflammatory arthritis.

Tu106. Phenotype-based Isolation of Antigen-specific CD4+ T Cells in Autoimmunity: A Study of Celiac Disease

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The pathogenic immune response in celiac disease (CeD) is orchestrated by phenotypically distinct CD4+ T cells that recognize gluten epitopes in the context of disease-associated HLA-DQ allotypes. Cells with the same distinct phenotype, but with elusive specificities, are increased across multiple autoimmune conditions. Here we test whether in CeD sorting of T cells based on their distinct phenotype (Tphe cells) yields gluten-reactive cells. The method`s efficiency is benchmarked by parallel isolation of gluten-reactive T cells (Ttet cells), using HLA-DQ:gluten peptide tetramers. From gut biopsies of 12 untreated HLA-DQ2.5+ CeD patients, Ttet+/Tphe+, Ttet-/Tphe+ and Ttet-/Tphe- cells are sorted for single cell T-cell receptor (TCR)-sequencing (n=8) and T-cell clone (TCC)-generation (n=5). The generated TCCs are TCR sequenced and tested for their reactivity against deamidated gluten. Gluten-reactivity is observed in 91.2% of Ttet+/Tphe+ TCCs, 65.3% of Ttet-/Tphe+ TCCs and 0% of Ttet-/Tphe- TCCs. Interestingly, only 55% of gluten reactive Ttet-/Tphe cells were reactive to known gluten epitopes, suggesting an existence of yet undiscovered ones. TCR sequencing reveals clonal expansion and sequence sharing across patients, features reflecting antigen-driven responses. The feasibility to isolate antigen-specific CD4+ T cells by sole use of phenotypic markers in CeD outlines a potential avenue for characterizing disease-driving CD4+ T cells in autoimmune conditions. It also allows for effective and simple strategy for isolation of cells for the purpose of antigen/epitope discovery.

Tu107. Increased Monocyte Activity in Patients with Primary Progressive Multiple Sclerosis

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Background. Emerging evidence suggests a prominent role of the innate immune system in the pathogenesis of multiple sclerosis (MS). With this study, we therefore investigated monocyte prevalence and activation in patients with relapsing remitting MS (RRMS) and primary progressive MS (PPMS).
Methods. Peripheral blood monocytes from healthy controls (n = 35), untreated patients with RRMS (n = 35), untreated patients with PPMS (n = 38), and patients with RRMS treated with ofatumumab (n = 17), dimethyl fumarate (n = 20) or alemtuzumab (n = 15) were analyzed by flow cytometry. Classical monocytes were defined as $CD14^{+}CD16^{-}$, non-classical monocytes as $CD14^{+}CD16^{+}$, and activated monocytes as $CD40^{+}$.
Results. This analysis showed an increased absolute count of classical monocytes in blood in patients with PPMS compared to healthy controls and, furthermore that the activation of both classical and non-classical monocytes in patients with PPMS was increased. No difference in monocyte phenotype or activation was observed in patients with RRMS compared to healthy controls. Following treatment of patients with RRMS with either of the three disease-modifying therapies included, the activation of non-classical monocytes was reduced. Conclusion. Altogether, our data provide evidence of increased blood monocyte counts and activation in patients with PPMS but not in RRMS. Furthermore, we find that the activity of blood monocytes is reduced by various disease-modifying therapies. Consistent with previous findings, our study therefore indicates an involvement of innate immunity in the pathogenesis of progressive MS.

Tu109. SQZ® TAC Cell Therapy Platform Induces Antigen Specific T-regs and Prevents Onset of Type 1 Diabetes in Adoptive Transfer Models

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Antigen specific tolerance is a preferred therapeutic mode of promoting durable disease remission in autoimmune diseases with well-defined antigens and could overcome the problems associated with broad immunosuppression. The Cell Squeeze® technology utilizes microfluidic mechanical deformation to engineer cell function. To induce tolerance, red blood cells (RBCs) are engineered to deliver target antigens to generate tolerizing antigen carriers (TACs). The Cell Squeeze technology allows the SQZ®TACs to resemble senescent RBCs, enabling them to be cleared by eryptosis, the physiological mechanism of RBC clearance. Herein, we demonstrate SQZ TACs biodistribute to phagocytic cells in the spleen and liver where antigens are processed and presented to antigen specific T-cells in a tolerogenic manner. SQZ TAC treatment in several accelerated murine models of T1D led to significant delay or prevention of hyperglycemia. In both CD4+ (BDC2.5) and CD8+ (NY8.3) NOD adoptive transfer T1D models, co-delivery of TAC loaded with T1D autoantigens provided protection from early disease onset characterized by increased T-cell apoptosis, tissue specific T-reg induction and decreased effector T-cell pancreatic infiltration. In addition, administration of a CD4+ directed TACs with the peptide p31 protected T1D onset in NOD mice co-transferred with both BDC2.5 and NY8.3 T cells, thereby demonstrating TAC induced bystander suppression. Recent preclinical efforts have expanded to include development of a TAC-deamidated gliadin formulation to ameliorate gluten sensitivity in patients suffering with Celiac Disease.

Tu110. Understanding Neuro-Immune Interactions in Multiple Sclerosis Brain by Single Nucleus Profiling

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Multiple sclerosis (MS) is an autoimmune, neurodegenerative inflammatory disease characterized by the demyelination of neurons in the human central nervous system (CNS). Recent studies have increasingly pinpointed neuroinflammation as a crucial contributor to the pathogenesis of MS where activated non-neuronal cells, such as microglia, help drive and perpetuate an immune response within the CNS through altered transcriptional changes. However, there is still a lack of known MS-specific neuroinflammatory biomarkers involved in the activation of these pro-inflammatory pathways. We address this issue by using single-nucleus RNA sequencing (snRNA-seq) to profile MS and healthy control donor brain samples (3 MS and 3 HC) in order to develop a comprehensive and detailed perspective of the role that microglia play in the induction of neuroinflammation in MS. Using snRNA-seq, we analyzed a total of 37,533 brain cells and identified major brain cell types, including 4,796 microglial cells. Within the detected microglia, we compared MS and control brains and identified 1456 differentially expressed genes between MS and controls. We performed pathway analysis, using Ingenuity Pathway Analysis (IPA) and Gene Ontology (GO) Enrichment Analysis, and determined the canonical pathways of the microglia changes, with an upregulation of pathways involved in cellular stress and inflammation as well as a downregulation in normal neurodevelopmental pathways. Moreover, we identified 5 MS-specific microglial subclusters and their cluster-specific markers. Taken together, our study indicates that there are likely unique subgroups of microglia with specific neuroinflammatory pathways of communication that are involved in the pathogenesis of MS.

Tu123. IL-23 Signaling Induces Autoimmune Disease Genes in Mucosal-associated Invariant T (MAIT) Cells

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Genome-wide association studies and mouse models of autoimmune disease have demonstrated an important role of the IL-23 signaling pathway in the pathogenesis of psoriasis, Crohn's disease (CD) and axial spondyloarthritis (axSpA). This notion has been validated in the clinics by the successful treatment of psoriasis and CD with IL-23 blockers. However, despite a strong genetic association and pre-clinical models arguing in favor of IL-23 as a valid pharmaceutical target, IL-23-blockers failed in axSpA. This unexpected finding raised questions about the role of IL-23 in the pathogenesis of axSpA and psoriasis.
The goal of this project is to determine which human T cell populations can respond to IL-23 and how IL-23 signaling regulates immune functions. We found that mucosal-associated invariant T (MAIT) cells expressed higher level of the IL-23R mRNA and protein than other immune cell populations. To better define the effects of IL-23 on MAIT cells, we used a MHC related molecule 1 (MR1) tetramer presenting the 5-OP-RU antigen. Compared to anti-CD3/CD28 T cell stimulation, we observed that the tetramer induced specifically MAIT cell activation and proliferation. Activation of MAIT cells in the presence of IL-23 enhanced secretion of several cytotoxic molecules, however, did not increase IL-17A/F production by these cells. Of note, disease and KEGG pathway overrepresentation analysis of RNA-seq data revealed significant enrichment of autoimmune disease genes in MAIT cells cultured in the presence of IL-23. Ongoing single-cell analyses investigate the contribution of these IL-23-induced genes to pathogenic functions of MAIT cells.

Tu130. Central Nervous System-specific Chimeric Antigen Receptor Regulatory T Cells for the Therapy of Multiple Sclerosis

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Multiple sclerosis (MS) is a chronic demyelinating autoimmune disease targeting the neurons of the central nervous system (CNS). So far, there is no cellular therapy for this incurable disease. Therefore, we are developing an approach using the immunosuppressive abilities of regulatory T cells (Tregs) combined with the antigen specificity of chimeric antigen receptors (CARs) to induce tolerance against self-antigens directly at the inflamed site. This strategy has the advantage of no systemic inhibition of the immune system compared to the currently used immunosuppressive drugs. Utilizing antibody phage display libraries we screened for single chain variable fragments (scFvs) binding to a human extracellular target protein of the CNS, which plays a pivotal role in MS pathology. To secure cross-reactivity we tested the binding ability of the generated scFvs on murine brain sections using immunohistochemistry. Binding scFvs were cloned into different second generation CAR constructs with a CD28 and a CD3zeta signaling domain and subsequently tested their functionality in a NFAT-GFP reporter T cell line. This assay demonstrated that our CARs were able to specifically recognize not only the antigen presented on transfected HEK cells but also the naturally occurring antigen in murine brain lysates. In contrast, CAR signaling was not activated on untransfected HEKs or murine liver lysates, which both do not express the target of interest. Generated CAR-Tregs were also antigen-specific, had a normal Treg phenotype, were suppressive and were stable *in vivo*. Thus, target-specific CAR-Tregs represent a promising option for tissue-specific immune regulators without compromising the general immune response.

Tu132. Induction of Antigen-Specific Immune Tolerance in Type 1 Diabetes by Antigen-Expressing Red Cell Therapeutics

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We are developing allogeneic Red Cell Therapeutics (RCT) that are genetically engineered in vitro from hematopoietic stem cells to express antigenic peptides with the goal of inducing antigen-specific tolerance in T cell-mediated diseases such as type 1 diabetes (T1D). For T1D mechanistic and POC studies we used both the BDC2.5 adoptive transfer model and the spontaneous T1D NOD model. We show in the BDC2.5 model that a single treatment with mouse RBCs (1e9) carrying a peptide agonist of BDC2.5 T cells (mRBC-p31) confers long term protection from diabetes (90+ days). Even longer protection (160 days) was observed when mice were treated 3 times with a 10-fold lower dose. Moreover mRBC-p31 administered after insulitis has set in were equally effective in halting the disease for at least 120 days. Tolerance induced by mRBC-p31 was resistant to rechallenge with BDC2.5 T cells, suggesting the emergence of regulatory T cells. Indeed, using single cell RNA-Seq we observed that mRBC-p31 mediated tolerance involved induction of both Foxp3+ and Tr1 regulatory T cells and additionally uncovered an anergic population. In the NOD model, two treatments at weeks 10 and 14 with mRBCs containing two epitopes from insulin and the 2.5HIP hybrid insulin peptide delayed disease induction up to 25 weeks of age, a finding that supports the notion that the RBC-based therapy induces bystander suppression in this complex disease model. In summary, we report on the potential utility of antigen-expressing RCTs as a novel modality to treat T1D and potentially other HLA-linked autoimmune diseases.

Tu133. Mass Cytometry Demonstrates an Extrafollicular T and B cell Signature that Correlates with Disease Activity in Pediatric Lupus Nephritis

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Systemic lupus erythematosus (SLE) is a multi-organ rheumatologic disease with heightened severity in children. A majority of childhood-onset SLE (cSLE) patients develop lupus nephritis (LN), resulting in significant morbidity and mortality. Current diagnostics are inadequate for detecting incipient renal disease or anticipating severe manifestations. Incomplete knowledge regarding immune dysregulation in LN poses a *significant hurdle* for the development of biomarkers to monitor disease activity and/or guide specific therapy. We performed mass cytometry (CyTOF) on peripheral blood from cSLE patients (n=22, 7 with LN). These single-cell immune phenotypic and functional data were complemented by whole blood gene expression and clinical data. We applied traditional supervised and novel unsupervised analytical tools to identify immunological signatures common to cSLE, and those unique to LN.

cSLE patients exhibit a broadly activated immune system, with an increased interferon gene expression signature and increased pro-inflammatory CD14hi monocyte cytokine production. Their T cells exhibit an activation/exhaustion phenotype with an increased frequency of T follicular and peripheral helper cells (Tfh and Tph). Consistent with an increase in these T cell subsets, there is an increased frequency of plasmablasts, memory B cells, and extrafollicular B cells contained within the anergic and age-associated B cell subsets—supporting a complementary B cell activation state. For LN patients, SLEDAI score correlates with i) Tph frequency and ii) CD38 and PD1 expression on CD8 T cells. These data suggest that extrafollicular T:B interactions are processes unique to LN and may constitute i) biomarkers for disease monitoring; and ii) targets for novel therapeutic approaches.

Tu134. High CD96 Expression Marks an IL-22 Producing CD4 T cell Population Reduced in Lupus Patients

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Systemic lupus erythematosus (SLE) is an autoimmune disease that remains an important diagnostic and therapeutic challenge. Manipulation of activating or inhibitory receptors on T cells represents a promising therapeutic strategy in diseases such as SLE. Our goal is to define patterns of immunomodulatory receptor expression on T cells and to identify alterations in immunomodulatory receptor expression on T cells in patients with SLE. Method: We analyzed PBMC from SLE patients and controls (n=20 each) with a 35-markers mass cytometry panel. To identify subpopulations altered in SLE, we used FlowSOM to evaluate memory CD4+ T cells in a multidimensional manner. We compared the abundances of the metaclusters in SLE and control samples. Next, we performed low-input bulk RNA-seq to characterize the altered populations. We validated RNA-seq results by flow cytometry. Result: We identified a CD4 population that is decreased in frequency in SLE patients and marked by high expression of CD96 (and lack of PD-1 and TIGIT). RNA-seq analysis revealed that the CD96 population highly expressed CCR6, RORA, IL-22, IL-2, oncostatin M and is enriched for a Th22, not Th17 transcriptomic signature. Functional in vitro stimulation experiments demonstrated that CD96+ T cells comprise a population that is preferentially enriched for IL-22, with intermediate capacity for IL-17A and IFN-gamma production.Conclusion: We have identified a population represented by high expression of CD96 that is reduced in frequency in PBMC of SLE patients, with high capacity for IL-22. Mechanisms involved in the depletion of this population in SLE are currently being investigated.

Tu136. Intravenous Ofatumumab Treatment of Multiple Sclerosis and Related Disorders: An Observational Study

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Ofatumumab is an anti-CD20 monoclonal antibody approved for subcutaneous administration for the treatment of relapsing multiple sclerosis (MS). Intravenously administered ofatumumab has been used off-label for MS and related disorders, but data is limited to a single trial of 38 MS patients receiving one infusion series.

The objective of the present study was to assess disease activity and side effects in relation to longer-term intravenous ofatumumab treatment in MS, neuromyelitis optica spectrum disease (NMOSD) and myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD). We conducted a retrospective study of patients treated off-label with intravenous ofatumumab for MS, NMOSD or MOGAD at the Danish Multiple Sclerosis Center. Data was retrieved from the Danish Multiple Sclerosis Registry and medical chart review. Fifty patients were identified (4 with NMOSD, 4 with MOGAD and 42 with MS) with a median treatment duration of 2.2 years. Median annualized relapse rate decreased from 1.0 at baseline to 0.3 during ofatumumab treatment. At 24 months, the probability of having experienced a relapse was 55% and confirmed worsening of disability 7%. Infusion-related reactions were observed in 81% during the first infusion and 35% during the last infusion. Six patients experienced infections requiring hospitalization. De novo low IgM was detected in 10/36 patients, and de novo low IgG in ≤3/35. Extended follow-up for a median of 2.1 years after treatment termination showed no cases of late onset neutropenia. Our data indicate that intravenous ofatumumab treatment of inflammatory demyelinating disorders reduces frequency of relapses, stabilizes disability, and has an acceptable safety profile.

Tu145. STAT3 Gain-of-function Variants and T Cell-mediated Inflammation

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STAT3 is a transcription factor that plays a role in the proliferation, survival, and function of many different cell types. In T cells, STAT3 is required for the polarization and function of Th17 cells, and can inhibit development of peripheral Tregs. Patients with germline gain-of-function variants in STAT3 present with early-onset poly-autoimmunity, including psoriasis. We hypothesized that STAT3 GOF variants enhance Th17 responses following an inflammatory stimulus, leading to autoimmune disease. To examine this, we generated a mouse model of STAT3 GOF using an amino acid substitution identified in patients (p.G421R). Heterozygous STAT3 GOF mice display delayed STAT3 de-phosphorylation and increased Th17 polarization of naïve T cells in vitro. To test if this would drive disease susceptibility In vivo, WT and STAT3 GOF mice were treated with topical imiquimod. Following treatment, STAT3 GOF mice demonstrated increased ear swelling and an increased Th17 response in the skin and periphery. Adaptive lymphocytes and IL-22 mediated this response; however, γδ T cells are not required. Using bone marrow chimeras, we identified a role for both radiosensitive and resistant cells in this inflammatory response. We also observed a competitive advantage for STAT3 GOF Th17 cells in the skin. Collectively, these data demonstrate that STAT3 GOF results in a cell-intrinsic skewing of T cells with a Th17 phenotype, suggesting a potential mechanism for the development of autoimmunity in patients with STAT3 GOF syndrome.

Tu149. Plasma from Pediatric Acute-Onset Neuropsychiatric Syndrome (PANS) Induces Increased BBB Permeability Through Disruption of Tight Junction Proteins

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Pediatric acute-onset neuropsychiatric syndrome (PANS) is characterized by abrupt onset of obsessive-compulsive disorder (OCD) or severe food restriction and concurrent abrupt onset of other neuropsychiatric symptoms- thought to be caused by a post-infectious inflammation. Diagnosis of PANS is particularly challenging due to lack of specific biomarkers.

We have identified increased circulating inflammatory and anti-inflammatory monocyte subsets, the latter also in cerebral spinal fluid (CSF) in PANS in various clinical states (e.g., flare, improvement). Immune cells, including monocytes, are impermeable to the healthy blood brain barrier (BBB). Anatomically, brain microvascular endothelial cells (BEC) is the main constituent of the BBB, forming a selective semipermeable border that prevents solutes in the blood circulation from non-selectively crossing into the extracellular fluid of the central nervous system. BECs are tightly regulated by tight junction proteins (TJPs), specifically claudin 5, claudin 12, occludin and Zonula occludens-1 (ZO1), and act as gatekeepers of the central nervous system. Disruption of the BBB allows infiltration of leukocytes, resulting in brain edema and inflammatory injury. In the present study, we found that PANS flare plasma at 1% volume increased the permeability to 10 kDa dextran of a human brain endothelial cell (hBEC) monolayer by >2-fold in 24hr as compared to healthy control plasma. Furthermore, PANS flare plasma at 1% volume decreased expression of TJPs (claudin 5, ZO-1) and adherent junction protein, VE-Cadherin, in 24hr. Decreased TJP expression is the hallmark of compromised BBB function and may allow blood leukocytes to cross the BBB in diseased conditions.

Tu150. Autoantibodies to Perilipin-1 Define a Subset of Acquired Generalized Lipodystrophy

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Acquired lipodystrophy is often characterized as an idiopathic subtype of lipodystrophy. Despite suspicion of an immune-mediated pathology, biomarkers such as autoantibodies are generally lacking. Here, we used an unbiased proteome-wide screening approach to identify autoantibodies to the adipocyte-specific lipid droplet protein Perilipin-1 (PLIN1) in a murine model of Autoimmune Polyendocrine Syndrome 1 (APS1). We then tested for PLIN1 autoantibodies in human subjects with acquired lipodystrophy with two independent severe breaks in immune tolerance (including APS1) along with controls using a specific Radioligand Binding Assay and indirect immunofluorescence on fat tissue. We identified autoantibodies to PLIN1 in these two cases, including the first reported case of APS1 with acquired lipodystrophy and a second patient who acquired lipodystrophy as an immune-related adverse event following cancer immunotherapy. Lastly, we also found PLIN1 autoantibodies to be specifically enriched in a subset of patients with acquired generalized lipodystrophy (17/46; 37%) particularly those with panniculitis and other features of autoimmunity. These data lend additional support to new literature that suggests that PLIN1 autoantibodies represent a marker of acquired autoimmune lipodystrophies and further link them to a break in immune tolerance.

Tu151. GAD65 Mediated Inflammatory Immune Response In HLA-DR3/DQ2+ Type 1 Diabetes Patients

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Aim and Objective: This study was planned to define diabetogenic GAD65 peptides possibly presented by HLA-DR3/DQ2 molecules to CD4+ T-cells in type 1 diabetes patients. Methods: Top 30 GAD65 peptides, found to strongly bind *in silico* with HLA-DR3/DQ2 molecules, were selected for this study and grouped into 4 pools according to their amino acid positions. The peptides were used to stimulate CD4+T-cells of study subjects in 16 hours culture. CD4+ T-cells' stimulation in terms of IFN-γ, TNF-α, IL-17 and IL-10 expression was analysed using flow-cytometry. Results: While, all GAD65 peptide pools resulted in significant increase in IFN-γ ($p=0.003$, $p< 0.0001$, $p=0.03$ and $p=0.04$ respectively), pool 1-3 showed significant increase in the expression IL-17 ($p=0.003$, $p< 0.001$ and $p=0.004$ respectively) in T1D vs. controls. However, the expression of TNF-α and IL-10 by CD4+ T-cells was comparable between patients and controls. Inter-peptide group comparison for immunogenicity revealed significant increase in IFN-γ and IL-17 producing CD4+T-cells for pool 2 as compared to other groups ($p< 0.0001$ in both cases) in patients but not in controls. Further, group 2 GAD65 peptides yielded significant increase in IFN-γ ($p=0.002$) and IL-17 ($p=0.003$) and significant decrease in IL-10 ($p=0.04$) expression by CD4+T-cells in HLA-DR3/DQ2+ patients as compared to DR3/DQ2+ healthy controls. Conclusion: GAD65 peptides, particularly those belonging to group 2, induced CD4 T-cells to produce inflammatory IFN-γ and IL-17 cytokines in patients, suggesting that, group 2 GAD65 peptides possibly presented by HLA-DR3/ DQ2 molecules to CD4 T cells shift immune balance towards inflammatory phenotype in T1D patients.

Tu152. Senescence Associated DNA Damage in Lymph Node and Synovial Tissue Biopsies of Early Rheumatoid Arthritis Patients

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Increased DNA damage is a physiological hallmark of senescence which has been observed alongside dysfunctional DNA repair machinery in T cells of patients with Rheumatoid Arthritis (RA). T cells closely interact with resident stromal cells within lymph node and synovium tissue, an interaction that might play an important role in initiating chronic inflammation. We hypothesize that DNA damage is already present in synovial and lymph node stromal cells as well as T cells before clinical inflammation presents itself as a result of cellular senescence. A cohort of RA patients and RA-risk individuals underwent ultrasound guided lymph node and synovial tissue biopsies. Biopsies were analyzed by immunofluorescence staining for T cells, stromal cells and the DNA damage marker, gamma H2AX which accumulates at double stranded DNA breaks. Preliminary analysis shows the presence of DNA damage in both stromal cells and T cells within synovial tissue sections of RA patients as well as RA-risk individuals. In line with this, ex-vivo expanded synovial and lymph node stromal cells from RA-risk and RA patients also displayed the presence of DNA damage at higher levels when compared with stromal cells from healthy individuals.. DNA damage is already present in RA-risk individuals before development of clinical inflammation. It is interesting to postulate whether DNA damage correlates with future arthritis development and what effect possible DNA damage in lymphoid organs may have on the break in tolerance observed in RA.

W3. Anti-CD45RC Antibody Immunotherapy Prevents and Treats Experimental Autoimmune Poly Endocrinopathy Candidiasis Ectodermal Dystrophy Syndrome

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Targeted monoclonal antibody (mAb) therapies show great promise for the treatment of transplant rejection and autoimmune diseases by inducing more specific immunomodulatory effects than broadly immunosuppressive drugs routinely used. We recently described the therapeutic advantage of targeting CD45RC, expressed at high levels by conventional T cells (Tconv, CD45RC^{high}), their precursors and terminally differentiated T (TEMRA) cells, but not by regulatory T cells (Tregs, CD45RC$^{low/-}$). We demonstrated efficacy of anti-CD45RC mAb treatment in transplantation but its potential has not been examined in autoimmune diseases. APECED is a rare genetic syndrome caused by loss-of-function mutations of the key central tolerance mediator, autoimmune regulator (AIRE) leading to abnormal auto-reactive T cell responses and autoantibodies production. Herein, we showed that, in a rat model of APECED syndrome, anti-CD45RC mAb was effective both as prevention and treatment of autoimmune manifestations and inhibited autoantibody development. Anti-CD45RC mAb intervention depleted CD45RC^{high} T cells, inhibited CD45RC^{high} B cells, and restored the Treg/Tconv ratio and the altered Tregs transcriptomic profile. In APECED patients, CD45RC was significantly increased in peripheral blood T cells and lesioned organs from APECED patients were infiltrated by CD45RC^{high} cells. Our observations highlight the potential role for CD45RC^{high} cells in the pathogenesis of experimental and human APECED syndrome and the potential of anti-CD45RC antibody treatment.

W8. The B Cell Repertoire in Multiple Sclerosis Reveals Molecular Mimicry between EBV EBNA1 and GlialCAM

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Infection with Epstein-Barr virus (EBV) is highly associated with multiple sclerosis (MS). Molecular mimicry of EBV antigens and antigens of the central nervous system (CNS) has been hypothesized to be a driver of neuroinflammation, but mechanistic evidence is scarce. To identify anti-viral antibodies in MS, we single-cell sequenced the antibody repertoire of B cells in the cerebro-spinal fluid (CSF) and blood of MS patients and identified clonal expansions of activated B cells that carry the hallmarks antigen-specific activation. A selection of these antibodies were tested against a spectrum of viruses implicated in MS pathogenesis. One antibody was identified that binds the EBV transcription factor EBNA1 at an epitope known to elicit high antibody reactivity in MS patients. We demonstrate that this EBNA1-binding antibody cross-reacts to the glial cellular adhesion molecule GlialCAM. We provide structural evidence for the evolution of GlialCAM-reactivity from an unmutated B cell binding only EBNA1. Cross-reactivity between the two antigens is facilitated by a post-translational modification of GlialCAM. EBNA1-GlialCAM cross-reactive antibodies are more prevalent in MS patients than in healthy controls. EBNA1 peptide immunization aggravates the mouse model of MS. Together, our results suggest that EBNA1-reactive antibodies can cross-react with the CNS-specific membrane protein GlialCAM and induce neuroinflammation, thereby exacerbating MS. Our results provide a long sought mechanistic link for the association between MS and EBV.

W10. Intrathecal CD8+CD20+ T Cells in Primary Progressive Multiple Sclerosis

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Despite accumulating evidence of intrathecal inflammation in patients with primary progressive multiple sclerosis (PPMS), most anti-inflammatory therapies to treat PPMS have failed. With this study, we investigated the involvement of $CD20^+$ T cells in the pathogenesis of PPMS and their involvement in the failure of the disease-modifying therapy treatment dimetyl fumarate. Assessing $CD20^+$ T cells in the blood and cerebrospinal fluid (CSF) of patients with PPMS and controls showed an increased percent of $CD20^+$ T cells in blood of untreated patients, and a strong enrichment in the CSF. We also found a positive correlation between intrathecal $CD8^+CD20^+$ T cells and concentrations of myelin basic protein in the CSF and white matter brain lesion volume, and a negative correlation with thalamus volume. Recently, we performed a randomized controlled trial investigating dimethyl fumarate treatment in patients with PPMS. Unfortunately, we found no improvement in neurodegeneration or demyelination after 48 weeks of treatment. In this study, we show that despite a decrease in intrathecal T cells following dimethyl fumarate treatment, the $CD8^+CD20^+$ T cell population was unaffected by the treatment. In conclusion, this study shows an association between intrathecal $CD8^+CD20^+$ T cells and myelin injury as well as neurodegeneration in the CNS of patients with PPMS. Furthermore, it suggests that failure to suppress intrathecal $CD8^+CD20^+$ T cells may contribute to the lack of efficacy of treatment with dimethyl fumarate in PPMS. We conclude that $CD8^+CD20^+$ T cells likely play a role in the pathogenesis of PPMS.

W21. Reemergence of Pathogenic B Cell Clones in Autoimmune Myasthenia Gravis Following B Cell Depletion Therapy

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Myasthenia gravis (MG) is an autoantibody-mediated autoimmune disorder of the neuromuscular junction. Some patients with MG have pathogenic mAbs that recognize muscle-specific tyrosine kinase (MuSK). These autoantibodies are predominantly IgG4, recognize the Ig1-like domain of MuSK and originate from plasmablasts. Patients respond well to CD20-mediated B-cell depletion therapy (BCDT), but relapse. The mechanisms underlying relapse are not well understood. We developed a monomeric fluorescently labelled antigen to enrich for MuSK-specific B-cells and found that MuSK-reactive B-cells were rare within the circulation (around 1-2 per 10 million cells). We isolated two MuSK mAbs (2E6 and 6C6) that were of the IgG4 subclass and originated from plasmablasts. Both mAbs recognized the Ig1-like domain of MuSK and showed in vitro pathogenic capacity. Single cell gene expression profiling paired with B-cell receptor sequencing showed that CD20 was expressed by 25% of plasmablasts. Persistent clones were identified with B-cell repertoire tracing. Clonal variants of 2E6 were detected at several timepoints over 79 months and reemerged after BCDT-mediated remission, appearing several months prior to relapse. In summary, a subpopulation of plasmablasts express CD20 and are potential targets of CD20-mediated BCDT. A reservoir of very rare pathogenic MuSK autoantibody expressing clones survives treatment. These clones reemerge as autoantibody-producing plasmablasts, during B-cell re-population and can be detected in the circulation prior to manifestation of clinical relapse, thus, providing both a mechanistic understanding of relapse and a valuable candidate biomarker for relapse prediction.

W22. Kv1.3−/− Mice Do Not Develop CIA: A Novel Target with Small Molecules for Autoimmune Arthritis

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Engagement of the T cell receptor triggers Ca^{++} influx. This Ca^{++} influx is only possible with a counterbalancing K^+ efflux through Kv1.3 and/or KCa3.1 channels. Given its regulatory role in the effector memory-T cells (TEM), Kv1.3 is likely to play a critical role in T cell mediated autoimmune arthritis (e.g.; rheumatoid arthritis(RA), psoriatic arthritis). Here using $Kv1.3^{-/-}$ mice we are reporting that functional Kv1.3 channels are critical for induction of collagen induced arthritis(CIA). $Kv1.3^{-/-}$ mice are on the C57BL/6 background so we used C57BL/6 mice to induce CIA. CIA was induced in $Kv1.3^{-/-}$ mice and in C57BL/6 wild-type (n=10,each). $Kv1.3^{-/-}$ mice did not develop arthritis; whereas, about 60% of the wild-type C57BL/6 mice had arthritis. Clinical and histopathological scores for arthritis on day-60 was zero in the $Kv1.3^{-/-}$ mice. In wild-type mice the respective scores were 2.8±0.5 and 6.2±1.5 ($p< .01$). Evidence of arthritis was further confirmed/quantified by micro-PET imaging. We also observed that knocking out of Kv1.3 renders T cells resistant to activation. A marked proliferation of the mouse splenic $CD3^+$ T cells with CD3/CD28 ab in the wild type C57BL/6J was noticed compared to the Kv1.3 KO ($p< .01$); this was demonstrated by more generations of cells and an increased number of cells in each generation. These observations substantiate a critical role of the Kv1.3, a voltage-gated K^+ channel in the pathogenesis of T cell mediated autoimmune arthritis including RA. These results provide a platform to develop small molecule-based novel Kv1.3 antagonists (e.g., PAP-1) for treatment of RA.

W30. Underlying T Cell Receptor Methylation Defects in Type 1 Diabetes Associated with Quantitative Defects

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The structural organization and signaling of the T cell receptor (TCR)/CD3 complex underlies T cell development. T cell maturation defects are associated with autoimmunity, suggesting an underlying TCR selection defect. To explore quantitative defects in the TCR/CD3 protein complex as a cause for altered TCR selection in autoimmunity, we compared TCR expression densities on CD4 T cells from subjects with type 1 diabetes (T1D) (n=80) and non-diabetic controls (n=37) and looked for possible underlying overmethylation in T1D. Significant quantitative defects in TCR and CD3 proteins were observed in CD4+ T cells from T1D vs controls. TCR complex genes and associated CD3 genes were overmethylated in T1D, resulting in downregulated cell surface expression. Evaluation of TCRαβ expression in CD4+ T cells at the protein level confirmed methylation patterns, showing that the population of TCRαβ+ cells was significantly reduced in T1D vs controls (p=0.005). The MFI density of TCRαβ antibody in T1D was also significantly decreased vs controls (p=0.01). All CD3 genes except CD3ε had hypermethylated patterns in T1D, confirmed by RNAseq analysis. Both the percentage CD4+ T cell population (p=0.04) and the MFI of CD3+ T cells (p=0.02) were reduced in T1D vs controls. Patients with T1D have quantitative defects in the TCR/CD3 protein complex, resulting in decreased expression. It is recognized that TCR triggering is affected by intermolecular distance (density) between TCR proteins; activation diminishes with increased proximity between TCR surface proteins. In T1D, this might be a novel mechanism for failed T cell selection leading to autoimmunity.

W32. Therapeutic Effects of Anti-IFNg Therapy in a Spontaneous Mouse Model of Myositis

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Myositis are autoimmune and inflammatory disorders leading to skeletal muscle weakness and disability. Human studies suggest a role for type I and II interferons in the pathogenesis of the disease. Few clinical trials used anti-IFNg blocking antibodies in autoimmune conditions such as Crohn's disease (HuZAF), systemic Juvenile Idiopathic Arthritis and Adult-onset Still's Disease (Emapalumab), but their therapeutic relevance in myositis has not been studied. The scarcity of myositis murine models has been a roadblock in therapeutic research. ICOS signaling pathway invalidation on the diabetes-prone NOD mouse background switches their phenotype from spontaneous diabetes to myositis. *Icos*$^{-/-}$ NOD mice display reduced muscle strength and impaired locomotor activity but no diabetes. Muscles exhibit extensive immune cell infiltration with abundant macrophages and TCD4 cells. Molecular analyses revealed myofiber mitochondrial abnormalities, ROS production and extensive oxidative stress. We found that IFNg and IFNg-induced (*Cxcl9*, *Cxcl10, Gbp2*) gene expressions were significantly elevated in muscles from these mice with established myopathy, which led us to evaluate the therapeutic potential of IFNg blockade. Anti-IFNg administration strikingly haltered myositis clinical progression, with reduced muscle strength loss and preservation of normal locomotor activity (*Catwalk* gait analysis). Moreover, anti-IFNg significantly reduced chemokine expression, immune cell infiltration and free radical production. *Ex vivo* peripheral immune cell restimulation showed that Th1/Th2/Th17 profiles were not altered by the treatment. This may suggest that anti-IFNg therapy blocked effector mechanisms rather than on T cell priming. This work supports the relevance of IFNg in the pathogenesis of myopathy and demonstrate the relevance of IFNg-blocking antibodies.

W36. Plasma Levels of the MiR-193b/365a Cluster in Combination with Clinical Parameters Predict Response to Lactococcus Lactis-based Antigen-specific Combination Therapy

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Combining systemic immunomodulation with disease-relevant antigens could provide longer-term solutions for preventing and even reversing autoimmune type 1 diabetes (T1D). Our team established that a combination therapy (CT), composed of a short-course low-dose anti-CD3 treatment with oral delivery of genetically-modified Lactococcus lactis (L. lactis) bacteria secreting full proinsulin plus the anti-inflammatory cytokine IL-10 (LL-PINS+IL-10), was effective in reversing T1D in the non-obese diabetic (NOD) mouse model. Overall disease remission was 45% by the CT (n=110) compared to 0% in untreated controls (n=13; P< 0.01) that remained hyperglycaemic. Here, we aimed to identify robust peripheral biomarkers for prediction of CT response using circulating cell-free microRNAs (miRNAs). Using a TaqMan™ miRNA array, we identified a miR-193b/365a cluster that was significantly overexpressed in plasma of non-responders compared to responders mice before CT initiation. Combining plasma expression of the miR-193b/365a cluster with clinical parameters (i.e., age and glycaemia at CT initiation) allowed prediction of CT outcome with 83% specificity and 89% sensitivity. Furthermore, we exploited CITE-sequencing (CITE-seq), a multimodal phenotyping method that simultaneously measures RNA and cell surface proteins at single cell level, to investigate the immune cell types regulated by the identified miRNA signature. We selected high-confidence miR-193b/365a target genes and scored their expression in CITE-seq profiled immune cells. Interestingly, increased levels of miR-365-3p and miR-193-3p in NR mice could influence several lymphoid and myeloid cell types. In conclusion, the miR-193b/365a cluster may serve as a novel circulating biomarker that provides additional support for individualized therapy with the L. lactis-based CT.

W37. JAK Inhibition in a Patient with a STAT1 Gain-of-function Variant Reveals STAT1 Dysregulation as a Common Feature of Aplastic Anemia

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W38. Antigen-driven Ocular Immune Responses Distinguish Granulomatous and Non-granulomatous Uveitis

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Non-infectious uveitis is a heterogeneous group of blinding ocular inflammatory diseases in which pathophysiologic mechanisms are poorly understood, resulting in empiric treatments that are ineffective for many patients. We therefore set out to identify immunologic mechanisms that would differentiate uveitis subtypes and reveal therapeutic targets. We performed single-cell V(D)J sequencing in parallel with single-cell RNA sequencing in order identify antigen-expanded T cells and characterize the gene expression of ocular immune cells from 18 patients with diverse types of uveitis. We identified clonal expansion of ocular CD4 T cells and gene expression consistent with IFNg response and effector memory cell state in a subset of patients with granulomatous clinical features, suggesting that antigen triggered ocular immune responses in these patients. Clonal CD4 T cell expansion was also associated with more type 1 conventional dendritic cells (DC1s), but fewer type 2 conventional dendritic cells (DC2s), suggesting that DC1 may direct antigen-driven ocular immune responses in granulomatous uveitis. In the subset of patients without granulomatous features or clonal expansion, CD4 T cells differentially expressed genes associated with IL4, IL-7 and the unfolded protein response, suggesting alternative immunologic mechanisms may be active in these patients. Conclusion: Antigen-driven immune responses may be a discriminatory pathologic feature in uveitis patients, particularly those with granulomatous features, while alternative mechanisms including unfolded protein responses may distinguish other uveitis subtypes.

Role of Peptide Modification and Multivalent Delivery on the Efficacy and Safety of Peptide-based Immunotherapy in Type 1 Diabetes

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Peptide-based immunotherapy offers a more targeted approach to reestablish tolerance in several human autoimmune diseases, but issues of bioavailability, enzymatic degradation and anaphylaxis may limit their clinical application. We previously showed that soluble antigen arrays (SAgAs), made of multiple antigenic peptides grafted onto a hyaluronic acid polymer, efficiently deliver p79 and 2.5HIP peptides and protect NOD mice against autoimmune diabetes. However, some mice developed anaphylaxis following repeated dosing. Thus, we further investigated two SAgA variants and their corresponding modified peptides for their effect on both disease prevention and anaphylaxis in NOD mice. To that end, two different N-terminal peptide modifications were used for grafting the peptides to make SAgAs: aminooxy (AO) for hydrolysable linkers and homopropargyl (HP) for non-hydrolysable linkers. AO-functionalized peptides and corresponding SAgAs led to significantly accelerated anaphylaxis compared to HP-functionalized peptides and SAgAs. Moreover, anaphylaxis occurred significantly faster when using free peptides than with SAgAs. Anaphylaxis was dose-dependent and mainly mediated by IgG1 against p79. Importantly, both SAgA variants efficiently prevented the development of spontaneous diabetes while free peptides failed to provide any significant protection at equivalent peptide doses. The protection appears to be mediated by a combination of induced regulatory T cells, exhaustion and/or deletion of peptide-specific T cells, depending on the time of analysis and forms of SAgA. In sum, we provide evidence that SAgAs are more effective than free peptides to induce tolerance and block autoimmune diabetes, and those made with HP-modified peptides are best to minimize risks of anaphylaxis.

W43. Combined Germline and Somatic FADD Mutations as a New Cause of Autoimmune Lymphoproliferative Syndrome

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Autoimmune lymphoproliferative syndrome (ALPS) is characterized by splenomegaly, lymphadenopathy, hypergammaglobulinemia along with autoimmune cytopenias. Most patients with ALPS harbor FAS mono-allelic mutations, but few examples of germline bi-allelic mutations of FAS, FALG, or FADD have also been reported. To date, only six patients exhibiting FADD deficiency have been described, all with bi-allelic germline mutations. They exhibit a clinical phenotype partially overlapping with ALPS-FAS patients. We previously discovered that germline FAS haplo-insufficient mutations can be associated with somatic events on the second FAS allele in the patients, but not in the healthy relatives carrying the germline mutation only. These second genetic events could be a somatic mutation in the second allele of FAS, or a somatic uniparental disomy resulting in a loss of heterozygosity (LOH). Those somatic events are mostly detected in the TCRab+ CD4-CD8- T cells called double negative T cells (DNTs). Here, we report on 3 unrelated patients presenting with clinical features fulfilling the classical ALPS criteria. Whole exome sequencing revealed germline mono-allelic mutations of FADD affecting the same codon (p.R117) in all 3 patients. By analogy with our previous studies on ALPS-FAS, we sequenced DNA from sorted DNTs of 2 patients. We identified a somatic LOH resulting in homozygous FADD mutations in the DNTs subset. This is the second description indicating that auto-immune diseases can developed as the consequence of the accumulation of one germline and one somatic event.

W47. Immunometabolism Effect of Follicular Helper T Cells in Systemic Lupus Erythematosus

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The generation and function of T follicular helper (Tfh) cells is critical for protective immune responses against foreign antigens, but uncontrolled generation and altered functionality of Tfh cells appears to be critically involved in the development of some autoimmune diseases, such as Systemic Lupus Erythematosus (SLE). Although increased metabolism in total CD4+ T cells has been described in lupus, the metabolic profile of Tfh cells is not described. We characterized glycolysis/TCA in Tfh subsets and investigate whether intervention of metabolism regulates function of Tfh cells from SLE. We analyzed glucose uptake, lactate, mitochondrial mass and mitochondrial membrane potential in CD4+ T, Tfh (CXCR5+), Tfh1, Tfh2 and Tfh17 cells purified from peripheral blood of SLE patients comparing them to healthy controls. Increased glucose uptake by stimulation in total SLE CD4+ T cells, but was not in Tfh subsets. No difference in the level of lactate in Tfh subsets. Different to CD4+ T cells, Tfh cells do not display increased glycolysis. However, Tfh17 cells have an increased mitochondrial activation which is depending on glucose as a fuel source. Blockage of glycolysis and TCA by STF-31 and IACS-10759, Tfh cells activation (CD69+) and costimulatory molecules ICOS and PD1 were down-regulated. Analyzing in vitro B cell activation by Tfh17, there was not difference in the plasmablast differentiation and Ig secretion by PB cells in Tfh17 SLE treated with inhibitors. Thus, this study elucidates the importance of metabolism reprograming that T cells exerts on effector T cells through modulation of glycolysis and TCA cycle.

W50. A Dysregulated CXCR3+CCR6+ Adaptive Immune Rheostat Exists in Patients with Active Enthesitis-related Arthritis

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Enthesitis-related arthritis (ERA), a common subtype of juvenile idiopathic arthritis (JIA) in Asia, carries a poor prognosis. Current evidence has implicated CD4+ T cells in its immunopathogenesis. Using a high-dimensional interrogative strategy, we sought to deconvolve the CD4+ T cell landscape in ERA to define immune signatures for augmenting disease management. We performed mass cytometry on circulating (active ERA, n=30; healthy paediatric controls, n=30) and synovial (active ERA, n=10) CD45+ immune cells using a T-cell centric antibody panel. Cells were stimulated with PMA-ionomycin to capture their cytokine profiles. We analysed the CD4+ T cell compartment and identified subsets differentially present in ERA patients. Memory CD4+ T cells (CD45RA-CD45RO+) were significantly enriched in the ERA synovium, of which the IFN-γ+TNF-α+ and IL-17A+TNF-α+ effector T cells (Teffs) predominate. CXCR3+CCR6+ CD4+ T cells and memory regulatory T cells (Tregs) were also enriched in the ERA synovium. By further examining the Ki67+ fraction as a surrogate for an antigen-specific response, we detected a significantly lower CXCR3+CCR6+ Treg-Teff ratio in the ERA circulation and synovium. However, this was not seen for the CXCR3-CCR6- Treg-Teff ratio. The ERA synovial microenvironment is enriched with pathogenic and protective memory CD4+ T cell subsets, which include Teffs bearing the Th1 and Th17 phenotypes and memory Tregs. The lower Ki67+C+ Treg-Teff ratio in ERA suggests reduced suppression of CXCR3+CCR6+ Teffs in disease, which may drive ERA immunopathogenesis. Future work will have to address the relationship between Th1/Th17 and CXCR3+CCR6+ Teffs and the efficacy of Treg suppression in disease.

W59. Deep Biology Approach for Development of Novel Therapeutics for Autoimmunity and Inflammation

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Tissue-specific immune processes control treatment outcomes in cancer and inflammation. We developed a deep understanding of human tissue Tregs behavior to enable a next generation of therapeutics aimed at activating or disarming these regulatory circuits at the location where the disease manifests. Single-cell RNA sequencing and computational tools are used on various healthy and multiple inflammatory diseases, focusing on barrier tissues to generate a high-resolution map of immune-regulatory pathways that prioritize pathways and targets. This in silico and multi-dimension functional interrogation of tissue Treg pathways allowed us to identify novel regulatory nodes and forms the foundation of our growing pipeline of a new class of tissue Treg focused therapeutics for autoimmune and inflammatory diseases. We will present data of how this approach enabled us to identify novel drug targets to alter biological Treg regulatory functions to promote productive immunity.

W64. Association of rs763361 C>T and rs727088 G>A SNPs of DNAM-1 with CRP Levels and BASDAI Index in Ankylosing Spondylitis Patients

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Ankylosing Spondylitis (AS) is an inflammatory autoimmune disease of unknown etiology. In Mexico the prevalence is estimated at 0.09% and the overwhelming risk factor for developing AS is the presence of HLA-B27. However, others genes could also be involved in AS development. DNAM-1, an activating receptor for CD8+ T lymphocytes and NK cells, participates in the regulation of cytotoxicity and the release of cytokines. Several reports have associated single nucleotide polymorphisms (SNPs) rs.763361 C>T and rs.727088 G>A in the DNAM-1 gene with different autoimmune pathologies. These SNPs are associated with decreased expression of both mRNA and protein, and decreased cell activation; however, the role of DNAM-1 in AS is currently unknown. For this reason, the aim of this work was to analyze the association of these SNPs with the BASDAI index, CRP levels and AS. We enrolled 35 patients with AS and 75 healthy subjects; genotyping of the polymorphisms rs.763361 C>T and rs.727088 G>A was performed using end-point PCR. Our results showed that rs.763361 C> T and rs.727088 G> A are not associated with the susceptibility to develop AS ($p>0.005$). However, the haploytpe analysis showed the T+A haplotype as a protective factor (OR = 0.35, 95%CI = 0.15-0.78, $p= 0.012$) for AS development. Also, the TT genotype of rs.763361 C> T was associated with an increased BASDAI index ($p< 0.005$). In conclusion, DNAM-1 could be involved in the physiopathology of AS.

W67. Novel Therapy with Neddylation Inhibitor Delays the Progression of Disease in Pre-Diabetic Non-Obese Diabetic Mice by Restoring Endogenous Treg Function

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Autoimmune diseases, including type 1 diabetes, can result from a defect in peripheral regulatory T cells (Tregs). We found that the Tregs of autoimmunity patients exhibit a defect where IL-2R signaling is turned off too soon. Normally, IL-2R signaling is maintained by inhibition of IL-2R desensitization. This is accomplished by blocking degradation of pJAK1 (associated with IL-2Rb) via inhibition of neddylation at the SOCS3/Cul5 cullin ring ligase (CRL) that normally ubiquitinates and degrades pJAK1. We showed that Treg function can be restored using a neddylation activating enzyme inhibitor (NAEi) that blocks neddylation at Cul5 CRL. Here, we demonstrate that the NAEi, MLN4924, can be used in combination with low dose IL-2 to treat 12 week old late-stage pre-diabetic NOD mice when administered at a relatively high dose, daily for 3 weeks. Disease was reduced to 24% vs. 56% in MLN/IL-2-treated mice vs. mice treated with IL-2 alone. The onset of disease, however, was only delayed by 2 weeks, to 15 weeks of age, and systemic administration of the NAEi had toxic effects. To obviate this, we generated an IL-2 protein-drug conjugate (PDC) for targeted delivery of MLN4924, allowing lower amounts of drug to be utilized. The IL-2 activity of the PDC was comparable to IL-2, and 5 daily treatments of PDC was sufficient to delay disease onset until 23 weeks of age, with biweekly or monthly single maintenance doses delaying disease further to 27 weeks of age. Together, these data support the potential use of NAEi-containing drugs for immune therapy.

Single-cell Transcriptomics of Human Alopecia Areata Reveals Clonally Expanded and Activated CD4+ and CD8+ T Cell Populations

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Alopecia areata (AA) is a common autoimmune disease that causes hair loss from the scalp and body. Treatment of AA with intralesional and systemic immunosuppressants is limited by incomplete clinical responses and sequelae associated with chronic immunosuppression, highlighting the need for new targeted treatments. CD4+ and CD8+ T-cells are known mediators of AA, however the cellular states of autoreactive disease driving T-cells is not well understood. Here, we use paired single-cell RNA and T-cell receptor sequencing to profile cutaneous T-cells of AA and healthy controls. Compared to healthy subjects, we find that active lesions of AA contain enriched populations of CD4+ and CD8+ T-cells that are clonally expanded, express markers associated with chronic antigenic stimulation, and display an inflammatory Th1 phenotype. Bioinformatic clustering of T-cell receptor sequences revealed shared clonotypes unique to AA patients that represent putative autoreactive receptors. Overall, our data identifies pathogenic T-cell populations and clonotypes associated with alopecia areata and immune mechanisms leading to disease pathogenesis.

W164. Aging and Autoimmunity: Down Syndrome Gives a Master Class

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Down syndrome (DS, trisomy 21) is the most common chromosomal abnormality. People with DS exhibit clinical and cellular features of advanced immune aging (inflammaging), especially increased predisposition to autoimmunity. To better define how immune architecture changes with age, we immunophenotyped 28

participants with DS across the lifespan using mass cytometry. We also included age- and sex-matched typical controls and, as a representative autoimmune disease, participants with type 1 diabetes. We built new analytic software (IMPACD, Iterative Machine-assisted Permutational Analysis of Cytometry Data) to execute rapid, exhaustive permutational analysis of immunophenotyping data.

We found broad overlap between immune remodeling observed in DS and in many other autoimmune diseases. Further, we successfully used IMPACD's highly granular data to build very accurate linear models of age using only immune subset data from typical control participants. These models allowed us to quantitatively demonstrate for the first time advanced immune aging in participants with DS of 5.2 to 16.9 years. These findings were supported by contemporaneous RNAseq studies, which pointed to heme metabolism and BACH2 as candidate drivers of immune aging. Notably, a DS-informed immune subset model suggested advanced immune aging in participants with T1D, the magnitude of which correlated with earlier age of onset of T1D. Inflammatory cytokines were also elevated in people with DS, supporting increased inflammaging. Together, these findings highlight novel relationships between immune dysregulation associated with DS, autoimmunity and immune aging. Elucidating these common underlying mechanisms is likely to advance therapeutic strategies for autoimmunity in people with and without DS.

W173. Rosnilimab, a Novel PD-1 Agonist Monoclonal Antibody, Demonstrated Modulation of Peripheral T Cell Activity and Reduction of Circulating PD-1high Expressing CD4 and CD8 T Cells in a Phase 1 Healthy Volunteer Clinical Trial

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Genetic studies have demonstrated that PD-1 pathway mutations increase human susceptibility to multiple autoimmune diseases and insufficient PD-1 signaling can lead to dysregulated T cell responses. Rosnilimab is a PD-1 agonist antibody designed to modulate activated T cells for the treatment of inflammatory diseases. Rosnilimab's pharmacodynamic activity resulted in rapid and sustained reduction in the quantity and functional activity of PD-1+ T cells, which are known to be pathogenic drivers of inflammatory diseases. Conventional T (Tcon) cells expressing PD-1 were reduced, on average through Day 30 in single ascending dose cohorts where full receptor occupancy was sustained following rosnilimab treatment, by approximately 50%, including in both CD4+ and CD8+ subsets, in a dose-dependent manner and in correlation with receptor occupancy. This reduction was maximized on high PD-1 expressing Tcon cells, with approximately 90% reduction relative to baseline. Rosnilimab did not modulate total T cells, total Tcon cells, or total regulatory T (Treg) cells, resulting in a favorable shift in the ratio of PD-1+ Tcon cells to total Treg cells post-treatment. No effect was observed on any of the aforementioned cell types in placebo-dosed subjects. In addition, an antigen-specific functional T cell assay measuring *Ex vivo* interferon-gamma released in response to antigen challenge, was inhibited to a maximum of approximately 90% relative to baseline within 30 days following single rosnilimab dose, while placebo administration had no effect. Based upon these data, we believe rosnilimab's *in vivo* mechanism has the potential to treat T-cell driven human inflammatory diseases.

Basic Science of Immunology - Adaptive Immunity

Th77. Postnatal Depletion of Maternal Cells Shifts Splenic NK and CD8+ T Cell Profiles Toward Mature and Activated Phenotypes

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The maternal cells transferred into the fetus during gestation persist long after birth in the progeny. These chimeric maternal cells have been hypothesized to promote the maturation of the fetal immune system in utero but there are still significant gaps in our knowledge of their potential roles after birth. To provide insights into these maternal cells' postnatal functional roles, we set up a transgenic mouse model to specifically eliminate maternal cells in the neonate by diphtheria toxin injection and confirmed significant depletion in the spleens. We then performed immunophenotyping of the spleens of two-week-old pups by mass cytometry to pinpoint the immune profile differences driven by the depletion of maternal cells in early postnatal life. We observed a decreased proportion of CD4+ T cells and naive CD8+ T cells and a heightened expression by NK cells and effector and memory CD8+ T cells of markers related to activation and maturation. We hypothesize these results to indicate a potential postnatal suppression of cytotoxic effector responses by maternal cells. Together, our findings highlight the immunological influence of maternal microchimeric cells postnatally, possibly protecting against adverse hypersensitivity reactions of the neonate at a crucial time of new encounters with self and environmental antigens.

W39. Identification of a Transcription Factor Network that Regulates Anti-TNF Mediated IL-10 Expression by Human CD4+ T-Cells

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CD4+ T-cells are key players in the pathogenesis of rheumatoid arthritis (RA) through their production of pro-inflammatory mediators. Anti-TNF therapy has revolutionised the treatment of RA and we previously demonstrated that anti-TNF treatment in vitro promotes anti-inflammatory IL-10 expression in multiple subpopulations of CD4+ T-cells. Here we investigate the transcriptional mechanisms underlying the IL-10 induction by TNF-blockade in in vitro stimulated CD4+ T-cells. CD4+ T-cells were isolated from PBMCs of healthy volunteers and stimulated polyclonally for 3 days in the absence or presence of anti-TNF. After culture, CD45RA+ cells were depleted. RNA was extracted for gene expression profiling by RNA-seq and cell nuclei were isolated for chromatin accessibility analysis by ATAC-seq. Gene expression analysis of memory CD4+ T-cells showed a distinct gene signature of 183 genes (q-value < 0.05) conferred by anti-TNF treatment. Pathway enrichment analysis of the differentially expressed genes revealed multiple pathways related to regulation of cytokine signalling and production; in particular, IL10 was the most upregulated gene by anti-TNF, while the proinflammatory cytokines and chemokines IFNG, IL9, IL22 and CXCL10 were significantly downregulated (q-value < 0.05). Transcription factor (TF) motif analysis at the differentially open chromatin regions after anti-TNF treatment revealed 58 shared TF motifs enriched at the IL10 locus. We identified 7 TF candidates that were either differentially expressed or whose locus was differentially accessible upon anti-TNF stimulation. We postulate that this TF network, which includes MAF and PRMD1, drives transcriptional regulation of IL-10 in anti-TNF treated T-cells. Supported by Versus Arthritis Grant #21139.

Th108. T Cell Cross Reactivity Between Viral and Self-peptides in the Coronary Atherosclerotic Plaque

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Background: Vascular thrombosis has been described in patients actively infected with influenza and, more recently, with the coronavirus. The risk may persist during the convalescence period. The underlying mechanisms mediating these events remain unclear. Objective: To determine whether human coronary plaque T cells reactive to influenza virus are also activated by self-proteins. Methods/Results: Using single cell targetRNAseq, we mapped the T cell repertoire in 19 patients with a range of coronary artery disease severity. To determine the potential peptides activating coronary plaque T cell receptors (TCRs), we matched TCR alpha and beta identity with HLA restriction to a database of known specificities. We found that plaque TCRs were potentially activated by viral epitopes, including those from the influenza M1 protein. We used a computational algorithm to identify self-proteins in the endothelial and vascular smooth muscle cells that may have peptides with similar homologies to viral epitopes predicted to bind our plaque TCRs. We then introduced plaque TCRs into the Jurkat T cells, exposed these cells to T2 (HLA-2 antigen presenting cells), viral peptides (e.g., M1) and self-epitopes (e.g., TSPAN17; ZIP9), and demonstrated T cell cross reactivity in vitro (Figure 1). TSPAN17 is a transmembrane protein that regulates VE-cadherin expression and promotes T cell transmigration. Zip9, in contrast, is a protein that regulates zinc homeostasis, serves as an androgen receptor on endothelial cells, and has been shown to promote endothelial proliferation. Conclusion: Taken together, our findings suggest that T cell cross reactivity may be a potential mediator of viral-induced arterial thrombosis.

Th115. Barcode Enabled Antigen Mapping (BEAM) Enables Next-Generation Systems Immunology Analysis of the Post-COVID-19 Immune Landscape

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A complete understanding of immune responses to disease requires consideration of multiple different cell types across both the innate and adaptive branches of the immune system along with the detection of multiple analytes. We used Barcode Enabled Antigen Mapping (BEAM) and Immune Profiling technology to perform simultaneous multimodal profiling at single cell resolution in hundreds of thousands of peripheral blood mononuclear cells (PBMCs) from a human donor following recovery from COVID-19. In addition to measuring gene and protein expression, we generated full-length, paired sequences of rearranged T- and B-cell receptors while also screening their specificity for a wide range of antigens from SARS-CoV-2 and other viral pathogens. These data provide insights into the entirety of the immune landscape after recovery from acute viral disease. The scale and throughput of our experiments gave us high-resolution data from all cell types from the innate and adaptive immune systems. We identified antigen-specific clones of both B and T lymphocytes, with the high cellular throughput enabling detection of rare clones. Analysis of all PBMC cell types allowed us to place these antigen-specific clones within the overall transcriptional landscape of the post-viral immune system.

Experiments like these will underpin new systems immunology approaches and will continue to reveal the complex interplay between components of the immune system. We envisage that these methods will be valuable in the analysis of the immune response to vaccination, infectious disease, cancer, allergy, autoimmune conditions, and ageing that can potentially lead to the development of novel diagnostic and therapeutic approaches.

Th122. Mutation of a Single Critical Region in the IgV of Butyrophilin 3 Reveals That One Chain Functions as a Sensor and the Other as a Ligand for the Sensing of Prenyl Pyrophosphates by Vγ2Vδ2 T Cells: Implications for Cancer Immunotherapy

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γδ T cells expressing Vγ2Vδ2 TCRs play critical roles in human immunity to tumors and microbes. These cells use their TCRs for pattern recognition to monitor phosphorylated metabolites in isoprenoid biosynthesis in a process requiring butyrophilin (BTN) 3A1 and BTN2A1. Metabolites are sensed by binding to the intracellular BTN3A1 B30.2 domain. The role played by the extracellular domains (ECD) of BTN3 is unclear. To define this role, ECD residues were mutagenized to alanine and expressed in 293T cells lacking BTN3A1/2/3, and the transfected cells used to stimulate Vγ2Vδ2 T cells. Mutation of 13 IgC and 23 IgV residues on the inner and side faces had no effect. However, 4 residues on the IgV outer face diminished stimulation along with. Others have identified 2 additional residues in this region. Identification of the critical BTN3A ECD region allowed us to investigate the contribution of BTN3A isoforms. Co-expression of wt BTN3A2 or BTN3A3 with wt BTN3A1 results in higher T cell responses due to heterodimerization. Furthermore, expression of wt BTN3A2 or -A3 with IgV-mutant BTN3A1 also results in similar high T cell responses whereas IgV-mutant BTN3A2 with IgV-mutant BTN3A1 does not stimulate. Unexpectedly, IgV-mutant BTN3A2 with wt BTN3A1 decreases stimulation to near background levels. This suggests that wt BTN3A1 protein is expressed with mutant BTN3A2 in a non-functional heterodimer. Thus, in BTN3 dimers, one chain functions as the sensor (using the B30.2 domain) and the other functions as the ligand to activate Vγ2Vδ2 T cells.

W5. Human Aging, Autoimmunity, and Palmitate: The Fatty Acid Palmitate Induces Human B Cell Metabolic Reprograming and Autoimmunity in Aging and Obesity

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This study demonstrates that increased palmitate in vitro mimics obesity-accelerated age defects including auto-antibodies in human B cells. Participants were young (30-50 years) and elderly (≥65 years), both lean (BMI< 24.9) and obese (BMI≥30). Plasma and stimulated B cells from young lean (Y_L), elderly lean (E_L), young obese (Y_O), and elderly obese (E_O), analyzed for malondialdehyde (MDA) and adipocyte-derived (AD) "self" protein-specific IgG antibodies showed E_L greater than Y_L, Y_O equivalent to E_L, E_O greater than Y_O and E_L for both AD and MDA-specific antibodies, as well as for lipid accumulation, as evaluated by staining with LipidTOX. We have previously shown that the secretion of autoimmune antibodies relies on an effective metabolic reprogramming needed to support B cell function. Therefore, we evaluated by mitostress test and Seahorse technology the metabolic profile of B cells from Y_L and E_L individuals, stimulated with CpG in the presence or absence of the fatty acid, palmitate.

Results demonstrated that palmitate increased age defects in B cells from Y_L individuals inducing the secretion of IgG autoimmune antibodies and mRNA expression of T-bet, the transcription factor associated with autoimmunity, similar to levels observed in E_L individuals. Palmitate also induced an increase in the RNA expression of metabolic enzymes and extracellular acidification rate (ECAR) in CpG-stimulated B cells from both Y_L and E_L individuals, consistent with a metabolic switch to anaerobic glycolysis, necessary for expansion of pathogenic B cells. This study emphasizes the role of lipids/fatty acids in decreased B cell function in human aging and obesity.

W24. Interferon Gamma Induction of TH1-like Tregs Dampens Anti-viral Responses

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We have previously shown that T_{reg} response to interferon gamma (IFNγ) in the tumor microenvironment is required for response to immunotherapy. We aimed to determine the role of T_{reg} response to IFNγ during viral infection and the impact on suppression of the anti-viral T cell response. Mice, which have IFNγ receptor (IFNGR1) deleted from T_{regs} (*Ifngr1*$^{L/L}$*Foxp3*$^{Cre\text{-}YFP}$) were infected with an acute (Armstrong), or chronic (Clone 13), strain of lymphocytic choriomeningitis virus (LCMV) to study *Ifngr1*-deficient T_{regs} compared to controls (*Foxp3*$^{Cre\text{-}YFP}$ mice). Additionally, mice which have IL12 receptor β2 subunit (IL12Rβ2) deleted from T_{regs} (*Il12b2*$^{L/L}$*Foxp3*$^{Cre\text{-}YFP}$) were infected with Clone 13 to study *Il12rb2*-deficient T_{regs} compared to controls. Surprisingly, T_{reg} response to IFNγ, but not IL12, induced helper T cell 1 (T_H1)-like polarization (expression of T-bet, CXCR3 and IFNγ) in LCMV Clone 13, which was required for adequate suppression of effector T cells. In LCMV Armstrong and Clone 13 infected mice IFNGR1 deletion from T_{regs} prevented T_H1-like polarization and reverted to a T_H2-like state (expression of GATA-3, CCR4 and IL-4). scRNAseq of viral antigen-specific $CD8^+$ T cells from Clone 13 infected mice showed that *Ifngr1* deletion from T_{regs} promoted $CD8^+$ T cell memory. These findings were confirmed in an Armstrong/Listeria monocytogenes-GP33 memory model. Further, *Ifngr1* deletion from T_{regs} enhanced response vaccination when challenged with Clone 13. These findings provide fundamental insight on how T_{regs} sense inflammatory cues from the environment (IFNγ) during chronic viral infection to control the effector T cell response to prevent overt tissue damage, and shape the memory response.

W41. Profound Abnormalities In Thymic Epithelial Cells In Rag1 Mutant Mice: Implications For Immune Reconstitution After Stem Cell Transplantation

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Thymic epithelial cells (TEC) are a heterogeneous population of stromal cells which orchestrate T cell development and selection. Although recent reports highlighted TEC complexity, their developmental origin, hierarchy and differentiation remain ill-defined. We compared TEC distribution and gene expression in wild-type (WT) mice and in mice carrying *Rag1* hypomorphic mutations (*Rag1*^{mut}) observed in patients with immunedeficiency and immune dysregulation. Single cell RNA-seq analysis of TECs isolated from *Rag1*^{mut}mice revealed an excess of cortical TECs (cTECs). Medullary TECs (mTECs), albeit reduced in number, showed a similar distribution in mature subsets. To address whether TEC abnormalities in *Rag1*^{mut} mice might result from a developmental block, we compared them to TECs from neonate WT mice. Indeed, we observed a similar TEC distribution in adult *Rag1*^{mut} mice and newborn WT mice, although we identified peculiar features in cTECs of *Rag1*^{mut} mice.

Transplantation of WT hematopoietic stem cells (HSC) into *Rag1*^{mut} mice was able not only to correct thymocyte development, but also to revert TEC phenotype to normalcy. However, in a competitive transplantation setting where 10% of WT and 90% mutant HSCs were used, only partial restoration of thymocyte subsets was observed, associated with incomplete rescue of TEC phenotype. In summary, we show that abnormalities of TEC composition in *Rag1* mutant mice correlate with impaired development of thymocytes, highlighting the role of lymphostromal cross-talk in TEC maturation. Moreover, these data point to the need to achieve full donor chimerism after HSC transplantation in order to rescue the thymic compartment and prevent defects of immune tolerance.

W44. An Automated Approach to High-Content Cytometric Immune Profiling of Human PBMC and Blood Samples

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CyTOF® mass cytometry is a single-cell analysis platform using isotope-tagged antibodies to resolve 50-plus markers per panel without signal compensation, making CyTOF ideal for routine high-content immunophenotyping. The Maxpar® Direct™ Immune Profiling Assay™ includes a 30-marker panel in a dry, single-tube format for staining human whole blood or PBMC, designed for use on CyTOF systems. Maxpar Pathsetter™ software automates identification of 37 canonical immune cell populations including reporting of population statistics and stain assessments. This immune profiling system has been shown to provide highly reproducible results run-to-run and site-to-site[1] when using the Helios™ mass cytometer. CyTOF XT™, the latest CyTOF system, differs substantially from Helios in that CyTOF XT features automated sample acquisition while the Helios system requires manual sample resuspension and loading by an operator. We compared performance of the manual Helios system to that of the automated CyTOF XT by acquiring the same human whole blood or PBMC samples stained with various panels requiring different workflows and data analysis approaches. These included the Maxpar Direct Immune Profiling Assay, which contains only markers for cell surface epitopes as well as protocols that include cytoplasmic, nuclear and phosphoprotein staining. Overall, these studies demonstrated no significant difference in population frequencies analyzed by the two systems. On average, samples acquired on CyTOF XT had better resolution between positive and negative populations compared to Helios. 1. Bagwell, C.B. et al. *Cytometry Part B Clinical Cytometry* 98 (2020): 146–160.

W58. Transcriptomic Comparison of Pediatric Acute Myeloid Leukemia Cells Sensitive or Resistant to Engineered Tr1-like Cell Mediated Killing

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Type 1 regulatory T (Tr1) cells are pivotal players in maintaining peripheral tolerance. These IL-10 secreting, FOXP3^{-} regulatory T cells develop in the periphery and exert their suppressive function via secretion of IL-10 and granzyme B-mediated killing of myeloid cells. The suppressive properties of Tr1 cells make them a unique immunotherapy option for autoimmune, inflammatory and transplant-related disorders. Additionally, due to their myeloid-killing capability, they could be a cancer immunotherapy tool. However, Tr1 cells are low in frequency in peripheral blood and hard to expand *in vitro*, thus limiting their use as cell therapeutics. To overcome this, our lab generated a cell therapy product by engineering human CD4^{+} T cells to overexpress *IL10* (CD4$^{IL\text{-}10}$). CD4$^{IL\text{-}10}$ are functionally similar to Tr1 cells, with high suppressive capacity and cytotoxic properties against myeloid cells. We tested the killing of CD4$^{IL\text{-}10}$ against a set of 40 primary pediatric AML blasts and grouped these blasts as sensitive, intermediate-resistant or resistant to killing.

We performed bulk RNA sequencing and selected 2 sensitive and 2 resistant blasts as well as $CD4^{IL\text{-}10}$ cells from two donors to compare the transcriptional changes in both cell types before and after *in vitro* co-culture. Our analysis revealed differentially expressed markers specific to resistant blasts that could be predictive of $CD4^{IL\text{-}10}$-mediated killing resistance. In conclusion, $CD4^{IL\text{-}10}$ are cytotoxic cells that can kill majority of AML blasts. Sensitive and resistant pediatric AML blasts have distinct transcriptional profiles which can be used to further predict the sensitivity vs resistance to immunotherapy in the future.

W61. Expression of TNFR2 and CD29 Define a Highly Suppressive Subset of Human CD8+ T Regulatory Cells

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$CD8^+$ regulatory T cells (Tregs) were the first suppressive cells reported in 1970, but they were put aside for years due to a lack of markers to properly define them. Our team demonstrated that $CD8^+$ Tregs identified by low and/or negative expression of CD45RC show potent suppressive activity *in vitro* and *in vivo,* while cells expressing high levels of CD45RC do not. Herein, we addressed the heterogeneity within $CD8^+CD45RC^{low/-}$ Tregs and identified new markers. We performed single cell RNA-sequencing and a CD screen analysis of 360 membrane molecules. Using these technologies, we were able to characterize the transcriptomic heterogeneity at a single cell level and the proteomic heterogeneity from non-stimulated $CD8^+CD45RC^{low/-}$ Tregs. We thus identified sub-populations of cells and one cluster of particular interest expressing critical markers and molecules involved in tolerance. Further work on two of these promising membrane markers, i.e., TNFR2 and CD29, using cell sorting and suppressive assays highlighted $CD8^+CD45RC^{low/-}$ $TNFR2^+CD29^{low}$ Tregs subset as the most suppressive subsets within $CD8^+CD45RC^{low/-}$ Tregs. Here, we reveal the importance of the combination of TNFR2 and CD29 as highly specific membrane markers for human $CD8^+$ Tregs and highlighted their potential roles as targets for treatments in tolerance. Other new promising markers identified in our study showed an interesting role in $CD8^+$ Tregs function; their precise roles are still under investigation. 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.

W81. Mass Cytometry to Define Healthy Immunotypes

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Healthy adult immune systems are composed of cell populations that are longitudinally stable within individuals over time, but highly variable between individuals. We hypothesize that these intrinsic inter-individual differences may help explain response to immunotherapy or propensity for development of immune-mediated disease. Here, we have identified and correlated immune cell populations to describe stable immune baseline phenotypes, or immunotypes, in younger and older healthy adult cohorts. We obtained longitudinal whole blood samples from 51healthy adults between 25 and 35 years of age and 49 healthy adults between 55 and 65 years of age. All participants reported no history of chronic disease. 10 samples per participant were collected; 4 at timepoints that were not associated with any known immune challenge, and 2 sets of 3 timepoints surrounding vaccination for seasonal influenza. We used mass cytometry to identify major innate and adaptive immune cell populations. Cell populations were selected based on high within- and low between-individual stability, to identify clusters of features that reliably enable grouping of individuals by immunotype. We describe associations between immunotype and many demographic variables including age, sex, substance use, BMI, and residence history to parse their influence on immunotype. Furthermore, we investigate how response to seasonal flu and COVID-19 vaccination may be influenced by immunotype. These data contribute to our understanding of human immune system variability and lay the groundwork for future studies of immunotype's influence on disease course and treatment.

W88. IL-21 is a Key Regulator of Chronic Germinal Centers in Autoimmune Settings

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Abundant experimental evidence has linked defective B cell and T cell interactions with the development of autoimmunity, highlighting the importance of further investigations into pathways underpinning B cell and T cell collaboration in health and disease. Elevated levels of the archetypal follicular helper T-cell cytokine interleukin 21 (IL-21) have been reported in many autoimmune conditions including type 1 diabetes, rheumatoid arthritis, and systemic lupus erythematosus, and IL-21 has been widely shown to promote pathology in their corresponding animal disease models. However, the underlying mechanisms of IL-21-dependent regulation of humoral immunity as well as their contribution to the development of autoimmunity remain poorly understood. Previous work by our group has demonstrated that, alongside systemic autoimmunity, mice rendered deficient for T-cell peripheral regulator CTLA-4 present with a significant upregulation of IL-21 as well as a formation of exacerbated germinal centers (GC). We have taken advantage of this model to home in on the role of IL-21 in the regulation of chronic GC responses in autoimmune settings. By studying GC in CTLA-4-/- mice that were crossed to IL-21R-/- animals we were able to demonstrate that IL-21 represents a key regulator of light zone GC B cell selection and that IL-21 signaling is essential for an efficient formation of the GC dark zone compartment. Our study provides novel insights into IL-21 involvement in the regulation of GC processes that are central to the development of robust humoral immunity but that may also aid disease pathogenesis in the context of autoimmunity.

W103. IRF5 Regulation of CD4+ T Cell Metabolism Controls CD40L Expression

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Systemic Lupus Erythematosus (SLE) is a devastating autoimmune disease that results from failure of the immune system to distinguish “self” from “non-self”. Studies in our lab and others demonstrated that human SLE CD4+ T cells have elevated levels of IRF5 and increased metabolism, while Irf5 knockout murine CD4+ T cells show diminished oxidative phosphorylation and glycolysis. However, how IRF5 contributes to CD4+ T cell support of B cell function and dysfunction has not been fully elucidated. Using IRF5 KO C57BL/6J mice, we show that loss of IRF5 in CD4+ T cells directly contributes to defects in plasmablast generation. Examination of the CD40-CD40L interaction revealed significant decreases in CD40L expression in Irf5 KO T cells. Analysis of Cd40lg transcript expression showed no difference in mRNA expression in Irf5 KO CD4+ T cells. This finding indicated a novel post-transcriptional regulatory role for IRF5. Examination of the canonical mTOR pathway, a key translational regulator, showed decreased phosphorylation of the S6 ribosomal protein with loss of Irf5, indicating diminished activity of the mTOR pathway. mTOR inhibition with rapamycin in turn resulted in decreased CD40L expression, supporting a role for mTOR in CD40L regulation. As mTOR signaling is known to be regulated by metabolism, we examined if loss of IRF5 resulted in altered T cell metabolism. We found that Irf5 KO T cells have decreased glucose metabolism and increased glutamine metabolism. This data has increased our understanding of how metabolism influences CD4+ T cell function and provides insight into novel post-translational regulatory roles for IRF5.

W122. Developing Trajectories of Immunosenescence in Diverse Adults

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Immunosenescence (ISC) is the natural aging of the immune system, involving gradual changes in immune-cell proportions and functions. Genetic variation, sexual-dimorphism and previous viral infections may influence it. Thus far, limited large-scale studies are available, mostly involving European descends, although clear multi-modal ISC-trajectories remain unknown. To characterize ISC phenotypic/functional changes in a large-scale manner, we used PBMC samples (n=257, age: 18-84 years) part of two Columbia aging-cohorts. Multiparametric-cytometry profiles, single-cell-RNA-sequencing, bulk-PBMC RNA-sequencing and T-cell proliferation were captured. First, we used standard FlowJo bi-dimensional gating to analyze cell-proportions and MFI of main innate and adaptive immune-cells, plus other markers: CD11c, CD57, PD-1/PD-1L & KLRG-1. Second, we processed .fcs files to create a multidimensional data matrix analyzed using PhenoGraph algorithm to empirically cluster PBMCs into 20 discrete cell-subtypes. Preliminary analyses of a cohort representative (n=60), confirms previous findings: decrease in naïve CD8 T-cells (p=0.000054), increase of CD8 TEMRA T-cells (p=0.0027), and decrease of $CD20^{+}$B-cells (p=0.0028), while $CD11c^{+}CD20^{+}$ B-cells increased with advancing age. No meaningful age-associated changes were noted in IgD^{+}naïve, nor $IgD^{-/+}CD27^{+}$memory B-cells. We further found a population of $CD56^{+}CD8^{+}$NKT-cells increasing with age (p=0.0019). Next, our clustering analysis uncovered an unexpected subset of $CD56^{+}$PD-$L1^{+}$NK-cells increasing with age (p=0.000096). We report several aging-related cell populations including well-known ones. ISC signatures included NKT-cells and $CD11c^{+}$B-cells. Surprisingly, we identified an intriguing senescent PD-$1L^{+}$NK population, perhaps contributing to higher cancer incidence in elderly. Results from n=257 will be presented, enabling ISC-trajectories assessment amongst African-American, Latin-x, and non-Hispanic Whites in an ongoing effort to establish personalized ISC-trajectories.

W155. Integrating Spatial Transcriptomics and Single-Cell RNA Sequencing to Spatially Characterize Human Lymphatic Tissue

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Advances in single cell analysis technologies have revolutionized efforts to understand cell state and heterogeneity in conditions of health and disease. However, tissue dissociation procedures do not always encompass the full cellular constituents within a tissue, particularly for many stromal subsets. Moreover, cell:cell interactions and localization within functional units are often lost upon tissue digestion. Emerging spatial transcriptomic (ST) technologies address this limitation by assessing gene expression across a tissue section while preserving positional information that is otherwise lost with single cell RNA-seq approaches. Despite further advancements, ST approaches currently do not offer the degree of throughput and resolution that is achieved with scRNA-seq, thushighlighting the need to integrate single-cell and spatial data.Here, we present spatial transcriptomics data (Visium, 10x Genomics) generated from fresh frozen, OCT-embeddedthymus, spleen, and pancreatic lymph node obtained fromcontrol human organ donors (N=2, age=1.5, 32 y.o.).Furthermore, we integrated these datasets with reference gene expression signatures derived from scRNAseq datasets to deconvolute the relative cell type abundances across the tissue section. We then employ the TRUST4 algorithm to extract candidate TCR and BCR reads from Visium data in order to reconstruct the immune receptor repertoire of ab and gd T cells as well as B cells to assess their clonal diversity and relative positions in situabgd. Together, this multi-modal analysis is expected to enable detailed studies capturing the cellular heterogeneity, spatial organization, and clonal diversity withincomplex tissues during health and disease.

Basic Science of Immunology – Innate Immunity

Tu35. Single-cell Transcriptomic Analysis of Peripheral Blood Reveals the Disparate Subsets of Innate Immune Cells in Kidney Transplant Recipient with BK Virus Infection

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Purpose: BK virus (BKV) infection is a common problem in kidney transplant recipients receiving immunosuppressive therapy, resulting in a serious complications including BKV-associated nephropathy and subsequent allograft loss. The purpose of this study was to characterize the disparate blood subsets in BKV infection/nephropathy at the single-cell transcriptional level. *Methods: We isolated peripheral blood mononuclear cells from three kidney transplant recipients with stable status, BK viremia and BK nephropathy. Single-cell libraries were generated using the 10x Genomics Chromium Platform. We analyzed multiplexing data in Cell-Ranger Pipeline and used R and "Seurat" packages for downstream analysis. *Results: A total of 14,031 cells were analyzed, including 5473 cells from stable patients, 3976 cells from patients with BK viremia, and 4582 cells from patients with BK nephropathy obtained from Illumina HiSeq X. We characterized 17 distinct clusters representing different cell types. Of these, 13 clusters had differentially expressed transcripts for each sample, and the most differentially expressed markers were S100A8, CCR7, LTB, GNLY, GZMK, GZMH, MT-CO1, LINC02446, IGKC, AIF1, HLA-DRA, STMN1. Stable patient had more mRNA upregulated in B cells compared to patients with BK virus infection. In BK viremia, NK cells and monocytes were downregulated, whereas in BK nephropathy, mRNA expression was high in gamma delta T cells. *Conclusions: Our study revealed that BK virus infection/nephropathy induce unique characteristics in lymphocytes, which could be confirmed through single-cell RNA analysis. Further studies are needed to characterize the innate immune cells involved in the progression of BK nephropathy.

Tu45. Isocaloric Ketogenic Diet Modulates NLRP3 Inflammasome of Macrophages via β-hydroxybutyrate and Fibroblast Growth Factor 21 in Humans

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A ketogenic diet (KD) is known to have beneficial health effects. Various types of KD interventions have been applied to manage metabolic syndrome based on modification of diet parameters such as duration of intervention, macronutrient components, and total calories. Nevertheless, the beneficial health impact of isocaloric KD is largely unknown, especially in healthy subjects. The present study investigated the acute effects of a 3-day isocaloric KD. In this non-randomised intervention study, we recruited 15 healthy volunteers aged 24-38 years (7 men and 8 women) and placed them on an isocaloric KD restricting intake of carbohydrates but not energy (75% fat, 20% protein, 5% carbohydrate) for 3 days. Biochemical profiles and laboratory measurements were performed. Peripheral blood monocular cells were cultured, and measured cell stimulated cytokines. After short-term isocaloric KD, subjects lost body weight and serum free fatty acid levels were increased. These results accompanied elevated serum β-hydroxybutyrate (BHB) concentration and fibroblast growth factor 21 (FGF21) levels and improved insulin sensitivity. Regarding the direct effect of BHB on inflammasome activation, interleukin-1β (IL-1β) and tumor necrosis factor-α secretion in response to adenosine triphosphate or palmitate stimulation in human macrophages decreased significantly after isocaloric KD. In ex-vivo experiments with macrophages, both FGF21 and BHB synergistically reduced IL-1β secretion compared to either BHB or FGF21 alone.

The inhibitory effect of FGF21 on IL-1β secretion was blunted with bafilomycin treatment, which blocked autophagy flux. In conclusion, isocaloric KD for 3 days is a promising approach to improve metabolic and inflammatory status.

Tu49. Multi-OMICs Analysis of Metabolites, Proteins, and Histone Modifications During Human Macrophage Polarization Reveals Novel Regulatory Pathways

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Background: Immune system uses a highly intriguing mechanism to polarize naïve macrophages (MΦs) into pro-inflammatory, antimicrobial M1-MΦs and anti-inflammatory, tissue repair mediating M2-MΦs. To develop therapeutic strategies for uncontrolled MΦ polarization, we analyzed the interplay between metabolism and epigenetics using a highly sensitive, multiplexed 'triomics'. Hypothesis: We hypothesized that a crosstalk between 'metabolism and epigenetic modifications' determines MΦ polarization essential for infection and inflammation control. Methods: A novel 'triomics' method was used to analyze metabolites, histone modifications and protein expression concurrently from the same samples. Results: (a) Acetylation and M1-MΦ phenotype: TCA cycle maintained by replenishment of OAA through glutamate/aspartate metabolism, upregulated de novo synthesis of NAD+ from tryptophan metabolism and increased NAD+ production by oxidation of NADH via the mitochondrial respiratory complex I-derived Sirtuin-type histone deacetylase activity. Lower acetyl-CoA production from glycolysis and blocked acetyl release from NAA, NAG and NAO, and increased NAD+ synthesis or production appeared to be the major causes for reduction of histone global acetylation in M1-MΦs. (b) Accumulation of Nα-Acetyl-Aspartate (NAA), -glutamate (NAG), and -ornithine (NAO) resulted in the trap of acetyl-CoA, which contributed to hypoacetylation of histones measured in M1-MΦs. (c) We found that blocked one-carbon metabolism led to histone demethylation in M1-MΦs. Conclusions: Our data indicate that metabolite-altered histone acetylation, histone and RelA arginine methylation are promising targets for polarizing macrophages to control infection or inflammation.

Tu51. Pim1 Positively Regulates Interferon Production by Interacting with IRF3

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The Pim (proviral integration site for Moloney murine leukemia virus) proteins form a serine threonine kinase family that regulates cell proliferation, migration and cell survival. However, the role of Pim kinases in innate immune response is largely unknown. Here we demonstrate for the first time that a Pim1 kinase plays an essential role in the expression of IFN-b induced by pathogen-associated molecular patterns (PAMPs). Specifically, Pim1 is quickly upregulated after Toll-like receptor (TLR) stimulation by PAMPs in a NF-kB-dependent manner. Pim1 deficiency resulted in reduced IFN-b and IFN stimulated genes (ISGs) production. Mechanistically, Pim1 interacts with TRIF-signaling molecules and specifically regulates IFN-b induction by regulating IRF3 phosphorylation and nuclear translocation in a kinase activity-independent manner. Our study uncovers a previously unrecognized role for Pim1 in IFN-b production, thus providing a new target for controlling antiviral innate immune responses.

Tu84. Diverse Functional Profiles of Individual Human Gamma Delta T and iNKT Cells Revealed With a 32 Color Intracellular Cytokine Staining Spectral Flow Cytometry Panel

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Populations of innate T cell subsets such as gamma delta T cells and iNKT cells are unique in their ability to shift effector function profiles from inflammatory (Th1/Th17) to immunoregulatory/suppressive (Th2/Treg). The nature of innate T cell lineage commitment versus functional plasticity is unclear to date. Previous limitations in flow cytometry panel size disallowed for proper analysis of higher numbers of cytokines and other effector functions from these unique T cell populations with the single cell resolution required to address these questions. However, with the advancement of spectral cytometry, the design and optimization of larger scale flow cytometry panels is now feasible, thus allowing for the expansion of emission detectors and a wider optical spectral range to accommodate panels with 30 or more colors. Using the full optical spectrum, a 32-color Intracellular Cytokine Staining (ICS) panel was successfully optimized, enabling measurement of 11 cytokines, as well as Ki67, granzyme B, and CD107a from several immune cell subsets in PBMCs (CD4+ T cells, CD8+ T cells, NKT cells, iNKT cells, Vd1 gd T cells, Vd2 gd T cells, regulatory T (Treg) cells, and NK cells). Use of this panel revealed differences in polyfunctionality of gamma delta T cells between younger versus older subjects, and that individual innate-like T cells were found to express a 'Th0' phenotype, producing both Th1 and Th2 lineage cytokines after short term mitogenic stimulation. The application of large ICS panels will help elucidate innate T cell functional signatures in the context of different diseases and disorders.

Tu92. TLR-4 Agonist Induces IFN-gamma Production Selectively in Pro-inflammatory Human M1 Macrophages Through the PI3K-mTOR and JNK-MAPK Activated p70S6K Pathway

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IFN-g, a pro-inflammatory cytokine produced primarily by T cells and NK cells, activates macrophages and engages mechanisms to control pathogens. Although there is evidence of IFN-g production by murine macrophages, IFN-g production by normal human macrophages and their subsets remains unknown. Herein, we show that human M1 macrophages generated by IFN-g, and IL-12 and IL-18-stimulated monocyte-derived macrophages (MDM-M0) produce significant levels of IFN-g. Further stimulation of IL-12/IL-18-primed macrophages or M1 macrophages with agonists for Toll-like receptors (TLR)-2, TLR-3 or TLR-4 significantly enhanced IFN-g production in contrast to the similarly stimulated M0, M2a, M2b and M2c macrophages. Similarly, M1 macrophages generated from COVID-19-infected patient's macrophages produced IFN-g that was enhanced following LPS stimulation. The inhibition of M1 differentiation by Jak inhibitors reversed LPS-induced IFN-g production suggesting that differentiation with IFN-g plays a key role in IFN-g induction. We subsequently investigated the signaling pathway(s) responsible for TLR-4-induced IFN-g production in M1 macrophages. Our results show that TLR-4-induced IFN-g production is regulated by the ribosomal protein *S6 kinase* (p70S6K) through the activation of phosphoinositide-3-kinase (PI3K), the mammalian target of rapamycin complex-1/2 (mTORC-1/2) and the c-Jun N-terminal kinase (*JNK*) mitogen-activated protein kinase (MAPK) pathways. These results suggest that M1-derived IFN-g may play a key role in inflammation that may be augmented following bacterial/viral infections. Moreover, blocking the mTORC-1/2, PI3K and JNK MAPKs in macrophages may be of potential translational significance in preventing macrophage-mediated inflammatory diseases.

W1. Aryl Hydrocarbon Receptor Activity is Required Downstream IL-10 Signaling to Promote Regulatory Functions in Human Dendritic Cells

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Peripheral immune tolerance is a key physiological mechanism preventing immune reactions to self and harmless antigens. Interleukin (IL)-10 plays key roles in this system by inhibiting effector cells and instructing regulatory cells. Here, we investigated the mechanisms underlying IL-10-mediated induction of regulatory activity in human dendritic cells (DC). Using chromatin and transcriptomic studies in tolerogenic IL-10-induced monocyte-derived DC, we show that IL-10 induces a pattern of accessible enhancers that are exploited by Aryl Hydrocarbon Receptor (AHR) to promote the expression of a set of core genes. For the first time, we demonstrate that AHR acts downstream of IL-10 signaling in myeloid cells and that its activity is required for the establishment of IL-10-induced core genes and induction of tolerogenic activities in DC. Analyses of tolerogenic DC in peripheral blood show that the IL-10/AHR genomic signature is active In vivo in healthy conditions. In multiple sclerosis patients we instead observe significant alterations in this signature that correlated with functional defects and reduced frequencies of IL-10-induced tolerogenic DC in vitro and In vivo. Our studies reveal previously undescribed IL-10-induced molecular mechanisms required for establishing tolerogenic activities in human myeloid cells and may help in designing new therapies to re-establish immune tolerance.

W23. Sex Differences in NK Cells Mediated by the X-linked Epigenetic Regulator UTX

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Viral infection outcomes are sex-biased, with males generally more susceptible to human cytomegalovirus (HCMV) and other viral infections compared to females. These differences may reflect sexual dimorphism in immune cell composition and function. As such, it is surprising that numbers of natural killer (NK) cells, a first line of anti-viral defense, are increased in males compared to females. Here we show in mouse models and human samples that while males harbor increased NK cell numbers, they concomitantly produce less IFN-γ, a key proinflammatory cytokine for NK-mediated effector responses. This difference is not due solely to divergent levels of gonadal hormones, since these differences persist even with gonadectomy. Instead, these differences are attributable to lower male expression of the epigenetic regulator Kdm6a (UTX), a X chromosome gene which escapes X inactivation in both human and mouse NK cells. NK cell-specific UTX deletion phenocopied multiple features of male NK cells, which include increased numbers and reduced IFN-γ production. Moreover, NK cell UTX is critical for optimal anti-viral immunity, since mice with NK cell UTX deficiency show increased lethality to mouse cytomegalovirus (MCMV) challenge. Integrative ATAC-seq and RNA-seq analysis revealed a critical role for UTX in maintaining open chromatin accessibility and gene expression of Ifng, Csf2, Thy1, and other gene loci important in NK cell homeostasis and effector function. Taken together, these data implicate UTX as a critical molecular determinant of NK cell sex differences and suggest enhancing UTX function may be a new strategy to boost endogenous NK cell anti-viral responses.

W28. A SLIT3 Deficiency Promotes M1 Macrophage Polarization and Inflammation

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Macrophages primarily function in the detection of infection or tissue injury to induce immune responses. However, little is known about the signaling factors that regulate their action and thereby control inflammatory responses. We show here that the neuronal guidance factor SLIT3 is induced by bacterial lipopolysaccharide (LPS) through toll-like receptor 4 (TLR4) activation in bone marrow macrophages. Depleted SLIT3 suppressed the augmented phagocytic activity of macrophages in LPS response and their anti-microbial effects in vitro. A SLIT3 deficiency also promoted a macrophage 1 (M1) profile accompanied by an upregulation of pro-inflammatory cytokines. SLIT3-deficient mice displayed increased M1 populations in peritoneal cells compared with wild-type mice upon LPS exposure. We determined whether a direct SLIT3 administration In vivo during endotoxemia by E. Coli bioparticles would control the inflammatory disease state. As expected, SLIT3-treated mice recovered from the acute endotoxic inflammatory response though enhanced phagocytosis by Gr-1+ granulocytes and F4/80+ macrophages. This diminished the deleterious aspects of the host response to the pathogen-induced cytokines IL-1β or IL-18 in the peritoneal lavage and blood. These findings suggest that SLIT3 regulates innate immunity in a manner relevant to inflammation, and that this may provide a potential treatment strategy for excessive inflammation or a sustained hyperinflammatory response.

W69. Effect of Chronic Exposure to Abrocitinib on Immune Homeostasis in Mice

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Abrocitinib (PF-04965842) is an oral JAK1 selective inhibitor and in development for type 2 inflammation disorders such as atopic dermatitis. Despite the broad impact of JAK inhibition on signaling mechanisms, the effect of abrocitinib on immune homeostasis has not been well studied. Therefore, we administered abrocitinib at 100mg/kg daily for three weeks to 3-month-old C57BL/6 mice and performed a comprehensive immunologic profiling to determine the impact of chronic JAK1 inhibition on immune homeostasis in adult mice. Pharmacokinetic profile at 30mg/kg showed that abrocitinib had a short half-life (45min) and a complete clearance 8 hours post-dose. Target engagement analysis indicated the effect of abrocitinib on JAK1 inhibition lasted less than 6 hours. Immunological analysis revealed an impact in splenic NK cells, macrophages, and monocytes. In thymus, data pointed out modification in double negative thymocytes CD4-CD8- subset. As to the B cells subset, we noticed a decrease in precursors B cells in bone marrow and subsequently a decrease in T1 and T2 transitional B cells in spleen. Altogether, these data show that JAK1 chronic inhibition in adult mice causes some delay in maturation of B cells and thymocytes and confirmed a decrease in splenic NK cells and macrophages populations, similar to results obtained in preclinical chronic studies with baricitinib (JAK1/JAK2) and tofacitinib (JAK1/JAK3 inhibitor). Further studies, such as a genomic profiling to assess abrocitinib effects on the chromatin modification and a transcriptomic analysis, may help to understand the mechanisms underlying the delay in the maturation and terminal differentiation of these cell subsets.

W93. Selective Induction of Cell Death in Human M1 Macrophages by Smac Mimetics is Mediated by cIAP-2 and RIPK-1/3 Through the Activation of mTORC

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Inflammatory macrophages have been implicated in many diseases including rheumatoid arthritis and inflammatory bowel disease. Therefore, targeting macrophage function and activation may represent a potential strategy to treat macrophage-associated diseases. We have previously shown that IFN-γ-induced differentiation of human M0 macrophages towards pro-inflammatory M1 state rendered them highly susceptible to the cytocidal effects of second mitochondria-derived activator of caspases (SMAC) mimetics (SMs), the inhibitors of apoptosis (IAPs) proteins, whereas M0 and anti-inflammatory M2c macrophages were resistant. Herein, we investigated the mechanism governing SM-induced cell death during differentiation into M1 macrophages and in polarized M1 macrophages. IFN-γ stimulation conferred on M0 macrophages the sensitivity to SM-induced cell death through the Jak/STAT, interferon regulatory factor-1 (IRF-1) and the mammalian target of rapamycin complex-1/ribosomal protein S6 kinase (mTORC-1/S6K) pathway. Interestingly, mTORC-1 regulated SM-induced cell death independent of M1 differentiation. In contrast, SM-induced cell death in polarized M1 macrophages is regulated by the mTORC-2 pathway. Moreover, SM-induced cell death is regulated by cellular IAP-2 (cIAP-2), receptor-interacting protein kinase-1 (RIPK-1) and RIPK-3 degradation through mTORC activation during differentiation into M1 macrophages and in polarized M1 macrophages. In contrast to cancer cell lines, SM-induced cell death in M1 macrophages is independent of endogenously produced TNF-α as well as the NF-κB pathway. Collectively, selective induction of cell death in human M1 macrophages by SMs may be mediated by cIAP-2, RIPK-1and RIPK-3 degradation through mTORC activation. Moreover, blocking cIAP-1/2, mTORC or IRF-1 may represent a promising therapeutic strategy to control M1-assoiated diseases.

W101. Notch2 Regulates The Homeostasis Of Lung Interstitial Macrophages

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Macrophages reside in every organ yet adapt to the needs of their microenvironment through interactions with other cells and soluble factors. Notch signaling plays a crucial role in the lineage specification of cells, including those of the immune system such as T cells, B cells and dendritic cells. The role of Notch receptors on macrophage differentiation has been blurred by the lack of proper models to study their individual contribution. Here we found that Notch1 and Notch2 have different roles in lung macrophages, with a Notch2-Jag1/2 signaling axis acting as a negative regulator to prevent differentiation of interstitial macrophages. Notch2 acted in a macrophage-intrinsic fashion to control macrophage number as well as a cell-extrinsic fashion to polarize the niche. scRNA-Seq analysis showed that Notch2 blockade did not skew existing IM subtypes, but instead induced the presence of a new macrophages that transcriptionally resembled those in inflamed tissues. Interestingly, Notch2 blockade largely attenuated disease in a bleomycin model of fibrosis, shedding light on the dynamic and versatile functional role of macrophage.

W150. Natural Killer Cells may be Functionally Altered due to Simulated Microgravity Exposure

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Immune dysregulation has been reported due to spaceflight, including impacts on Natural Killer (NK) cell function. As cytotoxic cells of the innate immune system, NK cells target virus-infected and malignant cells. NK cells are an important cell type to study in this context, given the increased risk of cancer development in deep space missions due to radiation exposure, and reported viral reactivation during spaceflight. Microgravity is the condition of "weightlessness" due to low gravitational forces in the space environment and may contribute to the reported changes in astronaut immune function. Here, we cultured the NK92 cell line in simulated microgravity (SMG) for 72 hours. Our preliminary results indicate a 12.8% and 8.0% decrease in NK cytotoxicity against K562 and MCF7 cancer cell lines, respectively. Interestingly, we measured a 32.3% and 27.0% increase in mRNA expression of cytotoxic molecules, perforin and granzyme B. Our secretory data of these molecules indicate a 13.5% decrease in perforin secretion, coupled with a 209% increase in granzyme B secretion. Taken together, our preliminary results suggest that NK cells may be functionally altered due to exposure to simulated microgravity. The measured decrease in cytotoxicity after SMG exposure may be preceded by 1) dysregulation of the NK cell degranulation process and/or 2) NK-target cell interactions. Our next steps will focus on these parameters. Understanding the effects of microgravity exposure will become increasingly important as astronauts complete future deep space missions. This research also has the potential to inform us about age-related immune dysregulation experienced on Earth.

W170. TMEM176B is a Target to Control Pathogenic Inflammasome Activation in Obesity

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Inflammation is thought to play a pathogenic role in obesity and related morbidities. However, targeting inflammatory players has not shown a significant benefit in the clinics. Thus, identification of novel druggable targets and anti-inflammatory strategies is needed. We speculated that the cation channel TMEM176B, a physiological inhibitor of the NLRP3 inflammasome, may protect the host against obesity and insulin resistance. Here we show that high fat diet (HFD) down-regulates TMEM176B expression in white adipose tissue (WAT). In WAT from HFD-fed *Tmem176b*$^{-/-}$ mice, inflammasome activation was enhanced versus WT animals. HFD also led to increased weight gain and insulin resistance in *Tmem176b*$^{-/-}$ mice. This metabolic phenotype was partially reversed by blocking IL-1β or deletion of *Casp1*. Having shown that TMEM176B is a potential target in obesity, we then wished to pharmacologically modulate this channel. We show that a salicylic acid nitroalkene (SANA), known to control diet-induced obesity, triggered TMEM176B-dependent ion transport. Accordingly, SANA inhibited inflammasome activation *in vitro* and in HFD-fed animals. Finally, genotypic studies show that *TMEM176B* single nucleotide polymorphisms are associated with obesity and type 2 diabetes in a cohort of 288 obese individuals. Thus, TMEM176B is a druggable target to control inflammasome activation in obesity and insulin resistance.

Inflammatory Responses Conserved Across Human and Murine Neutrophils

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Neutrophils are central to immune defense and pathologic inflammation, yet how their transcriptome is conserved across inflammatory conditions and species is poorly understood. Here we show that in neutrophils, a larger fraction of expressed genes possess one-to-one orthologs across humans and mice compared to other leukocytes. Within the most abundant orthologous genes, neutrophils display less transcriptional diversity and a higher correlation of lineage-specific gene expression across species compared to other lineages. Transcriptomes of inflamed neutrophils deviate markedly from resting blood neutrophils and are highly heterogenous, with most variability attributable to species, source of inflammation and tissue. Nevertheless, we identify a program of features conserved across a broad range of conditions and between humans and mice, including genes encoding IL-1 family members, CD14, IL-4R and PD-L1, suggesting the existence of a canonical inflammatory response program in neutrophils. Key elements of this program were validated experimentally by *in vitro* stimulation. Transcription factor enrichment analysis implies NF-κB family members, AP-1 complex members, NFIL3, CSRNP1 and PLSCR1 as key drivers, consistent with strong enrichment for GO terms associated with NFκB activation and cytokine response in inflamed neutrophils. Neutrotime scores trended higher in inflammation, implying more advanced maturation as neutrophils become activated. Taken together, we demonstrate that the neutrophil transcriptome is substantially conserved in homeostasis and that inflamed neutrophils display an inflammatory response program conserved across conditions and species.

COVID-19

Tu5. Serum IL-6 and PTX3 Predict Severe Outcome from Covid-19 in Ambulatory Subjects

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SARS-CoV-2 infections lead to a wide-range of outcomes including mild or asymptomatic illness to serious disease and death. While studies have been reported to characterize hospitalized patient responses leading to death from SARS-CoV-2, we were interested in immune changes in ambulatory subjects. In this post-hoc analysis, baseline samples from 56 SARS-CoV-2 infected individuals that went on to hospitalization or death were compared to 132 individuals in the same study who did not require medical intervention to evaluate if nasal interferon signature gene transcripts, serology titers, as well as 184 serum cytokines and chemokines could help predict severe outcome. Baseline demographics, including age, baseline normalized viral load, duration from symptom onset and BMI have a predictive capability of an adverse event with an AUC of ROC = 0.77. The predictive performance of the model increased when the serum protein markers were included. In fact, our analysis indicated that there were 51 individual proteins (including known markers of inflammation like IL-6, MCP-3, CXCL10, IL-1Ra and PTX3) that increased the AUC of ROC ranges from 0.78 to 0.88. Moreover, a two-marker model of IL-6 and PTX3 further improved the prediction to an AUC of ROC = 0.91 over adding a single protein marker. Further combination of analytes or analysis of baseline serology or nasal interferon signature gene transcripts with demographics did not improve the prediction. This analysis demonstrates the potential additive value of serum IL-6 and PTX3 concentrations to predict hospitalization or death in an ambulatory population infected with SARS-CoV-2.

Th27. Clinical Analysis of SARS-CoV-2 Seropositivity and Neutralization Capacity in Patients Infected with COVID-19

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Background: Since the dawn of the 2019 outbreak of the novel beta coronavirus SARS-CoV-2, rapid spread of COVID-19 disease has resulted in a pandemic. Despite wide availability of vaccines, many are unvaccinated or under vaccinated and are vulnerable to severe disease. Methods: We utilize a serum database consisting of 63 patients who visited the UC Irvine Douglas Hospital and received a diagnosis of COVID-19 in the emergency department. Whether the patient was held for single overnight observation or several weeks in the intensive care unit, daily finger-pricks were taken and stored to characterize patient serology against a robust SARS-CoV-2 antigen microarray. A commercially available surrogate virus neutralization test for SARS-CoV-2 was also used in every sample to further characterize the neutralization capacity of these antibodies. Results: Most patients reach a 50% signal inhibition rate by 5-10 days of their disease course, with a majority plateauing at 95% signal inhibition by 14 days. A small cohort of patients reach 50% signal inhibition before day 5, while others never reach 50% signal inhibition despite being hospitalized over the course of several days. We also report the presence of 11 different SARS-CoV-2 antibodies, as well as their correlation with neutralization capacity. We are able to correlate clinical biomarkers with disease severity and seropositivity. Up to date research will be presented. Conclusion: The COVID-19 virus induces a variable sero-response in patients afflicted with minor and severe disease, with some patients receiving 95% neutralization capacity within the first week of infection.

Tu15. Elevated anti-PEG Antibody Levels Amongst Covid-19 Vaccinated Donors

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Beginning in early 2021, without any change to our flow cytometry antibody products, we observed an increase in the number of donors showing unusual non-specific staining of lymphocytes and granulocytes. This unusual staining pattern was limited to workflows where antibody staining was performed in whole blood, using Pegylated (PEG) fluorophore antibody conjugates. Not all the donors tested showed this staining pattern and some of them had been previously tested and did not exhibit this aberrant staining pattern. The only difference we could identify was most if not all of our donors recently received the COVID-19 vaccine. Since PEG is a component of some COVID-19 vaccine, we assayed the Plasma samples from 70 donors for the presence of anti-PEG antibody using a commercially available ELISA kit assay. Comparing with normal-staining donors, we observed elevated anti-PEG antibodies in the plasma of those donors with aberrant staining. All the donors who had elevated anti-PEG antibodies had received a Covid-19 vaccine. All non-specific staining can be corrected by pre-washing blood or adding PEG in blood. Further study should be conducted to address this finding.

Tu21. Monitoring of Naturally Acquired and Vaccine-induced SARS-CoV-2 Specific Cellular Immune Responses

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The current global COVID-19 pandemic has emphasized the importance of widespread cellular immune-monitoring programs for evaluation of immunity upon natural infection and vaccination. Cellular immune-monitoring tools are important for understanding the SARS-CoV-2-induced immune responses and can help provide guidance to clinicians and health authorities about the type, degree, and duration of cellular immunity and thus improve the decision-making about future vaccine programs and the roll-out of booster doses.
We have developed SARS-CoV-2-specific immune-monitoring tools based on the Dextramer® technology to detect and characterize virus-specific $CD4^+$ T cells, $CD8^+$ T cells and B cells. The Dextramer® reagents were clinically evaluated by longitudinal assessment of immunity in individuals previously infected with or vaccinated against SARS-CoV-2. Inspection of the T cell epitopes included in the Dextramer assay showed a high degree of conservation between Wuhan, Delta, and Omicron variants and are minimally affected by mutations. Our results show that SARS-CoV-2-specific Dextramer® reagents can detect SARS-CoV-2-specific immune cells in patients previously exposed to SARS-CoV-2 or vaccinated individuals. We found that both COVID-19 infection and vaccination elicit strong immune responses towards SARS-CoV-2, which declines over time. These results are encouraging and will help understanding the longevity of immunity after infection and vaccination. Moreover, the results support the idea of using immune monitoring as a widespread tool for tailoring the vaccine schedule based on the individual's immunity. The tailored vaccine approach will be further investigated by analysing T-cell specificities using the DNA barcode-based dCODE Dextramer® technology in combination with single cell multi-omics systems.

Tu36. SARS-CoV-2-specific T Cells in Unexposed Adults Display Broad Trafficking Potential and Cross-react with Commensal Antigens

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The baseline composition of T cells directly impacts later response to a pathogen, but the complexity of precursor states remains poorly defined. Here we examined the baseline state of SARS-CoV-2 specific T cells in unexposed individuals. SARS-CoV-2 specific $CD4^+$ T cells were identified in pre-pandemic blood samples by class II peptide-MHC tetramer staining and enrichment. Our data revealed a substantial number of SARS-CoV-2 specific T cells that expressed memory phenotype markers, including memory cells with gut homing receptors. T cell clones generated from tetramer-labeled cells cross-reacted with bacterial peptides and responded to stool lysates in a MHC-dependent manner. Integrated phenotypic analyses revealed additional precursor diversity that included T cells with distinct polarized states and trafficking potential to other barrier tissues. Our findings illustrate a complex pre-existing memory pool poised for immunologic challenges and implicate non-infectious stimuli from commensal colonization as a factor that shapes pre-existing immunity.

Tu38. CLEC5A and TLR2 are Critical in SARS-CoV-2-induced Immunothrombosis and Lung Inflammation

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Coronavirus-induced disease 19 (COVID-19) infects more than three hundred and sixty million patients worldwide, and people with severe symptoms frequently die of acute respiratory distress syndrome (ARDS). Autopsy demonstrates the presence of thrombosis and microangiopathy in the small vessels and capillaries. Recent studies indicated that excessive neutrophil extracellular traps (NETs) contributed to immunothrombosis, thereby leading to extensive intravascular coagulopathy and multiple organ dysfunction. Thus, understanding the mechanism of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-induced NET formation would be helpful to reduce thrombosis and prevent ARDS. It has been shown that sera from individuals with COVID-19 triggered NET release in vitro, and spleen tyrosine kinase (Syk) inhibitor R406 inhibited NETosis caused by COVID-19 plasma. However, the serum components responsible for NET formation are still unknown. In this study, we found that virus-free extracellular vesicles (EVs) from COVID-19 patients (COVID-19 EVs) induced robust NET formation via Syk-coupled C-type lectin member 5A (CLEC5A). Blockade of CLEC5A inhibited COVID-19 EVs-induced NETosis, and simultaneous blockade of CLEC5A and TLR2 further suppressed SARS-CoV-2-induced NETosis in vitro. Moreover, thromboinflammation and lung fibrosis were attenuated dramatically in clec5a-/-/tlr2-/- mice. These results suggest that COVID-19 EVs play critical roles in SARS-CoV-2-induced immunothrombosis, and blockade of CLEC5A and TLR2 is a promising strategy to inhibit SARS-CoV-2-induced intravascular coagulopathy and reduce the risk of ARDS in COVID-19 patients.

Tu40. Humoral Response to Heterologous Prime-boost Vaccination with CoronaVac and BNT162b2 in Healthy Individuals and Solid Organ Transplant Recipients

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Vaccination against SARS-CoV-2 has proved to be a successful strategy for preventing severe COVID-19. CoronaVac and BNT162b2 are the most used vaccines worldwide, but little is known about their use in heterologous vaccination schedules. Based on local results and international evidence, Uruguay has become one of the first countries to authorize a booster with BNT162b to all the population previously vaccinated with either BNT162b or CoronaVac, and BNT162b2 complete re-vaccination in immunosuppressed individuals previously immunized with CoronaVac. In this work, we have evaluated the specific humoral response in a cohort of healthy individuals and in kidney and liver transplant recipients after fully vaccination with CoronaVac or BNT162b2, and after 3rd-dose or re-vaccination with BNT162b2. In the healthy group, we also evaluated anti-RBD IgGs levels 3 months after full vaccination and after 3rd-dose, along with their neutralizing activity, binding kinetics, and Fc-mediated functions (ADCC and ADCP). Healthy individuals displayed full seroconversion after two doses of CoronaVac. Antibody levels increased significantly after the third dose with BNT162b2 and exhibited a fall 73 days after the booster. Immunoglobulin functionalities showed similar behaviors. In transplant recipients, only a percentage seroconverted after full vaccination (41,2% in LTR and 29% KTR) and antibody levels were significative low. After booster, although antibody levels increased in responders and some non-responders seroconverted, a subgroup of non-responders still remained seronegative. This behavior is probably related to the previous state of immunosuppression. Our work also highlights the impact of researchers, clinicians, and authorities´ coordinated work to face the COVID-19 pandemic.

Tu46. Quantitative Analysis of SARS-CoV-2 Antibody Titer After the 3rd Dose of COVID-19 Vaccination

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Background: We performed quantitative analysis of SARS-CoV-2 antibodies after completing two doses of AstraZeneca (AZ) ChAdOx1 nCoV-19 vaccine and a booster dose of Pfizer BNT162b2 vaccine (Pfizer). Methods: A total of 160 serum samples were obtained from 80 healthcare workers 4 weeks and 4 months after a three-dose AZ-AZ-Pfizer vaccination. Twenty-seven (33.7%) of 80 participants experienced COVID-19 infection during this 4-month study period, and participants in the infected group stated that infection occurred 8 to 37 days before the second blood draw. Total antibody titers against the spike protein (anti-S) and nucleocapsid protein (anti-N) of SARS-CoV-2 were measured using the Elecsys anti-SARS-CoV-2 S and Elecsys anti-SARS-CoV-2 assays (Roche), respectively. Results: The median anti-S antibody titer at 4 months after the 3rd dose were significantly decreased in both the infected groups and non-infected groups compared with those after 4 weeks, and the infected group showed significantly higher antibody titers than the non-infected group (Infected group: from 21,178 to 18,108 U/mL vs. non-infected group: from 17,777 to 3,068 U/mL). The median titer of anti-N antibody titer at 4 months after the 3rd dose was 2.21 cut-off index (COI) (range: 0.186–37.4, 74.1% positivity) in the infected group. Conclusions: After a three-dose AZ-AZ-Pfizer vaccination, the median anti-S antibody titers decreased in both the infected and non-infected groups 4 months after the 3rd dose compared with those of 4 weeks after the 3rd dose. These data will provide useful information for determining quarantine strategies including the fourth vaccination.

Tu53. Rapid Waning and Unique Response Kinetics of Humoral and Cellular Immune Responses to SARS-CoV-2 Vaccination in Patients on Immunomodulatory Drugs

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Autoimmune diseases such as rheumatoid arthritis (RA) and scleroderma (SSc) are often treated with immunomodulatory drugs, reducing the strength and longevity of immune responses. Given the vulnerability of these populations to SARS-CoV-2 infections, understanding the efficacy of SARS-CoV-2 vaccination in patients with RA and SSc is critical for their protection. We examined the longevity and magnitude of serum antibody and memory T cell responses following SARS-CoV-2 vaccination in patients with RA or SSc, who are on immunomodulatory drugs, compared to age- and sex-matched controls. Blood and serum were collected from participants. SARS-CoV-2 spike- and receptor binding domain (RBD)-specific antibodies were quantified by ELISA. Spike-specific memory T cell responses were quantitated by flow cytometry using an activation induced marker assay. Following the second COVID-19 vaccination, participants with RA trended towards lower levels of spike-specific antibodies and CD4+ T cells than controls. Before dose 3, participants with RA exhibited rapidly waning antibody levels, which were rescued by the third COVID-19 vaccination. One month after dose 2, half of participants with SSc had low anti-RBD and anti-spike IgG, a deficit not observed in their spike specific T cells. By 3 months after dose 2, 3/4 participants with SSc who previously had low antibody levels exhibited increases in anti-spike IgG. In conclusion, participants with RA, who are on immunomodulatory drugs, may require shorter intervals between booster doses to maintain sufficient protection. Participants with SSc respond to two doses of a COVID-19 vaccine but can take longer to reach adequate antibody levels.

Tu56. Analysis of Antigen-Specific Cellular and Humoral Immune Response in COVID-19 Patients

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BACKGROUND: The cellular and humoral immune responses are essential towards COVID-19 recovery and protection against SARS-CoV-2 reinfection. To this day, the evaluation of the immune protection to SARS-CoV-2 has mainly been focused on the detection of antibodies; However a large body of evidence suggest T lymphocytes have a greater role to resolve an established infection.
OBJECTIVE: Compare the magnitude of the cellular and humoral immune response in 252 convalescent COVID-19 from mild to severe patients. METHODS: 252 individuals with COVID-19 (202 mild, 20 moderate and 30 severe COVID-19) were enrolled in the study. The study inclusion criteria included individuals age ≥18 years diagnosed by SARS-CoV-2 positive real-time reverse transcriptase PCR (RT-PCR) tests from nasopharyngeal swabs. Blood samples were collected two weeks after the first RT-PCR positive test. The IgG, IgA and IgM were assessed by an in-house Luminex-based assay. The N- and S-reactive T cells were evaluated by in-house ELISpot and CoVITEST assay. RESULTS: SARS-CoV-2-specific T cells were identified in 100% of mild COVID-19, 100% of moderate COVID-19 and 81% in severe COVID-19. On the other hand, anti-SARS-CoV-2 immunoglobulins were identified in 95% of mild COVID-19, 90% of moderate COVID-19 and 87.5% of severe COVID-19. We observed a strong SARS-CoV-2-specific T cell responses in mild COVID individuals compared to moderate and severe COVID-19. CONCLUSION: We show that the T-cell responses, unlike antibodies, are detected in practically all patients with COVID-19. Reinforcing the need of assessing cellular immunity, especially in vulnerable patients, instead of the humoral response.

Tu96. Epitope Profiling of COVID-19 Convalescent Plasma and Vaccine Elicited Antibodies

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Background: Immune responses to SARS-CoV-2 following infection or vaccination are highly variable, which may contribute to reinfection or vaccine breakthrough infections. A comprehensive evaluation of antibody binding to SARS-CoV-2 (CoV2) and endemic human coronaviruses (HCoVs) may identify individuals at increased risk for future infection and highlight the role of coronavirus cross-reactivity in antibody response potency. Methods: We generated all possible 56 amino acid peptide epitopes that could be produced by in-frame or out-of-frame translation of the CoV2 or HCoV genomes. We then quantified antibody reactivity to these epitopes via phage immunoprecipitation sequencing (PhIP-seq) of serum antibodies from 38 COVID-19 recovered individuals, 21 vaccinated individuals, and 13 vaccinated individuals who subsequently developed breakthrough infections. We additionally performed coronavirus focused PhIP-seq on 126 potential COVID-19 convalescent plasma (CCP) donations with known SARS-CoV-2 neutralization capacity. Results: CoV2 reactive antibodies from vaccinated and infected individuals rarely, if ever, targeted out-of-frame translation products. Spike epitope reactivities post-vaccination were well correlated to those following infection. Antibody reactivity to an immunodominant region of HCoV HKU1 spike occurred with significantly greater frequency in vaccinated individuals who went on to experience breakthrough infections compared to vaccinated individuals who did not become infected. Furthermore, potential CCP donations with antibody reactivity to this HKU1 epitope had relatively diminished SARS-CoV-2 neutralizing. Conclusions: Coronavirus epitope profiling revealed interplay between endemic coronavirus antibody reactivity and the CoV2 antibody response, supporting a role for exposures to HCoVs in shaping the CoV2 immune response at the epitope level.

Tu111. Severity of SARS-CoV-2 Infection is Linked to Double Negative (CD27- IgD-) B Cell Subsets Numbers

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Background: The role of B cells in COVID-19, beyond the production of specific antibodies against SARS-CoV-2, is still not well understood. Here, we describe the novel landscape of circulating Double Negative (DN) CD27- IgD- B cells in COVID-19 patients, representing a group of atypical and neglected subpopulations of this cell lineage. Methods: Using multiparametric flow cytometry, we determined DN B cell subsets (frequencies and absolute numbers) from a cohort of 91 COVID-19 patients, correlated those with cytokines, clinical and laboratory parameters, and segregated them by principal components analysis. Results: We detected significant increments in the DN2 and DN3 B cell subsets, while we found a relevant decrease in the DN1 B cell subpopulation, according to disease severity and patient outcomes. These DN cell numbers also appeared to correlate with pro or anti-inflammatory signatures respectively and contributed to the segregation of the patients into disease severity groups. Conclusion: This study provides insights into DN B cell subsets' potential role in immune responses against SARS-CoV-2, particularly linked to the severity of COVID-19. Supported by CONACyT A3-S-36875 and UNAM-DGAPA-PAPIIT IN212122.

Tu126. Efficacy and Safety of mRNA SARS-CoV-2 Vaccination in Hematopoietic Stem Cell Transplant Recipients: Single Centre Experience

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Background: Hematopoietic stem cell transplantation (HCST) has been considered for priority vaccination. However, as patients usually remain immunosuppressed for months after HSCT, vaccine efficacy might be compromised. The characterization of the immune response of transplanted patients could help to design more efficacious vaccination programs. Methods: SARS-CoV-2 vaccination with mRNA1273 vaccine (Moderna) was prospectively evaluated in haematological patients after HSCT and serological response was assessed after complete vaccination. In patients without specific anti-SARS-CoV-2 antibodies, we reassessed humoral and cellular response after receiving a third booster dose. Results: Eighty-seven patients (56 auto-HSCT and 31 allo-HSCT) received the complete vaccination regimen with two doses between March and May 2021. At 2-4 months, 86% of patients presented seroconversion. Median antibody response rate was higher for the allo-HSCT group (2578 UI/mL) compared to auto-HSCT group (1675.5 UI/mL). Twelve patients presented a persistent negative serological status after vaccination. We found an association between negative serological response and low B-lymphocyte count (< 113.5 cells/uL), low IgG (< 700 mg/dL) and treatment with anti-CD20 antibodies in the last year before vaccination. 11/12 patients without seroconversion received a third dose of the vaccine after which 5/11 patients achieved seroconversion and 6/11 presented cellular response. Conclusions: Seroconversion rate after mRNA SARS-CoV-2 vaccination in HSCT recipients is elevated although lower than in the general population (>90%). Anti-CD20 therapy, low B-lymphocyte count and hypogammaglobulinemia are associated with no serological response after vaccination. Specific immune monitoring after SARS-CoV-2 vaccination is indicated to identify booster dose candidates.

Tu137. Allogenic and Autologous Stem Cell Transplant (SCT) Recipients have Detectable Levels of SARS-CoV-2 Antigen-specific T Cells One Month After mRNA Vaccination

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Introduction: The recent outbreak of SARS-CoV-2 has posed a serious threat to immune-compromised individuals. Concerns have been raised over the effectiveness of mRNA vaccinations in cancer patient populations. This small, cross-sectional study evaluates the ability of SARS-CoV-2 mRNA vaccines to elicit T cell responses in leukemia, lymphoma, and myeloma patients post-allo or auto-SCT. Methodology: 7 allo- and auto-SCT recipients from the BC Cancer Agency were enrolled. Whole blood was collected at the time of SARS-CoV-2 vaccination (T1) and 3-4 weeks post dose 2 (T2). Antigen-induced marker assays were used to measure the magnitude of T cell responses overtime. Whole blood was stimulated for 48-hours in the presence of SARS-CoV-2 spike peptides at 37 C, and stained for CD25 and OX40 to measure $CD4^+$ antigen-specific T cells and CD69 and CD137 to measure $CD8^+$ antigen-specific T cells. Phenotyping of antigen-specific $CD4^+$ T cells was conducted to measure the frequency of Tregs, Th1, Th1.17, Th2, Th9, and Th17 T cell subsets. Results: All 7 individuals had detectable levels of antigen-specific $CD4^+$ T cells at T2. 4 individuals (57%) had detectable levels of antigen-specific $CD8^+$ T cells at T2. 4 individuals had sufficient antigen-specific $CD4^+$ T cell frequencies to conduct phenotyping. The response skewed towards Th2, with 50.85% of antigen-specific $CD4^+$ T cells being $CCR4^+ CCR6^-$. Conclusions: This promising preliminary data suggests allo- and auto-SCT recipients are capable of mounting antigen-specific T cell responses against the SARS-CoV-2 spike protein following mRNA vaccination. Additional timepoints are needed to evaluate the durability of this response.

Tu138. Sequence Similarity Between SARS-CoV-2 Nucleocapsid and CNS Proteins Provides Mechanistic Insight into Viral Neuropathogenesis Following Infection

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The novel coronavirus SARS-CoV-2 emerged in 2019 and continues to cause death and disease throughout the world. To date, there have been 272 million infections and 5.3 million deaths worldwide, underscoring the necessity of understanding the virus and host immune response as well as developing novel therapeutics for severe disease. From the start of the pandemic, a prominent pattern of central nervous system (CNS) pathologies – including cases similar to Multiple Sclerosis (MS) - has emerged that suggest a related, underlying mechanism of viral mimicry to proteins expressed by CNS tissues. We hypothesized that immunodominant epitopes of SARS-CoV-2 share homology with proteins typically associated with MS. Using Pepmatch, a bioinformatics package which predicts peptide similarity in the context of MHC presentation, we discovered that Nucleocapsid protein, but not Spike or Membrane proteins, of SARS-CoV-2 shares significant overlap with 22 MS-associated proteins. To provide further physiological relevance, we used an MHC binding prediction tool to determine whether these Nucleocapsid peptides would bind preferentially to MS-associated alleles. While we did not find any significant binding pattern among MS-associated alleles, we did discover that Nucleocapsid peptides which overlap with CD99 Antigen were among the top HLA-peptide predictions. This finding provides a plausible mechanism by which the targeting of CD99 by lymphocytes leads to compromised blood-brain-barrier integrity and exacerbated viral neuropathogenesis. This work provides a deeper understanding of the unique viral dynamics with the human immune system and corroborates the targeting of CD99 to ameliorate neurological complications of SARS-CoV-2.

Tu158. TNFα-producing CD4+ T Cells Dominate the SARS-CoV-2-specific T Cell Response in COVID-19 Outpatients and are Associated with Durable Antibodies

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SARS-CoV-2-specific $CD4^+$ T cells are likely important in immunity against COVID-19, but our understanding of $CD4^+$ longitudinal dynamics following infection, and specific features that correlate with the maintenance of neutralizing antibodies, remains limited. We characterized SARS-CoV-2-specific $CD4^+$ T cells in a longitudinal cohort of 109 COVID-19 outpatients. We observed that the quality of the SARS-CoV-2-specific $CD4^+$ response shifted from cells producing IFNγ to TNFα from five days to four months post-enrollment, with $IFN\gamma^-IL21^-TNF\alpha^+$ $CD4^+$ T cells the predominant population detected at later timepoints. These $IFN\gamma^-IL21^-TNF\alpha^+$ cells highly correlated with activation induced marker $(AIM)^+$ $CD4^+$ T cell populations, including $ICOS^+$ circulating T follicular helper cells (cTfh). Greater percentages of $IFN\gamma^-IL21^-TNF\alpha^+$ $CD4^+$ T cells on day 28 correlated with SARS-CoV-2 neutralizing antibodies measured seven months post-infection (ρ=0.4, P=0.01). Similar positive correlations were observed with activated $ICOS^+$ cTfh and neutralizing antibodies. mRNA vaccination following SARS-CoV-2 infection boosted both IFNγ and TNFα producing, spike protein-specific $CD4^+$ T cells. These data suggest that SARS-CoV-2-specific, TNFα-producing $CD4^+$ T cells may play an important role in antibody maintenance following COVID-19.

Tu228. Comprehensive Assessment of SARS-CoV-2 Antibodies After COVID-19 Infection and Vaccination (BNT162b2 or ChAdOx1 nCoV-19) Using Multiplex Bead Assay

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Comprehensive assessment of SARS-CoV-2 antibodies is essential for monitoring after infection or vaccination. Thirty-two COVID-19 patients and forty naïve healthcare workers (HCWs) following two doses of Oxford–AstraZeneca (AZ) (n=20) or Pfizer–BioNTech (Pfizer) (n=20) vaccines were sequentially tested using multiplex bead-based assay (One lambda) (anti-full S, N, S1, S2, RBD), Roche (S1 RBD total Ig) and GenScript (neutralizing IgG). For COVID-19 patients, anti-S, S1 and RBD levels were higher than anti-S2 or N for 15-40 days after infection. Severe disease showed higher anti-S, S1, S2, and RBD levels than mild disease ($p < 0.05$). Comparing post-infection and post-prime vaccination, anti-S2, N and RBD levels were higher post-infection than post 1st-dose AZ vaccination. After 2nd-dose vaccination, anti-S, S1, S2 and RBD levels were higher in vaccinated HCWs ($P < 0.05$). Neutralizing antibodies levels were not different between post-infection and post-2nd-dose AZ group ($P > 0.05$). Comparing AZ and BNT group, 1st-dose BNT showed higher anti-full S, S2 and neutralizing antibody levels than 1st-dose AZ ($P < 0.05$). After boost vaccination, anti-full S, S1, RBD and neutralizing antibody levels were higher in BNT than AZ ($P < 0.05$). Evaluating intra-individual changes from post-prime to post-boost vaccination showed that AZ boost increased anti-S2 (3.5-fold) and neutralizing antibody (1.5-fold), and BNT boost increased anti-S1 (1.8-fold), S2 (1.5-fold), RBD (1.3 fold) levels. The dynamics and antibody levels assessed in natural infection and vaccination were assay-dependent. Understanding of vaccine versus infection-induced antibody responses will contribute to adjusting vaccination strategies and accelerating vaccination efficacy.

Evidence of Induction and Sustained Expression of Autoantibodies Six Months After SARS-CoV-2 Infection

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Autoantibodies are commonly present during acute COVID-19. If these antibodies are pre-existing, induced post-infection, and/or maintained months into convalescence is unclear. Also, if autoantibody trajectories post-infection differ between inpatient and outpatient subjects is unknown. We measured the expression of 17 autoantibodies linked to autoimmune connective tissue diseases from five subject groups: (1) pre-pandemic, (2) SARS-CoV-2+ inpatient, (3) SARS-CoV-2+ outpatient, (4) scleroderma, and (5) systemic lupus erythematosus. Autoantibody levels from SARS-CoV-2+ subjects were followed over time from initial symptom onset for an average of six months. Cross-sectional analysis revealed that six of the 17 autoantibodies measured were higher on average in inpatient and/or outpatient SARS-CoV-2 subjects six months after symptom onset compared with prepandemic controls. Autoantibody trajectories over time from longitudinal sample sets were differentially scored based on expression changes and two key findings were revealed: (1) a 'newly induced and sustained' expression pattern was found for at least one autoantibody in 18% of the outpatient and 63% of the inpatient subjects, indicating initiation and durable expression of new, self-reactive memory immune reponses post-infection; and (2) 64% and 83% of positively scored autoantibodies from the inpatient and outpatient groups, respectively, exhibited an 'always positive' pattern beginning from the initial days of symptom onset, suggesting pre-existing self-reactive immunity as a putative driver of symptomatic COVID-19 after viral exposure. As positivity to individual autoantibodies precedes presentation of clinical autoimmune disease, SARS-CoV-2 may create a potent "fertile field" of self-reactive immune cells that will spur autoimmune disease with a future immune trigger.

W65. Longitudinal Dynamics of SARS-CoV-2 Specific Cellular Immunity to Different Vaccination Schemes

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Evaluation of the longitudinal immune responses induced by various SARS-CoV-2 vaccination regimens is still lacking. We aimed to investigate the cellular immunities in uninfected immunocompetent after different COVID-19 vaccinations. SARS-CoV-2 specific T cell responses were evaluated using 309 serial samples from 53 vaccinated healthcare workers (35 AZD1222/BNT162b2, 11 mRNA-1273, 7 Ad26.COV2.S). INF-γ enzyme-linked immunospot (ELISpot) and two commercial INF-γ release assays (QuantiFERON SARS-CoV-2, Covi-FERON) were used. The highest levels of SARS-CoV-2 specific T cell immunity were detected at 8 weeks after 2nd mRNA-1273 vaccination (mean 312.0 spots/106 PBMC), and after 4 weeks from booster shot (mean 329.3 spots/106 PBMC). The AZD1222 vaccine showed the highest level at 4 weeks after the first vaccination (mean 142.8 spots/106 PBMC), while Ad26.COV2.S showed peak response at 8 weeks after vaccination (mean 64.6 spots/106 PBMC). QuantiFERON showed a higher level of IFN-γ at 4-8 weeks after the mRNA-1273 2nd vaccination. Covi-FERON showed an increased IFN-γ level at 4 weeks after the mRNA-1273 2nd shot and after the booster shot. AZD1222 induced increased IFN-γ until 12 weeks after 2nd vaccination, and after the BNT162b2 booster shot. Ad26.COV2.S showed a continuously increased level up to 12 weeks after vaccination. Strong correlations were shown between two INF-γ release assays, whereas ELISpot showed weak correlations with INF-γ release assays. All of ELISpot, QuantiFERON and Covi-FERON showed significantly higher median results as the neutralizing antibody response increased (all $P < 0.05$). We found a slightly different SARS-CoV-2 specific T cell response was observed depending on the vaccines.

W75. A 10-Month Period Evaluation of Cellular Responses After ChAdOx1-nCoV-19 and BNT162b2 Vaccinations

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Background: Since the outbreak of COVID-19, vaccines against SARS-CoV-2 were developed. While many studies evaluated the humoral responses to the vaccinations, data on cellular responses are sparse. We evaluated T-cell responses after ChAdOx1-nCoV-19 and BNT162b2 during 10 months. Methods: From March to December 2021, interferon (IFN)-gamma was measured with Covi-FERON ELISA (SD Biosensor Inc., Suwon, South Korea) in blood samples from 91 individuals. For the ChAdOx1-nCoV-19-vaccinated group (N=36), three weeks/three months/five months after the second dose and three weeks after the BNT162b2 booster dose were measured. For the BNT162b2-vaccinated group (N=30), three months/six months after the second dose and three weeks after the booster dose were measured. Control group (N=25) consisting of unvaccinated individuals without history of COVID-19 infection and negative for SARS-CoV-2 anti-nucleocapsid IgG was also included. Results: The median (IQR) IFN-gamma value (IU/mL) of the control group was -0.01 (-0.03–0.00). In ChAdOx1-nCoV-19, medians were 0.31 (0.12–0.69), 0.14 (0.04–0.24), 0.14 (0.06–0.41), and 1.92 (0.73–5.01) for three weeks, three months, five months, and three weeks after booster, respectively. In BNT162b2, medians were 0.54 (0.36–1.01), 0.31 (0.16–0.86), and 1.93 (1.13–4.00) for three months and six months after the second dose, and three weeks after booster, respectively.
Conclusions: Both ChAdOx1-nCoV-19- and BNT162b2-vaccinated group showed high T-cell responses in contrast to unvaccinated group. The responses were declined afterwards but were highly increased after booster. This could be a baseline data on T-cell responses after SARS-CoV-2 vaccination and IFN-gamma assay would be useful in monitoring immunogenicity in vaccinated population.

W87. A Versatile New Monoclonal Antibody for Elucidating Human TMPRSS2 Expression and Function

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Transmembrane Serine Protease 2 (TMPRSS2) is a cellular type II transmembrane serine protease expressed in human respiratory epithelium that cleaves and activates the SARS-CoV-2 spike protein. This activation is essential for viral infectivity. Blocking TMPRSS2 activity with protease inhibitors inhibits SARS-CoV-2 infection. TMPRSS2 is overexpressed in human cancers and promotes metastasis by cleavage of extracellular matrix proteins. In prostate cancer, increased expression of TMPRSS2 is associated with progression, invasion and metastasis. However, the normal physiological role for TMPRSS2 remains poorly understood. Methods for inhibiting TMPRSS2 are urgently needed to further understand TMPRSS2 function and for potential therapy. Current tools for the study of TMPRSS2 function are limited to protease inhibitors which have low specificity for individual proteases, limiting their use for therapeutic approaches and functional studies of TMPRSS2. We developed a mouse monoclonal antibody against human TMPRSS2 (clone S20014A) and performed specificity testing by flow cytometry, immunofluorescence and in functional assays. Clone S20014A specifically binds human TMPRSS2 and shows positive signal by flow cytometry in on human colon and prostate carcinoma cells and on human platelets. Clone S20014A detects expression of TMPRSS2 in human colon tissue sections by immunofluorescence. Furthermore, this antibody can inhibit the protease activity of recombinant TMPRSS2 similar to the broad-spectrum protease inhibitor Nafamostat. Clone S20014A blocks invasion of the human colon carcinoma cell line Caco-2, with potency similar to Nafamostat. In conclusion, anti-human TMPRSS2 (clone S20014A) is a valuable tool for the study of TMPRSS2 function and expression in human cells.

W90. CSF Inflammatory and Neuroaxonal Damage Biomarkers in COVID-19 Associated Encephalopathy and Encephalitis

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The frequency and spectrum of neurologic manifestations of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection have been described, but many questions remain regarding the underlying mechanisms related to central nervous system involvement. Sixty patients with COVID-19 and new-onset neurological symptoms were included in the study. We measured 14-3-3 protein, IgG oligoclonal bands, intracellular and neuronal surface autoantibodies and a panel of chemokines, cytokines and other soluble factors in serum and CSF. We then compared the results obtained with age-matched healthy control subjects. Compared to HC, in the COVID cohort, IL-18, IL-6, and IL-8 levels were elevated in both serum and CSF; IL-10, IL-1RA, IP-10, MIG and NfL were elevated in serum only, and MCP1 was elevated in CSF only. Antibodies against neural antigens were all negative. No differences were found in serum and CSF levels of cytokines, NfL or 14-3-3 protein when comparing patients according to their neurologic diagnosis. 14-3-3 and NfL CSF levels in addition to serum levels of IL-18, IL-1RA, IL-8 and NfL, were significantly associated with the severity of the acute COVID-19 systemic disease. Furthermore, 14-3-3 protein and NfL levels in CSF significantly correlated with the long-term functional outcome measured by the mRS. Several proinflammatory cytokines are significantly associated with acute COVID-19 severity. Furthermore, levels of 14-3-3 and NfL in CSF could be useful as biomarker of long-term functional outcome.

W112. B Cell Subsets as Severity-associated Signatures in COVID-19 Patients

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SARS-CoV-2 infection represents a global health problem that has affected millions of people. The fine host immune response and its association with the disease course have not yet been fully elucidated. Consequently, we analyze circulating B cell subsets and their possible relationship with COVID-19 features and severity. Using a multiparametric flow cytometric approach, we determined B cell subsets frequencies from 52 COVID-19 patients, grouped them by hierarchical cluster analysis, and correlated their values with clinical data. The frequency of $CD19^+$ B cells is increased in severe COVID-19 compared to mild cases. Specific subset frequencies such as transitional B cell subsets increase in mild/moderate cases but decrease with the severity of the disease. Memory B compartment decreased in severe and critical cases, and antibody-secreting cells are increased according to the severity of the disease. Other non-typical subsets such as double-negative B cells also showed significant changes according to disease severity. Globally, these differences allow us to identify severity-associated patient clusters with specific altered subsets. Finally, respiratory parameters, biomarkers of inflammation, and clinical scores exhibited correlations with some of these subpopulations. The severity of COVID-19 is accompanied by changes in the B cell subpopulations, either immature or terminally differentiated. Furthermore, the existing relationship of B cell subset frequencies with clinical and laboratory parameters suggest that these lymphocytes could serve as potential biomarkers and even active participants in the adaptive antiviral response mounted against SARS-CoV-2.
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W125. SARS-CoV-2 Antibody Kinetics in Healthcare Workers in Northern Metropolitan Barcelona

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Background: Understanding humoral responses and seroprevalence in SARS-CoV-2 infection is essential for guiding vaccination strategies in both infected and uninfected individuals. Methods: We determine the kinetics of IgM against the nucleocapsid (N) and IgG against the spike (S) and N proteins of SARS-CoV-2 in a cohort of 860 health professionals (healthy and infected) in northern Barcelona. We model the kinetics of IgG and IgM at nine time points over 13.5 months from infection, using non-linear mixed models by sex and clinical disease severity. Results: Of the 781 participants who were followed up, 478 (61.2%) became infected with SARS-CoV-2. Significant differences were found for the three antibodies by disease severity and sex. At day 270 after diagnosis, median IgM(N) levels were already below the positivity threshold in patients with asymptomatic and mild-moderate disease, while IgG(N, S) levels remained positive to days 360 and 270, respectively. Kinetic modelling showed a general rise in both IgM(N) and IgG(N) levels up to day 30, followed by a decay whose rate depended on disease severity. IgG(S) levels increased at day 15 and remained relatively constant over time. Conclusions: We describe kinetic models of IgM(N) and IgG(N, S) SARS-CoV-2 antibodies at 13.5 months from infection and disease spectrum. Our analyses delineate differences in the kinetics of IgM and IgG over a year and differences in the levels of IgM and IgG as early as 15 days from symptoms onset in severe cases. These results can inform public health policies around vaccination criteria.

W175. Immunological Profiles of HIV-infected and Uninfected Patients Admitted with COVID-19 in Tshwane

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People living with HIV (PLHIV) could have worse COVID-19 outcomes due to persistent immune deficiency and dysregulation. This study determined and compared the immunological profiles and clinical outcomes of PLHIV and HIV-uninfected patients admitted with COVID-19 to hospitals in Tshwane, South Africa. One hundred and thirty SARS-CoV-2 PCR-positive patients with moderate to severe COVID-19 were recruited from April 2020 to July 2021. Whole blood samples were obtained during the first day of hospitalization and T-cell and monocyte phenotyping performed on a CytoFlex flow cytometer. Data were analyzed using FlowJo v10.7.1 and the Cytobank platform. Twenty-six patients were PLHIV: median CD4 count 212 cells/mm^3; suppressed HIV viral load in 61%. PLHIV were younger (45.5 ±10.81versus 53 ±13.34 years), more likely to be female (73.08% versus 40.38%) and had less severe disease (ROX score 16.2 [IQR 8.8- 22.62] versus 7.92 [IQR 4.35-14.12]) than HIV-uninfected patients. Mortality was similar between the groups: 15.38% versus 7.69% in PLHIV (p=0.838). PLHIV had lower CD4+ (p=0.0001) and higher CD8+ (p=0.0001) T-cell counts, higher CD8+ naïve (p=0.049) and lower CD8+ effector (p=0.021) and exhausted T-cells (p=0.0079), and lower CCR2 expression on monocytes (p=0.0065). After adjusting for ROX, PLHIV had fewer CD4+ effector T-cells (p=0.0006). Differences in CD8+ T-cells remained together with lower CD8+ effector memory-1 (p=0.028), PE2 (p=0.028) and TEMRA (p=0.0098), higher effector memory-2 (p=0.0042) subsets, higher proportions of non-classical monocytes (p=0.0041) and lower CCR2 expression (p=0.0058) in PLHIV. We suggest that skewed CD8+ T-cell maturation could be protective against development of hyper-inflammation during COVID-19 in PLHIV.

W185. Fc Structure and Function of Antibodies Elicited by COVID-19 mRNA Vaccine and Infection

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The outbreak of the COVID-19 pandemic, with its associated tremendous health and economic consequences, has sparked a worldwide effort to design vaccines using different platforms. Messenger RNA-based vaccines against COVID-19 induce a robust anti-SARS-CoV-2 antibody response with potent viral neutralization activity. Antibody effector functions are determined by its constant region subclasses as well as by its glycosylation patterns, but their role in vaccine efficacy is unclear. Moreover, whether vaccination induces antibodies similar to those in patients with COVID-19 remains unknown. This study was designed to investigate the overall IgG response, IgG subclass distribution, subclass-specific Fc glycosylation patterns and effector functions of IgGs produced following administration of the SARS-CoV-2 mRNA-based BNT162b2 vaccine and to compare these to the response to natural infection with SARS-CoV-2. For this purpose, we analyzed plasma samples from adults (aged ≥ 18-year-old) from the vaccine cohort 2 weeks post each vaccine dose and from patients with mild or severe symptoms at corresponding time points from diagnosis. We analyze BNT162b2 vaccine-induced IgG subclass distribution and Fc glycosylation patterns and their potential to drive effector function via Fc-gamma receptors and complement pathways. We identified unique and dynamic pro-inflammatory Fc compositions that are distinct from those in patients with COVID-19 and convalescences. Vaccine-induced anti-spike IgG is characterized by distinct Fab- and Fc-mediated functions between different age groups and in comparison, to antibodies generated during natural viral infection. These data highlight the heterogeneity of Fc responses to SARS-CoV-2 infection and vaccination and suggest that they support long-lasting protection differently.

Immuno-engineering and Cellular Therapies

Th1. Clinical Grade CD8+T Regulatory Cells Moving Forward in Human Transplantation

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We have demonstrated the therapeutic potential of human polyclonal and antigen-specific CAR-modified CD8$^+$Tregs cell therapy to prevent allogeneic human skin transplantation rejection and xenogeneic GvHD in humanized NSG mice (Bézie et al., Front Immunol.2017, Blood Adv.2019). However, their potential has never been evaluated in a clinical trial. We are thus preparing the launch of a phase I/IIa human clinical trial using polyclonal CD8$^+$Tregs cell therapy in living donors kidney transplant patients. To do so, we develop a new GMP-compatible cell manufacturing process. First, we determined a new method of isolation of CD8$^+$Tregs from peripheral blood using a safe closed system. Next, we identified the optimal clinically compatible culture medium, refined cytokines doses and stimulation methods to preserve a high proliferation rate, phenotypic profile and suppressive function of CD8$^+$Tregs. Using this process, we were able to efficiently expand CD8$^+$ Tregs from peripheral blood of patients with renal failure, with high purity, while preserving their regulatory function. We confirmed the absence of cytotoxicity. In addition, we showed that CD8$^+$Tregs were phenotypically and functionally stable for 4h after conditioning, which is important for the logistic delay between cell harvest and patient infusion. Finally, we showed that CD8$^+$Tregs persist for more than 90 days in NSG mice without inducing any signs of xenogeneic GVHD, and could withstand combined immunosuppressive drug therapy administered to the patient. To conclude, we designed a clinically compatible manufacturing process to isolate and culture CD8$^+$Tregs from renal failure patients that preserves their function and patient safety.

Th26. CRISPR-Mediated Deletion of PD1 to Enhance the Efficacy of Human CAR Tregs

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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. This is a particularly important consideration in the context of type I diabetes and islet transplantation as β-cells are known to express PDL1, a ligand of PD1. 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. Upon stimulation with $HLA\text{-}A2^+$ cells, PD1-deficient CAR Tregs upregulated activation markers (e.g. 4-1BB) to a greater degree than PD1-sufficient CAR Tregs. Thus, PD1-deficient CAR Tregs are more sensitive to antigen stimulation and may be more therapeutically efficacious upon adoptive transfer. Future work will explore the functional consequences of removing PD1 in CAR Tregs and test the therapeutic efficacy of these cells in a humanised mouse islet transplant model.

Th33. Developing Islet Antigen-Specific Regulatory T Cell For Therapy in Type 1 Diabetes

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Early-phase clinical trials of *Ex vivo* expanded polyclonal $CD4^+$ T regulatory cells (Tregs) in Type 1 Diabetes (T1D) have shown persistence of the infused Tregs and promising safety profile. However, efficacy in stopping islet destruction has not yet been demonstrated, likely due to the very low frequency of islet antigen-specific Tregs among the polyclonal Treg preparations. Chimeric antigen receptor (CAR) is one potential strategy to redirect polyclonal Tregs to islet antigens. We thus constructed islet-specific CARs using previously validated nanobodies for human DPP6, a protein preferentially expressed by human islet endocrine cells. Nanobodies derived from camelidae antibodies have the advantages of being smaller, having a more stable structure, lower immunogenicity, and better access to target antigens than single chain variable fragments of antibodies that are commonly used in CAR construction. DPP6-CARs with IgG4 hinge, CD28 transmembrane, and CD28 and CD3 zeta signaling domains are expressed at the cell surface of primary human T conventional cells (Tconvs) and Tregs. When co-cultured with human islets, DPP6-CAR expressing T cells are strongly and specifically activated. In addition, DPP6-CAR Tregs suppress *in vitro* Tconv proliferation more efficiently than polyclonal Tregs. Since mouse islets do not express DPP6, we have developed a humanized T1D mouse model in which transplanted human islets or pluripotent stem cells-derived beta-like cells (BLC) are rejected by islet antigen-specific human Tconvs. Ongoing experiments are evaluating the *in vivo* suppressive capacity of DPP6-CAR Tregs. Results from this study will inform future efforts in translating this strategy to the clinic.

Th39. The Role of Chimeric Antigen Receptor (CAR) Versus Endogenous Co-stimulation in the Function of Alloantigen-specific Tregs

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Cell therapy with regulatory T cells (Tregs) can diminish rejection and their efficacy can be improved by expression of an alloantigen-specific CAR. In pursuit of optimized CARs for use in Tregs, we and others explored the effect of CAR-encoded co-stimulatory domains finding that inclusion of a CD28 co-stimulatory domain is optimal for human CAR-Treg. However, a limitation was the use of immunodeficient mice which lack a full complement of antigen presenting cells. Here we aimed to re-visit the question of optimal CAR design in an immunocompetent Bl/6-based mouse model of skin transplantation. CARs specific for HLA-A2 encoding CD3z with or without intracellular domains from CD28, PD1, ICOS, GITR, OX40 or 41BB were generated and expressed in mouse Tregs. In vitro proliferation and cytokine production revealed superior effects of the CD28-encoding CAR, confirming data from human CAR-Tregs. Surprisingly, In vivo CAR-Treg function showed no significant difference between CD28 and CD3zeta alone CAR-Tregs to extend allograft survival. Moreover, we found that CD28 and CD3zeta CAR-Tregs were equal in their ability to suppress expression of CD86 and CD80 on HLA-A2+ splenic DCs. Hypothesizing that co-stimulation from the DCs could influence CAR-Treg function, we tested effects of CD86+ or CD86- HLA-A2+ cells, finding that CD86-mediated stimulation through endogenous CD28 can partially replace the requirement for a CAR-encoded CD28 domain. Our results demonstrate that CAR-Tregs require co-stimulation via CD28, which can be delivered via the CAR or DC-CAR-Treg interactions. Optimization of CAR-design should consider signals mediated by physiologically-relevant cell-cell interactions and use fully immunocompetent models.

Th50. Elucidating Inflammation-induced Cell Fate Decisions in Primary Human Tregs

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Adoptive regulatory T cell (Treg) therapy is an emerging therapeutic paradigm for promoting immune tolerance in transplant and autoimmune disease patients. However, accumulating evidence has exposed a susceptibility for murine Tregs to undergo epigenetic reprogramming within chronically inflamed tissue environments, resulting in acquisition of proinflammatory functions and the capacity to exacerbate tissue damage. Despite the safety implications of Treg lineage decommitment for cell therapy applications, inflammation-driven human Treg cell fate decisions remain poorly understood. Here, we investigate the stability of human Treg identity during chronic *in vitro* exposure to proinflammatory cytokines. Over the course of repeated anti-CD3/28 stimulations amid sustained IL6, IL1β, and IL23 exposure, primary human Tregs progressively downregulated FOXP3 and HELIOS, re-methylated the *FOXP3* conserved non-coding sequence (CNS)2 enhancer region, lost *in vitro* suppressive function, and acquired proinflammatory cytokine expression. To gain insight into the gene regulatory networks driving Treg dysfunction, we used single-cell multiome (RNA + ATAC) sequencing to profile primary human Tregs maintained in the presence or absence of IL6, IL1β, and IL23. Unbiased clustering revealed a dysfunctional Treg population with an epigenetic signature characteristic of murine Treg to “exTreg” conversion, including altered chromatin accessibility at the *IFNγ*, *IL17A*, and *FOXP3* CNS2 loci. Inference of transcription factor (TF)-associated chromatin accessibility indicated a key role for E26 transformation-specific (ETS) family members. Ongoing experiments involving CRISPR-mediated perturbations aim to dissect the roles of specific TFs in safeguarding or unraveling the Treg epigenome, enabling the development of strategies to ensure safe implementation of Treg therapies.

Th61. The Tolerogenic Effects of IL-2 on T Regulatory Cells (Tregs) are TGF-β Dependent

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We and others have previously shown that IL-2 is essential for TGF-β to convert naïve $CD4^+CD25^-$ T cells into $CD4^+CD25^+Foxp3^+$ Tregs. Here we extend this finding by showing that the tolerogenic effects of IL-2 on Tregs are TGF-β dependent. Using IL-2-loaded nanoparticles (NPs) coated with anti-CD2 antibody (Ab) to target both T cells and NK cells, we recently identified the critical importance of TGF-β-producing NK cells in the induction of $CD4^+$ and $CD8^+$ $Foxp3^+$ Tregs which prevented murine lupus-like disease. Here we used anti-CD2 Ab-coated NPs to inhibit a human anti-mouse graft versus host disease (GVHD) that develops after transfer of human PBMC into immunodeficient NOD/SCID mice. We found that NPs containing only IL-2 protected mice from GVHD similarly to NPs containing both IL-2 and TGF-β. Remarkably, the blockade of TGF-β signaling with an ALK-V inhibitor not only abolished the protective effects of the NPs but also reduced the survival of the mice. Thus, in the absence of TGF-β, IL-2 induces pathogenic T effector cells instead of promoting the induction of protective Tregs. This key role of TGF-β in the induction of Tregs is relevant to ongoing clinical trials that employ low-dose IL-2 or IL-2 muteins in SLE and other autoimmune diseases to numerically expand functional Tregs. Because lymphocyte production of TGF-β is decreased in SLE, this study suggests the need to correct the deficits of both IL-2 and TGF-β for the optimal induction and expansion of functional Tregs in the immunotherapeutic management of SLE and other immune-mediated disorders.

Tu71. Synthekines: A Novel Platform for Combinatorial Engineering of Cytokine Receptor Agonists

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Cytokines are secreted immunomodulatory proteins that activate key signaling pathways by receptor dimerization. While many cytokines have been approved as therapeutics, native structure restricts their therapeutic potential to pathways activated by their cognate cytokine receptors. At Synthekine we have implemented a platform to dimerize native and non-native pairs of cytokine receptors using single domain (VHH) antibodies, allowing us to design synthetic cytokine mimics that generate targeted and modulated signals on key cell types to improve on first generation cytokine therapeutics.

Tu76. A Robust, Xeno- and Feeder-Free Culture System for Expansion of Human Peripheral Blood NK Cells

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Natural killer (NK) cells are lymphocytes that play an important role in innate immunity against tumors and viral infections by secreting proinflammatory cytokines and exhibiting cytotoxicity. NK cells hold promise for the development of cancer immunotherapies against solid tumors. We have developed a culture system for expansion of peripheral blood NK cells without feeder cells, serum, or xeno components. Freshly isolated NK cells were cultured for two weeks in ImmunoCult™ NK Cell Expansion Medium and on ImmunoCult™ NK Cell Expansion Coating Material. $CD56^+CD3^-$ NK cells expanded 88 ± 17-fold (n = 34, mean ± SEM), with an average frequency of 87 ± 1%. To assess functionality, the expanded NK cells were stimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin, or co-cultured with K562 cells. On average, 75 ± 4.0% of NK cells stimulated by PMA/ionomycin and 48 ± 3.6% of NK cells stimulated by K562 cells expressed intracellular interferon-γ (n = 13). Stimulated NK cells also expressed surface CD107a, a marker of degranulation (88 ± 4.8% [PMA/ionomycin] and 74 ± 5.8% [K562 co-culture]). Directed NK cell killing of target K562 cells was measured in co-cultures. K562 cells were treated with a substrate that is cleaved by activated caspase-3/7 resulting in the release of a fluorescent dye. At an effector:target ratio of 1:1, an average of 48 ± 2.4% of K562 cells were killed (n = 9). Therefore, this culture system enables xeno- and feeder-free generation of large numbers of functional NK cells.

Tu80. Discovery of T Cell Receptors for Adoptive T Cell Therapy Against Solid Tumors

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Many solid tumors do not respond well to available therapies and are associated with poor survival. A promising treatment strategy is the adoptive transfer of T cells engineered to overexpress tumor-specific T cell receptors (TCRs). At the heart of this strategy are clinically relevant, safe and functional TCRs specific for antigens expressed by tumor cells. Discovery of TCRs has been challenging in the past due to the low frequency of tumor-reactive T cells in tumor samples and in peripheral blood. Addressing this challenge, ImmunoScape's discovery efforts are focused on isolating rare cancer-targeting TCRs, primarily by mining peripheral blood samples from healthy donors and cancer patients. Our Deep Immunomics platform leverages a multiplexed tetramer technology that allows high-sensitivity and high-throughput screening of PBMC for reactivity against >350 T cell antigen specificities in one experiment. Downstream single cell sequencing technology enables the combined analysis and mining of phenotype, transcriptome, TCR specificity, and TCR sequence to build our library of TCRs against both novel and well characterized tumor antigens. We will present the identification and assessment of select TCR candidates that validate our discovery workflow and are part of a portfolio of potential clinical candidates.

Th127. Engineered Single Amino Acid Substitutions Protect Therapeutic Cells From CD123 Targeted Immunotherapy in vitro

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Therapeutic depletion of diseased cells using monoclonal antibodies, T cell engagers or chimeric antigen receptor (CAR) cells is very effective. Whereas B cell targeted immunotherapies are highly specific, the co-expression of myeloid markers such as CD33 or CD123 on healthy hematopoietic stem and progenitor cells (HSPCs) bears the risk for unintentional myelotoxicity. In order to enable therapeutic targeting of such proteins it was proposed to transplant engineered HSCs in which the target was removed. However, since removing the protein abolishes its function, this approach is limited to redundant proteins and bears a certain risk for antigen-negative tumor relapse. Here, we show feasibility for shielding the therapeutic cells from targeted therapy while preserving the function of the targeted receptor. Structure-guided computer modeling identified 28 single amino acid (aa) substitutions in CD123 designed to interfere with CSL362 (talacotuzumab) binding but preserve CD123 function. Using flow cytometry of cells expressing the variants we identified variants for which the single aa substitution resulted in complete non-binding while CD123 remained normally expressed. Cells expressing non-binding variants were shielded from antibody dependent cellular cytotoxicity (ADCC), T cell engager or CAR T-mediated killing. Protein characterization of select variants demonstrated normal biophysical properties of most variants. Further functional investigation is ongoing for variants with normal binding of Interleukin-3. Thus, our data demonstrates the feasibility to engineer proteins harboring single aa substitutions that shield therapeutic cells from all tested cell depleting modalities while preserving their function. We propose that this concept could be broadly applicable to targeted immunotherapies.

Th139. Engineering Optimized Autoreactive T Cells for the Study of Type 1 Diabetes Pathogenesis

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A hallmark histopathological feature of Type 1 Diabetes (T1D) is infiltration of β-cell specific T cells into the insulin producing Islets of Langerhans. These cells are key mediators of disease, but due to scarcity in circulation there remains a paucity of information regarding their phenotype and function. This has been circumvented by the generation of antigen specific T cells through lentiviral vector-mediated gene transfer, though the main caveat to this approach is the potential for T cell receptor (TCR) chain mispairing. The consequences of chain mispairing can range from reduced antigen specific functionality to off-target reactivity, thus complicating functional testing, and ultimately reducing therapeutic potential in the case of antigen-specific regulatory T cell (Treg) adoptive cell therapy applications. Our lab has extensive experience generating GAD-specific Tregs and IGRP-specific $CD8^+$ T cells by lentiviral transduction. With the goal of extending this work, we deleted the endogenous TCR a-chain gene (*TRAC*) via CRISPR/cas9 in these cell subsets. We observed enhanced antigen specificity as shown by increased peptide MHC-dextramer staining, as well as CD69 and CD25 upregulation after stimulation with cognate antigen. TRAC KO in $CD8^+$ T cells increased antigen-specific killing of b-cell lines when compared to unmodified cells. Importantly, TRAC KO Tregs maintained purity and epigenetic stability. Hence, we present a TCR-gene editing protocol that results in efficient deletion of endogenous TCR and robust enhancement of antigen-specific T cell function, which could be used for the study of autoreactive T cells or downstream development of antigen-specific Treg therapeutics with enhanced antigen affinity.

Th140. CAR-T Cells Mediated B Cell Depletion in EAE-induced Disease

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Chimeric antigen receptor (CAR)-T cells are T cells expressing non-MHC antigen specific receptor. We tested whether antiCD19 CAR-T cells could recapitulate the beneficial effects of B cell depletion in experimental autoimmune encephalomyelitis (EAE) mouse models. Female wild-type C57BL/6 mice were treated with cyclophosphamide (preconditioning for CAR-T treatment) and anti-CD19 CAR-T cells or activated control T cells. EAE was induced by immunization with recombinant human (rh) myelin oligodendrocyte protein (MOG) (B cell-dependent) or MOG peptide (p) 35-55. Mice were evaluated daily for clinical signs of EAE and weekly for peripheral B/T cell counts. B cell levels, T cell immune modulation, and histopathology were assessed at termination. In rhMOG-induced EAE, clinical scores were reduced in mice treated with cyclophosphamide and anti-CD19 CAR-T cells or control T cells in comparison to WT or cyclophosphamide alone treated mice. B cell depletion occurred only in the anti-CD19 CAR-T cell treated group, including within brain/spinal cord. There was no difference in T cell modulation including Th1, Th17, or Treg populations. Clinical scores did not differ between treatment groups in p35-55-induced disease. Histopathology is pending. T cell therapy (CAR-T and control) resulted in less severe disease in B cell dependent EAE, but its effect was independent of B cell depletion or endogenous T cell. The mechanism for this observation has not been explained. The capability of anti-CD19 CAR-T cells to penetrate the CNS and more thoroughly deplete B cells than monoclonal antibodies suggest they may hold promise for depletion of CNS B cells implicated in progressive MS.

Th146. Chimeric Antigen Receptor Regulatory T Cells (CAR-Tregs) Provide Significantly Greater Modulation of Alloimmune Responses than Alternative Alloantigen-Specific Treg Strategies

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Conferring alloantigen-specificity to *Ex vivo* expanded CD4+CD25+Foxp3+ Tregs increases their capacity to counteract effector alloimmune responses following adoptive transfer into transplant recipients. This has been achieved in humans by: 1) expanding Tregs in the presence of donor B cells (donor-reactive DR-Tregs); 2) culturing Tregs in the presence of donor material and co-stimulation blockade (CSB-Tregs); and 3) transducing Tregs with a HLA-A2-specific chimeric antigen receptor specific (CAR-Tregs). All 3 modalities of manufactured Treg products are currently undergoing clinical development. We lack, however, a clear understanding of the relative potency of each of these strategies. Our goal here was to directly compare the 3 manufacture approaches both i*n vitro* and In vivo*.* Antigen-specificity and suppressive function of the cell products were evaluated *in vitro* by performing activation, proliferation, and T cell suppression assays. Therapeutic efficacy was assessed *in vivo* in a xenogeneic GvHD model in humanized NSG mice. As compared to polyclonal Tregs, all 3 strategies increased the proportion of alloantigen-specific Tregs within the manufactured cell product, with the highest level being observed in the CAR-Treg products. The differences in the precursor frequency of alloreactive Tregs were consistent with the results of the suppression assays, in which on a cell-per-cell basis CAR-Tregs exhibited the strongest effects, followed by DR-Tregs and CSB-Tregs. In vivo, infusion of CAR-Tregs together with HLA-A2+ PBMCs into irradiated NSG mice resulted in most prolongation of recipient survival. Results conclude that the CAR technology is a more powerful strategy in conferring alloantigen specificity to *Ex vivo* expanded Treg products.

Tu105. A Screening Platform for Drug Effects on Vaccine Responses Using Human Tonsil Immune Organoids

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The SARS-CoV-2 pandemic has reaffirmed the importance of vaccines in public health; however, efficacy is variable. Patients receiving immunosuppressive medications are at particularly high risk of poor responses, though there is no rapid mechanism to screen vaccines in the presence of drug compounds to inform patient care. We recently developed an *in vitro* model of the human immune system using dissociated tonsil cells. These cultures mimic hallmarks of *in vivo* vaccine effects, including somatic hypermutation, class switching and secretion of high affinity antibodies. We adapted the system as a screening tool to detect adverse drug effects on vaccination efficacy. We established immune cultures growing in 96-well plate format. Vaccination using a seasonal Live Attenuated Influenza Vaccine (LAIV) resulted in influenza-specific IgG antibody production with robust Z' factor of 0.87 and signal to noise ratio (S/B) of 22, suitable for high-throughput screening. We tested the system using dilution series of clinically relevant but mechanistically diverse immunosuppressive medications. Methotrexate and mycophenolic acid (MPA) potently inhibited influenza-specific IgG at nanomolar concentrations, whereas hydroxychloroquine and cyclosporine A impaired responses in the micromolar range. In depth mechanistic profiling of organoids vaccinated in the presence of MPA using flow cytometry revealed impaired germinal centre B cell and plasmablast differentiation, though no changes in CD4+ T cell activation. In summary, the system functions as a rapid and efficient method to screen how various medications impact antigen specific responses during vaccination. It provides a platform for testing novel and existing compounds to better predict clinical vaccine efficacy.

Tu108. Immunogenicity Induced by Full Academic CD19 Chimeric Antigen Receptor (ARI0001) in Patients Recruited into the CART-BE-01 Clinical Trial

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Background: ARI-0001 cells express a second generation CAR19, which combines the anti-CD19 scFv originated from A3B1, a murine monoclonal antibody, and intracellular domains. It has been approved for the treatment of acute lymphoblastic leukemia (ALL) in patients older than 25 years of age by the Spanish Agency of Medicine in 2021 under hospital exemption. Even though its safety and efficacy have been demonstrated, there is still lack of data about the relevance of the immunogenicity it triggers. Methods: Regarding the humoral immune response, the presence of human anti-murine antibodies (HAMA) in patients' sera was assessed at different time-points. Afterwards, cytotoxicity assays were performed to appraise whether these antibodies diminished the efficacy of ARI-0001 cells. About the cellular immunogenicity, CAR19 peptides were predicted *in silico* in relation to the HLA of the patients. Results: A total of forty-seven patients received ARI-0001 cells. Approximately twenty-five per cent of the patients tested positive for the presence of anti-CAR antibodies a hundred days after the first or second infusion. Even though twenty-five per cent had anti-CAR antibodies, only some of them were diminishing the effectiveness of CAR-T19 *in vitro*. In relation to the prediction *in silico*, a set of 103 prioritized immunogenic peptides were selected to assess the T-cell response. Conclusions: The presence of HAMA diminishes the efficacy of ARI-0001 cells *in vitro* and the most immunogenic peptides are derived from the scFv. So, these data suggest that the assessment of immunogenicity induced by CAR-T19 could be important, especially before considering a second infusion.

Tu117. Deep Molecular Comparison Between Lymphoid Progenitor Cells Differentiated from CB or mPB CD34+ Hematopoietic Stem Cells Cultivated on Notch Ligand DLL4 Through a Clinically Compatible Culture Procedure

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Ex vivo generated Human lymphoid progenitors ($CD34^-CD7^+$) have demonstrated their ability to seed patient thymus and to achieve a polyclonal T cell repertoire reconstitution faster and more efficiently than classical $CD34^+$ hematopoietic stem and progenitor cell (HSPC) treatment. Currently under investigation in 3 clinical trials, this approach aims to improve significantly patient prognosis after Hematopoietic Stem Cell Transplantation (HSCT). $CD34^+$ HSPC used as starting material are isolated from mobilized peripheral blood (mPB) or from Cord Blood (CB) units. In preclinical studies, both products demonstrated similar T lymphoid potential *in vitro* and *in vivo*. However, impact of $CD34^+$ HSPC source was obvious in terms of production efficacy. CB-derived cells were indeed associated with significantly higher expansion and $CD34^-CD7^+$ cell purity (>90%). Here, we describe a comparative analysis between lymphoid progenitors produced from CB or mPB using a complementary single-cell RNA sequencing (scRNAseq) and mass cytometry (CyTOF) approach. This in-depth characterization revealed a diversity of the $CD7^+$ subset, as well as the presence of non-lymphoid cell subsets, differentially represented according to the $CD34^+$ HSPC source used. Molecules associated with a known or putative role in thymus homing and function were also differentially expressed between CB and mPB-derived $CD34^-CD7^+$ lymphoid progenitors. The potential impact of these differences in the clinics will be assessed. Altogether, our study provides interesting insights on lymphoid cells differentiation and highlights the potential impact of $CD34^+$ cell source on advanced therapies production.

Tu229. Two-dimensional Materials for Biomedical Applications: MXenes Immune Profiling

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Two-dimensional (2D) transition metal carbides/carbonitrides (MXenes) are rapidly growing as nanoplatforms in biomedicine. Considering the central importance of the immune response for any clinical translation of nanomaterials, we evaluated the effects on human peripheral blood mononuclear cells (PBMCs) of three MXenes: $Mo_2Ti_2C_3$, Nb_4C_3, and Ta_4C_3. We assessed immune cell functionality by measuring 40 cytokines by Luminex technology. The materials exhibited only a slight modulation on the inflammatory mediators analyzed. Next, we investigated their effects on PBMC by mRNA sequencing. By performing principal component analysis on all analyzed transcripts (N=19,959), we found that MXene-treated samples clustered close to the controls, and separately from concanavalin A (ConA)- and lipopolysaccharide (LPS)-treated samples, thus indicating that only minimal perturbations were induced by the materials. Overall, we did not observe genome-wide changes induced by MXenes. All materials showed modest effects on PBMCs, modulating the expression of fewer genes than the positive controls ConA and LPS. In particular, even if all materials had similar effects on gene expression modulation, Ta_4C_3 had slightly greater effects than $Mo_2Ti_2C_3$ and Nb_4C_3. The expression of 142, 46, and 83 genes was modulated by Ta_4C_3, $Mo_2Ti_2C_3$, and Nb_4C_3, respectively, whereas the positive controls ConA and LPS modulated the expression of 4,340 and 710 genes, respectively. Furthermore, pathway-enrichment analyses on genes coherently modulated by the materials showed only modest enrichment of immune-related pathways without a clear indication of activation or inhibition. Our results provide a compendium of knowledge on the biocompatibility of MXenes for the safe development of nanosystems in biomedicine.

W4. Tolerogenic Dendritic Cells Engineered with a Lentiviral Vector Encoding for IL-10 and Antigen Control T Cell Responses to Pathogenic Antigens

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Tolerogenic dendritic cells (tolDC) play a crucial role in promoting tolerance and represent the cells of choice to fulfill the goal of restoring antigen(Ag)-specific tolerance in autoimmunity. Effective DC-based therapy should dampen autoreactive T cell responses, inducing pathogenic T cell exhaustion, and restore long-term tolerance*via* Ag-specific regulatory T cell (Treg) induction. Here we show that *in vitro* differentiation of DC *via*transduction with bidirectional lentiviral vectors encoding for the Ag of interest (Ovalbumin or Matrix Protein 1 as model Ags, or insulin-/gliadin-derived peptides, as disease-specific Ags) in combination with IL-10 from both human and murine precursors allows efficient generation of tolDC ($DC^{IL\text{-}10/Ag}$), which secrete high amounts of IL-10 in the absence of pro-inflammatory cytokines. $DC^{IL\text{-}10/Ag}$ efficiently modulated *in vitro* activation and proliferation of Ag-specific $CD4^+$ and $CD8^+$ T cells. Repetitive administration of $DC^{IL\text{-}10/Ag}$ *in vivo* promoted OVA-specific $CD4^+$ type 1 regulatory T (Tr1) cells, $CD8^+$ T cells with an exhausted phenotype ($CD44^+PD\text{-}1^+LAG\text{-}3^+TIM\text{-}3^+$) and prevented T1D development in two distinct murine models of disease. Human $DC^{IL\text{-}10/Ag}$ promoted the *in vitro* induction of Ag-specific $CD49b^+LAG\text{-}3^+$ T cells, which displayed the Tr1 cell gene signature and modulated gliadin-specific $CD4^+$ T cell responses of Celiac Disease patients. We developed engineered tolDC that effectively modulate pathogenic $CD4^+$ and $CD8^+$ T cell responses *in vitro* and *in vivo,* by promoting Ag-specific Tr1 cells and $CD8^+$ T cells with an exhausted phenotype. These results lead to the definition of an innovative approach based on the use of autologous DC to restore tolerance in T cell-mediated diseases.

W11. Combination of ImmTOR Tolerogenic Nanoparticles and IL-2 Mutein Induces Massive Expansion of Antigen-specific Regulatory T Cells

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We have previously demonstrated that ImmTOR nanoparticles encapsulating rapamycin induce selective immune tolerance to co-administered antigens. Unlike Treg-selective IL-2 muteins, ImmTOR does not expand total Tregs; however, we hypothesized that ImmTOR could act synergistically with IL-2 muteins. Here we demonstrate that a single dose of ImmTOR co-administered with IL-2 mutein resulted in increased and more durable total Tregs. We next evaluated the expansion of antigen-specific Treg in mice with adoptively-transferred ovalbumin-specific OTII T cells. As expected, ImmTOR + ovalbumin did not expand total Treg, but increased the percentage of Foxp3+ OTII cells from ~3% to ~15%. IL-2 mutein + ovalbumin resulted in a modest increase that was similar to that observed with ovalbumin alone. However, the combination of ImmTOR + IL-2 mutein + ovalbumin showed a profound synergistic effect, with ~45% of OTII cells expressing Foxp3. We tested whether ImmTOR and IL-2 mutein would enable more durable inhibition of antibody responses to co-administered AAV gene therapy vectors. Mice treated with AAV8 vector on Days 0 and 56 developed a robust anti-AAV IgG antibody response. Treatment with IL-2 mutein showed only a modest reduction in anti-AAV IgG antibodies. ImmTOR showed dose-dependent inhibition of anti-AAV antibodies; however some mice showed delayed development of antibodies. In contrast, the combination of ImmTOR + IL-2 mutein completely inhibited antibody formation through Day 117, even at sub-optimal doses of ImmTOR. These results show that the combination of ImmTOR and IL-2 mutein has the potential to provide more durable antigen-specific immune tolerance to mitigate unwanted immune responses.

W19. MHC-deficient CD8+ Tregs as Universally Compatible Cell Therapy

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Adoptive Treg therapy has shown to be effective in a variety of immune diseases in preclinical studies (Bézie et al., Front Immunol.2017, Blood Adv.2019) and is currently moving to phase I and II to demonstrate its effectiveness. However, autologous Tregs are expensive and slow to produce, which is why we are considering using ready-to-use allogeneic Tregs for future cell therapy. First, to explore the feasibility of off-the-shelf $CD8^+$Treg-cell based therapy, we investigated the capacity of $CD8^+$Tregs to control allogeneic immune responses. Interestingly, $CD8^+$Treg could inhibit third-party APC-induced allogeneic effector T cell proliferation *in vitro* as efficiently as syngeneic cells. In addition, $CD8^+$Tregs still control the development of GVHD induced by PBMCs from of foreign donor *in vivo* in humanized NSG mice. However, as $CD8^+$Tregs express high levels of MHC I and II after *Ex vivo* expansion, we evaluated their immunogenicity against allogeneic T cells. As expected, $CD8^+$Tregs activated and initiated the proliferation of allogeneic $CD8^+$ and $CD4^+$ effector T cells. Thus, we developed a method to invalidate HLA molecules using CRISPR/Cas9 technology. By targeting *B2M* and *CIITA* genes encoding the proteins required for the expression of MHC-I and II respectively, we succeeded in generating MHC-I and MHC-II deficient $CD8^+$Tregs with preserved suppressive function, and which, unlike MHC^+CD8^+Tregs, did not activate effector T cell proliferation *in vitro*. To conclude, we have shown the therapeutic potential of MHC-deficient $CD8^+$Tregs for off-the-shelf cell therapy. Experiments are underway to explore the impact of MHC-I deficiency of $CD8^+$Tregs on their susceptibility to NK cell lysis.

Regulation of Allogeneic T Cells by Human Group 2 and Group 3 Innate Lymphoid Cells and Applications for Cell Therapy

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Harnessing regulatory immune populations in cell-based therapies for transplantation is a promising tolerance-promoting approach. Innate lymphoid cells (ILCs) are a family of lymphocytes that rapidly respond to tissue signals and orchestrate immune responses to maintain tissue homeostasis. In mice, group 2 ILCs (ILC2s) and group 3 ILCs (ILC3) were shown to limit allogeneic T cell responses and enhance regulatory T cell function, supporting their potential in promoting immune tolerance in transplantation. To assess human ILC2 and ILC3's cell therapy potential, we developed methods to isolate and expand human ILC2s and ILC3s from blood in a manner that maintains expression of signature cytokines and transcription factors. Single cell RNAseq and flow cytometric analysis revealed both ILC2s and ILC3s expanded using our approach also express high levels of the regulatory cytokine IL-10 and the tissue repair factor amphiregulin, consistent with dual immunoregulatory and reparative potential. Using a xenograft model of graft-versus-host disease (GVHD), we show that adoptive transfer of human ILC2s and ILC3s reduces the severity of xenograft GVHD and prolongs survival of NOD-scid-IL2Rγ^{null} mice. ILC2s and ILC3s reduce proliferation of allogeneic CD4$^+$ and CD8$^+$ T cells and ILC2s limit inflammatory T cytokine production. Ongoing studies are delineating mechanism harnessed by allogeneic ILC2s and ILC3s to limit T cell responses, but *in vitro* studies support human ILC2s directly regulate allogeneic T cells through secreted factors. Our findings support that human ILC2s and ILC3s limit harmful T cell responses and may have applications in adoptive cell-based therapies for transplantation.

W62. Osteosarcoma Tumorigenesis is Affected by Macrophage Behavior in 3D Engineered Bone Marrow Model

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Osteosarcoma (OS) is the most common primary malignant bone cancer in children and adolescents, yet treatment has remained unchanged for 4 decades and offers less than a 25% 5-year survival rate for those with metastatic disease. This underscores a critical lack of understanding of OS progression and necessitates the study of this disease in a novel system. Here, we adapt an engineered bone marrow (eBM) construct for use as an *in vitro* model for OS research. We first optimized eBM synthesis by testing the influence of the anatomical implantation site on the quality of generated tissue. Compared to constructs formed subcutaneously, eBM formed subfascially better recapitulated native mouse bone marrow. Next, homotypic cultures of highly metastatic K7M2 and less metastatic K12 OS cell lines were loaded into the eBM. eBM was also loaded with heterotypic cultures comprised of each cell line and macrophages at an 85:15 ratio and cultured under 21% and 5% O_2. Flow cytometry for OS cell number and macrophage polarization revealed that both K7M2s and K12s under heterotypic culture exhibited significantly decreased cell number only under 21% O_2. However, M1 and M2 macrophage behavior were not consistent between K7M2s and K12s, suggesting that the inflammatory status of the immune microenvironment does not dictate OS behavior as previous studies often conclude. The eBM in this project will advance the treatment of patients afflicted by OS by mimicking the complex environment in which OS arises, thereby surpassing other *in vitro* models that fail to account for these key parameters.

W68. Human CD4+CD25+ Regulatory T Cells Deficient of CD226 Demonstrate Increased Purity and Lineage Stability Following *Ex vivo* Expansion for Adoptive Treg Therapies

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Regulatory T cell (Treg) therapy is an emerging strategy for restoring immune tolerance in autoimmune diseases. Tregs are commonly purified as $CD4^+CD25^+CD127^{lo/-}$ T cells, yielding a population enriched in the expression of the canonical thymically-derived Treg (tTreg) transcription factors FOXP3 and Helios. Previous work has identified the type 1 diabetes risk-associated locus and costimulatory molecule CD226 as being highly expressed on both effector T cells and interferon-γ-producing peripheral Tregs (pTregs). Thus, we sought to determine whether isolating $CD4^+CD25^+CD226^-$ Tregs yields a Treg population with increased purity and suppressive capacity relative to $CD4^+CD25^+CD127^{lo/-}$ cells. After 14d of culture, expanded $CD4^+CD25^+CD226^-$ Tregs displayed a decreased proportion of pTregs relative to $CD4^+CD25^+CD127^{lo/-}$ cells as determined by the $FOXP3^+Helios^-$ expression and the epigenetic signature at the *FOXP3* Treg-specific demethylated region (TSDR). Furthermore, $CD226^-$ Tregs exhibited decreased production of the effector cytokines, IFN-γ, TNF-α, and IL-17A, along with increased expression of the inhibitory cytokine TGF-β1 as well as a higher suppressive capacity *in vitro* compared to their $CD127^{lo/-}$ counterparts. To corroborate the pathogenic role of CD226 in Treg stability, we conducted CRISPR-Cas9 gene knockouts (KOs) of *CD226* on $CD127^{lo/-}$ Tregs. Following expansion, *CD226* KO Tregs demonstrated higher proportions of total Tregs and tTregs, decreased proportions of pTregs, as well as increased expression of CD25 and TIGIT compared to non-edited controls. Overall, these data suggest the elimination of CD226 signaling during in vitro expansion generates a tTreg population with increased purity, lineage stability, and suppressive capabilities for adoptive cell therapies for the treatment of autoimmune diseases.

W118. *Ex vivo* Production Of Immunotherapeutic NK Cells From Cord Blood Or Mobilized Peripheral Blood CD34+ Cells Using Notch Ligand Delta-Like 4 Culture System

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NK cell immunotherapy is becoming an attractive alternative to T-cell immunotherapy because they do not cause graft-versus-host disease, and not induce cytokine release syndrome. However, the limited availability of efficient clinical-grade *Ex vivo* NK cell expansion, purification and genetic modification methods restricts the application of NK cell immunotherapy. Here, we developed an efficient, scalable, feeder cell-free and clinically applicable method for generating large numbers of immunotherapeutic NK cells from $CD34^+$ cells based on our recently published immobilized DL-4 culture system supplemented with TNFα. The $CD7^+$ progenitors obtained from DL-4/+TNFα cultures of CB or mPB $CD34^+$ cells were able to efficiently differentiate into NK cells with frequencies up to >90% with an efficient transduction at 1 and 2 weeks upon subjecting them to feeder cell-free NK cell differentiation. A single CB HSPC could give rise up to 10000 NK cells. The NK cells expressed the activation receptors and NK cell transcription factors, but not expressed the inhibitory KIRs and KLRG1. They expressed the cytotoxic molecules perforin and granzyme B and could be induced to express IFNg and TNFα, and undergo degranulation upon stimulation with myelogenous leukaemia cell line K562 cells. They could kill both K562 and THP1 cells. Therefore, the combination of our DL-4 culture system with NK cell differentiation/expansion procedure may provide a feeder-cell free efficient and clinically applicable method to produce large numbers of immunotherapeutic pure NK and CAR NK cell population. These results lay a groundwork towards an effective NK cell therapy platform for cancers and viral infections.

W119. Neurofilament Light Chain Levels as a Potential Biomarker of Immune effector Cell-Associated Neurotoxicity Syndrome (ICANS) in Patients Treated with CAR T Cell Therapy

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The Immune effector Cell-Associated Neurotoxicity Syndrome (ICANS) is an adverse event associated to Chimeric Antigen Receptor (CAR) T-cell therapy. Neurofilament light chain (NfL) levels have been proposed as a biomarker of neuroinflammation. We hypothesize that NfL could be employed as a biomarker of ICANS risk and severity in CAR T-cell therapy. We retrospectively measured serum or plasma NfL levels in a cohort of 29 patients who received CAR T-cells against CD19 (n=24) or BCMA (n=5), and 53 age matched healthy donors (HD). Fifteen patients presented ICANS of any grade. Blood samples were obtained at pre, intra and post ICANS episodes in ICANS study group; and at day 0, +7 and +28 after CAR T-cell infusion in patients without ICANS (NO-ICANS). Both ICANS and NO-ICANS showed higher levels of NfL in comparison to HD prior to CAR T-cell infusion (median: 36.65pg/mL; 52.84pg/mL; 14.96pg/mL). Grade 3-4 ICANS and grade 1-2 ICANS had a NfL mean of 57.2pg/mL and 28.5pg/mL during episodes, respectively; while NO-ICANS showed a mean of 43.6pg/mL at day+7. Significant differences comparing grade 3-4, 1-2 grade ICANS and NO-ICANS were not observed. Differences in NfL levels between ICANS (47pg/mL) and NO-ICANS (53.5pg/mL) were not detected post ICANS resolution and at day+28, respectively. High baseline NfL levels were present in both ICANS and NO-ICANS groups before CAR-T cell therapy. Differences in NfL levels were not observed in patients with ICANS of any grade compared to NO-ICANS group. NfL levels may not help to assess ICANS risk or severity in our cohort.

W121. Modulating Innate Immune Signaling with Nanomaterials to Drive Antigen-Specific Tolerance

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Autoimmune diseases like multiple sclerosis (MS) occur when the immune system targets self-antigens, attacks and damages host tissues. Therapies that promote antigen-specific tolerance are a transformative goal to specifically counter this attack while leaving protective immunity intact. One exciting possibility to promote antigen-specific tolerance is to modulate innate immune signaling pathways and regulate the immune response against self-antigens delivered as a therapeutic. Toll-like receptors (TLRs) control innate signaling pathways that direct the first line of defense against invading pathogens. Remarkably, activation of TLR pathways has also been observe in MS. Thus, control of TLRs by MS therapeutics during antigen-delivery may promote tolerance. We hypothesized that therapeutics self-assembled from a TLR9 regulatory cue (GpG) and myelin self-antigen (MOG) would promote tolerance and counter autoimmunity. To test this hypothesis, a small library of therapeutics was self-assembled through electrostatic interactions to form nanostructured microcapsules. Excitingly, therapeutics containing both MOG and GpG reversed established paralysis in a pre-clinical model of MS, while leaving healthy immunity intact (Oakes RS, *et al.*, *ACS Nano*, 2021). We then isolated MOG and GpG into separate formulations, which showed that MOG was required for therapeutic efficacy and GpG enhanced efficacy. Gene expression analysis of disease and treatment draining lymph nodes showed that GpG inclusion in microcapsules decreased *Tnf* and *Myd88* (a TLR master regulator) expression *in vivo*, but soluble GpG alone did not. These data indicate that composition and biophysical presentation (soluble vs. microcapsule) are important design parameters for manipulation of immune signaling to drive therapeutic tolerance.

W157. Segregating the Regional Effects of Aging and Blood Immune Factors on Age Related Brain Athrophy

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Immunology and neuroscience are historically distant fields with limited intercommunication partially justified by the observation that the brain blood barrier keeps the brain isolated while microglia provide its immune defense. The newly developing science of aging has revealed that the chronic presence of proinflammatory immune factors has effects on accelerating the aging process in multiple tissues including the central nervous system. Noninvasive volumetric MRI scans have identified the loss of brain volume as a hallmark of the aging brain. Here we study a cohort of 554 subjects using structural MRI scans and blood immune proteins concentrations. We identify a set of age predictive proteins whose concentrations change with age (TNF_I, IL-6, MCP-1, IP-10, Eotaxin, VEGF-D, VEGF, PLGF and Vcam-1). From these we extract a cytokine clock (CyClo) that can estimate the subject age (±6 years). The proteins with the highest contributions to the clock are known for roles in vascular growth (VEGF, VEGF-D and PLGF), general levels of inflammation (TNF_I, IL-6), chemoattraction for monocytes/macrophages (MCP-1, IP-10), recruitment of eosinophils into sites of inflammation (Eotaxin) and promote the interaction between vasculature and immune cells (Vcam-1). Using machine learning we correlate the variance in volume of multiple functionally determined cortical networks with the age, sex and CyClo of our subjects. We find that the variability in volume of the different networks and these three factors associate differentially thus suggesting targeted vulnerabilities of certain functional networks (default mode, limbic, dorsal attention network) to circulating levels of immune markers of aging and chronic inflammation.

Immunogenetics

Tu57. Genotype-Phenotype Correlation of T cell Subtypes Reveals Senescent and Cytotoxic Genes in Alzheimer's Disease

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Following the discovery of several key risk variants for Alzheimer's disease (AD) in genes related to immune function, much has been done to catalogue the genetic landscape and transcriptomic profiles of microglia and T cells in health and disease. Several recent studies investigate expression quantitative trait loci (eQTL), which correlate risk variants with gene expression changes, in T cells. However, these studies primarily involve healthy young donors, which may preclude detection of age- or disease-related phenotypic changes. We have collected whole-genome and RNA sequencing data to detect eQTL in four T cell subsets from 96 participants in the Religious Orders Study/Memory and Aging Project (ROSMAP) cohort of aged and AD subjects. Gene co-expression modules reveal several genes related to IL-10 signaling in one module, and genes related to cytotoxicity and immunosenescence in another. We also compared single nucleotide polymorphisms (SNPs) from the trans-eQTL to disease-associated SNPs from the GWAS catalog database. We discovered that several dozen SNPs among the four T cell subsets matched GWAS catalog SNPs previously correlated with traits related to immune function, neurodegenerative disease, autoimmune disease, and psychiatric disorders. Running pathway analysis on genes that included SNPs from the trans-eQTL between their transcription start site and end site returned top biological pathways related to synaptic function, axon guidance, and nerve cell development, suggesting that neurodevelopmental processes could regulate adaptive immune responses. We are currently investigating key genes and variants in cell culture and mouse models to lend mechanistic relevance to our findings and identify additional therapeutic targets.

Tu59. Multicohort Meta-Analysis of Bronchial Epithelial Cells Classifies Asthma from Healthy Subjects and Identifies Pathways in Asthma

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Individuals with asthma exhibit a broad range of symptoms, triggers, and responses to treatments despite carrying the same diagnosis. The current view describes two general endotypes, “T-helper cell 2 (T2)-high” and “T2-low”. However, these two classes incompletely describe the spectrum of asthma and the true number of endotypes remains unclear, particularly within the Th2-low population. We hypothesized a robust set of genes that accurately distinguishes patients with asthma from healthy subjects can be identified by integrating publicly available data sets. We used seven transcriptome data sets of 366 bronchial epithelial cell (BEC) samples from 148 healthy controls (HC) and 218 patients with asthma used as “discovery cohorts.” Using a Bayesian multi-cohort analysis framework, we identified 505 differentially expressed genes. A BEC score, defined as the difference between the geometric mean of over-expressed genes and under-expressed genes, distinguished patients with asthma from HCs with high accuracy with summary area under the receive operating characteristic curve (AUROC) of 0.906 (range: 0.841-0.986) in the discovery cohorts. We used 311 BEC samples from the Severe Asthma Research Program (SARP), an intensive and comprehensive characterization study, as validation cohorts. The BEC score distinguished patients with asthma with AUROC of 0.844 (range: 8.22-8.70), demonstrating it is robust to the real-world heterogeneity. Importantly, in 4 cohorts (2 discovery, 2 validation) with samples from each severity group, the BEC score significantly correlated with severity (JT trend test p-value < 1e-06).

Tu98. Development of a Classification Criteria-based Polygenic Risk Score for Systemic Lupus Erythematosus

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Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by a heterogenous clinical phenotype that may present differently over time and between patients, which can create challenges for identifying cohorts of patients with SLE. The Systemic Lupus International Coordinating Clinics (SLICC) classification criteria characterize the myriad of manifestations and laboratory tests that collectively define SLE. Previously published algorithms for the identification of SLICC attributes and SLE International Classification of Disease (ICD) instances were adapted to the Electronic Medical Records and Genomics (eMERGE) Network, which combines biorepository and electronic health record data at multiple health centers across the US. Cases were defined by satisfying of four or more SLICC attributes or both a positive antinuclear body and renal SLICC attribute. A cohort of cases and controls were identified within eMERGE and stratified into African (n = 4,152) and European ancestry (n = 33,036) groups. A genome-wide association study utilizing the criteria-based case definition was conducted for each ancestry group and summary statistics from each were used to calculate the best fit polygenic risk scores (PRS). PRS for patients with ≥3 ICD SLE instances at least 30 days apart were significantly higher than patients with < 3 SLE ICD instances in both European Americans (4.19e-4 versus -9.54e-5, p=3.24e-245) and African Americans (4.16e-3 versus 1.12e-3, p=1.44e-138). The results suggest that a rule-based algorithm consisting of individual disease components can be successfully utilized as a case definition for population genetics studies.

Tu114. Prediction of HLA Genotypes from Single-cell Transcriptome Data

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The human leukocyte antigen (HLA) locus plays a central role in adaptive immune function and has significant clinical implications for tissue transplant compatibility and allelic disease associations. Studies using bulk-cell RNA sequencing have demonstrated that HLA transcription may be regulated in an allele-specific manner and single-cell RNA sequencing (scRNA-seq) has the potential to better characterize these expression patterns. However, quantification of allele-specific expression (ASE) for HLA loci requires sample-specific reference genotyping due to extensive polymorphism. While genotype prediction from bulk RNA sequencing is well described, the feasibility of predicting HLA genotypes directly from single-cell data is unknown. Here we evaluate and expand upon several computational HLA genotyping tools by comparing predictions from human single-cell data to gold-standard, molecular genotyping. The highest 2-field accuracy averaged across all loci was 76% by arcasHLA and increased to 86% using a composite model of multiple genotyping tools. We also developed a highly accurate model (AUC 0.93) for predicting *HLA-DRB345* copy number in order to improve genotyping accuracy of the *HLA-DRB* locus. Genotyping accuracy improved with read depth and was reproducible at repeat sampling. Using a metanalytic approach, we also show that HLA genotypes from PHLAT and OptiType can generate ASE ratios that are highly correlated (R^2 = 0.8 and 0.94, respectively) with those derived from gold-standard genotyping.

Tu156. A GATA3 Frameshift Mutation Alters DNA-binding Causing Loss of Th2 Polarization in a Patient with Hypoparathyroidism, Sensorineural Deafness, and Renal Dysplasia Syndrome

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GATA3 is a highly conserved transcription factor important in development and in the immune system. It is the master transcription factor driving T helper type 2 (Th2) polarization. GATA3 acts as a homodimer with each monomer containing transactivation domains, zinc finger domains, and an uncharacterized C-terminus. Heterozygous loss-of-function mutations in GATA3 cause hypoparathyroidism, sensorineural deafness and renal dysplasia (HDR) syndrome. GATA3 mutations causing loss of one functional allele decrease Th2 function. However, the impact of GATA3 frameshift mutations on Th2 function has not been assessed. We identified a heterozygous frameshift elongation mutation, $GATA3^{M401Vfs*106}$, in a patient with HDR syndrome and juvenile idiopathic arthritis. $GATA3^{M401Vfs*106}$ has intact DNA binding domains and disrupts the poorly understood C-terminus. An *in vitro* Th2 polarization assay was performed on patient cells. The Th2 polarized cells were examined for GATA3 expression, gene transcription, cytokine production, and GATA3 DNA-binding. $GATA3^{M401Vfs*106}$ Th2 cells expressed GATA3 and $GATA3^{M401Vfs*106}$ by Western blot. $GATA3^{M401Vfs*106}$ Th2 polarized cells had decreased Th2 function identified by lower production of the cytokines IL-5 and IL-13. Chromatin immunoprecipitation sequencing analysis of genome wide GATA3 DNA-binding in $GATA3^{M401VfsX106}$ Th2 polarized cells identified unique and overlapping binding peaks throughout the genome with enrichment of non-GATA3 associated motifs. This study identified that frameshift mutations in the C-terminus of GATA3 decrease Th2 function by altering GATA3 DNA binding, gene expression, and production of cytokines. This study reveals that the GATA3 C-terminus has an important role in Th2 polarization.

W63. DiSiR: A Software Framework to Identify Ligand-receptor Interactions at Subunit Level from Single-cell RNA-sequencing Data

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Most of cell-cell interactions and crosstalks are mediated by ligand-receptor interactions. The advent of single-cell RNA-sequencing (scRNA-seq) techniques has enabled characterizing tissue heterogeneity at single-cell level. Over the past recent years, many methods have been developed to study ligand-receptor interactions at cell type level using scRNA-seq data. However, the packages depend on databases and do not provide a convenient way to query specific user-defined signaling pathway and produce quick visualizations. Here, we present Dimer Signal Receptor (DiSiR), a fast and easy-to-use software framework to investigate how individual cells are interacting with each other by analyzing signaling pathways of multi-subunit ligand-activated receptors from scRNA-seq data, even for the genes that are not listed in available databases. DiSiR produced publication-quality heatmaps, as well as bubble plots, for the genes of interest.

W66. Association of CTLA-4 (AT)n Genetic Variants in Basal Cell Carcinoma and Squamous Cell Carcinoma in Patients from Western Mexico

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Introduction: The incidence of nonmelanoma skin cancer (NMSC) is constantly increasing, becoming a major public health problem. Genetic variants in several genes that codify immune proteins are highly related to NMSC. CTLA-4 is a key player in the control of immune regulation. It has been established that short repeats alleles in (AT)n genetic variants of the CTLA-4 gene are associated with a higher susceptibility to some types of cancer. This study aims to establish the possible association between some (AT)n genetic variants in the CTLA-4 gene with the susceptibility of NMSC carcinogenesis in people from western Mexico. Methods: A total of 450 individuals participated in this study: 150 patients with BCC, 150 patients with SCC, and 150 individuals as the reference group. For the analysis of the (AT)n genetic variants of the CTLA-4 gene, an endpoint PCR was performed, followed by electrophoresis on 7% polyacrylamide gels to genotype the samples. Results: in BCC, the statistical analysis showed that 104/104 bp genotype may be associated as a risk factor (OR=2.92, p=0.03), whereas 106/106 bp genotype may be associated as a less risk factor (OR=0.13, p=0.01). In SCC, the 106/106 bp genetic variant may also be associated as a less risk factor (OR=0.32, p=0.01). Discussion: our results show that in the western Mexican population, short repeats of (AT)n genetic variants in the CTLA-4 gen are associated with a risk factor in BCC and SCC, while long repeats may be a lower risk factor in BCC.

W78. Autoimmunity Risk Loci Tagged to CIITA and MHC Class II Impact CD8+ T Cell and Monocyte HLA-DR Expression

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Despite significant progress in defining genetic risk loci that contribute to autoimmunity, our understanding of the impact for such variants on composition and function of the immune system is limited. Hence, we performed flow cytometric immunophenotyping and microarray-based precision medicine genotyping on 464 unrelated subjects (non-autoimmune, n=290; type 1 diabetes (T1D) autoantibody (AAb)-positive, n=14; T1D, n=160). Quantitative trait loci analysis, including AAb or T1D status, age, sex, and population structure covariates, revealed 8 significant independent loci:phenotype associations after multiple-testing correction. Monocyte HLA-DR mean fluorescence intensity was significantly associated with *rs601945* (p=7.60e-13), a broad autoimmunity locus previously reported to modulate HLA class II gene expression. The HLA-DR$^+$CD38$^-$ percentage of CD8$^+$ T cells (bulk [p=5.18e-11], T central memory [Tcm, p=2.06e-10], T effector memory [Tem, p=8.62e-13], and Tem re-expressing CD45RA$^+$ [Temra, p=7.93e-14]) were associated with a risk locus (*rs4781011*) for inflammatory bowel disease, reported to regulate *CIITA* expression. Lastly, the HLA-DR$^+$ percentage of Tc1 (T cytotoxic type 1, p=2.79e-13), Tc2 (p=3.79e-13), and Tc17 (p=2.26e-11) were also associated with *rs4781011*. These data suggest that these two risk loci may contribute to autoimmunity by increasing *HLA-DR* and *CIITA* expression by monocytes and CD8$^+$ T cells, respectively. We propose that increased HLA-DR expression augments antigen presentation by monocytes and that elevated HLA-DR on CD8$^+$ T cells indicates increased activation by these cells. Ultimately, these data may inform future efforts to elucidate underlying mechanisms of genotype:phenotype associations in the immune system and to reverse these inflammatory phenotypes in individuals at-risk for autoimmunity using precision therapeutic strategies.

W79. A Hypermorphic Variant in CARD11 Leads to A Novel Inflammatory and Immune Regulation Disorder

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Background: CARD11 is a lymphocyte scaffold protein required for antigen receptor signaling. Complete loss of CARD11 causes severe combined immune deficiency. While rare, dominant negative loss-of-function mutations lead to CARD11-associated atopy with dominant interference of NF-kB signaling (CADINS). Germline constitutive CARD11 gain-of-function (GOF) mutations cause B-cell expansion with NF-kB activation and T-cell anergy (BENTA) disease. These disorders underscore the CARD11 role in immune regulation, and suggest that discrete phenotypes can emerge from different functional classes of variants. Methods: We examined 6 patients from 4 families with a heterozygous variant in the membrane-associated guanylate kinase domain of CARD11 (c.2542C>T, p.Arg848Cys, MAF=0.00005), presenting with variably penetrant symptoms of inflammation and immune dysregulation (autoimmune cytopenia, colitis, psoriasis and bacterial infection). T cell function and phenotyping were measured in primary patient cells and controls, and in Jurkat T-cells transfected with patient-derived CARD11 variants. Results: Ectopic expression of CARD11 R848C into CARD11-deficient Jurkat T-cells results in enhanced NF-kB signaling only upon T cell receptor (TCR) stimulation, distinct from the constitutive NF-kB activation in BENTA-derived CARD11 GOF mutations without stimulation. Primary patient T cells had enhanced mTOR activation and cellular activation as measured by CD25 upregulation, but normal proliferative response. Patient CD4/CXCR5+ T-follicular cells and IL21 production were elevated, while CD4/CD45RA-FOXP3+CXCR5+ T-follicular regulatory cells were diminished, and Th1/Th2/Th17 cytokine production was similar to controls. Conclusion: A relatively rare, non-private CARD11 variant leads to a unique immune dysregulation and TCR hyperactivation phenotype distinct from other known pathogenic CARD11-related diseases, illuminating a broader spectrum of pathogenic CARD11 mutations.

W131. Altered Innate and Adaptive Immune Landscape in Pediatric Patients with Glycogen Storage Disease 1b

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Patients with Glycogen Storage Disease 1b (GSD1b), resulting from SLC37A4 mutations, suffer from recurrent episodes of hypoglycemia, hepatosplenomegaly and recurrent infections attributed to chronic neutropenia. However, little is known about immune defects associated with the disease beyond abnormal neutrophil development. Utilizing a systems immunology approach with Cytometry by Time of Flight (CyTOF) analysis of blood samples from pediatric GSD1b patients aged 0.7-13.8 yrs and controls, we were able to characterize the peripheral immune landscape of GSD1b and investigate crucial changes in both adaptive and innate cell populations apart from the widely reported neutropenia: We show a decrease in macrophages, monocytes and natural killer cells as well as a shift from effector to central memory in T cells. Additionally, we observed both population-specific and global changes in expression of surface markers central to immune function such as CXCR3 and CD38, among others. These findings shine light on the complex dysregulation of the immune system accompanying GSD1b and uncover additional explanations for the observed clinical symptoms. Thereby our work contributes to our understanding of the disease and paves the way for future discoveries offering more specific and refined treatment methods.

W161. Genome-wide Prioritization of Autoimmune Disease-associated Genetic Variants in T Cells Identifies a Risk Variant that Elicits Effector T Cell Differentiation

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Genome-wide association studies have uncovered hundreds of autoimmune disease-associated loci; however, defining the causal genetic variant(s), most of which are in non-coding regions, and determining their effects in disease-relevant cell types remains a substantial challenge. Here, we test >18,000 autoimmune disease-associated non-coding variants for five autoimmune diseases for variant-modulated cis-regulatory activity in massively parallel reporter assays. We identify 60 putative disease-causal variants that likely play a role in altering T cell function. For one highly conserved non-coding variant within a putative enhancer, we used base editing to confirm a reduction in expression of the target gene *BACH2* in a human T cell line, and we introduced a small deletion of the orthologous non-coding sequence in mice, resulting in reduced *BACH2* expression in naïve CD8 T cells. T cells from these mice also had reduced expression of genes that suppress activation and maintain T cell stemness, a key function of *BACH2*, and, upon acute viral infection, variant-deleted naïve T cells are more likely to differentiate into effector cells. Our results represent an example of defining disease-causal variants and studying their immunologically relevant effects, allowing for discovery of disease-risk mechanisms.

W167. CRISPR-based Functional Genomics in Primary Human T Cell Subsets Decode Cis and Trans Regulation of the CD28, CTLA4, and ICOS Costimulatory Locus

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Costimulation is essential for complete T cell activation, requiring the coordinated expression of various surface proteins including those encoded by CD28 family members CD28, CTLA4, and ICOS. Interestingly, these three genes lie adjacently in a topologically-associating domain (TAD) and are believed to have arisen through duplications of CD28, though they have evolved opposing functions and exhibit expression differences across cell states and T cell subsets. We hypothesized that distinct cis- and trans-regulatory circuits emerged to uniquely govern the expression of each gene. Therefore, we tiled CRISPR interference (CRISPRi) across the 1.44Mb TAD in primary human conventional (Tconv) and regulatory T (Treg) cells to functionally profile cis-regulatory elements (CREs) controlling expression of CD28, CTLA4, and ICOS. We find that inhibiting the transcriptional start site of one gene (but not gene KO) enhances the expression of an adjacent gene, potentially due to redirection of shared CREs to the un-inhibited promoter. Moreover, we find distinct CREs responsible for constitutive expression of CTLA4 in Treg cells but stimulation-responsiveness in Tconv cells. We also find evidence of a single CRE that negatively regulates CD28 at baseline but positively regulates CTLA4 upon restimulation. Lastly, we characterize mechanisms of CRE control by identifying upstream regulators of target genes via CRISPR knockout screening. Our work nominates ZNF217, a zinc finger protein with no known function in human T cells, as a novel negative regulator altering chromatin accessibility in the locus. We present a framework for functional characterization of modules of gene regulation in primary human CD4+ T cells.

Immuno-oncology

Th6. A CXCR4/PLC-mediated Cell Survival Pathway is Involved in Steroid Resistance of B-acute Lymphoblastic Leukemia

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The initial response to glucocorticoids (GC) is an important predictor of outcome in B acute lymphoblastic leukemia (B-ALL). We recently showed that intracellular Ca^{2+} or downstream ERK signaling mediates dexamethasone (Dex) resistance in B-ALL cells. We now hypothesize that PLC-mediated Ca^{2+} signaling contributes to Dex resistance and show herein that GC paradoxically promote a CXCR4/PLC-mediated cell survival pathway in B lymphoblasts. Dex provoked a decrease in CXCR4 membrane expression via internalization, resulting in activation of PLC dependent-Ca^{2+} signaling. The PLC inhibitor U73122, the CXCR4 antagonist AMD310 and CXCR4 gene silencing decreased Dex-induced calcium signaling. Exposing B-ALL cells to the CXCR4 ligand SDF-1 induced rapid increase in $[Ca^{2+}]i$. SDF-1 potentiated GC resistance in B lymphoblasts. This resistance could be overcome by AMD3100. Also, U73122 and CXCR4 gene silencing allowed overcoming GC resistance, leading to a significant increase in Dex-induced reactive oxygen species production, cytochrome c release, caspase-3 activation and eventually apoptosis. Treatment with Dex and AMD3465 also significantly delayed Nalm6 leukemia development in NSG mice. Bioinformatic analysis of B-ALL datasets revealed that CXCR4 expression is higher in GC-resistant as compared to GC-sensitive ALL patients, further supporting a role of CXCR4 in sensitivity to GC. In this line, patients with high expression of CXCR4 had a poorer clinical outcome than low-expressors. Taken together, these data indicate that GC paradoxically induce their own resistance in B lymphoblasts by stimulating a CXCR4-mediated pro-survival pathway through the activation of PLC.

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Th20. An Academic BCMA-directed CAR-T Therapy (ARI0002h) with Fractionated and Booster Dose in Patients with Relapsed/Refractory Multiple Myeloma: First Data About Efficacy and Safety

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ARI0002h is an autologous and academic CAR T-cell product with a 4-1BB co-stimulatory domain and a humanized single chain variable fragment targeting BCMA. Here, we report safety and efficacy results of the CARTBCMA-HCB-01 multicenter clinical trial for patients with relapsed/refractory multiple myeloma (NCT04309981) receiving ARI0002h in 5 Spanish center, while productions were done in 2 of them, by using CliniMACS Prodigy. Patients (pts) eligible had measurable disease and refractory to previous conventional treatments. Targeted dose was 3x106/kg CAR+ cells, being administered in fractionated manner (10%/30%/60%). A second dose (3x106 CAR+ cells/kg) was planned at least 4 months after the first dose in pts with any grade of response without serious complications after first administration. 30 pts received ARI0002h cells. Median CAR-T cell production time was 11 days (range 9-14) with a 100% manufacture success. The ORR of 30 evaluable pts was 100%, with a stringent complete remission (sCR) plus very good partial response (VGPR) rate of 90%. Median time to first response was one month. Of 26 MRD-evaluable pts at day +100, 92% were MRD-negative in bone marrow. 53% of patients were alive and without progression at 16 months. Median overall survival (OS) was not reached and the 16-month OS rate was 80%. ARI0002h cells demonstrated peak expansion on day 14. 24 out of 28 eligible pts (86%) received the second dose. 7 pts (29%) improved their response after reinfusion. A second cohort of additional 30 pts has been started, trying to contrast with the first 30 patients.

Th32. A Case of μ Heavy and λ Light Chain Amyloidosis in a Patient with Bi-clonal (IgM κ and λ) Gammopathy Successfully Treated with Daratumumab

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Lymphoplasmacytic lymphoma (LPL) causes 3% of amyloidosis cases, primarily amyloidosis with light chains (AL). Amyloidosis with heavy chains (AH/AHL) is an uncommon form of immunoglobulin-derived amyloidosis with the μ heavy chain variant known to be the rarest type. The anti-CD38 monoclonal antibody, Daratumumab has recently propelled itself in the forefront of treatment of AL amyloidosis after the publication of the Andromeda study. We present a case of an elderly male with a history of IgM κ lymphoplasmacytic lymphoma who underwent a renal biopsy revealing the presence of μ heavy and λ light chains. His bone marrow biopsy demonstrated κ light chain restriction by flow cytometry accompanied by amyloid deposition. The patient's serum had elevated free κ and λ light chains with a free light chain (FLC) ratio of 3.17. Serum immunofixation was positive for IgM κ and λ light chain clones. He completed 6 cycles of Cyclophosphamide, Bortezomib, Dexamethasone, and Rituximab (CyBorD+R) resulting in the normalization of the FLC ratio, but he continued to present with persistently elevated M protein and IgM κ and λ light chains on immunofixation. Thereafter, Daratumumab was initiated which led to a negative immunofixation study after 2 cycles of the monoclonal antibody accompanied with reduction in the excretion of protein in the urine. The patient achieved a complete hematological response with daratumumab. To date, our case is the only reported μ heavy and λ light chain amyloidosis patient with bi-clonal (IgM κ and λ) gammopathy to be successfully treated with daratumumab.

Th34. Performance Comparison of the Spectral Signature, Spillover Spreading Matrix, and Data Unmixing Quality Between the Spectral Cytek Aurora and the Spectral BD FACS Symphony A5 SE by a 35-Color Human Broad Immunophenotyping Panel

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Spectral flow cytometry allows the collection of the entire spectral signature across all lasers. This aids in the overall workflow including improvements in data collection, fine-tuned visualization of autofluorescence and panel design. Moreover, by the process of spectral unmixing we can take the sum of the fluorescence and mathematically separate out each individual color, allowing us to further discriminate between the variations within the spectral signature of a molecule and use novel fluorochrome combinations that were historically not used in the same panel. In recent years many cytometry manufacturers have developed their own instrumentation to perform such multicolor spectral cytometry experiments, most notably Cytek with their Aurora system as well as more recently by BD Biosciences with their FACS Symphony A5 SE (Spectrally Enabled). In this study, we independently compare the performance between the Cytek Aurora and the FACS Symphony A5 SE. First, we assessed human peripheral blood mononuclear cells (PBMCs) individually stained with anti-human CD4 antibodies (35 different colors, clone SK3) to compare the staining index for each fluorescent channel as well as the spillover spreading matrix, which visualize the interactions between pairs of fluorochromes and estimate the spread. Secondly, we designed a 35-color broad human immunophenotyping panel to stain PBMCs from 3 donors and compared the data quality and resolution of canonical immune cell populations between the instruments. The outcome of this study will help inform and elucidate the selection of appropriate platforms to support immunological research.

Th42. Next-generation Immunostimulatory Antibody-drug Conjugate (iADC) Combines Direct Tumor Killing and Innate Immune Stimulation to Provide Protective Anti-Tumor Immunity

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In recent years, combination therapies of antibody-drug conjugates (ADCs) and immune system activators have garnered much interest due to their potential to unlock greater durable clinical benefit. Recently, we demonstrated that STRO-002, our novel anti-folate receptor alpha (FolR) ADC currently in Phase I clinical trials, can engage the immune system through induction of immunogenic cell death (ICD). We further showed that by complementing the ICD-inducing property of STRO-002 with combination treatment with Avelumab, we could enhance infiltration of CD8+ T cells into the tumor and generate potent anti-tumor immunity. To develop the combination concept further, we leveraged Sutro's breakthrough XpressCF+TM cell-free technology, which utilizes precise site-specific conjugation to generate complex molecules, to engineer the immunostimulatory ADC (iADC), a next-generation ADC molecule that combines tumor-targeted cell killing and immune activation in a single modality. Using murine MC38 tumors engineered to express human FolR , we demonstrated that delivery of a hemiasterlin/TLR agonist dual-conjugate anti-FolR iADC resulted in a more robust anti-tumor response compared to either modality administered alone. This improved response was associated with innate immune cell activation and increased CD8+ T cell infiltration in the tumor. Moreover, a greater number of complete responses was also observed following treatment with the anti-FolR iADC, and complete responders developed broad and robust immune memory that was able to reject MC38-hFolR rechallenge in a CD8+ T cell-dependent manner and parental MC38 rechallenge. Thus, the iADC concept unites two complementary mechanisms of tumor control in a single molecule to attain greater therapeutic benefit.

Th47. Single Cell and Spatially Resolved Analysis of Tissues by Integrated RNA and Protein Multi-omics Identifies Novel Tumour-infiltrating Lymphocyte Phenotypes

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The development of single-cell methods to incorporate detection of cellular protein epitopes via barcoded antibodies has enabled advances in immunophenotyping but its application to investigating tissue immunology is limited. Here we present an optimised experimental and analytical framework for the phenotyping of human tissues by integrated transcriptome and barcoded antibody clustering analysis of single cell suspensions and tissue sections. Using a dataset of 6 breast cancer patients, we demonstrate how the integration of proteomic data with RNA enhances cellular stratification across cell types within the tumor microenvironment, leading to the identification of unique clinically relevant lymphocyte subsets. We characterize tumor-infiltrating lymphocyte subsets indistinguishable by transcriptomics alone. We reveal patterns of RNA and protein co-expression across lymphocytes, identifying new protein markers of resting and activated tissue resident lymphocytes including innate lymphoid cells, T cells and NK cells. We characterize a CD4 Tfh subset previously not described in breast tissues, one associated with markers of tissue residency and exhaustion, found to be strongly associated with prognosis and response to checkpoint immunotherapy across multiple cancers. We further investigate these phenotypes spatially, developing Spatial Indexing of Transcriptomes and Epitopes (SITE-Seq), a method for spatially resolved joint transcriptome and epitope analysis of snap frozen tissues using a modified 10X Visium protocol. We show that the integration of RNA and protein resolves immune subsets at higher resolution than either modality alone. This work highlights the importance of multi-omic methods for the phenotyping of cell states and the emergence of novel cellular states in the tumor microenvironment.

AO. Immune Response to GFP in GFP Expressing Pan02 and CT26 Tumor Models

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Immune mechanisms of antitumor response are poorly understood. The aim of this study was to compare cellular and humoral immune responses in two murine tumor models with opposite growth characteristics, Pan02 and CT26. Both pancreatic Pan02 and colorectal CT26 cells were stably transfected with GFP and transplanted s/c onto C57BL/6 or BALB/c mice accordingly. Humoral response to tumor cells was analyzed by cell-ELISA. Cellular response was estimated by intracellular production of IFNg and TNF-a by CD45+CD3+ T-cells collected from Payer's patches of tumor bearing mice in response to cleared CT26, Pan02 cell homogenates or GFP as was studied by flow cytometry. CT26-eGFP cells quickly produced large, soft, nonfibrous, and steadily growing tumors while Pan02-eGFP cells grew In vivo much slower, developed 10-20 times smaller and highly fibrous tumors which never reached the CT26-eGFP tumor size. Analysis of immune response was conducted at 1 mo post tumor transplantation. Cell-ELISA using parental non GFP cells or GFP protein as coatings demonstrated that sera from CT26-eGFP but not Pan02-eGFP bearing mice recognized GFP but not tumor cells showing that IgG response depends on tumor mass and focuses to exogenous but not self antigens. Analysis of T-cell response also demonstrated that CD3+ T-cells from Payer's patches of CT26-eGFP but not Pan02-eGFP mice recognized significantly only GFP (p=0.36) while there was a non significant tendency to recognize tumor antigens. These data show that immune system recognizes well exogenous but not self antigens. This work was supported by RFBR grant 20-04-00521

Th72. Engineering of Alpha/Beta Selective IL-2 Muteins for Antigen Activated T cells in Solid Tumors

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Interleukin-2 (IL-2) potently stimulates cytotoxicity, survival, and the proliferation of T and NK-cells, but it also stimulates regulatory T-cells (Treg). High dose IL-2 monotherapy induces complete responses in cancer patients, but severe acute toxicities including capillary leak syndrome (CLS) limit its application. Here we describe new IL-2-partial agonists selective for the high affinity, trimeric IL-2 receptor, IL-2Ra/b/g (CD25/122/132) expressed on activated T cells. These a/b-IL2 do not activate $CD25^-$ NK cells allowing continuous treatment without acute toxicities in non-human primates and mice. This enables improved efficacy and single agent complete responses in mouse tumor models and a significant increase of tumor infiltrating CD8+ T-cells (TIL) and the TIL/Treg ratio in tumors, compared to wt or IL-2Rb/g-selective IL-2s. a/b-IL2 may improve clinical success of IL-2 in cancer patients.

Th80. The Role of Mucosal-Associated Invariant T (MAIT) Cells in Glioblastoma Immunity

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Glioblastoma (GBM) is a devastating disease, and its 5-year survival rate is approximately 10%. Immunotherapies have not been shown to be effective against GBM, so a better understanding of GBM immunity is needed. Mucosal-Associated Invariant T (MAIT) cells are MHC class I related-1 molecule (MR1)-restricted T cells and regulate tumor immunity. Although MAIT cells were reported to exist in GBM tissue, their roles are unknown. We examined their function in GBM. Since MAITs' T cell receptors (TCRs) use limited combinations of gene segments, we searched the characteristic sequence combinations in the TCGA-GBM transcriptomic data and calculated the MAIT cell infiltration levels (MIL). Results showed that 8.6% of samples were MAIT positive, and immune-suppressive genes were up-regulated in those positive samples. There was no appropriate method available to study co-expressing genes with MIL comprehensively. Thus, by taking advantage of our observation of a significant correlation between MIL and MR1 gene expression ($r = 0.22$, $p = 0.0045$), we extracted all genes highly co-expressing with MR1. The tumor-immunosuppressive "Neutrophil degranulation (ND)" pathway was enriched in those genes. The genes sorted into this pathway contributed to the exocytosis of Arginase 1 (Arg1), which can impair T cell functions. It was also confirmed that 90% of those genes and Arg1 co-expressed with MIL, and all the detected ND-related genes and Arg1 tended to inversely correlate with the patient survival. Our study suggested that infiltrated MAIT cells in GBM may suppress tumor immunity by activating ND and Arg1 release.

Tu52. NKG2D Co-stimulation Differentially Reprograms CD8 T Cells for Enhanced Memory Formation and Homeostasis in Mouse Prostate Tumors

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Patients with metastatic castration resistant prostate cancer (mCRPC) have poor prognosis and overall survival (OS). Although immunotherapies such as anti-PD-1/L1 checkpoint blockade have produced significant tumor regressions in melanoma and lung cancer, treatment efficacy is significantly impaired in prostate cancer tumors. High expression of NKG2D receptor agonists MICA/B is associated with poor prognosis in human carcinomas. Soluble MIC (sMIC) shed by tumor cells suppresses CD8 T cell activation and accelerates tumor progression. In "humanized" TRAMP/MICB mice, sMIC-neutralizing anti-MIC mAb (B10G5) causes regression of prostate tumors by 1) sequestering sMIC, and 2) stabilizing/sustaining NKG2D and CD3ζ signaling and CD28 expression, re-invigorating CD8 T cell anti-tumor responses. NKG2D signaling modifies expression of epigenetic factors, and is essential in certifying CD8 T cell stemness necessary for tumor control. In this preliminary study, we used scRNA-seq of CD8 TILs from TRAMP/MICB mice treated or untreated with B10G5 to investigate the hypothesis that B10G5 differentially reprograms CD8 T cells at the genetic and epigenetic level via sustained NKG2D pathway co-stimulation, thereby optimizing TCR-dependent effector and memory responses. We found that B10G5-treated mouse tumors were enriched in central memory CD8 TILs expressing high levels of NKG2D and TCF7, as well as GZMKhi PD-1+ CD8 TILs upregulating epigenetic modifiers associated with H3K4 methylation and H3K27 demethylation and maintaining homeostasis of activated CD8 T cells, such as KMT2A/E, EZH2, and CREBBP. These results establish a groundwork for identifying targets for epigenetic alteration via NKG2D co-stimulation of CD8 TILs within the prostate tumor microenvironment.

Tu74. CD3+CD56+ Cells in Cervical Cancer Patients: Evaluation of Immune Checkpoint Expression
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Cervical cancer is one of the leading causes of death in developing countries. Similar to other types of tumors, the immune response plays an important in controlling the development of cervical neoplasia. However, malignant cells can often escape from immuno surveillance. For this reason, many of the newest therapies against cancer cells are directed at restoring immune responses. NKT cells (CD3+CD56+) share characteristics of T and NK cells and thus possess both innate and acquired immune functions. However, the contribution of these cells in immune checkpoint blockade therapy remains to be investigated. In this study we evaluated the expression of immunocheckpoint receptors (PD-1, TIGIT and Tim-3) in CD3+CD56+ lymphocytes. Peripheral blood cells from patients with cervical carcinoma (CC, n = 21), high-grade lesions (HG n = 9), low-grade lesions (LG,n = 24) and healthy donors (HD n = 24) were analyzed by multiparametric flow cytometry. Our results showed that CD3+CD56+ cells frequencies were increased in CC patients. We saw a significant increase in the percentages of these cells expressing inhibitory PD-1 or TIGIT, and a significant increase of TIGIT+DNAM+ cells, in the CC patients. A deeper characterization of these cells may help to clarify their role in cervical cancer.

Tu81. Multiplexed Single-cell Analysis Reveals Dysfunctional CD8+ CD103+ T Cells and Negatively Prognostic CD38+ Regulatory T Cells in Human Colorectal Cancer
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Although T-cell infiltrates correlate with clinical outcomes in CRC, different T cell types, their functions and their influence on clinical outcomes remain unclear. Using a droplet-based single cell sequencing, we profiled 37,931 single T cells from tumors and adjacent normal colon of 16 treatment-naïve CRC patients with respect to transcriptome, TCR sequence, and 23 cell surface markers. Gene set enrichment analysis enabled us to link gene signature of each identified T cell type within tumors to the TCGA database. Among $CD8^+$ T-cell infiltrates, we found two distinct cytotoxic T cell types differentiated into clonally-expanded exhausted T cells. *GZMK*$^+$ $KLRG1^+$ cytotoxic T cells with a less dysfunctional phenotype were enriched in CRC patients with good outcomes. Strikingly, *GNLY*$^+$ $CD103^+$ cytotoxic T cells, including intraepithelial lymphocytes (IELs), were not associated with good clinical outcomes, despite high co-expression of CD39 and CD103, markers which denote tumor-reactivity, possibly due to its dysfunctional phenotype. Among $CD4^+$ T cell-infiltrates, we found two distinct regulatory T cells (Treg) subtypes associated with opposite clinical outcomes. While total Tregs, predominantly $Helios^+$ cells, were associated with good outcomes, $Helios^-$ $CD38^+$ peripherally-induced Treg cells (pTregs) were strongly associated with bad outcomes independent of stage. $CD38^+$ pTregs, which shared gene signatures with Th17 cells, possessed a highly suppressive phenotype, suggesting they are the elusive Treg population that inhibits anti-tumor immunity in CRC. In summary, these observations support the potential for novel CRC therapies directed at $CD38^+$ pTregs or $CD8^+$ $CD103^+$ T cells to augment existing T cell-targeted immunotherapies.

Tu83. Anti-TIGIT Antibodies Promote Immune Activation in Combination with Immunotherapeutic Agents

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TIGIT is an inhibitory receptor expressed on natural killer (NK) cells, $CD8^+$, $CD4^+$ T cells and regulatory T cells (T_{regs}). TIGIT competes with an activating receptor, CD226, for binding to CD155 expressed by antigen-presenting and cancer cells. TIGIT has emerged as a key inhibitor of anti-tumor responses and blocking TIGIT results in immune activation and restored anti-tumor immunity. We have two anti-TIGIT antibodies, AB308 (IgG1) and AB154 (IgG1 with Fc-silencing mutations), that are undergoing clinical evaluation and biomarker assessment. AB308 can promote Fc-mediated effector function, as evidenced by human NK cell-driven ADCC against TIGIT-expressing target cells. In mice, while combining Fc-silent or Fc-enabled anti-TIGIT with anti-PD-1 enhanced tumor growth inhibition, Fc-enabled anti-TIGIT was associated with intratumoral T_{reg} depletion. Anti-TIGIT also increased signaling in Jurkat T cells and IL-2 secretion in PBMC cultures both alone and in combination with zimberelimab (anti-PD-1). In addition, combination of AB308 with the dual A_{2A}/A_{2B} adenosine receptor antagonist etrumadenant is required for maximal signaling through the CD155-CD226 axis in the presence of adenosine. Finally, TIGIT and its co-expression with CD226 correlated with PD-1 expression on human tumor-infiltrating $CD8^+$ T cells. Importantly, TIGIT and CD226 were co-expressed on circulating $PD\text{-}1^+$ cells that are characterized by granzyme K expression and may represent a pre-dysfunctional stem-like T cell population that has been described as central to the therapeutic benefit of anti-PD-1 agents. The data presented here support use of anti-TIGIT for cancer treatment and provides a rationale for combination with zimberelimab and adenosine pathway blocking agents such as etrumadenant.

Tu86. Rescuing Antitumor Responses Mediated by Immune Checkpoint Inhibition Through Jak Inhibition

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Immune checkpoint blockade (ICB) represents an established treatment for many cancers. However, most patients treated with ICB ultimately show disease progression. We have previously demonstrated that combining anti-PD1 and anti-CTLA-4 can induce activation-induced cell death of tumor reactive T cells leading to compromised anti-tumor efficacy and immunologic memory. We have also shown that this selective deletion of T cells is mediated by interferon g (IFNg). Since IFNg is also pivotal for the mediating antitumor responses, we investigated approaches to rescue tumor reactive T cells without compromising tumor efficacy. Because IFNGR1 signals through Jak1, we examined whether a JAK inhibitor (JAKi) could be used to enhance the effectiveness of combination of anti-PD-1 and anti-CTLA4. We found that administrating JAKi in a specific sequence leads to significantly increased rates of tumor rejection and survival. We also observed that T cell activation is maintained and even increased with time in the mice treated with JAKi. Importantly, tumor-reactive T cells are effectively expanded in the tumor-draining lymph nodes of those mice, potentially contributing to the observed antitumor efficacy. We also observed partial depletion of regulatory T cells in responding mice, indicating that activation induced cell death (AICD) is reduced in the presence of JAKi. Our results demonstrate that temporal modulation of IFNg can enhance anti-tumor immune responses leading to improved outcomes.

Th111. Metastatic melanoma (MM) patients with stable disease display reduced Mo-MDSCs levels in peripheral blood

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Abstract Text:

Introduction: Myeloid-derived suppressor cells (MDSCs) are increased in late-stage tumors promoting metastasis by exerting their immunosuppressive functions. Recently, treatment with anti-PD1 and/or anti-CTLA-4 has improved the prognosis of metastatic melanoma (MM). In this regard, the immune effect of cancer immunotherapy on MDSCs is poorly understood. The aim of this study was to examine Mo-MDSCs frequencies in peripheral blood in relation with clinical outcomes in MM patients under immunotherapy.Material and methods: Peripheral blood from 38 MM patients included in a clinical trial authorised by the Ethics Committee of the University of Regensburg was collected before administration of the first dose of Nivolumab and/or Ipilimumab. Patients were classified according to the iRECIST criteria into complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). Samples were acquired with a NaviosTM cytometer. Mo-MDSCs were defined as $CD14^+$ $CD33^+$ $CD11b^+$ $CD15^-$ $HLA\text{-}DR^{-/low}$ lin^- cells. Results: We found no association between Mo-MDSCs levels and checkpoint blockade adverse events (hepatitis and colitis), sex, presence of IgG anti-CMV and stage of the disease. On the contrary, we observed that Mo-MDSCs frequencies in patients with stable disease (n=7, median 50.92 IQR 49.63-71.49) were lower compared to those who progress (n=15, median 77.95 IQR 67.24-83.39) (p=0.026) and those who have partial response (n=11, median 78.98 IQR 66.84-80.35) (p= 0.0185). Conclusion: Further studies are required to understand the immune response to immunotherapy and if MDSCs could serve as a biomarker to predict prognosis in MM patients under immunotherapy treatment.

Th142. Evaluation of the MAPK and PI3K Signaling Pathways in Cancer Stem Cells Isolated from Prostate Cancer Tumor Tissues, a Preliminary Study

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Prostate cancer (PCa) has become a major threat to men's health to date, being the fifth-leading cause of death worldwide for men. Nowadays, studies have demonstrated that cancer stem cells (CSCs) play a decisive role in cancer relapse, including the formation, metastasis, drug resistance, and recurrence of cancer. These cells have dual capacities: self-renewal and differentiation. It is known that PI3K/AKT and MAPK/ERK signaling pathways can lead CSCs to proliferate into forming a prostate tumor. Currently, not much is known about these types of cells, which highlights the importance of researching its biology to understand the mechanisms underlying cancer resistance and metastasis. Aim: Isolate prostate cancer stem cells from patient's prostate tumors and evaluate the expression of MAPK and PI3K signaling pathways.Methods: The prostate cancer biopsies were obtained from the Angeles del Carmen general hospital in Jalisco, Mexico. Five samples were disintegrated via the use of collagenase and left to grow with DMEM F-12 medium. Immunophenotyping was performed by flow cytometry. Subsequently, protein extraction was done and the expression of signaling pathways was analyzed by Western Blot. Results: The cells were isolated after six weeks of culture and the main CSC markers were observed. Our results confirm that both ERK and AKT were expressed by these cells. ERK is observed to be significantly overexpressed in most samples while AKT is observed to have weak expression. Conclusion: ERK1/2 was highly expressed in stem cells from PCa patients and AKT to a lesser extent.

Th165. Induction of a T Cell Intrinsic DNA Damage and Repair Response is Associated with Clinical Response to PD-1 Blockade

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Despite the success of immune checkpoint blockade (ICB), many patients still fail to achieve durable clinical benefit, and the underlying immunological mechanisms of response versus progression remain poorly understood. Here, we investigated immune reinvigoration by ICB in chemotherapy-resistant hypermutated or microsatellite instability-high (MSI-H) uterine cancer patients treated with nivolumab. Upon initiation of treatment, CD8 T cells underwent rapid pharmacodynamic proliferation 2-4 weeks after starting PD-1 blockade. This immunological response, however, did not correlate with clinical response. We hypothesized that the T cell-intrinsic response to proliferative and genotoxic stress might contribute to the disparity between immunological and clinical response. To address this question, we developed a high-dimensional platform that enables simultaneous analysis of T cell differentiation state with changes in major DNA damage and repair (DDR) pathways at single cell resolution. This DDR-Immune platform revealed consistent T cell subset specific patterns of DDR, as well as specific DDR pathways induced by different types of DNA damage. Applying this DDR-Immune platform to MSI-H or hypermutated uterine cancer patients revealed a signature of DDR exemplified by rapid increase in phosphorylated-ATM (pATM) intrinsic to proliferating CD8 T cells that distinguished clinical responders and non-responders. Furthermore, transcriptional analysis with CRISPR knockout of ATM in human T cells suggested that the ability to induce ATM regulated transcriptional circuits in T cells upon PD-1 blockade is associated with better clinical response. These findings highlight a previously unrecognized role for T cell-intrinsic DDR as a potential determinant of immune fitness and clinical outcome to PD-1 blockade therapy.

Th178. The CDKN2A Deletion Influences the Establishment of the Tumor Immune Microenvironment of Pancreatic Ductal Adenocarcinoma

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The immune TME of pancreatic ductal adenocarcinoma (PDAC) is heterogeneous between patients but it is well established that poor T cell infiltration and high frequency of immunosuppressive cells affect the prognosis of PDAC. Since CDKN2A deletion in tumor cells participates to the immunomodulation in cancer we sought to investigate its role in the establishment of the tumoral immune microenvironment of PDAC. We injected into the pancreas of healthy mice two murine cell lines of PDAC: KPC cells or KPC carrying CDK2NA deletion (KPC-CDKN2A). Tumor infiltrated lymphocytes (TILs) were analyzed by flow cytometry to compare immune populations between the two mice models of PDAC. KPC-CDKN2A tumors were characterized by a lower proportion of $CD8^+$ T cells and a higher proportion of regulatory T cells (CD4+Foxp3+). T cells into KPC-CDKN2A tumors harbored an exhausted phenotype (higher PD1+/CTL4+%), while Treg were more activated (CD25+/TNFR2+%). CDKN2A is a gene locus encoding for the tumor suppressor p14/p16 proteins, when deleted the checkpoint is bypassed and CDK4/6 are activated. Administration of a chemical inhibitor of CDK4/6 in KPC-CDKN2A tumor bearing mice impaired tumor growth. CD8+ T cells infiltration increased and TIGIT expression on TILs decreased in treated mice. In conclusion, CDKN2A deletion seemed to promote infiltration of immunosuppressive cells and exhaustion of T cells in the TME. Restoring CDKN2A downstream pathways promoted CD8+ T cells infiltration. Studying the impact of CDKN2A deletion on the tumor immune microenvironment will allow improving therapeutic approaches and understanding the cause of immune infiltration heterogeneity in patients.

Tu120. Spatial Whole Transcriptome Profiling of the Tumor Microenvironment in FFPE Prostate Carcinoma using the Visium Platform

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Despite years of prostate cancer research, balancing the risks of treatment complications and tumor progression remains a challenge. The inability to dissect tumor microenvironments (TME) and immune compartments contributes to a knowledge gap. Spatially resolved molecular profiling allows understanding these complexities for potential personalized treatments. We used 10x Genomics Visium Spatial Gene Expression for FFPE tissue for spatial whole transcriptome analysis of two prostate cancer sample TMEs. The tissues were processed over~5,000 molecularly barcoded, spatially encoded capture probes in spots for spatially resolved transcriptional readout. We resolved whole transcriptome tumorigenic profiles in sections of normal, stage III adenocarcinoma, and stage IV acinar cell carcinoma FFPE human prostate tissues. Computational clustering identified spatial gene expression patterns that aligned with pathologist annotations. The data revealed spatial disorganization of basal and luminal cells in tumor samples. T lymphocytes were dispersed throughout the whole tissue in the adenocarcinoma, while plasma B cells were in the peritumoral region impacting patient prognosis. Computational methods inferred copy number variation, identifying aneuploidy regions and specific loci driving the genomic profile of the cancerous regions.
We demonstrated that spatial whole transcriptome analysis resolves FFPE prostate samples. Our data rapidly confirms known cell type patterns and tumor region-specific gene expression while enhancing understanding of the TME for drug target or biomarker discovery for tailored therapies and patient stratification.

Tu128. Enhanced Function of Vaccine Dendritic Cells from Obese Donor upon Inhibition of Lipid Metabolism

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In the last decade tumor immunotherapy has been revolutionized by the implementation of checkpoint inhibitors that “remove the brake” posed by the tumor microenvironment onto the immune system. There exists increasing evidence that for optimal patients´ outcome an active vaccination to “start the engine” of the immune response is also needed. For this reason, it is important to optimize the protocol for the production of dendritic cells (DC)-based vaccines. In light of the link found between metabolism and function of immune cells, we focused on the manipulation of metabolic pathways during the *in vitro* differentiation process and implemented monocytes from obese donors, a population for which impaired immune response as well as enhanced risk for cancer development have been reported. Treatment of monocytes with different inhibitors of lipid metabolism resulted in mature DC with a lower expression of costimulatory molecules on their surface, but with an enhanced capability to induce the cytotoxic activity and IFNγ secretion of autologous effector cells. Mechanistically, the inhibition of the lipid metabolism drastically reduced the otherwise high levels of IL-10 secretion by obese mature DC leading to enhanced IL-12p70 to IL10 ratio and type1 polarization of the immune response. Thus, an enhanced immune function can be obtained in obese patients by treating their DC with a” lipid diet” during the *in vitro* protocol for their production.

Tu141. Correlation of GPER with GLI1 and GLI3 in Tumor and Stromal Tissue with Different Prognosis Groups of Patients with Prostate Cancer

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Prostate cancer is the most common non-skin malignancy in men and second-leading cancer-related disease worldwide. Gleason scoring assigns a score from two different locations of the sample specifying the degree of malignancy. GPER may drive cancer progression via pathway signaling activation; among these is Hedgehog, implicated in cell differentiation and growth control with the participation of GLI factors, whose aberrant expression and activation contribute to cancer by enhancing survival, stemness, metastatic potential, stimulation of stroma, and blood vessel formation. GLI1 is considered an activator, while GLI3 has dual activity. Nuclear and cytoplasmic expression of GPER is important because it can interact with molecules involved in carcinogenesis. Their correlation with GPER-GLI has not been evaluated in different Gleason degrees in prostate tissues. Aim: Evaluate the correlation of nuclear and cytoplasmic GPER, GLI1, and GLI3 protein expression in tumoral and stromal tissues in prostate cancer having different Gleason scores. Methods: Immunohistochemistry assays were performed for GPER, GLI1, and GLI3 (total and phosphorylated) in tumoral/stromal prostate cancer samples with different Gleason scores. Results: GPER is mainly expressed in the nucleus and enhanced as the Gleason score increases. GLI1/GLI1p are expressed in cytoplasm but this is not affected by the Gleason score. However, GLI3 reduces its nuclear expression in stromal tissue and GLI3p is expressed more as the Gleason score increases in tumoral and stromal tissue.
Conclusion: An inverse correlation is observed between GPER and GLI3 as the Gleason score increases in stromal tissue and positively correlates with pGLI3 in tumoral tissue.

Tu147. Regulatory T Cell Functional Dynamics During The Response To PD-1 Blockade

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Checkpoint inhibitors have been a major breakthrough in cancer therapy, inducing durable remission in patients. Yet, roughly 2/3 of patients do not respond to treatment in melanoma, the solid tumor type with maximal efficacy. Positive clinical outcomes are associated with higher degrees of pre-existing inflammation in the tumor microenvironment, while non-responding tumors are "cold". Regulatory T cells (T_{reg}) play a dominant role in tumor-induced immunosuppression. However, it is still unclear how their homeostasis differs in these different tumor microenvironments and how this impacts the outcome of checkpoint blockade. We hypothesize that checkpoint inhibitors dysregulate the homeostasis and the fate of T_{reg} cells, thus increasing anti-tumor responses. Using various syngeneic mouse models of "cold" (D4M.3A) and "hot" (YUMMER1.7) melanoma and Foxp3-reporter strains, we mapped out local and systemic regulatory T cells dynamics throughout tumor growth and response to checkpoint blockade. Despite different potency, in both models anti-PD1 delayed tumor growth and increased local CD8 responses. However, this increased inflammation was associated with T_{reg} cells displaying reduced PD-1 expression and a more highly activated phenotype both in the tumor and systemically. Furthermore, we established that in a subset of high responders, tumor-infiltrating T_{reg} cells progressively acquire Th1-like characteristics, namely expression of T-bet and secretion of IFNγ. This phenotype was strongly correlated with both strong CD8 anti-tumor responses and reduced tumor volume. Nonetheless, T_{reg} cells isolated from these tumors highly suppress the proliferation of splenic T_{resp} cells *in vitro*. Thus, it remains to be determined which signals dampen their suppressive capacity *in situ*.

Tu153. Comprehensive Analysis of the Immune Landscape in the Peripheral Blood of Advanced Melanoma Patients Reveals Chronic Activation of Differentiated Cytolytic Cell Subsets

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Background: Many advanced melanoma patients are either not responsive or relapse after immune checkpoint therapy, hence, finding diagnostic or prognostic markers is essential. To identify a potential immune signature in the peripheral blood, we delineated the phenotypic and functional characteristics of the PBMCs from melanoma patients and compared them to healthy individuals. Methods: We used 1 dataset from metastatic melanoma patients (n=72) and 2 datasets from healthy individuals (n=31) generated by different mass cytometry (CyTOF) analyses in the lab. To accurately compare patient and healthy cohorts, we first normalized the FCS files using Fluidigm EQ™ calibration beads. Then we performed manual gating in Cytobank, excluding the markers that were not shared between the different panels. Results: Most of the major immune population in the PBMCs were unchanged in the patients compared to the healthy donors. However, in CD8 T cells from patients, we observed a decrease of the naïve subset and an increase of the terminally differentiated effectors (TEMRA), along with higher frequency of activated cells. Moreover, we also observed an increase of $CD56^{dim}CD57^{+}$ NK cells. These data indicate a higher differentiation of the cytotoxic subsets in the melanoma patients. Upon PMA/Ionomycin stimulation, higher proportion of CD8 TEMRA from patients expressed cytokines (IL-2, IFNg, TNFa, GM-CSF, MIP-1b) and the degranulation marker LAMP-1. We also detected higher expression of lytic granules (granzyme B, perforin) along with increased expression of PD-1. Altogether, these data provide insight regarding the immune landscape in melanoma patients which may affect their overall immune responsiveness.

W9. Glucose Metabolism Controls Human **γδ**-T Cell-mediated Tumor Immunosurveillance in Diabetes Through AMPK Pathway

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Patients with type 2 diabetes mellitus (T2DM) have a high risk of cancer. The effect of glucose metabolism on γδ-T cells and their impact on tumor surveillance remain unknown. Here, we show that high glucose induces a “Warburg effect”-like bioenergetic profile in Vγ9Vδ2-T cells, leading to excessive lactate accumulation, which further inhibits lytic granule secretion by preventing the trafficking of cytolytic machinery to the Vγ9Vδ2-T cell-tumor synapse through suppressing AMPK activation, and finally resulting in loss of their antitumor activity in vitro, In vivo and in patients. Strikingly, activating AMPK pathway by glucose control or metformin treatment reverses the metabolic abnormalities and restores the antitumor activity of Vγ9Vδ2-T cells. These results suggest that the impaired antitumor activity of Vγ9Vδ2-T cells induced by dysregulated glucose metabolism may contribute to the increased cancer risk in T2DM patients, and that metabolic reprogramming through targeting AMPK pathway by using metformin may improve their tumor immunosurveillance.

W17. Immune Remodelling in Advanced Adenocarcinoma by a CD40L-MUC1 Vaccine

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Understanding how immunotherapy effects the global immunome is a significant unmet need. We carried out a first-in-man Phase 1 study of Ad-sig-hMUC1/ecdCD40L (NCT02140996), an adenoviral-vector vaccine encoding the tumour-associated antigen MUC1 linked to CD40 ligand. The intervention demonstrated safety and tolerability in a cohort of 21 patients with advanced adenocarcinomas. High-dimensional mass cytometry revealed extensive dysfunction of the systemic immune system in cancer in 39 of 100 immune cell clusters identified compared to age and sex-matched healthy controls. Of the cell types that were deranged in cancer versus healthy, vaccination increased the frequency of cytotoxic GZMB+ IFNγ+ TNFα+ CD8 T cells as well as CD38+ CD14+ monocytes. Immunisation also reshaped the global immune network in cancer and partially restored the complexity of connections between nodes. By stratifying patients according to clinical outcome, we found that circulating GZMB+ CD8 T cells and B cells were increased in patients with stable disease after vaccination. Importantly, key differences were found in the baseline immunomes of patients with stable disease compared to progressive disease, with higher circulating CD45RA+ CD4 T cells associated with better clinical outcomes. Overall, our results suggest a broad, partially "restorative" effect of vaccination with Ad-sig-hMUC1/ecdCD40L. Our data highlights the importance of a functional immune system for response to immunotherapy, and the utility of high-dimensional analyses in understanding and predicting effective cancer therapies.

W56. Evaluation of GPER Activation Pathways Associated with Migration and Invasion in LNCaP Cells

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Prostate cancer (PCa) is clinical problem worldwide and its molecular mechanisms are still studied. Metastasis is common in these patients. Recently, G-protein coupled estrogen receptor (GPER) act as a growth modulator but it's reported to control metastasis, by inducing signaling pathways activation, mainly mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K) involved in migration and invasion. Agonist molecules like G1 was identified to induced a conformational change in GPER which prevents its activity depending on the type of tissue, therefore GPER on cellular processes is extremely heterogeneous and needs to be studied for each type of cancer, however, in PCa is not well elucidated. Evaluate ERK and AKT signaling expression and their relationship between migration and invasion in LNCaP prostate cell lines. LNCaP cell line were grown and stimulated with G1 and E2, to subsequently performed protein extraction and evaluate ERK and AKT phosphorylated expression using Western Blot technique. Also, with the same stimuli, migration and invasion assays were performed by transwell plates. The expression of phosphorylated molecules were observed. For LNCaP AKT3 isoform remains constant in all treatments while in pERK (ERK1) decreases with G1 treatment compare to control. LNCaP migration was observed to decrease 33% with G1 and 50% with E2 compare to control with a significant $p< 0.001$, meanwhile invasion prevailed for both treatments being 60% with G1 with a significant $p< 0.01$ and 93% with E2 compare to control. In this research, G1 treatment affect ERK1 activity which decrease migration and invasion in LNCaP cells.

W74. The Tumor Suppressor APC Regulates T Lymphocytes Migration: Insights from Familial Polyposis Patients

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Adenomatous polyposis coli (APC) is a tumor suppressor whose mutations underlie familial adenomatous polyposis (FAP) and colorectal cancer. While the importance of APC in epithelial homeostasis and its polarity regulator functions in non-immune cells are well established, its role in T cell biology and anti-tumor immunity remains poorly characterized. APC regulates cytoskeleton organization, cell polarity and migration in various cells types. Appropriate migration of T cells and the ability to infiltrate tumors are required for efficient anti-tumor immune surveillance. Here we address whether APC plays a role in T lymphocyte migration, a key step of anti-tumor immune responses. Using a series of cell biology tools, we unveiled that T cells from FAP patients carrying APC mutations display impaired adhesion and motility in constrained environments. We further dissected the cellular mechanisms underpinning these defects in APC-depleted CEM T cell line that recapitulate the phenotype observed in FAP T cells. We found that APC is critical not only for VLA-4-dependent adhesion but also for actomyosin and microtubule organization in migrating T cells. Finally, APC-silenced CEM cells preferentially adopt an ameboid-like migration featuring unstructured pseudopodia and blebbing. These findings underscore a role of APC in T cell migration via modulation of integrin-dependent adhesion and cytoskeleton reorganization. Hence, APC mutations in FAP patients not only drive intestinal neoplasms, but also impair T cell migration, potentially leading to inefficient T cell-mediated anti-tumor immunity.

Infectious Diseases

Th46. Calibrating T Cell 'Help' by Antigen Cross-linking as a Universal Vaccine Platform Against Rapidly Evolving Pathogenic Viruses

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The effectiveness of the seasonal influenza vaccine is limited by the very heterogeneous immune response it elicits. Factors such as age, sex, and pre-existing antibody titers only partially explain this heterogeneity. We characterized the response in a large cohort (n >500) of healthy, vaccinated volunteers over multiple seasons. Using a novel deconvolution analysis, we observed that a skewed, subtype-specific response that increases with age as a major source of inter-individual variation, exceeding all the previously known factors combined. This "subtype-bias" leads to poor responses to disfavored strains, leaving vaccinated individuals susceptible to infection. Analysis in a twin cohort suggested a genetic basis to this bias that is modulated by exposure history. We determined the source of this genetic component to the wide variation in influenza hemagglutinin (HA) peptide presentation by different HLA class-II alleles resulting in an imbalanced, subtype-specific $CD4^+$ T follicular help (Tfh) after vaccination. To address this, we developed a tunable, plug-and-play vaccine platform to better calibrate T cell help. Using a human tonsil organoid system, we demonstrated that the 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, the applicability of the platform to develop "universal" vaccines was tested by generating a pan-coronavirus vaccine.

Th135. Biomarkers of Disease Outcome in Lassa Fever

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Lassa virus (LASV) causes the acute disease Lassa fever (LF) in humans and is endemic throughout western Africa. Symptoms of LF include fever, body aches, hypovolemic shock, and multi-system organ failure, leading to high mortality in hospitalized patients. The progression of LF is not well understood, so it is difficult to predict which patients will most need critical care, or which types of care will best improve patient outcomes. We aimed to identify protein biomarkers in blood that could predict patient mortality over the course of hospitalization. Samples were collected longitudinally from 201 patients with LF in Nigeria and measured by Luminex assay for 62 serum cytokines and proteins associated with the immune response, inflammation, and hemostasis. Statistical analysis followed by logistic regression modeling revealed 18 host proteins significantly elevated at admission in patients that died, of which PAI-1 (ROC AUC=0.88), soluble thrombomodulin (AUC=0.84), and soluble TNFR1 (AUC=0.81) most consistently predicted fatal outcome. We then applied our statistical pipeline to later timepoints to further characterize the predictive ability of these biomarkers throughout the course of disease.

At 2-3 days post admission, when many of the fatal cases were close to death, PAI-1, sTM, and TNFR1 remained highly predictive, and the chemokines fractalkine and MCP-1 became highly predictive of mortality (all five AUCs >0.87). Lastly, we tested whether pairs of these markers could improve predictive accuracy over single markers. All top predictive pairs at admission included PAI-1, with the best combination showing slight improvement over PAI-1 alone (PAI-1 + M-CSF, AUC=0.90).

Th154. Diminished Vδ2+ γδ T cell Cytokine Production and Degranulation Following in vitro Malaria Exposure

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Natural immunity to *Plasmodium falciparum (Pf)* malaria provides some protection against symptomatic disease in older children and adults, but is unable to eliminate parasite replication. A major component of this incomplete immunity is attenuation of cytotoxic, pro-inflammatory responses by innate cells like Vδ2+ γδ T cells following repeated malaria exposure. We are utilizing several innovative approaches to identify mechanisms underlying Vδ2+ T cell dysfunction, including replicating this phenotype *in vitro* and leveraging samples from a longitudinal cohort in Uganda. Purified malaria-naïve Vδ2+ cells stimulated *in vitro* with *Pf*-infected red blood cells (iRBCs) or the phosphoantigen HMBPP produce less TNFα and IFNγ and degranulate less in response to secondary stimulation compared to unstimulated cells. In contrast, stimulation with iRBCs or HMBPP does not impact the ability of the cells to respond to control stimuli, indicating that the reduced response is *Pf*-specific. Addition of monocytes to Vδ2+ T cells –particularly at higher ratios—reduced responses compared to unstimulated cells or cells stimulated without monocytes. Together, these results support both cell-intrinsic and extrinsic mechanisms contributing to reduced Vδ2+ T cell responsivity following malaria exposure. In parallel, we are performing paired RNA-Seq and ATAC-Seq experiments among Vδ2+ cells from Ugandan children at multiple timepoints in order to define transcriptional and epigenetic changes underlying altered cell function following repeated malaria. Ultimately, this work could deepen our understanding of mechanisms driving inefficient acquisition of antimalarial immunity and could inspire novel therapeutic approaches that enhance parasite clearance and/or reduce disease severity.

Th159. A Nine-Gene Blood-Based Signature Meets the World Health Organization (WHO) Target Product Profiles for Diagnosis of Active Tuberculosis and Predicting Progression from Latent to Active Disease

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As part of its End TB strategy, the WHO has identified the need for non-sputum-based diagnostics that meet target product profiles (TPP) of 90% sensitivity and 70% specificity for diagnosis of ATB and 75% sensitivity and specificity for predicting progression from LTB to ATB. The successful translation of a 3-gene blood-based signature, identified using diverse datasets, into a prototype point-of-care diagnostic that meets the WHO TPPs, has demonstrated the power of integrating large amounts of heterogeneous data to identify generalizable disease signatures.

We hypothesized that integration of more diverse datasets, comprising patients with ATB or other inflammatory lung diseases (e.g., COPD, viral infections, sarcoidosis, lung cancer, etc.), would identify novel robust signatures, for diagnosing ATB and predicting progression from LTB to ATB, that meet the WHO TPPs. By integrating data from 3615 peripheral blood samples across 49 publicly available transcriptomic datasets, we identified a 9-gene signature for diagnosing ATB patients from healthy controls, or individuals with LTB or other diseases. The signature achieved 90.1% sensitivity and 81.7% specificity in retrospective validation cohorts (3836 blood samples, 28 datasets) and 90.2% sensitivity and 68.9% specificity in a prospective cohort from Moldova (360 blood samples). In a longitudinal cohort of adolescents, the 9-gene signature predicted progression from LTB to ATB up to 1 year prior to sputum conversion with 76% sensitivity and 83.3% specificity. Finally, the signature predicted prolonged lung inflammation in the Catalysis Treatment Response Cohort. Overall, the 9-gene signature meets the WHO TPPs required for the End TB strategy.

Th182. High-Throughput B Cell Epitope Determination by Next-Generation Sequencing

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Development of novel technologies for the discovery of human monoclonal antibodies has proven invaluable in the fight against infectious diseases. Among the diverse antibody repertoires elicited by infection or vaccination, often only rare antibodies targeting specific epitopes of interest are of potential therapeutic value. Current antibody discovery efforts are capable of identifying B cells specific for a given antigen; however, epitope specificity information is usually only obtained after subsequent monoclonal antibody production and characterization. Here we describe LIBRA-seq with epitope mapping, a next-generation sequencing technology that enables residue-level epitope determination for thousands of single B cells simultaneously. By utilizing an antigen panel of point mutants within the HIV-1 Env glycoprotein, we identified and confirmed antibodies targeting multiple sites of vulnerability on Env, including the CD4-binding site and the V3-glycan site. LIBRA-seq with epitope mapping is an efficient tool for high-throughput identification of antibodies against epitopes of interest on a given antigen target.

W26. Serum Levels of IgG Antibodies Against Oxidized LDL and Atherogenic Indices in HIV-1-infected Patients Treated with Protease Inhibitors

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Abstract: Antibodies against low-density lipoproteins (LDLs) that have been oxidized are associated with development of atherosclerotic lesions. In individuals infected with human immunodeficiency virus type 1 (HIV-1) with or without therapy, dyslipidemia and increased cardiovascular risk are observed. Methods: Serum levels of IgG antibodies against oxidized LDLs (IgG anti-oxLDL Abs) were determined by assay in 151 HIV-1-infected patients. Of these, 42 patients did not receive anti-retroviral therapy (ART-naïve), whereas 109 received highly active anti-retroviral therapy (HAART) consisting of lopinavir/ritonavir (LOP/r; n=50), efavirenz (EFV; n=30) and nevirapine (NVP; n=29) associated with nucleoside reverse transcriptase inhibitors. HIV-1 seronegative individuals (n=43) participated in the study. The following parameters were quantified: total cholesterol and its fractions, atherogenic indices (AIs), apolipoproteins A1 and B100, high sensitivity C-reactive protein, CD4+ and CD8+ T cells, and HIV-1-RNA. Results:Levels of IgG anti-oxLDL Abs were significantly higher ($p < 0.05$) in the LOP/r group compared with the EFV and/or NVP and the seronegative group: median 0.32 (0.15, 0.58; 95% confidence interval) vs. 0.25 (0.13, 0.53) vs. 0.18 (0.04, 0.38), respectively. HIV-1-infected ART-naïve patients (n=42) presented antibodies levels similar to those observed for the LOP/r group, 0.33 (0.13, 0.63; $p > 0.05$).

The levels of IgG anti-oxLDL Abs correlated with an increase in AIs (r=0.216; p=0.036) and triglycerides (r=0.220; p=0.044) in the LOP/r group, and AIs in the ART-naïve group (r=0.300; p=0.046).Conclusions: Patients treated with LOP/r showed higher levels of IgG anti-oxLDL Abs compared with patients treated with EFV or NVP regimens, and these levels were associated with an increase in AIs.

W94. Herpes Simplex Virus Type 1 Modulation of Neutral Lipid Metabolism in Dendritic Cells Affects the Function of These Cells

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Herpes simplex virus type 1 is a prevalent human pathogen that causes lifelong infection by latently infecting neurons. HSV-1 also infects dendritic cells (DCs), affecting their viability, maturation profile and capacity to present viral antigens to T cells, thus impairing an effective immune response against this virus. On the other hand, lipid droplets (LDs) are neutral lipid-rich organelles mainly composed of triglycerides and cholesterol esters, a phospholipid monolayer and surrounded by proteins associated with neutral lipid metabolism, among others. LD functions are mainly related to energy reserve sources; however, LDs have also been associated with immune system regulation, wherein LD accumulation impairs the DCs antigen presentation to T cells. A recent study indicated that early HSV-1 infection induces LDs accumulation in astrocytes and epithelial cells, a process associated with an increase in the interferon antiviral response. We observed by confocal and transmission electron microscopy that HSV-1 induces LD accumulation in DCs. Moreover, RT-qPCR analyses indicate that HSV-1 regulates the expression of neutral lipid biogenesis-associated genes. Further, we determined that the inhibition of neutral lipid biogenesis pathways reduces HSV-1 plaque-forming units (PFU), a process associated with reduced LDs accumulation in infected-DCs. Moreover, the pharmacological inhibition of neutral lipid synthesis pathways recovered DC viability and increased MHC-I as well as MHC-II expression on the surface of DCs. Our results indicate that HSV-1 modulates neutral lipid biogenesis inducing LD accumulation and impacting DCs function.

W95. Targeting Cholesterol Metabolism as a Novel Immune Checkpoint in Viral Infections and Cancer

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Metabolic targets may provide novel strategies to complement existing classical checkpoints in order to boost the highly exhausted T cells directed against viruses and tumours. Recent studies demonstrated that inhibition of acyl-coenzyme A:cholesterol acyltransferases (ACAT) can both enhance murine anti-tumour CD8+ T cell efficacy and mediate a direct antitumour effect. We showed that reduced formation of membrane lipid microdomains is a feature of PD-1^{hi} exhausted T cells. We therefore investigated the potential for rescue of exhausted human virus-and tumour-specific T cell responses by modulation of cholesterol metabolism and lipid microdomain formation. We found that ACAT inhibition could enhance the expansion of functional virus-specific T cells from donors with chronic hepatitis B virus (HBV) or acute SARS-CoV-2 infection. ACAT inhibition also boosted hepatocellular carcinoma (HCC)-specific T cell responses directly isolated from human liver and liver tumour lesions in the majority of patients. Responding T cells showed increased lipid microdomain formation, reduced lipid droplets, enhanced T cell receptor (TCR) signaling and TCR-independent bioenergetic reprogramming. ACAT inhibition had a complementary effect with other immunotherapies, with increased responsiveness to PD-1 blockade and enhanced functional avidity of T cells genetically engineered to recognize HBV and tumour cells. Taken together, modulating cholesterol metabolism by inhibition of ACAT is a promising therapeutic target, enhancing immune responses in acute and chronic infection and in cancer.

W113. Identification of Conserved Detrimental Host Immune Response Predicts Severity of Bacterial and Viral Infections

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Background: Host immune response has been repeatedly shown to diagnose the presence and type of infection. Recently, we described a 42-gene blood-based signature, conserved across viruses, that correlates with and predicts the severity of viral infection, irrespective of age, sex, and host or pathogen genetics. This 42-gene signature is composed of 4 modules (2 protective, 2 detrimental). We hypothesize that these modules are also associated with disease severity in patients with bacterial infection. Methods: We analyzed 58 publicly available datasets of blood transcriptome profiles from 4989 patients (2300 healthy, 1169 with bacterial infection, 1520 with viral infection), and co-normalized them using COCONUT. Severity was stratified into seven categories ranging from healthy to fatal. We evaluated our four module and composite severe-or-mild "SoM" scores, and applied our previously described 7-gene signature (7GS, distinguishing viral from bacterial infection) to both bacterial and viral samples. Results: Two detrimental module scores were positively correlated with severity of bacterial infections (module 1: r=0.64, module 2: r=0.53), and one of two protective modules was inversely correlated (module 4: r=-0.59). Module 3, protective in viral infections, was minimally positively correlated with severity of bacterial illness (module 3: r=0.20). SoM score distinguished non-severe bacterial infections from severe bacterial infection (r=0.63, AUROC=0.77, 95% CI:0.73-0.80). Conclusion: SoM score can distinguish patients with severe infection, irrespective of bacterial or viral etiology. When used with our 7GS, it may help decide whether a patient warrants (1) treatment with an antibiotic or (2) discharge or admission upon presentation to an emergency department.

W179. Platelet Activation Through TRPC6 Mediates Cystic Fibrosis Lung Inflammation and Injury

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Introduction. Cystic Fibrosis (CF) lung disease presents with an early, exaggerated inflammatory response, which impairs bacterial clearance. Platelets mediate inflammation through activation, signaling, and cross-talk with neutrophils. We previously showed that deletion of *CFTR* in platelets results in a hyperinflammatory yet impaired immune response in CF mouse models, which can be rescued by pharmacologic or genetic inhibition of the Transient Receptor Potential Cation Channel 6 (TRPC6). Here, we investigate the role platelet-neutrophil interactions such as neutrophil-platelet aggregation (NPAs) and NETosis in CF mouse models. Methods. We generated conditional CFTR knockouts in which $CFTR^{fl/fl}$ mice were crossed with LysM-Cre (CF-LysM) or MRP8-Cre (CF-MRP8) to delete CFTR in myeloid cells or neutrophils, respectively, and PF4-Cre (CF-PF4) to delete CFTR in platelets. We generated a global double knockout of *CFTR* and *TRPC6* ($CFTR^{-/-}$ $TRPC6^{-/-}$). Mice were challenged with intratracheal LPS or *Pseudomonas aeruginosa* (*PsA*). Markers of inflammation, bacterial cfu, NPAs, and NETs were quantified at 48 hrs. Results. $CFTR^{-/-}$ and CF-PF4 mice demonstrated a hyperinflammatory yet impaired immune response in LPS and *PsA* models as well as increased NPAs and NETs. CF-LysM and CF-MRP8 mice did not exhibit this hyperinflammatory response. Genetic inhibition of the TRPC6 in the $CFTR^{-/-}$ $TRPC6^{-/-}$ double knockout mice normalized inflammation, bacterial clearance, and decreased NETosis. Conclusions. *CFTR* deletion in platelets results in a pathologic and impaired inflammatory response, which is attenuated by TRPC6 inhibition. Platelet activation mediates lung inflammation through increased NPAs and NETs. Modulating platelet activation and TRPC6 may be new therapeutic targets for treating CF.

W184. CGC+ CD4+ T Cells are Cytomegalovirus Specific Cells that are Biomarkers of Cardiometabolic Disease Risk in Persons with HIV

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Cells of the innate and adaptive immune system are important drivers of cardiometabolic disease in the general population. In persons with HIV (PWH) on antiretroviral therapy, persistent inflammation partly due to coinfection with cytomegalovirus (CMV) may be important. We recently defined a subset of $CD4^+$ T cells that co-express three surface markers, CD57, GPR56, and CX3CR1 abbreviated as 'CGC'. We previously showed that CGC^+ $CD4^+$ T cells are increased with diabetes and a high burden of carotid artery plaque in PWH. In this ongoing study, we use CMV tetramers to define the specificity of CGC^+ $CD4^+$ T cells. In a cohort of non-diabetic, pre-diabetic, and diabetic PWH (n=105), we found that CGC^+ $CD4^+$ T cells in blood were positively correlated with waist circumference (*p*=0.01), hemoglobin A1C (*p=0.002*), and fasting blood glucose (*p=0.0006*) but not cholesterol. Using the select MHC class II tetramers against known immunogenic CMV epitopes, all the CMV-specific T cells were CGC^+ $CD4^+$. We found up to 14-21% of CGC^+ $CD4^+$ T cells recognized a single immunodominant gB epitope – DYSNTHSTRYV (DYS). Multiparameter flow cytometry and mass cytometry analysis showed that CGC^+ $CD4^+$ T cells consist of T effector memory (T_{EM}) and T effector memory CD45RA (T_{EMRA}) cells. Other markers co-expressed on CGC^+ $CD4^+$ cells include $ICOS^+$, $CXCR3^{+/-}$, $CD28^{+/-}$,$OX40^{+/-}$, and $CD27^-$. Our findings provide evidence that CGC^+ $CD4^+$ T cells are CMV-specific T cells associated with cardiometabolic disease and could be used to monitor the clinical response to treatments used to reduce inflammation and improve CVD outcomes.

Inflammatory Diseases

Th38. A Novel Ultrasensitive Assay Enables Measurement of IL-11 in Fibrosing Disease Patients and Healthy Donor Samples

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IL-11 is a pleiotropic cytokine recently implicated in fibrotic diseases of various vital organs. Neutralizing antibodies were developed to address the unmet medical need of several fibrosing diseases. Initial IL-11 assays lacked sufficient sensitivity to measure IL-11 in human serum/plasma. To address this lack of sensitivity, a novel ultrasensitive assay platform was developed the ultra-sensitive SP-X platform (Quanterix Corp). IL-11 levels were assessed in over 230 healthy donors and patient samples of several fibrosing disease indications. We were able to successfully detect IL-11 in more than 90% of our healthy donors in serum, plasma and bronchoalveolar lavage fluid allowing us to discern a significant increase in soluble IL-11 levels in patients with multiple fibrosing diseases, which has hitherto been challenging due to lack of assay sensitivity. IL-11 has been implicated in various diseases, and the ability to measure this cytokine in more than 90% of healthy donors and > 80% of patients with diverse fibrotic diseases as a biomarker, is pivotal in understanding the underlying pathobiology options for targeted therapy.

Th41. The Therapeutic Potential of Hematopoietic Stem Cell IL-1RA Gene Therapy for the Treatment of Hereditary IL-1-mediated Autoinflammatory Syndromes

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Systemic autoinflammatory diseases (SAIDs), including Cryopyrin-associated periodic syndrome (CAPS), are a group of devastating diseases characterized by recurrent episodes of systemic inflammation affecting various organs. There is no definitive cure for these patients. A desirable approach to treating these diseases may be the selective delivery of anti-inflammatory proteins to sites of inflammation. Thus, we used a lentiviral vector (LV) to target hematopoietic stem cell (HSC)-derived immune cells as vehicles for local delivery of the anti-inflammatory human IL-1 receptor antagonist (IL-1RA) protein in various mouse models of IL-1-mediated inflammatory diseases. Our gene-based approach may restore a balanced immune response and ameliorate disease. LV-derived IL-1RA production in mice and human HSCs did not alter their clonogenic and differentiation potential In vivo. We went on to demonstrate the efficacy of the IL-1RA gene therapy approach in various mouse models of IL-1-mediated inflammation. IL-1RA gene therapy suppressed the neutrophil recruitment induced by monosodium urate crystals, the etiological agent of gout. Moreover, mice receiving IL-1RA gene therapy were cured of CAPS-like symptoms, including weight loss, blood leukocytosis, and systemic and tissue inflammation. Lastly, IL-1RA gene therapy ameliorated the course of chronic progressive experimental autoimmune encephalomyelitis, reducing disease severity. Our data indicate that the HSC-based IL-1RA gene therapy approach can effectively correct tissue inflammation in different syndromes caused by IL-1 overproduction.

Th54. Pre-existing Cardiothoracic Comorbidity and Lung Disease in Still's

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Background: Still's disease is a rare, chronic inflammatory illness of unknown etiology. Parenchymal lung disease (LD) is not expected. In 2021, we reported a striking HLA association with severe delayed drug reaction, implicating inhibitors of interleukin (IL)-1 or IL-6. This reaction can include parenchymal LD. Five-year survival probability with co-occurring Still's and LD, regardless of drug exposure, is reported as 42%.[1] Here, we associate risk of LD to certain pre-existing comorbidities. Methods: A retrospective analysis of 117 multi-center, international cases of parenchymal LD in Still's patients, included regardless of IL-1 or IL-6 inhibitor exposure, was performed. Control data was the published population frequency for each co-morbidity, as available. Results: In the past two years, the annual rate of parenchymal LD in Still's has increased to >20 fold above the rate prior to the introduction of IL-1 and IL-6 inhibitors. Evaluation of the 17 Still's LD cases with no exposure to IL-1 or IL-6 inhibition revealed pre-existing comorbidity in 47% (8/17), specifically lung disease, congenital cardiopulmonary anomaly or Trisomy 21 (T21). A similar analysis of 100 cases with drug reaction-associated diffuse LD revealed enrichment above published frequencies for bronchopulmonary dysplasia of prematurity (4/100 vs 1.39/10,000), complex congenital heart disease (9/100 vs 12.9/10,000), or T21 (10/100 vs 1-2/1000 live births), including two T21 without another co-morbidity. Conclusions: Pre-existing cardiothoracic co-morbidities are associated with life-threatening parenchymal lung disease in the setting of Still's disease, with or without inhibitor exposure. Mechanisms are unknown.

[1]Saper et al ARD 2019

Th55. Impaired T Cell Mitochondrial Function and Metabolic Shifts in Glycolysis Characterize Axial Spondylarthritis

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CD4+ T cells have been implicated in the pathogenesis of axial spondyloarthritis (axSpA), and in rodent models are sufficient to drive disease. With the aim of discovering unique features within CD4+ T cells, we analyzed previously published single-cell RNA sequencing data of PBMCs from two individuals with axSpA and two controls. The two most impacted pathways in T cells were oxidative phosphorylation and mitochondrial dysfunction (P< 1e-10) driven by downregulation in axSpA of critical genes in complexes 1, 3, 4 and 5. To assess T cell mitochondrial function in axSpA, we measured mitochondrial reactive oxygen species (ROS) using the flow-based MitoSOX assay (N=15). T cells in axSpA had significantly increased MitoSOX staining compared to controls (P=.011) with CD3+CD4+ showing 2.8 fold (P=0.004) and CD3+CD8+ T cells a 2.0 fold (P=0.005) increased mitochondrial ROS production. Next, we sought to understand how changes in T cell mitochondrial function were reflective of the general metabolomic profile of axSpA. We screened metabolites in serum of 23 patients with axSpA and 24 controls. Oxalosuccinate of the TCA cycle was significantly increased in patients with axSpA (P=7.86e-9) and multiple omega-3 fatty acids (hexadecanoic acid, octadecanoic acid, and docosahexaenoic acid) were all decreased compared to controls (FDR corrected P< .05 across tested metabolites), suggesting an impaired switch from glycolysis to oxidative phosphorylation. Together our results suggest mitochondrial dysfunction in T cells as well as overall metabolic dysfunction in patients with axSpA, opening an avenue for further study into the pathophysiology of axSpA.

Th62. Bioengineering Probiotic Yeast to Secrete Anti-Proinflammatory Cytokine Antibodies for Resolving Gut Inflammation

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Gut chronic inflammation is the culprit of many serious diseases and disorders, such as metabolic disorders, cancers, and neurological diseases. The proinflammatory cytokines Tumor Necrosis Factor alpha (TNF-α) and Interleukin 17a (IL-17A) are the key immune mediators that contribute to the pathogenesis of gut inflammation. The goal of the project was to genetically engineer an over-the-counter probiotic yeast, Saccharomyces boulardii, for the constitutive secretion of a bi-specific antibody and to neutralize both of the inflammatory cytokines into a safe and convenient oral medicine for inhibiting chronic gut inflammation. We first designed two formats of the bi-specific antibody by fusing two anti-cytokine single-domain antibodies, or VHHs, with human IgG1 Fc (VHH1-Fc-VHH2 and VHH1-VHH2-Fc). Next, we tested two strong constitutive promoters for their ability to efficiently drive the expression of the bi-specific antibody in S. boulardii. Finally, we evaluated two non-antibiotic selection markers for the selection of chromosomal insertion of the bi-specific antibody genes. We found that the two promoters each drove a slightly different bi-specific antibody expression. The bioactivities between the two formats of bi-specific antibodies were similar, but the format of VHH1-VHH2-Fc yielded a higher expression in the probiotic yeast. The selection markers did not affect the overall screening nor the expression of the antibody. Based on these results, we selected a final engineered yeast strain, which was designated as FZ008, as our lead drug candidate. In the future, we will perform preclinical evaluations on FZ008 against gut chronic inflammation in transgenic mice, and eventually human trials against neurodegenerative diseases.

Th63. Non-Conventional CD8+IL-13+ T Cells in Refractory Atopic Dermatitis Patients

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Atopic dermatitis (AD) is a chronic skin disorder mediated by type-2 cytokines, of which IL-13 plays a central role. While the primary source of IL-13 in AD is $CD4^+$ TH2 cells , non-conventional $CD8^+IL\text{-}13^+$ T-cells are an increasingly recognized source of this pathogenic cytokine. Cyclosporine A (CsA), a calcineurin inhibitor used in the treatment of AD, promotes anergy in CD4+ T-cells, but may have less efficacy on CD8+ T-cell function. We hypothesize that $CD8^+IL\text{-}13^+$ T-cells may be differentially regulated, persist despite remission, and contributing to disease chronicity. In order to investigate $CD8^+$ T-cell IL-13 production, we utilized our *in vitro* model, whereby PBMCs from AD patients meeting Hanifen and Rajka criteria (N=13; mean EASI 16.2; mean age 38.5 yrs) were activated for 7 days in the presence of *S. aureus* superantigen (SEB) +/- TSLP. Using multiparametric flow cytometry to compare IL-13 production in CD4 and CD8 T-cell subsets, we found comparable proportions of $CD8^+IL\text{-}13^+$ T-cells expressing pSTAT6 and GATA3 in the untreated condition. Furthermore, the level of IL-13 production was similar between the two populations. In contrast, exposure to TSLP+SEB significantly expanded the number of $CD4^+$ - but not $CD8^+$ - $IL\text{-}13^+$ T-cells, despite a significant increase in activation status (CD69) and pSTAT6 in both subsets. Using a prospective cohort of adult AD patients treated with CsA, we next aim to investigate the persistence of $CD8^+IL\text{-}13^+$ T-cells during disease remission. Our findings will allow us to gain insight on pathophysiology and potential mechanisms responsible for relapse.

Th76. The Effect of TRPV1+ Neuron-derived Neuropeptides on Cytokine Production of Th1 and Th17 Cells

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In periodontitis, Th1 and Th17 cells in inflammatory sites mediate host defense against bacterial infection but are also involved in tissue destruction. TRPV1$^+$ neurons that sense noxious stimulus are densely innervated in oral mucosa and secrete substance P, CGRP and glutamate in inflammatory conditions. Recent studies have shown that calcitonin gene-related peptide (CGRP) promotes Th17 differentiation and Th17 cell functions. However, the effect of neuropeptides on Th1 cells has not been studied yet. In this study, we investigated the effects of neuropeptides on cytokine production in Th1 and Th17 cells. Th1 and Th17 cells were differentiated by in vitro culture of isolated CD4$^+$ T cells from C57BL/6 mice with polarizing cytokines in RPMI medium. Both Th1 and Th17 cells expressed CGRP receptor genes (receptor-activity-modifying protein 1 and calcitonin-like receptor) and a glutamate receptor gene Grina but no substance P receptor genes. In addition, calcium influx in response to CGRP and N-methyl-D-aspartate (NMDA), a glutamate receptor agonist, was confirmed in Th17 cells. To examine the effect of neuropeptides on cytokines production in T cells, TCR- or bystander-activated Th1 and Th17 cells were stimulated with either CGRP or NMDA. CGRP did not affect IFNγ production in Th1 cells and decreased TCR-activated IL-17 production (0.9 fold) but increased bystander-activated IL-17 production (4.8 fold) in Th17 cells. NMDA increased TCR-activated IFNγ production (1.5 fold) in Th1 cells and TCR-activated IL-17 production (1.3 fold) in Th17 cells. Taken together, TRPV1$^+$ neuron-derived CGRP and NMDA increased the function of both Th1 and Th17 cells in vitro.

Th163. The Minor Allele of a Single Nucleotide Polymorphism in SH2B3 Promotes CD8+ T Cell IFNg Production, Hypertension Development, and Renal Damage

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SH2B3 is an adaptor protein that negatively regulates growth factor and cytokine signaling. A common missense single nucleotide polymorphism in *SH2B3* (rs3184504) results in substitution of tryptophan (Trp) for arginine (Arg) at amino acid 262 and is a top association signal for hypertension in human genome-wide association studies. Whether this variant causally impacts hypertension development and end-organ damage is unknown. We used CRISPR-Cas9 to engineer mice homozygous for the major and minor alleles of this *SH2B3* polymorphism, resulting in Arg/Arg and Trp/Trp mice, respectively. Trp/Trp mice exhibited approximately 10 mm Hg higher systolic blood pressure with chronic angiotensin II (Ang II) infusion compared to Arg/Arg mice. Renal damage was also exacerbated in Ang II-treated Trp/Trp mice, as evidenced by significantly increased urinary albumin/creatinine ratio and renal perivascular fibrosis. In addition, renal CD8$^+$ T cells from Ang II-treated Trp/Trp mice produced significantly more IFNg compared to Arg/Arg controls, and *Ex vivo* stimulated splenic CD8$^+$ T cells from Trp/Trp mice made 2.7-fold more IFNg. Interleukin-12 (IL-12)-induced IFNg production was greater in Trp/Trp compared to Arg/Arg treated CD8$^+$ T cells. In addition, IL-12 enhanced Stat4 phosphorylation in Trp/Trp compared to Arg/Arg CD8$^+$ T cells, suggesting that Trp-encoding SH2B3 exhibits less negative regulation of IL-12 signaling to promote greater IFNg production. Taken together, these results suggest that the Trp encoding allele of rs3184504 is causal for blood pressure elevation and renal dysfunction, at least in part through loss of SH2B3-mediated repression of T cell IL-12 signaling leading to enhanced IFNg production.

W2. Conventional and Unconventional T-Cell Sources of IL-17A and IL-17F in Psoriatic Arthritis

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The immunopathology of psoriatic arthritis (PsA) is driven in part by IL-17A. IL-17A production is typically attributed to conventional CD4+ and CD8+ T-cells, however unconventional innate-like T-cells including MAIT, iNKT and γδT-cells can be additional sources of IL-17A as well as IL-17F. We determined IL-17A and IL-17F expression in conventional and unconventional T-cells from PsA synovial fluid (n=7). Mass cytometry was performed on untreated or 3-hour PMA/ionomycin/GolgiStop stimulated CD3+ T-cells, and samples were analysed using a customised CATALYST pipeline. FlowSOM clustering revealed that the dominant synovial T-cell populations were CD4+ (53%) and CD8+ conventional T-cells (39%), with lower frequencies of unconventional γδT-cells (2.1%) and CD8+ MAIT cells (4.6%). Very low frequencies of TCRVα24-Jα18 expressing iNKT-cells (0.6%) were found. Synovial IL-17A+ T-cells were predominantly found within CD8+ (51%) and CD4+ (48%) T-cells and characterised by high CXCR6 expression, with the majority of IL-17A+CD8+ T-cells displaying a CD103+ tissue-resident memory (TRM) phenotype. On average, 1.5% of IL-17A+CD8+ T-cells co-expressed high levels of TCRVα7.2 and CD161, suggestive of MAIT cells. Very few IL-17F producing T-cells were identified; these cells co-expressed IL-17A and clustered as conventional CD8+ TRM cells or CD4+ T-cells. We detected only sporadic TCRγδ or TCRVα24-Jα18 expressing cells amongst synovial IL-17 producing T-cells. Our data indicate that synovial IL-17A and IL-17F production is predominantly confined to conventional CD4+ and CD8+ T-cells including TRM cells. High CXCR6 expression on PsA synovial IL-17-expressing T-cells suggests a functional role for the CXCR6-CXCL16 axis in recruitment or retention of these cells in the inflamed joint.

W13. Vedolizumab Reduces Dendritic Cells and Naïve Lymphocytes in the Colon Mucosa

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Vedolizumab blocks integrin alpha4/beta7 interaction with MAdCAM-1 on intestinal vascular endothelial cells. It is believed to treat inflammatory bowel disease (IBD) by preventing alpha4/beta7+ cells in blood from entering the intestinal mucosa. By CyTOF and flow cytometry, we found high expression of alph4/beta7 on nearly all myeloid dendritic cells in the blood. Flow cytometry was also performed on colon biopsies from 71 IBD patients on vedolizumab matched 1:1 with similar biopsies from 71 matched IBD patients not on vedolizumab. In the colon, vedolizumab use was associated with significantly fewer intramucosal myeloid dendritic cells (CD1c+, CD14-, CD11c+, HLA-DR+, Wilcoxon p=0.000001) in patients responsive to vedolizumab, but not in non-responders, relative to their respective controls. Fewer intramucosal naïve B (IgD+, Wilcoxon p=0.0001) and CD4 T cells (CD45RA+, Wilcoxon p=0.001) were also observed, regardless of treatment efficacy. No differences were observed in the frequency of other T or B cell subsets, including CD8 T cells, effector T cells, plasma cells and memory B cells expressing different immunoglobin isotypes, suggesting that they have an alternative means of recruitment not present among naïve lymphocytes, such as integrin alpha4/beta1, which binds VCAM-1. Thus we find no evidence that that vedolizumab functions by preventing effector lymphocytes from entering the intestinal mucosa. We instead associated vedolizumab use with fewer colonic naïve lymphocyte and myeloid dendritic cells. The latter is of particular interest, as it alone correlates with clinical response to this therapy.

Exaggerated Inflammatory Response During Acute Lung Injury: Role of Mitochondrial ROS and NLRP3 Inflammasome

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Acute lung injury (ALI) caused by acid aspiration often accompanies bacterial components leading to exaggerated inflammation and can result in acute respiratory distress syndrome (ARDS), but the underlying mechanisms behind such an exacerbation remain unclear. NLRP3 inflammasome and mitochondrial ROS (mtROS) have been implicated in ALI but their role in exacerbation of the injury is not known. Therefore, the present study was designed to elucidate the role of mtROS-NLPR3 inflammasome upon 'two-hit' mediated ALI. Our data showed that 'two-hit' induced ALI results in aggravated lung inflammation as compared to either of single hit(s) as reflected by pronounced increase in inflammatory cells particularly neutrophils in bronchoalveolar lavage fluid (BALF). Further, enhanced inflammation was associated with steep increase inmtROS as reflected by enhanced number of MitoSOX^{+} neutrophils and macrophages in BALF derived frommice subjected to two-hit. Importantly, ALI results in increased expression of active caspase-1 and IL-1b at the protein level, suggesting involvement of NLRP3 activation. Interestingly, NLRP3 inflammasome inhibitor, MCC950 suppressed the lung inflammation remarkably. Further, Mito-tempo, a mitochondrial-targeted antioxidant, halted 'two-hit' mediated NLRP3 inflammasome activation and IL-1β release followed by amelioration of lung inflammation. Suppression in number of MitoSOX^{+} stained neutrophils and macrophages by Mito-tempo was associated with down-regulation of phospho-p65-NF-κB and its dependent genes (IL-1β/TNF-α/IL-6).Overall, our data suggest that NLRP3 inflammasome activation by mtROS plays a critical role in pathogenesis of exaggerated inflammation and therefore targeting mtROS-NLRP3 inflammasome axis may be an attractive option for combating ALI/ARDS.

W49. Brain-homing Monocytes Identified in Pediatric Acute-onset Neuropsychiatric Syndrome (PANS) are Immunosuppressive

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Pediatric Acute-onset Neuropsychiatric Syndrome (PANS) is characterized by an abrupt onset of obsessive-compulsive disorder and other psychiatric and somatic symptoms. We found elevated levels of circulating monocytes in PANS patients compared to healthy controls. Human monocytes in blood can be divided into 3 subsets, which can differentiate to tissue-homing macrophages and dendritic cells during inflammation. We compared the immunophenotypes of peripheral monocytes in PANS patients (n = 18) and controls (n = 10) to determine their pro- vs anti-inflammatory immunophenotypes. Using flow cytometry, we found an increased level of pro-inflammatory monocyte-derived DC in PANS flare vs healthy control. We also observed an increase in M1-macrophage-type cells at flare and M2-type cells at improved state. Additionally, we identified a brain-homing monocyte subset that was significantly reduced during PANS flare, with a subsequent increase when clinical features of PANS improved. We found these cells in the cerebrospinal fluid of new-onset, but not chronic, PANS patients, confirming their brain-homing and BBB-crossing potential. Interestingly, we found that PANS plasma induced the expressions of these "brain-homing" markers in healthy monocytes. Cluster distribution from scRNA-seq of myeloid cells revealed two distinct subsets that expressed our pre-defined brain-homing markers and a pseudotime trajectory analysis argued that these cells derive from CD14^{+} monocytes. Gene-set enrichment analysis showed that the CCR2^{hi} brain-homing subset expresses immunosuppressive genes, consistent with an anti-inflammatory role. The CCR2^{lo} subset expresses neuroprotective genes. Together, our data support a model in which distinct myeloid cell subsets contribute to PANS brain inflammation and its suppression.

W143. Dynamic Peripheral Immune Proteins in Extremely Premature Infants

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There has been a recent shift in the paradigm that susceptibility to inflammatory diseases in premature infants is due to a poorly developed immune system. Yet, how postnatal changes immunity profile result in disease is poorly understood. Serial analysis of immune proteins in plasma in preterm infants at risk of disease can provide insight into the trajectory of postnatal immune development.
Preterm blood samples (n=5) were obtained within the first three days of life, 7-10 days, 2 weeks, 1 month and 2 months of age (total n=22 samples). Cord blood samples obtained from term infants (TCB, n=5) and peripheral blood from adults (AB, n=5) served as controls. Protein expression from dried blood spots was analyzed. The peripheral immune profile of preterm infants was distinct from both TCB and AB. In premies, the immune profile in the first two weeks of life differed from that of samples obtained at 1 and 2 months of age. Expression of proteins involved in innate immunity IFNLR1(Interferon lambda receptor 1(IFNLR1), TREM1(triggering receptor expressed on myeloid cells 1) and adaptive immunity LAG3 (lymphocyte activating 3) and CD28 were increased in the first two weeks of life. Meanwhile, expression of IL6 was highly variable in the first two months of life. PTHR1 (parathyroid hormone receptor 1) was higher in preterm infants compared to CB and higher levels persisted through the first two months of life. The peripheral immune profile in extremely premature infants is distinct and dynamic in early life.

W166. Severe Mycobacterial Immune Reconstitution Inflammatory Syndrome can cause Secondary Hemophagocytic Lymphohistiocytosis with a Unique Immune and Genetic Profile

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People with HIV/AIDS and mycobacterial infections can develop immune reconstitution inflammatory syndrome (IRIS) after starting antiretrovirals. Mycobacterial-IRIS has an overlapping clinical phenotype with hemophagocytic lymphohistiocytosis (HLH). We applied HLH-criteria to 80-patients with AIDS and mycobacterial infections followed at NIH and evaluated clinical outcomes, biomarkers, immunophenotypes, and genetics. Overall, 32.5% developed IRIS and met HLH-criteria (HLH-IRIS group, n=26), whereas 35% had IRIS without meeting HLH-criteria (IRIS group, n=28). IRIS did not occur in 32.5% (Non-IRIS group, n=26). Clinical outcomes were evaluated by logistic/linear regression. We measured 23-biomarkers at baseline and IRIS-timepoints. Flow cytometry was performed for activated and regulatory T-cells. Protein-altering variants in cytotoxicity and immunoregulatory genes were evaluated for with next generation sequencing. HLH-IRIS patients required more steroids (OR 24.4 [$CI_{95\%}$6.0-138.5]) for a duration 24.9-weeks ($CI_{95\%}$14.0-35.8-weeks) longer than those not meeting HLH-criteria. At the IRIS-timepoint, but not baseline, HLH-IRIS patients had greater production of IFNγ-IL18 axis biomarkers (IFNγ, CXCL9, IL18, IL18BP) when compared to IRIS and Non-IRIS groups. Soluble CD25 and percentage of activated T-cells were increased in HLH-IRIS with decreased regulatory T-cells. Rare, protein-altering variants in cytotoxicity genes were found in 11/54-patients (20.4%) that developed IRIS compared to 1/26 (3.8%) without IRIS. HLH-IRIS patients had additional rare, protein-altering variants in other immunoregulatory genes including *LRBA*, *RLTPR*, and *TREX1*. Severe mycobacterial-IRIS and HLH have overlapping pathogeneses involving the IFNγ-IL18 axis and a dysregulated T-cell immunophenotype. Rare variants in cytotoxicity and immunoregulatory genes could impact mycobacterial-IRIS risk. This shared pathophysiology could be used to improve the diagnosis and management of mycobacterial-IRIS.

Mucosal Immunology

Th19. Regulation of Antigen Presenting Cells by Retinal Pigment Epithelial Cells

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One of the mechanisms of ocular immune privilege is the suppression of phagosome maturation within potential antigen presenting cells. This has implications on the presentation of antigen on MHC class II. The suppression is mediated by soluble immune regulators produced by retinal pigment epithelial cells (RPE). This regulation is diminished under experimental autoimmune uveitis (EAU) conditions but recovers following treatment with the anti-inflammatory neuropeptide α-melanocyte stimulating hormone (α-MSH). To see if there is an effect of RPE on APC activation of T cells, we in vitro treated APC processing antigen with the condition-media of RPE-eyecups from naive, EAU, and α-MSH-treated EAU mouse eyes. These APC were assayed for cytokine production and used in vitro to activate T cells from antigen-immunized mice. The T cells were assayed by ELISA for cytokines and by flow cytometry for changes in CD25 and FoxP3 cell populations. The APC treated with the RPE-eyecup conditioned media had significantly suppressed TNF-α production with induced IL-10 production. The conditioned-media of RPE-eyecup from any mouse eye did not suppress or enhance APC antigen-stimulated T cell production of IFN-γ, IL-17, or IL-10. However, there was suppression in the expansion of $CD25^{+}FoxP3^{-}$ T cells with no change in the $CD25^{+}FoxP3^{+}$ Treg cell population. These results suggest that the immune regulation mediated by RPE may not induce Treg cell activity but more about generating an anti-inflammatory environment that minimizes effector T cell expansion. In addition, the results suggest that melanocortin-based therapy works to maintain or return normal immune regulating activity by RPE.

Th25. IL-33 and IL-4 Synergize to Dysregulate TREG Cell Function and Promote Type 2 Responses in RSV-induced Asthma

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Respiratory syncytial virus (RSV) is a major cause of hospitalization in infants and is associated with asthma development. While the way neonatal infections induce long-term immune alterations remain ill-defined, there is evidence regulatory T (T_{REG}) cells fail in preventing pulmonary T_H2 responses. Since IL-33 is an alarmin involved in the exacerbation of T_H2 responses, we hypothesized that neonatal RSV infections impair the long-term suppressive function of IL-33-responding ($ST2^{+}$) T_{REG} while contributing to T_H2-induced pathology. Using an RSV A2 murine model of infection, we uncovered that, contrary to conventional T_H2 cells, $ST2^{+}$ T_{REG} fail to accumulate in the lungs at the time of neonate infection but expand robustly and express GATA3 and IL-13 upon re-infection in adult BALB/c mice. Through a Foxp3-specific ST2 conditional knock-out mouse sensitized to house dust mite, we observed that $ST2^{+}$ T_{REG} are required for controlling exacerbated T_H2 cell responses in mice naturally resistant to type 2 immunity. Indeed, while IL-33 potentiated the expression of the IL4Rα, IL-4, in turn, drove the production of IL-10 by T_{REG}. However, increasing amounts of IL-4 also contributed to dysregulate the suppressive capacity of T_{REG} cells *in vitro* and drive a T_H2-like conversion of T_{REG} ($IL\text{-}13^{+}$, $IL\text{-}5^{+}$, $GATA3^{+}$) in a STAT6-dependent manner, suggesting that tight control of IL-4 signaling is required for $ST2^{+}$ T_{REG} to suppress T cells. These initial studies suggest RSV induced IL-33 sensitizes local T_{REG} to T_H2 produced IL-4 that ultimately dysregulate their suppressive capacity. This work provides a novel target for the development of asthma therapies.

Th30. Use of *Ex vivo* Lung Perfusion to Study the Immune Response to Influenza Viral Challenge in Human Lung

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Lung tissue resident immunity is critical for protection from influenza. Studies of tissues from human organ donors have identified cells that are transcriptionally similar to murine resident immune cells but these studies are limited because they evaluate cells in tissues at a single time point only. *Ex vivo* lung perfusion (EVLP) is a clinical bridge-to-transplant modality that maintains human lung viable over 4-6 hours. We hypothesized that EVLP can facilitate longitudinal studies of the immune response to influenza A virus (IAV) in the human lung. To test this hypothesis, we selectively challenged the right middle lobe (RML) of N= 5 human lungs on EVLP with varying titers of pandemic H1N1 influenza A virus (A/California/07/2009) virus and assayed for viral PCR and immune cells in bronchoalveolar lavage fluid (BAL) over 18-24 hours. We amplified the M gene of H1N1 IAV only from RML BAL We identified an increase in BAL cellularity over time only in the RML that comprised CD4+ and CD8+ T cells. We found CD4+ and CD8+ T cells from the RML BAL alone were proliferating (Ki-67+). Next, we adapted the activation induced marker (AIM) assay, routinely used to identify antigen-specific T cells in human blood, to enumerate antigen-specific T cells in BAL. Using this modified AIM assay, we found antigen-specific CD4+ and CD8+ T cells as expanded over time in the RML following IAV. Collectively, these data suggest that we can successfully study the immune response to IAV in human lung over time using EVLP.

Th60. High Salivary IgA-coating Levels of the Oral Microbiota in Patients with Systemic Lupus Erythematosus (SLE)

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Immunoglobulin A (IgA) is the primary antibody isotype present in the body fluids. There are two subtypes of IgA in humans: IgA1, mainly present in blood and mucosal sites, and IgA2, preferentially expressed in mucosal sites like the colon. In clinical practice, immunoglobulins are typically measured in venous or capillary blood; however, alternative samples, including saliva, are now being considered given its non-invasive and easy collection nature. Several autoimmune diseases have been related to intestinal microbiota dysbiosis, such as rheumatoid arthritis, systemic sclerosis, and systemic lupus erythematosus (SLE). However, little is known about the oral microbiota, and the role of IgA in these diseases, specifically SLE, since about 40-70% of these patients present alterations in the oral cavity. Thus, we decided to evaluate the levels of both IgA subtypes in the saliva of SLE patients measured by ELISA in a cohort of 27 patients with SLE that were compared with antibody levels of healthy volunteers. At the same time, we evaluated the percentage of coating of oral microbiota by salivary IgA using flow cytometry. Surprisingly, our results indicated that the oral microbiota of SLE patients is high coverage by salivary IgA1, and both IgA subtypes were elevated, but IgA1 was significantly elevated. Interestingly, we also found that salivary IgA levels, specifically IgA1, positively correlate with serum anti-dsDNA IgG related to the SLEDAI values. According to our results, there may be an alteration in the oral microbiota of patients with SLE due to the increase in IgA1 coating. CONACyT A3-5-36875/UNAM-DGAPA-PAPIIT IN212122

Th144. Single Cell Analysis Reveals Altered Regulatory T Cells in Neonates with Necrotizing Enterocolitis

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Preterm infants are susceptible to necrotizing enterocolitis (NEC). This devastating disease remains poorly understood and treatment is non-specific. There is an urgent need to identify biomarkers and to develop targeted therapies. Single-cell analysis with spatial resolution of NEC-affected mucosa can identify altered signaling and cellular interactions that could serve as potential therapeutic targets. Small intestinal tissue from infants with NEC (n=20, gestational age (GA) 23-39 weeks) and in neonates with non-immune congenital anomalies (neonatal n=13, GA 34-40 weeks) were compared. Single cell RNA sequencing (scRNAseq), deconvolution of bulk sequencing data, and imaging mass cytometry (IMC) was performed.
scRNAseq identified 7 distinct populations of lymphocytes. Analysis of differentially expressed genes showed that genes required for appropriate T reg suppressive function (FOXP3, CTLA4) were decreased, and SELL (CD62L), CCR7, SOCS1 were increased in NEC compared to neonates. This is consistent with the upregulation of SOCS1, a downstream target of STAT3 signaling and IMC analysis showed an increase in pSTAT3 in NEC associated Tregs. Interactions between T regs and macrophages were altered in NEC signaled with increased ligand-receptor interactions of IL1R1/IL1B, CAV1-TGFB1, and ABCA1-CALM1. Furthermore, IMC highlighted altered interactions in NEC Tregs. Specifically, decreased interaction with other T cells ($p< 0.01$), and increased interaction with inflammatory macrophages. In summary, we characterize altered cellular interactions and transcriptional signature in NEC-associated regulatory T cells that contribute to disease pathogenesis and could serve as potential targets for future therapies.

Th162. Commensal Myeloid Crosstalk in Neonatal Skin Regulates Long-term Cutaneous Type 17 Inflammation

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Early life immune interactions help shape longer-term skin health and homeostasis. Commensal microbes facilitate neonatal skin accumulation of innate and adaptive T cells, thereby promoting fundamental needs such as immune tolerance to commensals and wound healing. Comparatively little is known about commensal-myeloid cell crosstalk in neonatal skin and the functional consequences of these interactions. Using CyTOF and gnotobiotic mouse models we observed a population of classical monocytes uniquely enriched in the skin of microbially replete neonates. Corroborative studies revealed that skin monocytes rapidly accumulate between D1 and D3 of life, after which their numbers gradually decline. This early monocyte wave was prevented in antibiotic-treated SPF pups as well as in Myd88$^{-/-}$ but not IL1R1$^{-/-}$ mice, suggesting a key role for tonic TLR signaling in their accumulation. To dissect the functional relevance of these cells in cutaneous biology, we developed an antibody-based regimen to temporarily deplete monocytes in the first two weeks of life (NeoΔmono). scRNA sequencing revealed a heightened type 17 signature in D15 NeoΔmono skin T cells. Flow cytometry assays confirmed sustained elevation of IL-17A production by these cells through adulthood. This reflected a heightened response to commensals as IL-17 production was significantly reduced in antibiotic-treated NeoΔmono mice. While there was no visible skin pathology in NeoΔmono mice under homeostatic conditions, imiquimod treatment of the ears in adulthood led to significantly increased ear swelling and neutrophils. Taken together, our data demonstrate a previously unappreciated, commensal-dependent regulatory imprinting function of cutaneous classical monocytes in the early life window.

Th181. AMT-126: A Novel Oral GI-selective Fusion Protein Delivering IL-22 to Support Mucosal Homeostasis and Epithelial Barrier Integrity

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Background: IL-22 is a pleiotropic cytokine that mediates epithelial barrier integrity and mucosal homeostasis. In the intestine, it is produced largely by lymphocytes in response to proinflammatory cytokines and acts to induce epithelial cell proliferation, tight junction formation, and mucins & anti-microbial peptides production. IL-22 KO mice have weakened intestinal barriers, increased susceptibility to pathogens, and altered microbiome compositions, all of which can be reversed by providing exogenous IL-22. Treatment with parenteral human IL-22 in healthy volunteers, ulcerative colitis, and graft-versus-host disease patients shows clinical benefit but is associated with skin and mucosal toxicity. Approach: AMT-126 is a fusion protein of IL-22 and a carrier protein that mediates transcytosis through intestinal enterocytes into the lamina propria. Pre-clinical studies demonstrate that oral or colonic delivery of AMT-126 provides gut-selective exposure of biologically active IL-22 with minimal systemic pharmacokinetics (PK). In murine dextran sulfate sodium (DSS)-induced colitis, oral AMT-126 suspension attenuated overall disease severity. In non-human primates, oral dosing or colonic spray of AMT-126 showed little/no systemic PK, but pharmacological effects (4-hour fold induction vs. baseline: Reg3a – 1.8x; CRP – 10x; IL1Ra – 50x) comparable to those seen with parenteral delivery of IL-22. In all animal studies, AMT-126 was well tolerated with no adverse findings at any dose level. Conclusion: IL-22 is a clinically validated cytokine used to improve mucosal homeostasis but leads to toxicity when delivered intravenously. *In vivo,* AMT-126 (oral gut-selective IL-22 fusion protein) induces IL-22-associated mucosal repair mechanisms in the absence of toxicity, supporting advancement into the clinic.

Stem Cell and Organ Transplantation

Tu4. Molecular Signature of Myeloid Cells During Ischemia-Reperfusion Injury in Human Liver Transplantation

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schemia-reperfusion injury (IRI) is a significant clinical concern for recipients of liver transplantation, yet there are still no diagnostic or therapeutic strategies available to alleviate its significant influence on both short and long term allograft and patient survival. Myeloid cells are key players in the sustained tissue inflammation and damage associated with IRI, but mechanisms regulating these activities are unknown. Thus, there is a fundamental need for understanding the molecular pathways associated with myeloid cells present in either IRI- or IRI+ recipients of liver transplants. RNA-seq was performed on 80 longitudinal pre- and post-reperfusion biopsies from 40 human liver transplant recipients (23 IRI+, 17 IRI-). To further characterize myeloid involvement, we used transcriptional profiling and computational approaches to identify specific gene co-expression network modules which correlated with numbers of LYSO+ myeloid cells, MPO+ neutrophils and CD68+ monocytes/macrophages as determined by immunohistochemistry on sequential sections from the same patient biopsies. The global molecular map generated by this unique computational approach revealed markers of early myeloid activation in pre-reperfusion samples of IRI+ recipients coupled with subsequent lymphocyte activation post-reperfusion, which identified transcriptional programs associated with the transition to adaptive immunity. IRI- recipients had a less inflammatory profile pre- and post-reperfusion than IRI+ recipients, with pre-reperfusion samples dominated by myeloid genes related to a response to injury, followed by regeneration or repair signaling post-reperfusion. Strategies to therapeutically target these myeloid-specific genes and signaling pathways, as well as improve donor-recipient matching are urgently needed to limit IRI in the clinical setting of liver transplantation.

Th14. Crosstalk Between Alloimmunity and Ischemia-Reperfusion Injury in Human Liver Transplantation

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Recipient sensitization to donor HLA antigens and ischemia-reperfusion injury (IRI) are key challenges in liver transplantation (LT), increasing waitlist times and/or risk of acute and chronic cellular and antibody-mediated rejection. Yet it is still unresolved whether pre-transplant alloreactivity potentiates IRI or if IRI enhances post-transplant alloimmunity. We evaluated donor-specific HLA class I/II antibodies (DSA) pre- and at two timepoints post-transplant (early=within 3 months, late=4 months–2 years) in LT recipients with or without biopsy-proven IRI (n=78; IRI-=37, IRI+=41) using single antigen bead-based HLA antibody testing and intermediate resolution HLA typing. Only 25% of LT recipients pre-sensitized to HLA-DSA experienced IRI, suggesting pre-transplant exposure to HLA can tolerize against IRI. The number of IRI- patients producing allo-antibodies significantly decreased from pre- to early post-transplant, continuing to decrease late post-transplant. Further, only IRI+ patients who were not pre-sensitized had an early memory response that persisted through late testing or produced de novo antibodies late post-transplant, with DSA being higher than in IRI- patients at both timepoints. IRI- patient sensitization was more frequently against class I or I/II whereas IRI+ sensitization was against only class II. Finally, although more females were pre-sensitized, IRI+ recipients experienced increased post-transplant alloimmunity irrespective of gender.

Our data reveal that pre-sensitized LT recipients, particularly those with DSA, are protected from IRI. Conversely, recipients lacking pre-sensitization are significantly more likely to experience IRI and produce DSA post-transplant. Clinicians should therefore consider increased baseline and post-transplant monitoring for LT recipients, especially those without evidence of HLA pre-sensitization.

Th16. Effects of Cytomegalovirus on T Cell Differentiation in Two Distinct Immunosuppressed Populations

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Infection with cytomegalovirus (CMV) induces remodeling of the immune system. Though healthy immunity controls CMV infection, the virus establishes latency and can periodically reactivate. Immunosuppressive drugs impair memory immune responses, which can lead to uncontrolled CMV reactivation and clinical viremia. CMV reactivation is known to drive a process termed memory inflation, in which the CMV reactive CD8 T cell population expands and adopts an aged highly differentiated phenotype. Recent studies have found that memory inflation is accelerated in recipients of solid organ transplantation. However, it is unknown whether this effect is due to immunosuppression, the end-stage organ disease preceding transplant, or some other factor. To address this question, we have analyzed T cell differentiation and memory inflation in rheumatoid arthritis (RA) patients both before and one year after initiation of immunosuppressive therapy. Preliminary data indicate that T cells in RA patients have a highly differentiated state pre-treatment that is maintained a year after initiation of immunosuppressive therapy. This includes a CD8 T cell population with evidence of immune aging. Differentiation states and aging were similar in CMV seropositive and seronegative RA patients. Overall these findings indicate that independent of CMV serostatus, RA is associated with highly differentiated and aged T cells. In contrast to this stability of differentiation state, there is evidence of memory inflation of CMV-responsive T cells in the CMV seropositive RA patients, suggesting that immunosuppression may induce memory inflation in this setting as well as transplantation. Further study is needed to determine how immunosuppression induces memory inflation.

Th18. Long Term Expanded Purified Allospecific Tr1 Cells Maintain a Suppressive Phenotype and Function in the Presence of Inflammatory Cytokines and Express a Chemokine Receptor Profile Involved in Allograft Tolerance.

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The development of new strategies based on the use of Tr1 cells has taken relevance to induce long-term tolerance, especially in the context of transplantation. Recent studies have shown the need for a more exhaustive characterization to ensure a bona fide Tr1 population to be used as therapy. It has been reported that the proinflammatory environment induced by the allograft could affect the suppressive function of Treg cells, although the stability of Tr1 cells *in vitro* and *in vivo* has been poorly investigated. Another important aspect that must be considered is the homing of Tr1 cells to achieve an efficient allograft tolerance, where key chemokine receptor expression plays an important role. Here, we established an optimized protocol that allows long term *in vitro* expansion of highly purified allospecific Tr1 (exp-allo-Tr1). After being expanded under proinflammatory conditions we observed that our population maintains a high co-expression of CD49b, LAG-3 (≈80%), which produce high levels of IL-10 (≈90%) and expressed co-inhibitory receptors (PD-1, CTLA-4, TIM-3, TIGIT, and CD39) and efficiently suppressed allospecific but third party T cell responses. Additionally, we observed that exp-allo-Tr1 expressed a high percentage of chemokine receptors involved in allograft tolerance, such as CCR2 (65.5%±19.75), CCR4 (77.5%±10.45) but not CCR7 (16.9%±7.75). Moreover, exp-allo-Tr1 also expressed CXCR3 (47.3 %), which could promote their homing to the kidney allograft. Our data indicate that exp-allo-Tr1 are stable and express relevant homing receptors supporting their potential use in long-term transplantation tolerance.

Th56. A Novel Multiplex Digital PCR Method Offering Rapid Quantitation of Donor and Recipient DNA After Transplantation

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Background Accurate and sensitive quantitation of donor and recipient DNA is clinically important in both hematopoietic stem cell and solid-organ transplantation. Next-generation sequencing can simultaneously measure thousands of targets however has a relativity long turnaround time and high cost. Real-time and digital PCR have offered faster and less expensive alternatives but are practically limited by number of targets per assay. Objectives Our aim was to develop an easy-to-use multiplex digital PCR (mdPCR) method that can detect more than 10 targets in a single reaction while achieving rapid, accurate, sensitive, and absolute DNA quantitation. Methods A novel prototype assay (multiplex PCR chemistry, Luminex, Austin TX) detecting 12 alleles in a single well was evaluated using a microfluidic digital PCR platform (Thermo Fisher Scientific Absolute Q Digital PCR). Performance characteristics were assessed for samples with 1,000 and 10,000 human genome equivalents. Results Each run (up to 16 samples) was completed in < 3 hours. With a sample input of 10,000 copies of genomic DNA, the LoB for 7 probes was 0 – 0.02% and the analytical LoD was 0 – 0.03%. When testing 1,000 copies of genomic DNA, for 7 probes LoB was 0 – 0.18% and the analytical was LoD 0 – 0.30%. The remaining probes require optimization. Conclusions Initial designs for the prototype mdPCR assay show high promise for providing accurate, sensitive, cost-effective, same-day quantitative DNA testing. This methodology offers exciting opportunities for chimerism and cell-free DNA testing after transplantation and will be useful in cancer and infectious diseases.

Th64. Renal Allograft Rejection: New Paradigms in T Cell Reactivity Revealed by Single Cell RNAseq Analysis

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Background: Transcriptomic definition of the T-cell landscape in renal allograft rejection (RAR) is complicated by the inability of CDR3 sequence data to distinguish allospecificity of TCRs in graft infiltration. Here, we have begun functional analysis of infiltrating TCRs in kidney allografts. Methods: Following 5' single cell RNAseq with paired TCRα/β sequencing of RAR biopsies, Seurat and Loupe V(D)J analysis identified individual T-cell clonotypes. Expanded clonotypes were defined as >2 cells and >0.5% of T-cell barcodes identified with identical CDR3a/b sequences. To further understand the alloresponse and potential cross-reactivity, 5 expanded clonotypes randomly selected from 1 patient were transfected into Jurkat76 cells, with 1 identified as having a TCRα chain with EBV reactivity via VDJdb. Jurkat clones were co-cultured overnight with donor vs. third-party APCs and IL-2 ELISA was performed to determine T-cell activation.
Results: Samples obtained at the time of RAR diagnosis revealed an impressive degree of TCR restriction (avg. 14 expanded CD8+ clones/patient). In patients who had subsequent biopsies following RAR diagnosis, >80% of the original clones were present in subsequent biopsies, suggesting a limited number of clonally expanded T-cells during rejection. However, expanded T cell clonotypes are eliminated following rejection resolution. Strikingly, all 5 TCRs subcloned into Jurkats displayed evidence of alloreactivity through production of IL-2 in response to donor but not third-party APCs. Conclusions: 1) RAR is characterized by a remarkably limited number of distinct CD8+ T-cell clones and 2) TCR subcloning can allow determination of TCR specificity and link alloreactive T-cells to their transcriptomes.

Th73. Pilot Study of a Novel Anti-CD5 Antibody-drug Conjugate for Combined Peripheral and Thymic Lymphocyte Depletion in a Cynomolgus Macaque Model

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Several tolerance protocols in pre-clinical models and clinical transplantation rely on peripheral T cell depletion and thymic irradiation to reduce donor-reactive T cells during the peri-transplantation period. Decreasing toxicity of these protocols would facilitate broader clinical application. A novel anti-CD5 antibody-drug conjugate (anti-CD5ADC) with a short half-life has been shown to be effective for peripheral T cell and thymocyte depletion in humanized mice. This study examines the efficacy and toxicity of anti-CD5ADC in a pre-clinical NHP model. Six macaques underwent hemisternotomy for thymic biopsy on day -10. Five animals were administered 2 mg/kg of anti-CD5ADC on day 0. A control animal did not receive the drug. Animals were euthanized on day 4. CBC, BMP and LFTs were measured daily. Cell counts per gram of thymic tissue were used to quantify thymocytes. Flow cytometry was used to characterize lymphocyte populations in the blood and thymus. The drug was well tolerated aside from mild, asymptomatic LFT elevations. The average percent reduction in thymocytes was 55.27% for experimental animals vs 14.99% for the control. Paired t-tests for reduction in thymocytes/gram demonstrated a p-value of 0.0567 for experimental animals. The average peripheral CD3+ count was 4147.30 cells/uL blood on day 0, declining to 244.62 on day 4 for treated animals. Memory and naïve T cell subsets were similarly depleted. The anti-CD5ADC is safe and effective at peripheral and thymic lymphocyte depletion and warrants further studies for its efficacy as a replacement for thymic irradiation and chemical T cell depleting agents in NHP tolerance models.

Tu20. Chimerism and Bidirectional Alloresponses in Patients Receiving Lung Transplantation

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Outcomes after lung transplantation (LuTx) are suboptimal mainly due to high rejection rates. Lung grafts carry T, B and antigen-presenting cells, which leading to graft-vs-host (GvH) and host-vs-graft (HvG) alloresponses. Dynamic repopulation and clonal distribution of T cells, the driver for alloresponses after LuTx, are largely under-researched. We performed temporal analysis of immune cells in the serial bronchoalveolar lavage (BAL) of two patients. We utilized a combination of flow cytometry to distinguish donor- and recipient-derived cells by HLA specificity, with high-throughput TCR sequencing within pre-Tx lymphoid tissues to identify GvH- and HvG-reactive T cells defined by mixed lymphocyte reactions. Donor T/B/monocytes in BAL post-Tx were gradually replaced by the recipient. The turnover dynamic was faster in Pt4 compared to that in Pt1, especially for T cells. Donor CD8 T cells showed persistent high expression of TRM markers (CD69/CD103/CD49a) and low expression of effector T cell (Teff) marker CD28. However, recipient graft-infiltrating CD8 T cells gradually downregulating CD28 during the process of acquiring TRM features. An enrichment of HvG- compared to GvH-reactive sequences were observed in post-Tx BAL of Pt4, which was opposite as shown in Pt1. However, histology on the lung transbronchial biopsies described absence of rejection for both patients. Dynamic replacement of donor T cells by the recipient, and the presence and dominance of GvH- and HvG-reactive T cell sequences in lung allograft are likely providing valuable information to avoid miss diagnosis of early rejection by histology and revealing the underlying mechanisms of graft rejection and acceptance after LuTx.

Tu31. Development of Proliferating Cell Nuclear Antigen Autoantibodies with Manifestation of Graft Versus Host Disease after Peripheral Blood Stem Cell Transplantation

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Chronic graft-versus-host disease (cGVHD) is one of the most significant autoimmune manifestation after allogenic peripheral stem cell transplantation (PBSCT). Anti-nuclear antibody (ANA) expression was frequently observed in patients after allogenic PBSCT. However, there has been no written report of proliferating cell nuclear antigen (PCNA)-like pattern of ANA expression in patients with cGVHD. We present a case of 30-year-old woman who developed antinuclear antibody formation against PCNA with development of systemic graft-versus-host disease after allogenic PBSCT. The patient was diagnosed with T-lymphoblastic lymphoma for her mediastinal mass. She had received chemotherapy for 8 months and underwent allogenic PBSCT from the human leukocyte antigen-matched sibling donor. ANA was negative at the time of PBSCT. Two months after PBSCT, the patient developed acute GVHD at colon. Chronic skin and pulmonary GVHD occurred at 6 months and 9 months, respectively, after PBSCT. Together with her pulmonary GVHD manifestation, ANA test displayed strong immunoreactivity (PCNA-like pattern, 1:640) in her serum and pleural fluid. Other autoantibodies were not detected. Pulmonary complications contribute to late mortality post PBSCT. Currently, pulmonary cGVHD is diagnosed with bronchiolitis obliterans with a disease severities ranging from subclinical PFT impairment to respiratory failure, and the pathophysiology is not fully understood. Because of the non-specific clinical symptoms, a standardized approach is necessary to detect pulmonary cGVHD early before irreversible structural changes occur. Therefore, our case indicates that laboratory work-up including autoantibody test can be helpful for differential diagnosis.

Tu44. Immunotherapy Using IL-34 for the Treatment of GvHD and Solid Organ Rejection: Strategies for its Optimisation

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The IL-34 cytokine is mainly described for its role in the differentiation and survival of macrophages, and in the regulation of immune responses. We previously showed that IL-34 is also a Treg-secreted cytokine that induces tolerance in conjunction with a sub-optimal dose of rapamycin in a model of rat cardiac allograft, and induces tolerogenic macrophages able to increase $CD8^+$ and $CD4^+$ Tregs generation *in vitro* in humans and *in vivo* in rodents. Studying the potential of IL-34 as a tool for immunotherapy in transplantation, we described that human IL-34 treatment in combination with a short-term sub-optimal dose of rapamycin efficiently delays acute xenoGvHD development and human skin graft rejection in hPBMC humanized NSG mice.
To improve the potency and/or half-life of IL-34 *in vivo*, modifications were introduced in the IL-34 structure, either by mutations in key residues of the IL-34 sequence or by producing an IL-34-Fc fusion protein. The capacity of IL-34 mutants to induce monocyte differentiation and activation was then evaluated. In a flow-cytometric assay, increased levels of pAkt and pERK1/2 were observed for some mutants following CSF-1R signalling. Furthermore, monocytes treated with some of these IL-34 mutants showed a tolerogenic phenotype and a better survival at lower concentrations in viability assays. Treatment of NSG mice with the different variants is under way in our models. Altogether, wild-type and mutated IL-34 have potential to be used in immunotherapy.

Tu82. Detecting Alloreactive T Cells in Transplant Recipients with a Trogocytosis Assay

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Organ transplantation success is dependent on prevention of alloimmune graft rejection with immunosuppressant drug therapy, which unfortunately also increases the risk of infection and cancer. Drug regimens are adjusted and minimized based on graft health, which is currently evaluated by infrequent and invasive biopsies. A less-invasive, blood-based assay to monitor allograft rejection could reduce the need for regular biopsies and allow for more frequent alloimmune monitoring. Mixed lymphocyte reactions co-culturing donor tissue and recipient blood show that alloreactive T cell abundance inversely correlates with graft health; however, these assays are challenging to standardize and require difficult-to-acquire donor material. We hypothesized that a new way to measure allo-HLA-reactive T cells could be developed using the principle of trogocytosis, whereby T cells acquire cognate HLA proteins from target cells and re-incorporate them as a part of their own membranes. We generated K562 cell lines that express a donor HLA protein fused to a fluorescent reporter and designed assays to quantify allo-HLA-specific trogocytosis, defining alloreactive T cells as cells that become positive for the fluorescent allo-HLA protein. Assay conditions were optimized with human CD8+ T cells engineered to express an HLA-A2-specific TCR, and background trogocytosis was minimized by removing K562 co-stimulation and supplementary IL-2. Blood collected from an HLA-A2-negative kidney transplant recipient during biopsy-confirmed graft rejection of an HLA-A2-positive graft had a higher frequency of T cells that trogocytosed HLA-A2 than blood collected prior to transplantation. Experiments are ongoing to test the sensitivity and specificity of the assay in longitudinal patient cohorts.

Tu85. Long-term Impact of Atopy in Kidney Transplantation

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Background. Atopy is a genetic condition predisposing individual to develop immunoglobulin E (IgE) against common inhalant allergens through Th2 polarization mechanisms. The impact of atopy on graft survival in solid organ transplantation is unknown. Methodology. We retrospectively analyzed 285 renal allograft recipients with a 10-year follow-up from the Swiss Transplant Cohort Study, a prospective multicenter cohort for solid organ transplantation. A positive Phadiatop (>0.35 KU/L) before transplantation defined atopic patients, a blood test measuring IgE antibodies to a mixture of common inhalant allergens (grass, tree, herbs, spores, animals and mite). Results. Of 285 kidney transplant recipients, 70 individuals were atopic (24.6%). Atopic patients were significantly younger (58.0 vs 49.6-year-old, P = 0.002). No significant difference was found for gender, cold/warm ischemia time, preformed donor specific antibodies (DSA), HLA mismatches, induction and maintenance immunosuppressive therapy, CMV serostatus, and kidney failure cause. Ten-year patient and graft survival was increased in the atopic group, respectively 94.7% versus 78.8% (P = 0.005), and 90.4% versus 80.4% (P = 0.063). Multivariate Cox analysis revealed that atopy predicted patient survival independently of age and living donation. Finally, cumulative DSA was significantly lower in the atopic group. Conclusion. Atopy was associated with longer recipients survival and graft outcomes independently of age and living donation, possibly by limiting DSA formation. Atopy may be a predictive factor for long-term kidney allograft survival.

A New Lens into the Tissue-Specific Landscape of the T Cell Repertoire in Human Graft-Versus-Host-Disease

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Investigating T cell immunity in tissues is critical for understanding immune responses at the site of pathology, such as in graft-versus-host-disease (GVHD), an adverse sequela of transplantation. This often-lethal complication arises from alloreactive T cells leading to inflammation within tissues, such as the skin or gastrointestinal (GI) tract. We profiled the TCR repertoire in GVHD tissues in mice after receiving a major MHC-disparate allograft (6-8 tissues/mouse, 129 total tissues), revealing the greatest clone sharing by similarity of the anatomic region, such as across GI tract sections. We found a significant frequency increase in dominant clones from day 7 to 14 post-transplant, consistent with expansion of TCRs within tissues. To investigate human T cells in tissues directly, we studied a unique patient cohort via prospectively collected autopsy material: seven patients with hematologic cancers who underwent curative treatment with bone marrow transplantation whose post-transplant course was complicated by refractory GVHD and three comparator cancer patients who did not undergo transplantation. We profiled multiple sites per individual (5-18 samples/patient, 98 total samples) via TCR sequencing and found greater repertoire sharing across anatomically similar regions, regardless of transplant history, despite heterogeneity in degree of overlap. A computational motif-based clustering approach found evidence of potentially shared antigen-specific TCR motifs clustering by tissue across patients. Furthermore, in mice and humans, the blood and/or spleen repertoire did not fully capture the TCRs in tissues. Taken together, our work provides new insights into the site-specificity of the T cell repertoire, highlighting the importance of analyzing tissue-resident T cells.

Th168. Differentiation of Human iPSc into Functional Thymic Organoids for in vitro T Cell Generation and Cell Therapy

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The thymus is a primary lymphoid organ that plays a pivotal role in immune tolerance by educating thymocytes through tight interactions with thymic epithelial cells (TECs). Deficiency in genes regulating TEC development or functionality results in a defective immune balance and severe autoimmunity as observed in the APECED syndrome caused by mutations in the AIRE gene. Cellular therapies based on induced pluripotent stem cells (iPSc) are promising approaches to treat those pathologies. Here we address the generation of functional human iPSc-derived TECs positive for AIRE expression and able to support T cell development *in vitro*. We refined previous protocols allowing generation of thymic epithelial progenitors (TEPs) through an unbiased multifactorial method based on optimal experimental design and RNA-seq profiling of the cells throughout their differentiation. Modulation of signaling pathways known to control the different stages of embryonic thymus development enabled us to obtain iPSc-derived TEPs expressing typical thymic markers. We achieved the maturation of TEPs in AIRE-expressing TECs and the generation of thymic organoids by setting up a hydrogel-based 3D culture supplemented with an optimized cocktail of cytokines including RANK ligand. Finally, to assess the functionality of the generated TECs, primary early thymocytes were cocultured with the obtained organoids, resulting in a significantly improved generation of CD4 and CD8 single-positive T cells. Hence, the generation of functional TECs and thymic organoids enabling T cell development *in vitro* offers a platform for the study of thymic cellular interactions and paves the way for the establishment of cellular therapies for autoimmune diseases.

Th169. Detection and Clonal Dynamics of Alloreactive T Cells in a Kidney Transplant Recipient Experiencing Acute Rejection After Immune Checkpoint Inhibitor Therapy

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Immune checkpoint inhibitors (ICI) have been established as an effective standard care for many cancers, though it is often complicated by immune-related adverse events. Kidney transplant recipients have 3-10x higher risk of cancer post-transplantation, but the use of ICI is challenging due to the high risk of acute allograft rejection. We hypothesized that this could be explained by a vigorous and rapid expansion of preexisting, allograft-specific memory T cells after the initiation of ICI therapy. Here we leveraged a unique set of tissue and blood samples from a patient who experienced kidney allograft rejection during ICI therapy. To first identify these alloreactive cells, we performed a mixed lymphocyte reaction (MLR) using recipient PBMCs and donor splenocytes. Proliferating recipient T cells were flow-sorted and paired single-cell RNA and TCR sequencing was performed. We observed a population of highly proliferative and clonally expanded CD8+ T cells, which we posited to be alloreactive clones. When examining the presence of MLR-identified clones in bulk TCR sequencing of tissue samples, we found these alloreactive clones in the rejected kidney but not in lymph node or skin biopsies. Similarly, examination of a PBMC time-course showed these alloreactive clones could not be detected before ICI initiation but were found in high numbers after therapy initiation. Combined, this work describes the identification and spatiotemporal dynamics of a set of alloreactive T cells in a patient experiencing acute rejection after ICI therapy and highlights the utility of this technique to track pathologic T cell responses augmented by ICI therapy.

Tu101. A Novel Organotypic Model of Skin Rejection

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Vascularized composite allografts (VCAs), such as hand, leg, abdominal wall, and face, involve the transplantation of multiple tissue types including skin, fat, bone, and muscle as a single anatomic unit. Despite similar immunosuppressive regimens as organ transplants, VCAs undergo significantly higher rates of rejection compared to solid organ allografts, a phenomenon attributed to the immunogenicity of the skin component of VCAs. To study this process, we have developed an organotypic model of skin rejection by adapting a recently described immune organoid system where dissociated human tonsils are used to form organoids capable of recapitulating primary and recall immune responses. Using this system, we co-culture intact human skin with tonsil organoids derived from an allogeneic donor. After seven days of co-culture we find that tonsil T-cells actively infiltrate into allogeneic skin and are located throughout the dermis and epidermis. Infiltrating tonsil T-cells express markers of recent activation (PD-1, HLA-DR), proliferate, and exhibit a restricted TCR repertoire. Further, infiltration of allogeneic tonsil T-cells is associated with skin tissue damage and cell death. Lastly, we find that clinically used immunosuppressive medications (cyclosporine, tacrolimus, and mycophenolate) reduce both infiltration of allogeneic tonsil T-cells into skin as well as subsequent tissue damage. Overall, we report a novel fully human in vitro model of skin rejection that can facilitate the screening and discovery of new therapeutic targets.

Tu116. Bac Transgenic Overexpression Mice Recapitulate the Immunologic Role of Exonic Variants of APOL1

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Exonic variants (G1,G2) in apolipoprotein A1(APOL1) increase the risk of kidney disease (CKD) in African-Americans (vs G0 major allele). While kidney cell expression of APOL1 is necessary for CKD, our recent findings showed that renal transplant recipients with G1 or G2 genotype also sustained increased allograft rejection rates. *Ex vivo*, NK cells, activated CD4/CD8 cells had higher *APOL1* among immune cell subsets, and in G1 or G2 individuals these subsets showed enhanced activation by single cell transcriptomics. Since APOL1 is restricted to primates, we studied APOl1 variants in immune cells using a novel Bac-transgenic mouse model for G0, G1 or G2 expression under human APOL1 promoter(Bac-Tg). At baseline, splenocytes from Bac-tg mice showed similar proportions of CD4+, CD8+, CD19+ and innate immune- cell fractions between G0, G1, G2 Bac-Tg and WT mice(N=3 each). CD44+ or CD69+ CD4-Tcells were also similar between all Bac-Tg mice and WT mice. At baseline, we found a higher proportion of CD44+ CD8+ Tcells in G1-Bac-Tg mice vs G0- or wild type (WT) mice (55% vs 18%; $P< 0.001$). CD8+CD44+ Tcells were similar in G2 vs G0 comparisons at baseline. Next, we performed CFSE labelled proliferation assays (72 hours) in isolated CD4 and CD8-Tcells. While CD4+ proliferation was similar between APOL1 Bac-Tg mice, we observed greater proliferation in G1 or G2 CD8-Tcells vs G0 or WT mice (ANOVA $P< 0.05$). Hence, we show that human Bac-tg APOL1 mice recapitulate the CD8+ activation observed in human data, and serve as a model to study APOL1-related immune phenotypes.

W60. Racial and Ethnic Disparities in Detection of Donor-Specific HLA Antibodies

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Antibodies against donor-specific HLA proteins can cause rejection after allogeneic organ transplantation. To determine how donor ethnicity influences detection of HLA antibodies, HLA proteins in antibody detection reagents were compared with HLA allele frequencies in seven populations: European (EUR), Hispanic (HIS), Native American (NAM), African American (AFA), Asian/Pacific Islander (API), Middle Eastern/North Coast of Africa (MENA), and unknown/mixed ethnicity (UNK). For each population, HLA-A, -B, -C, and -DRB1 allele frequencies were obtained (Common, Intermediate, and Well Documented HLA Alleles catalog v3.0) and used to determine the percentage of HLA proteins in each population that are not represented in LABScreen Single Antigen reagents (OneLambda). Based upon HLA frequency data, ethnicity influences the likelihood that an organ donor will have HLA proteins (and unique HLA epitopes) that are not represented in reagents commonly used to detect HLA antibodies (range 7.4%-34.4%). Overall, EUR had the best representation at the HLA-A, -B, -C and -DRB1 loci with 7.4%, 14.0%, 30.8%, and 8.5% of the HLA proteins missing, respectively. HLA coverage was lowest for the HIS population, with 15.5%, 28.4%, 31.6%, and 30.1% unrepresented at each respective locus. Representation of HLA-C proteins was low across all ethnicities (28.1%-34.4% unrepresented). Commercial reagents may fail to detect clinically significant HLA antibodies due to unrepresented alleles, especially from non-European populations. The possibility of missing donor-specific antibodies should be a consideration when evaluating patients with suspected antibody-mediated rejection and in virtual crossmatching.

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