



FOCIS 2021 Abstract Supplement

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Adaptive Immunity - Basic Science Advances

W24. Lymph Node Structure in Naïve T Cell Aging

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Naive CD8 T cell loss is a hallmark of immune aging, however naïve CD4 T cells appear to be more resilient, demonstrating less numerical decline with age. Contrary to that, naïve CD4 T cells display significant changes in antigen-specific responses with age, implying a potential phenotypic shift in older individuals. Thus, we initially assessed the heterogeneity of peripheral naïve CD4 T cells using mass cytometry in young and older individuals. We found that a unique CD45RA^{high} population was lost with age, converting to a CD45RA^{+low} population. The CD45RA^{high} population was highly enriched in young adult lymph nodes (LN) and bioengineered LN-like organoids, but not 2D culture, actively maintained this cell type. Moreover, CD45RA^{+low} naïve CD4 T cells from older individuals were able to convert back to a CD45RA^{high} phenotype within these LN-like organoids. Fibroblastic reticular cells (FRCs) were required and their ability to maintain the CD45RA^{high} phenotype was unaffected with age and cellular senescence. However, breakdown of the 3D structure, but not the numerical loss of T cells, caused conversion to an aged CD45RA^{+low} phenotype; implying that the structural integrity of LNs may be essential in the maintenance of the CD45RA^{high} population in youth and that its breakdown with age may directly mediate age-related naïve T cell dysfunction.

W29. IL-13 Controls IL-33 Activity Through Modulation of ST2

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Interleukin-33 (IL-33) is a multifunctional cytokine that mediates local inflammation upon tissue damage. IL-33 is known to act on multiple cell types including group 2 innate lymphoid cells (ILC2s), T_H2 cells, and mast cells to drive production of T_H2 cytokines including IL-5 and IL-13. IL-33 signaling activity through transmembrane ST2L can be inhibited by soluble ST2 (sST2), which acts as a decoy receptor. In this study, we used *Il13*^{-/-} mice to investigate whether IL-13 regulates IL-33 activity by modulating the transmembrane and soluble forms of ST2. In *Il13*^{-/-} mice, the effects of IL-33 administration were exacerbated relative to wild type (WT). *Il13*^{-/-} mice administered IL-33 i.p. had heightened splenomegaly, more immune cells in the peritoneum including an expanded ST2L⁺ ILC2 population, increased eosinophilia in the spleen and peritoneum, and reduced sST2 in the circulation and peritoneum. In the spleen, lung, and liver of mice given IL-33, gene expression of both isoforms of ST2 was increased in *Il13*^{-/-} mice relative to WT. We confirmed fibroblasts to be an IL-13-responsive cell type that can regulate IL-33 activity through production of sST2. IL-13 stimulation of 3T3 embryonic fibroblasts and primary mouse lung fibroblasts resulted in significant secretion of sST2. This study elucidates the important regulatory activity that IL-13 exerts on IL-33 through induction of IL-33 decoy receptor sST2 and through modulation of ST2L⁺ ILC2s.

W37. Regulatory T-cells in Colon Cancer: A Case of Multiple Personality Disorder

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Regulatory T-cells (T_{reg} s) are a heterogeneous population of thymic and extrathymic origin that have diverse immune suppressive functions. Expression of the lineage-determining transcription factor Foxp3 is essential for maintaining T_{reg} identity, but is not sufficient to account for their substantial functional diversity. In addition to FOXP3, T_{reg} s can express other transcription factors that are normally associated with T-helper cell functions, namely ROR γ t, GATA3, or TBET. More than half of gut-infiltrating T_{reg} s in healthy mice express ROR γ t and cMAF, and these T_{reg} s critically maintain immunotolerance and host microbe homeostasis. Using patient blood cells and mouse models of hereditary colon cancer, we demonstrate that expression of β -catenin and TCF-1 is altered in T_{reg} s during CRC. Genetic manipulation of T_{reg} s, epigenetic analysis, single cell RNAseq, and functional assays reveal mechanisms of gain of tumor promoting properties by T_{reg} s. Our data suggest that TCF-1 and Foxp3 co-bind active enhancers of pro-inflammatory pathway genes and limit their expression. Sustained Wnt- β -catenin activation induces newly accessible chromatin sites in these genes, upregulates their expression. We identify two mature and multiple memory T_{reg} s clusters with distinct molecular signatures, that gain of TH17 profile in response to β -catenin/TCF1 signaling. In this process, peripherally induced ROR γ t⁺ T_{reg} s gain potent CD8 T-cell suppressive activity while giving up their ability to suppress TH17 and TH1 inflammation. The outcome is deregulated inflammation and compromised T-cell cytotoxicity leading to unhindered tumor growth.

W51. Novel Interaction Partners of the SH2 Domain in T Cell Specific Adaptor Protein

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T cells play a crucial role in the adaptive immune system and depend on tightly regulated intracellular signalling pathways to respond in an appropriate manner. Following naïve T cell activation, T cell specific adaptor protein (TSAd) is upregulated and believed to mediate T cell receptor (TCR) signalling by modulating Src family tyrosine kinase signalling. As an adaptor protein, TSAd contains several protein interaction motifs, including the Src homology 2 (SH2) domain. Prototypic binding of phosphorylated tyrosines to SH2 domains suggests that TSAd is involved in the phosphotyrosine signalling pathway. However, the molecular details of the underlying mechanisms and the defined role that TSAd plays downstream of TCR stimulation remains elusive. Here, we perform pulldowns with exogenous TSAd in Jurkat T cells and subject it to mass spectrometry analysis, revealing novel as well as previously characterised ligands of the TSAd SH2 domain. Using various immunoprecipitation and YFP-based protein fragment complementation assays, we validate and further characterise the consequence of these interactions downstream of TCR stimulation. Furthermore, using TSAd CRISPR-knockout mutants and TSAd-deficient murine CD4⁺ T cells, we explore the affected signalling pathways that these interaction partners are implicated in. Taken together, these findings provide insight into the role of TSAd in T cell function and possible mechanisms for how TSAd acts to fine-tune T cell intracellular signalling, which may have implications in developments for immunotherapies.

W54. A Novel T Cell “Spheromer” Probe Shows Conserved SARS-CoV-2 Peptide-specific T Cells Correlate with Milder Disease

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A central feature of the SARS-CoV-2 pandemic is the wide spectrum of disease severity displayed by patients. While some individuals develop severe disease that may ultimately lead to fatality, others only present mild

symptoms or are asymptomatic. It has been suggested that this might have to do with other low pathogenic human coronaviruses that have been in general circulation, but the evidence for this and a direct link between disease trajectory and SARS-CoV-2 epitopes is limited. We have engineered a superior $\alpha\beta$ T cell staining reagent, with each functionalized maxi-ferritin “spheromer” displaying 12 peptide-MHC complexes. This novel platform offers several advantages: ease of production, defined site-specific conjugation of pMHC molecules that significantly reduces inter-batch variation, and compatibility with currently available pMHC molecules and streptavidin reagents that allows for facile translation. These reagents are able to stain specific T cells more efficiently and capture additional T cell receptors of a given specificity. Analyzing T cells from naïve individuals with spheromers, we find that peptides conserved amongst coronaviruses are more abundant and tend to have a “memory” phenotype, compared to those unique to SARS-CoV-2. Significantly, CD8⁺ T cells with these conserved specificities are much more abundant in COVID-19 patients with mild disease versus those with a more severe illness, suggesting a protective role.

W55. EGR2 and EGR3 Suppress the Accumulation of B1a and CD21^{low} CD23^{low} Age-associated B Cells *in vivo*

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The *Egr2* and *Egr3* gene paralogues encode early growth response (EGR) transcription factors, act as immediate early genes following B cell receptor (BCR) signalling and are highly up-regulated in anergic mouse B cells. Anergy preserves microbial epitope responsiveness from a finite pool of pre-immune B cells, but is also reversible – creating a risk of autoimmune disease. Pathological proliferation of self-reactive B cells can also cause chronic lymphocytic leukemia (CLL), 3.8% of which harbour somatic missense *EGR2* mutations resulting in loss- or change-of-function and correlated with poor prognosis even within aggressive *TP53*-mutant CLL. We analysed B cells in mice lacking one or both alleles of *Egr2* and/or *Egr3* and show that *Egr2* and *Egr3* deletion cause the cell-intrinsic accumulation in multiple lymphoid organs of populations enriched for self-reactive BCRs: B1a and CD21^{low} CD23^{low} age-associated B cells. Global single-cell RNA profiling of these expanded populations *in vivo* demonstrated their differential expression of genes involved in their survival and maintenance. We use chromatin immunoprecipitation sequencing (ChIP-Seq) to show that several of these genes are direct EGR2 transcriptional targets in human CLL cells. This is the first report on the cell-intrinsic roles of *Egr2/3* in B cells. It highlights a novel role for these transcription factors in suppressing the accumulation of B1a and age-associated or atypical memory B cells, and supports the hypothesis that EGR2 loss-of-function contributes to CLL pathogenesis.

W80. ROCK2 Specific Inhibition Attenuates AngII-induced Hypertension in Mice

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Hypertension, or an elevated blood pressure, is the primary modifiable risk factor for cardiovascular disease, the number one cause of mortality worldwide. We previously demonstrated that Th17 activation and IL-17A/IL-21 production is integral for the full development of a hypertensive phenotype as well as the renal and vascular damage associated with hypertension. Rho-associated coiled-coil containing protein Kinase 2 (ROCK2) serves as a molecular switch upregulating Th17 and inhibiting regulatory T cell (Treg) differentiation. We hypothesize that hypertension is characterized by excessive T cell ROCK2 activation leading to increased Th17/Treg ratios

and ultimately end-organ damage. We first showed *in vitro* that KD025, an experimental orally bioavailable ROCK2 inhibitor inhibits Th17 cell proliferation and IL-17A/IL-21 production. To determine if hypertensive stimuli such as endothelial stretch increases T cell ROCK2 expression, we cultured human aortic endothelial cells exposed to 5% (normotensive) or 10% (hypertensive) stretch with circulating human T cells and HLA-DR⁺ antigen presenting cells. Hypertensive stretch increased T cell ROCK2 expression 2-fold. We then tested the effect of ROCK2 inhibition with KD025 (50mg/kg i.p. daily) *in vivo* on angiotensin II (Ang II)-induced hypertension. Remarkably, treatment with KD025 significantly attenuated the hypertensive response within 1 week of Ang II treatment and this persisted for the duration of the 4 week study reaching blood pressures 20 mmHg lower than vehicle treated mice. Flow cytometric analysis of tissue infiltrating leukocytes revealed that KD025 treatment decreased Th17/Treg ratios in the kidney. Thus, T cell ROCK2 may be a novel therapeutic target for the treatment of hypertension.

W94. Characterization of Phenotype and $\alpha\beta$ TCR of HLA-B*27-restricted CD8 T-cell Clones Associated with Spondyloarthropathies

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The mechanism by which HLA-B27 induces Ankylosing Spondylitis (AS) and related disease are unknown, but a leading hypothesis is that it presents 'arthritogenic epitopes' to CD8 T-lymphocytes. Supporting this hypothesis, we have previously demonstrated clonal expansions of CD8 TRB clones adhering to the CASSVG(V/I/L)(Y/F)STDTQYF CDR3 motif in AS pathogenesis (DOI: 10.1002/art.41252), which have also been reported by 2 recent independent studies in AS, as well as in samples from patients with bacterial-induced reactive arthritis, using different methodologies. The presence of those clones exclusively in *HLA-B*27+ve* disease points to a common antigen engaged by receptors of similar structure. Therefore, the aim of our study was to obtain the paired $\alpha\beta$ TCR carried by these cells and phenotype them using single-cell RNA sequencing. For this, we used PBMCs of 2 AS donors screened in our previous study and flow-sorted memory CD8 T cells carrying the relevant TRBV genes to enrich for our low-frequency target population. We captured 8 different clonal expansions containing the same TRB consensus CASSVGL(Y/F)STDTQYF, and all using a paired TRAV gene. These cells were characterized by the expression of KLRB1, LGALS3, DUSP1 and GNLY whilst lacking HLA-II expression, identified as a marker of activation. However, higher expression of inflammatory genes was also observed, highlighting the upregulation of CD69 and expression of PDE4D, an AS-relevant therapeutic target. This is the first report of a deep profiling of HLA-B*27-restricted AS-associated CD8 clonotypic expansions, providing essential information for the identification of the antigenic priming occurring on the background of spondylarthritis.

W105. Defining the Phenotype, Specificity and Avidity of Anti-tumor CD8⁺ T Cells in Melanoma

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At the heart of antigen-specific anti-tumor immune responses are the interactions between T cell receptors (TCR) and their cognate peptide-MHC complexes, expressed on tumors. Tumor-specific T cells are critical for disease control, and yet the relationship between their phenotypic characteristics and TCR properties are not well-elucidated. By linking in vitro measured antigen-specificity of hundreds of TCRs to T cell clonality and cellular phenotype of melanoma-infiltrating lymphocytes detected at single-cell resolution and at large-scale, we show that tumor specificity shapes the phenotype of tumor-infiltrating CD8⁺ T cells. Melanoma-reactive lymphocytes displayed an exhausted phenotype; conversely, non-tumor reactive T cells, enriched for viral specificities, segregated in the non-exhausted compartment. The TCR clonotypes from intra-tumoral exhausted lymphocytes persisted in peripheral blood at higher levels in patients with poor response to checkpoint blockade compared to those achieving durable disease regression, consistent with chronic stimulation mediated by the presence of residual tumor antigen. Tumor-specific TCRs reactive against public melanoma associated antigens or personal neoantigens displayed shared an exhausted phenotype and exhibited a broad range of avidities that were inversely related to the expression level of cognate targets in melanoma cells and proportional to the binding affinity of peptide-HLA class I complexes. By revealing how the quality and quantity of tumor antigens drive tumor-specific TCR avidities and the downstream phenotype of tumor-specific CD8⁺ T cells, we gain insights into the rules governing the properties of TCRs recognizing tumor neoantigens and offer a roadmap for the selection of antigenic targets for cancer immunotherapy.

W110. An AP-1 Independent Gene Module Regulated by an NFAT2 Positive Feedback Loop Driven by Persistent TCR Stimulation is Required for Tfh Maintenance

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Follicular helper T (Tfh) cells are critical regulators of humoral immunity. Unlike Th1, Th2 or Th17 cells that drive inflammatory, pathogen-clearing responses in peripheral tissues, Tfh cells facilitate GC B cell development in a non-destructive and non-cytotoxic manner, suggesting Tfh gene programs may exhibit distinguishing features. NFAT family members regulate T cell activation. In canonical NFAT signaling, NFAT proteins cooperate with AP-1 proteins. Alternatively, AP-1 independent NFAT signaling is associated with hyporesponsive T cell states including anergy and exhaustion. Our transcriptome analysis of Tfh cells derived from a variety of immune settings reveals a common AP-1 independent NFAT gene signature. We tested whether this mode of NFAT signaling plays a critical role in Tfh development or function. Experiments with mutants of NFAT2 unable to interact with AP-1 or with overexpressed AP-1 proteins show that AP-1 independent NFAT signaling is necessary and sufficient for Tfh development

in vivo

We demonstrate NFAT2 autoregulation, a TCR-mediated and NFAT-dependent positive feedback loop, is essential for Tfh cell maintenance, providing a molecular rationale for the known dependence of Tfh cells on antigen persistence. We find NFAT signaling and autoregulation in Tfh cells is critical for development of murine lupus, suggesting these pathways underlie the therapeutic efficacy of calcineurin inhibitors. Unlike hyporesponsive cells, which rely on impaired costimulation to generate AP-1 independent signaling, the stoichiometry of NFAT:AP-1 governs NFAT signaling in Tfh cells. Thus, NFAT signaling mode, along with the molecular mechanisms which drive mode selection, are important variables for T effector cell fate determination.

W127. Alternative Oral Immunotherapy for Hemophilia A

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Prophylactic tolerance protocol are lacking to protect hemophilia A patients receiving factor VIII (FVIII) replacement therapy. We previously reported an Oral immunotherapy (OIT) based on repeated uptake of a mixture of lettuce plant cells transgenic for heavy chain (HC) or C2 domain of human FVIII fused to cholera toxin B (CTB) subunit [PMID:24825864; PMID: 29106782]. An alternative to OIT is the oral delivery of immune modulatory antibodies. Here, we compared the plant cell-based method with oral delivery of anti-CD3. Hemophilia A BALB/c mice received CTB-FVIII-HC/-C2 (1.0µg/antigen/gavage) expressing lettuce cells 2x/week for 9 weeks. Starting at 4 weeks, 1 IU/mouse of BDD-FVIII was given iv, 1x/per week for 5 weeks. Alternatively, 0.5µg or 5µg of anti-CD3 Fab fragment was given by gavage daily for 5 straight days, followed by BDD-FVIII injection. Control animals developed inhibitors with an average titer of 18.3 BU/ml. Inhibitor formation was significantly reduced in plant cell-treated mice, with 47% showing no or low-titer inhibitors. While a daily dose of 5µg anti-CD3 Fab/mouse was unsuccessful, a dose of 0.5µg greatly reduced titers. Combination of 2 approaches did not represent an improvement as just 33% of mice developed < 5 BU/ml. Lettuce-fed mice showed elevated frequencies in LAP⁺ Treg, including LAP⁺Foxp3⁺ Treg (2-fold higher than control or anti-CD3 treatment), which showed high expression of GARP and TIGIT. Whereas Treg cells induced by anti-CD3 delivery were Foxp3⁺ Treg with high expression of PD1⁺. In summary, both approaches are effective in reducing inhibitor formation but work through different Treg-mediated mechanisms.

Th1. Human B Lymphocyte Dysfunction and Leptin

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Leptin, an adipokine secreted primarily by the adipose tissue/adipocytes, has endocrine and immune functions. It has been shown to increase the secretion of pro-inflammatory cytokines by immune cells. We show that incubation of B cells from young lean (Y_L) individuals with leptin increases the frequencies of pro-inflammatory B cells and induces intrinsic B cell inflammation, characterized by mRNA expression of pro-inflammatory cytokines (TNF-α and IL-6), chemokines (IL-8), micro-RNAs (miR-155 and miR-16), TLR4 and p16, a cell cycle regulator associated with immunosenescence. We also show that the expression of these pro-inflammatory markers in unstimulated B cells is negatively associated with the antibody response of the same B cells after in vivo or in vitro stimulation. We have evaluated here the in vitro class switch and IgG secretion in CpG-stimulated B cells from Y_L individuals as compared to B cells from young obese (Y_O) and elderly lean (E_L) individuals. We found that B cells from Y_L individuals, after in vitro incubation with leptin, show reduced class switch and influenza vaccine-specific IgG production. Our results altogether show that leptin makes B cells from Y_L individuals similar to those from Y_O and E_L individuals, supporting leptin as a possible mechanism of immunosenescence in human B cells.

Th8. Thymus Hypoplasia Due to 22q11.2 Deletion Syndrome Corrected by Mesenchymal Cell Replacement

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Thymus hypoplasia occurs in several clinical conditions including 22q11.2 Deletion Syndrome (22q11.2DS). 22q11.2DS is the most common human microdeletion disorder known, affecting < 1/4000. Thymuses from 22q11.2DS patients are smaller in size than normal, producing fewer T cells resulting in more infections, much like patients with various mutations in the *FOXP1* transcription factor. In both clinical syndromes, an allogeneic thymus tissue graft is a preferred treatment option to resolve the stromal cell defects of the host tissue. To determine the molecular mechanisms contributing to a small thymus size in 22q11.2DS, we compared the development of the thymus in embryos from mouse models of this syndrome versus normal controls and those from *Foxn1* mutant mice. Reaggregate fetal thymic organ culture assays established that replacing mesenchymal cells from 22q11.2del hypoplastic lobes with normal ones enabled tissue expansion and restored thymopoiesis. Thymic epithelial cells, used as substitutes, could not. This is distinct from the *Foxn1* mutant mice, wherein defective thymic epithelial cell functions lead to thymus hypoplasia/aplasia. Single cell RNA sequencing of normal and hypoplastic thymus lobes revealed differential expression of transcripts that primarily impacted 5 distinct mesenchymal cell subsets in 22q11.2DS. These transcripts are involved in cell-cell interactions, collagen deposition and growth. This contrasted the transcriptome differences using hypoplastic lobes from *Foxn1* mutant mice, wherein TECs are significantly impacted. Our findings reveal novel approaches for regenerating a functional thymus rendered non-functional due to specific clinical conditions.

Th24. Neonatal T Helper 17 Responses are Skewed Towards an Immunoregulatory Interleukin-22 Phenotype

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Newborns are frequently affected by mucocutaneous candidiasis. Th17 cells essentially limit mucosal invasion by commensal *Candida spp.* Here, we sought to understand the molecular basis for the developmental lack of Th17 cell responses circulating blood neonatal T cells. Cord blood CD4 T cells stimulated in Th17-polarizing conditions inherently produced high levels of the interleukin-22 immunoregulatory cytokine, particularly in the presence of neonatal antigen-presenting cells. A genome-wide transcriptome analysis comparing neonatal and adult naïve CD4 T cells *ex vivo* revealed major developmental changes in gene networks regulating Small Drosophila Mothers Against Decapentaplegic (SMAD) and Signal Transducer and Activator of Transcription 3 (STAT3) signalling in neonatal T cells. These changes were functionally validated by experiments showing that the requirement for TGF- β in human Th17 cell differentiation is age-dependent. Moreover, STAT3 activity was profoundly diminished and overexpression of the *STAT3* gene restored Th17 cell differentiation capacity in neonatal T cells. These data reveal that Th17 cell responses are tightly regulated developmentally in humans in early life, at the gene expression level. These developmental changes may protect newborns against pathological Th17 cell responses, at the same time increasing their susceptibility to mucocutaneous candidiasis.

Th30. Follicular Helper T Cell Signature of Replicative Exhaustion, Apoptosis and Senescence in Common Variable Immunodeficiency

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Common variable immunodeficiency (CVID) is the most frequent primary antibody deficiency. CVID patients suffer from various manifestations of immune dysregulation, caused as well by an inappropriate signals to B cells from follicular T cells during the germinal center (GC) response.

We analyzed circulating follicular helper (T_{fh}) cells in the peripheral blood of 27 CVID patients (11 pediatric and 16 adult) with autoimmunity comparing them to 106 (39 pediatric and 67 adult) age-matched healthy controls. We applied Whole Exome and Sanger Sequencing to identify mutations possibly accounting for CVID development and associating with T_{fh} alterations.

A group of CVID patients ($n=9$) showed super-physiological frequency of T_{fh}1 cells and increased PD-1 and ICOS expression, plus a T_{fh} RNA signature consistent with highly active, but exhausted and apoptotic cells. Elevated plasmatic CXCL13 levels positively correlated with T_{fh}1 cell frequency, PD-1 levels, and an elevated frequency of CD21^{lo}CD38^{lo} autoreactive B cells. Four patients belonging to this group monoallelic variants in *RTEL1*, a telomere length- and DNA repair-related gene. Lymphocytes with shortened telomeres, and a T_{fh} signature enriched in genes involved in telomere elongation and response to DNA damage were seen. Histopathological analysis of the spleen in one patient showed reduced amount and size of the GC that, unexpectedly, contained more T_{fh} cells. These data indicate a novel pathogenetic mechanism in a group of CVID patients, whereby alterations in DNA repair and telomere elongation might be involved in GC-B cells, and acquisition of a Th1, highly activated but exhausted and apoptotic phenotype by T_{fh} cells.

Th35. ELF3 Activated by a Superenhancer and an Autoregulatory Feedback Loop is Required for High-level HLA-C Expression on Extravillous Trophoblasts

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HLA-C arose during evolution of pregnancy in the great apes 10 to 15 million years ago. It has a dual function on placental extravillous trophoblasts (EVTs) as it contributes to both tolerance and immunity at the maternal-fetal interface. Its mode of regulation is of considerable interest in connection with the biology of pregnancy and pregnancy abnormalities. First-trimester primary EVT in which HLA-C is highly expressed, as well as JEG3, an EVT model cell line, were employed. Single-cell RNA-seq data and quantitative PCR identified high expression of

the transcription factor ELF3 in those cells. Chromatin immunoprecipitation (ChIP)-PCR confirmed that both ELF3 and MED1 bound to the proximal HLA-C promoter region. However, binding of RFX5 to this region was absent or severely reduced, and the adjacent HLA-B locus remained closed. Expression of HLA-C was inhibited by ELF3 small interfering RNAs (siRNAs) and by wrenchnolol treatment. Wrenchnolol is a cell-permeable synthetic organic molecule that mimics ELF3 and is relatively specific for binding to ELF3's coactivator, MED23, as our data also showed in JEG3. Moreover, the ELF3 gene is regulated by a superenhancer that spans more than 5 Mb, identified by assay for transposase-accessible chromatin using sequencing (ATAC-seq), as well as by its sensitivity to (+)-JQ1 (inhibitor of BRD4). ELF3 bound to its own promoter, thus creating an autoregulatory feedback loop that establishes expression of ELF3 and HLA-C in trophoblasts. Wrenchnolol blocked binding of MED23 to ELF3, thus disrupting the positive-feedback loop that drives ELF3 expression, with down-regulation of HLA-C expression as a consequence.

Th39. Machine Learning Approaches to Developing TCR Repertoire-based Diagnostics for Pathogen Exposure

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T cells are an integral component of the adaptive immune system both in the response to foreign antigens, but also non-native self-antigens as may be generated in cancer. While T-cell repertoires are largely distinct between individuals due to the random generation of T-cell receptor proteins, examples of public T-cell receptors have been identified to common antigens including Flu and CMV. The current COVID-19 pandemic has created an urgent need to understand T-cell response to the virus during and after infection. We showed as a proof of concept that in a large cohort we could use machine learning approaches to identify CMV-specific TCR β sequences and develop a diagnostic for CMV status based only on immunosequencing. Validation experiments achieved a sensitivity of 90% and specificity of 88%. We further demonstrated that dominant HLA types for T-cell repertoires can be inferred based on the presence of certain TCRs. We applied a similar principle to identify COVID-19-specific TCR β sequences and to classify subjects as COVID-19 naïve or experienced. COVID-19 T-cell responses were detectable as early as 0-2 days post diagnosis, peaked at 15-28 days and were still present at 43+ days. Additionally, we characterized specific regions of the SARS-CoV-2 virus that elicit these responses using *in vitro* T-cell assays. These learnings can be applied in the future to identify tumor associated antigens or neoantigens that elicit strong immunogenic responses, find cancer specific TCR β sequences, and understand how these behave in different HLA settings.

Th42. NGS Characterization of Multiple Immune Receptors from a Single Multiplex PCR Reaction

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NGS analysis of immune cell repertoires has become a key tool in hematology and immuno-oncology research. Owing to primer-primer interactions and incompatibility of reaction conditions, current NGS immune cell assays require separate reactions for each immune receptor, depleting samples and extending time-to-answer. Two new highly-multiplexed NGS assays, based on Ion AmpliSeq technology, provide for efficient single-reaction library preparation for detection of rearrangements in the IGH, IGK, and IGL chains and the TCRB and TCRG chains, respectively. The combined B cell assay also includes primers to amplify rearrangements involving the kappa deletion and constant region intronic elements. To evaluate the performance of each assay, clonality detection assessment and sensitivity testing used gDNA from over 100 research samples representing common B and T cell malignancies (CLL, B-ALL, Multiple Myeloma, Burkitt's Lymphoma, NHL, DLBCL, and T-

ALL). Sequencing was performed using the Ion GeneStudio S5 System and analysis was completed using Ion Reporter 5.16. Clonality assessments carried out using gDNA collected from both cell line and clinical research samples show a >90% overall positive detection rate. Both the TCR and BCR multi-receptor assays perform as expected in linearity and sensitivity experiments, with linear response of detection to serial dilution, including the ability to detect clones of interest at 10⁻⁶. These results demonstrate the robustness of these novel Ion AmpliSeq assays for multi-receptor B and T cell repertoire analysis. These assays will cut the time-to-answer for translational research studies and offer simplification for clonality assessment and rare clone detection in hematology research.

Th51. Evaluating Humoral Primary Immunodeficiencies Using Coding Joint to Kappa-deleting Recombination Excision Circle (CJ:KREC) Ratio and Serum B-cell Activating Factor (BAFF) Level

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Background: Kappa-deleting recombination excision circles (KRECs) are excision products that do not replicate during B-cell division. The recombination site is the coding joint (CJ). The ratio of CJ:KREC DNA (via real-time quantitative PCR (qPCR)) estimates the number of B-cell divisions and number of newly formed B-cells. B-cell Activating Factor (BAFF) is a cytokine that promotes B-cell survival and proliferation. These data could assess severity of B-cell immunodeficiencies and predict risk of disease complication.

Methods: Patients with humoral primary immunodeficiencies (PID) aged 6 months to 22 years were eligible for inclusion. CJ and KREC levels were measured via qPCR. Serum BAFF levels were measured using Mesoscale®. Similar analysis was performed in healthy controls.

Results: 16 PID patients were included in analysis. Patients were separated by humoral PID diagnosis into two groups: antibody deficiency syndromes or common variable immunodeficiency (CVID). Mean CJ:KREC ratio in the PID cohort was 9.15, vs. 4.83 in controls. Mean CJ:KREC ratio in the antibody deficiency group was 5.25, vs. 13.04 in CVID (p=0.059). Mean serum BAFF in PID patients was 165.70, vs. 50.85 in controls. Mean serum BAFF in the antibody deficiency cohort was 107.88, vs. 216.30 in CVID (p=0.271).

Conclusions: Patients with humoral PIDs have increased CJ:KREC ratios and serum BAFF levels compared to controls. CVID patients have increased CJ:KREC ratios and increased serum BAFF, compared to those with antibody deficiency. Severity of humoral PIDs could be estimated using CJ:KREC ratio and serum BAFF level, and may involve an interplay between B-cell output and BAFF production.

Th55. Restoration of Follicular T Cell Compartment in Patients with Wiskott Aldrich Syndrome After Gene Therapy

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Background: Wiskott-Aldrich syndrome (WAS) is a rare X-linked primary immunodeficiency characterised by cellular and humoral defects and an increased risk of autoimmunity and lymphomas. It is caused by mutations in the WAS gene, expressed exclusively in hematopoietic cells and encoding for WAS protein (WASP). Gene therapy

(GT), an investigational treatment, may represent a potential alternative to allogeneic hematopoietic stem cell transplantation (HSCT), particularly for patients who do not have any suitable HSCT donor available.

Materials & Methods: Number, phenotype, and functional properties of follicular T cells (helper and regulatory – Tfh and Tfr) and CXCL13 plasma levels were determined, with flow cytometry and ELISA respectively, in 10 WAS pre-GT patients and 17 WAS patients post-GT.

Results: Pre-GT WAS patients displayed a lower frequency of circulating Tfh cells than age- and sex-matched healthy controls (HCs), but their frequency increased and reached physiological levels post GT. PD-1 and ICOS expression levels on Tfh were significantly higher before treatment compared to HCs. While Tfh PD-1 levels declined post GT, ICOS levels remained elevated. Tfr cells, the regulatory counterpart of Tfh, were low in pre-GT patients, but increased after GT. Finally, WAS patients had elevated levels of plasma CXCL13, that decreased after GT.

Conclusion: In summary, our findings indicate that GT may restore some of the defects observed in Tfh and Tfr cells of patients with WAS, contributing to the recovery of the humoral immune response. These data support GT as a potential treatment option for WAS patients with limited options for definitive therapy.

Th59. Priming of Long-Lived CD8⁺ T cell Responses in the Spleen During Influenza Virus Infection

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Initiation of viral CD8 T cell responses are important for killing virally infected cells at the site of infection. During influenza virus infection, initiation of these responses are thought to only occur in the lung draining, mediastinal lymph node (mLN). To start, migratory dendritic cells (mDCs) are activated in the lungs in response to infection and subsequently migrate to the mLN where they encounter cognate CD8⁺ T cells. As such, when mDCs are absent or when migration is inhibited, CD8⁺ T cell responses are compromised and the viral burden in the lungs increases. CD8 T cells have been appreciated for being a heterogeneous population of cells that are all primed in a single location. In contrast to the current paradigm, additional locally in the spleen has been hypothesized, however, mechanistically this hypothesis is unpopular because mDCs are thought to die in the mLN shortly after trafficking. Here we reconcile conflicting reports and show that in a mouse model of influenza infection, a portion of lung derived mDCs egress the mLN and subsequently migrate to the spleen to prime CD8 T cells, independent of the mLN. CD8 T cells primed in these locations show differences in longevity and ability to respond robustly to rechallenge. Altogether, our results demonstrate a novel DC trafficking pathway and support a new paradigm for how heterogeneous T cell responses to influenza are initiated.

Th98. Temporal Dissection of the T Cell-Intrinsic Factors that Maintain T Follicular Helper Cell Identity and Germinal Centers

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T follicular helper (Tfh) cells are crucial for the establishment of germinal centers (GCs) and potent antibody responses that are elicited during infection and vaccination. Despite their importance for humoral immunity, the T cell-intrinsic factors that are required for the maintenance of already established Tfh cells and GCs remain largely unknown. Here, we used temporally guided, tamoxifen-inducible CD4⁺ T cell-specific gene ablation to dissect the contributions of various factors, including CXCR5, Bcl6, and mature miRNAs, to the maintenance of Tfh cell

function and identity and its impact on B cells in ongoing GCs in viral infection and vaccination models. Induced ablation of *Cxcr5* in CD4⁺ T cells had only minor effects on the identity and function of established Tfh cells. Phenotypical and genome-wide transcriptional analyses revealed that *Cxcr5*-ablated cells still exhibited most features of CXCR5-positive Tfh cells. In contrast, continued *Bcl6* expression was essential to maintain the GC Tfh cell phenotype and GC reaction. CD4⁺ T cell-specific *Bcl6* ablation during acute viral infection resulted in transdifferentiation of “ex-Tfh” cells into Th1 cells. Finally, induced CD4⁺ T cell-specific depletion of all mature miRNAs resulted in the loss of the Tfh cell phenotype and resolution of GCs. By highlighting the high degree of Tfh cell plasticity, these studies provide novel insights into the mechanisms underlying T-B cell interactions and Tfh cell and GC maintenance.

Th114. An *in vitro* Neonatal Gut Model to Understand Necrotizing Enterocolitis

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De novo colonization of the immature neonatal intestine by microbes results in excessive inflammation and tissue damages. Mouse models showed the role of IL-17-producing T cells in the development of Necrotizing Enterocolitis (NEC), a severe intestinal inflammatory disease responsible for high mortality and long-term intestinal dysfunction in premature infants. However, our understanding of NEC is limited by the lack of models mimicking the developmental events of human neonatal gut.

Intestinal epithelial cells (IECs) educate T cells to combat intestinal pathogens, while this interaction is also required to promote a favorable intestinal commensal microbial flora in the gut. Recently, we showed that neonatal Th17 cells inherently produce a distinct cytokine profile, characterized by an abundance of the immunoregulatory cytokine IL-22 instead of the IL-17.

Here, we aim to establish an *in vitro* human neonatal intestinal organoids model to study the functional interactions between neonatal T cells and intestinal epithelial cells.

Intestinal organoids “mini-guts” comprising IECs were created *in vitro* from intestinal crypt biopsies obtained from neonates < 6 months for diagnostic or therapeutic indications. These organoids were then cultured in the presence of neonatal Th17 cell supernatants, and the effects IL-17 and IL-22 on IEC functions were determined using qPCR and immunostaining.

We successfully established an *in vitro* 3D gut “organoid” model comprised of primary IECs derived from neonatal intestinal biopsies. Our analysis indicated that high neonatal Th17 cell-produced IL-22 promoted IEC proliferation and mucus production, as shown by increased Ki67 and Muc2, respectively.

Th141. The Roles of BHLHE40 in Tr1 Cell Biology

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Type 1 regulatory T cells (Tr1) are a peripherally inducible, IL-10⁺FOXP3⁻ subtype of regulatory T cells. Tr1 cells can be identified by surface expression of CD49b and LAG-3 in addition to secretion of intermediate amounts of IFN- γ and high amounts of IL-10, and they are potent suppressors of autoimmune and inflammatory responses. Unlike other CD4⁺ T cell subtypes with known transcription factors (TFs) driving their differentiation, it is still largely unknown which TFs drive the differentiation and function of Tr1 cells. We have sequenced the transcriptomes of unstimulated FACS-sorted Tr1 cells and non-Tr1 memory CD4⁺ T cells from three healthy donors and identified several highly expressed TFs specific to Tr1 cells. Strikingly, amongst these TFs was basic helix-loop-helix family member e40 (BHLHE40), a known inhibitor of murine IL-10 production. Lentiviral-mediated overexpression of BHLHE40 led to upregulated IFN- γ production, suppression of IL-10, and co-expression of CD49b and LAG-3, suggesting a key role in Tr1 cell development. CRISPR/Cas9-mediated knockout of BHLHE40 in human naïve CD4⁺ T cells confirmed that BHLHE40 regulates the secretion of IFN- γ and IL-10. However, the presence of BHLHE40 was not required for Tr1 cell differentiation in our allo-antigen-specific Tr1 induction protocol. These studies indicate that BHLHE40 regulates the two key cytokines and phenotypic markers of Tr1 cells.

Th165. SuperNova v605 and v786: Next Generation Polymer Dyes- A Stellar New Way to See Dim Populations

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Beckman Coulter has introduced two new tandem polymer dyes, SuperNova (SN) v605 and v786 and associated conjugated antibodies. SN v605 and v786 have their maximum excitation at 414 nm and emission at 605nm and 786nm respectively, detected using 610/20 and 780/60 nm bandpass filters. These polymer dyes are the brightest in their respective channels, allowing the assessment of dim populations by flow cytometry with minimal nonspecific staining, attributed to the proprietary formulation, providing flow cytometry laboratories with greater confidence in their results. The compatibility of SN v605 and v786 with other fluorochromes has also been explored.

Three lots of SuperNova conjugates were stained with whole blood specimens. Median fluorescence intensity, % positive recruitment, stain index and non-specific binding were analyzed and compared with those of commercially available polymer dye conjugates, at recommended dose.

SN conjugates showed higher stain index, < 5% lot to lot positive delta recruitment and, most importantly, reduced non-specific staining in comparison with the commercially available polymer dye conjugates. SN dyes delivered equivalent performance both as single-color conjugates and in a multicolor panel consisting of conventional fluorochromes like FITC, PE, ECD etc, without affecting the characteristics of other fluorochromes.

This study demonstrates that, as compared to commercially available polymer dyes, SN dyes have exceptional brightness with minimal non-specific staining, thereby delivering better identification of low-density antigens and rare populations. They help to build brighter and higher-resolution cytometry experiments. SuperNova polymer dyes are a platform technology, allowing the development of additional dyes with different emission properties.

Allergy

W40. Association Between the Use of Aspirin and Obstructive Lung Diseases in Diabetic Patients

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Background: Many diabetic patients take a daily dose of aspirin, one reason being to protect their hearts. Aspirin has already shown a benefit in preventing cardiovascular diseases, but its role in obstructive lung diseases is less clear. This report presents data that contributes to establishing an association between obstructive lung disease and the use of aspirin in a diabetic cohort in the Northeast US.

Methods: A total of 1,003 subjects in community practice settings were interviewed at home at the time of enrolment into the Vermont Diabetes Information System, a clinical decision support program. Patients self-reported their personal and clinical characteristics, including any history of obstructive lung disease. Laboratory data were obtained directly from the clinical laboratory, and current medications were obtained by direct observation of medication containers. We performed a cross-sectional analysis of the interviewed subjects to assess a possible association between obstructive lung disease history and the use of aspirin.

Results: In a multivariate logistic regression model, a history of obstructive lung disease was significantly associated with use of aspirin even after correcting for potential confounders including gender, low income (< \$30,000/year), number of comorbidities, number of medications excluding aspirin, cigarette smoking, and alcohol problems (adjusted odds ratio (OR) = 0.66, P = 0.02, 95% confidence interval (CI) = 0.46-0.94).

Conclusion: These data suggest a negative correlation between use of aspirin and obstructive lung disease prevalence in patients with diabetes. Further studies are required to determine if this association is causal.

W48. Augmentation of Oral Immunotherapy with Tolerance-inducing Nanoparticles

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Roughly 15 million Americans suffer from food allergies which, in severe cases, can be life threatening. Presently, oral immunotherapy (OIT) is the most efficacious option to achieve sustained unresponsiveness (SU) in allergic patients but is limited by the risk of triggering anaphylaxis and requiring medical supervision. Several clinical studies indicate that SU after OIT is correlated with increased T regulatory cell populations, which suggests the supplementation of OIT with tolerogenic immunomodulatory factors will increase its efficacy. Polysaccharide A (PSA), a commensal molecule produced by the gut-symbiont *Bacteroides fragilis*, has been shown to have Treg-inducing capabilities within the gut. The tolerance-inducing capacity in combination with its polymeric structure makes PSA an intriguing biomaterial for the formulation of tolerogenic nanoparticles. We hypothesize that the encapsulation of allergen within PSA nanoparticles (NPs) will show significant improvements in efficacy and safety over traditional allergen-only oral immunotherapy due to PSA NPs ability to (i) induce Treg differentiation and deliver allergen simultaneously, and (ii) to shield the allergen from IgE receptor-mediated mast cell activation until internalization by intestinal dendritic cells. We show that PSA NPs can be readily fabricated using water/oil emulsification with chemical crosslinking and maintain immunoregulatory capability, including TLR2 stimulation and tolerogenic dendritic cell differentiation. Furthermore, in-vitro assays indicate that PSA NPs can effectively deliver encapsulated antigen to dendritic cells and mediate antigen-specific T regulatory cell

proliferation. PSA NPs have the potential to become a “plug-in-play” system to induce specific tolerance to any encapsulated allergen.

W123. Children and Adults with Eosinophilic Esophagitis Share a Conserved Interferon Signature

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Eosinophilic esophagitis (EoE) is an allergic inflammatory disease of the esophagus that occurs in 1 in approximately 1 in 2000 U.S. adults and children. The pathogenesis of EoE is still poorly understood, particularly in adults. Prior studies of esophageal biopsy gene expression have identified signatures of eosinophilia, type 2 inflammation, and epithelial cell dysregulation. In these studies, we examined esophageal biopsies from adults and pediatric EoE patients to determine if there is a shared inflammatory gene expression profile. Using RNA-sequencing we demonstrate a strong interferon signature that was significantly enriched in biopsy tissue from adult EoE patients as compared to non-EoE controls, and that was not found in the paired whole blood samples from the same patients. We observe that both type I and type II interferon responsive genes were upregulated in adult biopsies, and confirmed this in pediatric biopsy tissue using digital multiplex gene expression assays as well as independent analysis of a public pediatric and adult EoE patient biopsy RNA-sequencing dataset. Using a milk-peptide stimulation assay, we determined that antigen-specific peripheral CD4⁺T cells produce IFN γ upon activation with EoE-causal allergens. Together, this work identifies a novel interferon signature that is conserved in pediatric and adult EoE patients. These data highlight a role for non-type 2 inflammatory networks in EoE.

Autoimmune Diseases

W1. Immunological and Islet Cell Changes in Checkpoint Inhibitor-induced Diabetes

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Checkpoint inhibitors (CPIs) (anti-programmed death-1 (PD-1) or programmed death-ligand 1 (PD-L1)) have revolutionized cancer treatment but can also result in autoimmune complications such as diabetes (CPI-DM). We analyzed the mechanisms of CPI-DM utilizing a mouse model to interrogate the effects of CPIs on immune and islet cells. Non-obese diabetic (NOD) mice treated with anti-PD-L1 develop DM rapidly whereas anti-CTLA-4 mice do not, mirroring clinical experience. We treated NOD mice with anti-PD-L1 or anti-CTLA-4 and harvested islets for RNA sequencing to characterize differences in transcriptional changes between CPI treatments. RNA-seq of islet infiltrating immune cells revealed differences in IFN γ and other inflammatory pathways as well as upregulation of cytolytic mediators such as *Gzma/B* and *Fasl* in anti-PD-L1 treated mice. Changes in islet cells included IFN γ responsive genes such as *Irf1* and *Cd274*. We then tested the ability of anti-IFN γ and anti-TNF α to inhibit development of DM in anti-PD-L1 treated NOD mice. Anti-IFN γ alone delayed development of DM modestly, but inhibition of both cytokines resulted in significant inhibition of CPI-DM. We treated a patient with renal cell carcinoma who had worsening of DM following CPI therapy with anti-TNF mAb (infliximab) and found increased levels of C-peptide and reduced insulin requirements following treatment. CPIs targeting the PD-1/PD-L1 pathway results in unique transcriptional changes in islet cell and immune infiltrates that may lead to the

development of diabetes. Inhibition of inflammatory cytokines can reverse CPI-DM, pointing to a role for these inflammatory mediators in CPI-DM which could have important clinical implications.

W4. Peripheral Blood Lymphocyte Subsets in Arthritis in the Elderly

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Introduction: Lymphocyte subsets have been connected to joint infiltration and inflammation in rheumatoid arthritis (RA). Identification of leucocyte subsets dysregulated in arthritis development could provide insight into the aetiology of RA. Elderly-onset RA, EORA, is a RA starting at >60 years. Polymyalgia rheumatica (PMR) is another common rheumatic disease in the elderly. This study aimed to investigate the composition of the peripheral blood component.

Material and methods: Newly diagnosed arthritis in patients >60 years, with blood samples collected at baseline and 12 months after treatment. Compared with control individuals of same age and gender. T and B cell subsets in whole blood were determined with flow cytometry..

Results: 29 patients and 18 controls (HC) were analyzed (19 RA and 10 PMR). In patients with EORA, significant increase in percentage and numbers of CD4⁺ effector subset and significantly decrease in numbers of CD4⁺ central memory. In patients with PMR, a trend towards different B-cell subsets were observed with an increase in percentage of naïve B cells compared to HC and a significant increase in percentage and numbers of CD8⁺ central memory. Longitudinal analysis showed that several B and T cell subsets were significantly different at 12 months in both EORA and PMR patients suggesting an effect of therapy in the lymphocyte subsets.

Conclusion: Patients with EORA and PMR demonstrate a change in cellular immune parameters apparent in the periphery. More studies are needed to show whether these subsets can be related with clinical remission and response to treatment.

W5. Evidence for Impaired Thymic Negative Selection in Type 1 Diabetic Immune Systems

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Developing T cells undergo thymic negative selection to purge the T-cell receptor repertoire of autoreactive thymocytes. Despite this checkpoint, many patients suffer from T cell-mediated autoimmune diseases. Here, we used novel TCR-transgenic human immune systems to compare the efficiency of thymic negative selection of T cells generated from healthy control (HC) and Type 1 diabetic (T1D) hematopoietic stem cells (HSCs) in thymic organ cultures and human immune system (HIS) mice. We observed persistence of thymocytes that normally undergo negative selection and decreased thymocyte death following strong TCR stimulation in T1D compared to HC thymocytes. Further, negative selection and T-regulatory cell diversion of insulin-reactive T cells was impaired in some T1D immune systems. Impaired negative selection correlated with genotypic and single cell gene expression data implicating T1D-associated single nucleotide polymorphisms (SNPs) in Erk/MAP kinase pathway components. Our data also raise the hypothesis that T1D-associated SNPs in *SH2B3* may promote autoimmunity

by altering gene expression of crucial T-cell signaling genes involved in thymocyte selection. Single cell gene expression analysis demonstrated that T1D thymocytes have differential expression of multiple genes involved in thymic selection, including decreased expression of TCR signaling molecule Zap-70 and increased expression of anti-apoptotic genes compared to HC. These studies present the first evidence for T-regulatory cell (Treg) diversion of autoreactive thymocytes, for impaired thymic negative selection and Treg diversion in T1D immune systems, and suggest a genetic mechanism through which selection is impaired, providing insight into T1D pathogenesis.

W6. PD-L1 Expression by Thyroid Follicular Cells Can Modulate T Cell Function and Phenotype in Mixed Autologous; implications for Thyroid Autoimmune Diseases

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PD-L1 expression by parenchymal cells have been proposed as an important mechanism of peripheral tolerance that is utilized by neoplastic cells to escape anti-tumoral immune response, this being under intense investigation; however, but much less attention has been paid to its role in common autoimmune diseases.

PD-L1 expression by thyroid follicular cells (TFCs) in glands from patients with Hashimoto Thyroiditis (HT) and Graves' Disease (GD) has been just been reported by our group. Similar findings were recently reported in islet beta cells from human and mouse diabetic pancreas (Osum KC 2018; Colli ML, 2018).

Interestingly, PD-L1 expression by TFC from autoimmune glands is, however, much less intense than HLA class II "ectopic" expression but arises spontaneously in TFC as plated in culture and is increased by IFN-gamma, this pointing to PD-L1 *in vivo* inhibition. In order to better understand PD-L1 expression regulation, autologous mixed TFC-PBMC cultures have been set using samples from Graves' disease patients and organ donors. In this setting, TFCs have been shown to suppress CD3/CD28 induced T cell proliferation at ratios up to 1:4. Experiments to confirm these results and to demonstrate the role of PD1-PDL1 and IFN-gamma in this suppression are underway. Better understanding of the role of Checkpoint Receptors and their ligands in parenchymal-lymphocyte interactions may lead to reinterpretation the role of peripheral tolerance mechanisms in autoimmunity and the so called "aberrant" HLA-DR hypothesis of organ-specific autoimmunity.

W8. Single Cell Analysis of mTECs Carrying Dominant Mutations in Functionally Distinct Domains of AIRE Enable a Mechanistic Understanding of TSA Expression

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The autoimmune regulator (AIRE) is a large multi-domain protein that is required for the development of central immune tolerance through its regulation of tissue-specific antigen (TSA) expression in medullary thymic epithelial cells (mTECs). Aire-deficiency results in a failure of negative T cell selection and subsequent multiorgan

autoimmune disease in both humans and mice. The mechanisms by which the various structural domains of AIRE contribute to TSA expression remain poorly characterized. Recently, a kindred was identified with an autosomal dominant C311Y mutation in the PHD1 zinc finger domain of AIRE. To better understand PHD1 domain function, we generated a novel knockin (*Aire^{CY/+}*) mouse model and compared these mice to complete *Aire^{-/-}* and *Aire^{GW/+}* mice, which carry a previously described autosomal dominant mutation in the SAND domain. In general, *Aire^{CY/+}* mice had a unique pattern of affected peripheral organs and a milder blockade in terminal mTEC differentiation. Transcriptomic analysis by bulk RNA, scRNA and scATAC sequencing revealed that *Aire^{CY/+}* and *Aire^{GW/+}* mice shared many transcriptomic and epigenetic features, as expected. Strikingly, *Aire^{CY/+}* mice regulated fewer TSAs and had lower chromatin accessibility compare to *Aire^{GW/+}* mice. These results implicate both direct and indirect effects of the PHD1 and SAND domains on Aire-mediated gene expression and chromatin organization. In sum, our findings elucidate the role of the PHD1 domain in regulating TSA expression and describe a novel mouse model for a human autosomal dominant AIRE mutation.

W11. Do Global Changes Induced by T Cell-targeted Therapies Reflect Alterations in the Antigen-Specific CD8 T Cell Compartment? A Preliminary Report of T1D Autoantigen Response Profiles in Two Completed Clinical Trials

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Immune phenotyping studies during clinical trials in recent-onset type 1 diabetes have identified CD8 T-cell exhaustion profiles that correlate with better clinical outcomes in subjects who received teplizumab or alefacept therapy, targeting CD3 or CD2, respectively. We have now investigated whether these drug-induced phenotypic profiles are present in islet autoantigen-specific CD8 T cells, which represent a small fraction of the total CD8 T cell pool. We utilized a data clustering platform (DISCOV-R) to interrogate multiple cell characteristics determined by CyTOF analysis, utilizing MHC class I tetramers loaded with islet autoantigen-derived peptides, as well as 18 phenotyping markers. Prior to initiating therapy, low frequencies of T cells with exhaustion profiles were identified among islet-specific CD8 T cells in the peripheral blood. Increased expression of the teplizumab response-associated profile (TIGIT, KLRG1 and EOMES expression early after therapy) was observed in islet-specific cells from one of five teplizumab-treated subjects, an individual who was one of the two responders studied. Alefacept response-associated exhaustion profiles (high PD1 or CD57 expression late after treatment) increased for islet-specific cells in two of three responders and two of five non-responders following alefacept treatment. When changes in these exhaustion profiles among the total CD8 T cell population were compared with islet-specific subsets for each individual, we observed a frequent lack of concordance following therapy, although this result is limited by small numbers of subjects and cells available for study. These data illustrate the need for caution in extrapolating from global profiling to the antigen-specific fraction of cell populations.

W13. Upregulation of HLA Class II on Beta Cells in Pancreata from Organ Donors with Type 1 Diabetes

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Aim: HLA class II(HLA-II) expression in the islet β -cells of type 1 diabetic(T1D) patients has been historically controversial. We characterized and quantified HLA-II expression in human pancreas and analyzed its induction in human islets.

Methods: We immunostained human pancreatic sections with HLA-II, CD68, and insulin, from non-diabetic(n=5), auto-antibody positive(Aab+;n=5), and T1D(n=5)donors, obtained from nPOD. Images were acquired with widefield Zeiss Axioscan.Z1 (whole tissue section) and with Zeiss laser scanning confocal LSM880 (high resolution of 301 islets and enhanced resolution-Airyscan of selected β -cells). Image analysis was performed with QuPath,Zen and Imaris softwares. Further,we cultured healthy human islets (isolated-IIDP- and reaggregated-InSphero-)with varying concentrations of proinflammatory cytokines (IFN- γ ,TNF- α ,IL-1 β). Islet function was measured by glucose-stimulated insulin secretion, and HLA-II expression was evaluated with immunostaining and RNA-sequencing.

Results: We analyzed 7,415 islets that contained 339,480 cells. Insulin containing islets (ICIs) of T1D donors had a higher percentage of HLA-II positive area(24.31%) compared to T1D insulin deficient islets(0.67%), non-diabetic(3.8%) and Aab+(2.31%) donors. In T1D ICIs 45.89% of the insulin signal colocalized with HLA-II, and 27.65% of the β -cells expressed HLA-II. In isolated and reaggregated islets, upon stimulation with proinflammatory cytokines, we observed changes in insulin secretion, higher expression of HLA-II and upregulation of HLA-II RNA transcripts.

Conclusions: We provide definitive evidence that HLA-II can be expressed by pancreatic β -cells from T1D patients. This upregulation can be induced *in vitro* in human isolated or reaggregated islets by treatment with pro-inflammatory cytokines. Our findings support a role for HLA-II in T1D pathogenesis,HLA-II expressing β -cells can potentially become a direct target of autoreactive CD4⁺lymphocytes.

W25. The Role of Aire in the Selection of Regulatory T Cells in Diabetes

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In autoimmune diseases like T1D, it is proposed that failure in central tolerance mechanisms towards pancreatic islet self-antigens leads to the escape of pathogenic clones and loss of critical immunosuppressive antigen-specific Tregs. The importance of Aire in maintaining central tolerance against self-reactive antigens is evidenced by the multiorgan autoimmune disease seen in individuals with mutations within the *Aire* gene. When *Aire*-expression is specifically ablated in mouse mTECs, there is a dramatic increase in the number of self-reactive T cells that escape negative selection, in addition, a dramatic loss of Tregs. Utilizing novel high-throughput platforms and single-cell DNA barcoding technology has allowed us to assess several thousand CD4⁺ T-cell clones. The use of FoxP3-GFP reporter mice permits us to identify individual FoxP3⁺ Tregs that have undergone Aire-dependent selection in the thymus and compare the TCR repertoire to mice that have had Aire specifically ablated from the mTECs. Identification of unique clones and gene signatures within our large datasets has allowed us to start to qualitatively assess the functional role of the identified TCRs within the model of T1D. GLIPH2 clustering using our extensive insulin-specific TCRs have identified a number of motifs shared within our TCR dataset, as well as a unique V-gene usage that may predict TCR affinity. Our proposed study aims to expand our knowledge on TCR repertoire of Aire-dependent FoxP3⁺ Tregs and the antigen-specificity in T1D, improving our understanding of the respective function of thymic-derived antigen-specific Treg suppression in peripheral tissues associated with autoimmune T1D.

W27. Age-associated Immunity in Murine Atherosclerosis on Macro and Single-cell Level

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Healthy aging is one of the prime goals in today's society and atherosclerosis is among the greatest causes of morbidity in elderly. Although atherosclerosis, characterized by the formation of arterial plaques consisting of lipids and immune cells, already starts to develop at a young age, acute cardiovascular events often occur at advanced age. In order to gain better insights into age-driven atherosclerosis and to move one step closer towards tailored immunotherapies, we provide for the first time proteomic and transcriptomic insights in age-associated immunity in murine atherosclerosis.

Young (3 months old) and aged (18 months old) atherosclerosis-susceptible LDLr-deficient (*ldlr*^{-/-}) mice were fed a chow diet or atherogenic diet for 10 weeks, after which atherosclerosis and immune cell frequencies were measured. We report age-associated alterations, such as elevated circulating monocytes and a shift from naïve towards effector (memory) T cells with more extreme phenotypes, e.g. elevated IFN γ and IL-17-production, in our naturally aged atherosclerotic *ldlr*^{-/-} mice. Using single-cell RNA-sequencing on CD45⁺ leukocytes from aged *ldlr*^{-/-} aortas we identified 12 distinct immune cell clusters, which in contrast to young plaques, were dominated by T- and B cells followed by myeloid cell populations and natural killer cells. Subclustering approaches and subsequent flow cytometry revealed an age-associated increase in the frequency of several immune cells, including CD4⁺CD8⁺ T cells, GzmK⁺CD8⁺ T cells, and IL-17-producing $\gamma\delta$ T cells.

Collectively, we provide comprehensive profiling of aged immunity in atherosclerotic mice and reveal the emergence of age-associated cell subsets in the atherosclerotic aorta.

W35. HLA Autoimmune Risk Alleles Restrict the Hypervariable Region of T Cell Receptors

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Polymorphisms in the *human leukocyte antigen (HLA)* genes within the *major histocompatibility complex (MHC)* locus strongly influence autoimmune disease risk. Two non-exclusive hypotheses exist about the pathogenic role of *HLA* alleles; i) the central hypothesis, where *HLA* risk alleles influence thymic selection so that the probability of T cell receptors (TCRs) reactive to pathogenic antigens is increased; and ii) the peripheral hypothesis, where *HLA* risk alleles increase the affinity for pathogenic antigens. The peripheral hypothesis has been the main research focus in autoimmunity, while human data on the central hypothesis are lacking. Here, we investigated the influence of *HLA* alleles on TCR composition at the highly diverse complementarity determining region 3 (CDR3), where TCR recognizes antigens. We demonstrated unexpectedly powerful *HLA*-CDR3 associations. The strongest association was found at *HLA-DRB1* amino acid position 13 ($n = 628$ subjects, explained variance = 9.4%; $P = 4.1 \times 10^{-138}$). This *HLA* position mediates genetic risk for multiple autoimmune diseases. We identified multiple CDR3 amino acid features enriched by *HLA* risk alleles; for example, the risk alleles of rheumatoid arthritis, type 1 diabetes, and celiac disease all increase the hydrophobicity of CDR3 position 109 ($P < 2.1 \times 10^{-5}$). In the setting of celiac disease, the CDR3 features favored by *HLA* risk alleles are more enriched among candidate pathogenic TCRs than control TCRs ($P = 2.4 \times 10^{-6}$ for gliadin specific TCRs). Together, these results provide novel genetic evidence supporting the central hypothesis.

W41. Transfection of Vitamin D3-induced Tolerogenic Dendritic Cells for the Silencing of Potential Tolerogenic Genes. Role of CSF1R

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Background: Reestablishment of the immune tolerance by using tolerogenic dendritic cells (tolDC) is a promising strategy under investigation in several clinical trials. Identification of surface cell markers specific of tolDC is essential for their translation to the clinic.

Objective: To set up an easy and fast transfection methodology of monocytes during their differentiation to tolDC using vitamin D3 (VitD3-tolDC). To analyze the relevance of *CSF1R* (CD115) and *CD209* genes in their tolerogenic function.

Methodology: mDC and VitD3-tolDC were differentiated from monocytes cultured for 6 days in presence of IL-4, GM-CSF (and vitD3 for tolDC). At day 1, Viomer blue reagent combined with specific or control siRNA were added to VitD3-tolDC. Maturation was induced on day 4 with a pro-inflammatory cytokine cocktail (IL1 β +TNF α +prostaglandin E2). On day 6, phenotype, functionality, and gene and protein expression were determined.

Results: Optimization of transfection using 0.75 μ L of Viomer blue + 2.75 μ M of siRNA/ 10⁶ cells resulted in >80% of transfected cells without affecting phenotypical or functional characteristics of VitD3-tolDC. The transfection with specific siRNA for *CSF1R* and *CD209* genes allowed 80% gene silencing compared to non-transfected VitD3-tolDC, although only CD209 protein significantly reduced its expression.

A partial reduction in the proliferation induced by VitD3-tolDC to allogeneic cells was observed in siCD115-treated tolDC (p= 0.018, n=5). Additionally, reduction of glycolysis and lactate secretion in siCD115-tolDC was observed compared to untreated VitD3-tolDC (p= 0.010, n=3).

Conclusion: Viomer blue is an adequate transfection technology for VitD3-tolDC. *CSF1R* gene is related (but not essential) to the tolerogenicity of VitD3-tolDC.

W53. Organ Specific Immune Suppression via PD-1 Bispecific Agonists - A Novel Approach to Treat Autoimmunity

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The PD-1 pathway is a key immune checkpoint that limits T cell responses. Activating PD-1 to inhibit autoreactive T cells and treat autoimmunity is an appealing concept, but progress in this area has been limited, with few therapies entering early clinical trials. Moreover, limiting T cell suppression to the tissue or organs under autoimmune attack, thus avoiding systemic immunosuppression, would be desirable.

Effective PD-1-mediated inhibition of T cell activity requires co-engagement of PD-1 with the TCR and accumulation at the immunological synapse, a process stimulated by PD-L1 on the interacting target cell. To mimic PD-L1 function and create potent T cell inhibitors, we designed targeted PD-1 agonist bispecifics that contain a high affinity TCR moiety specific for pHLA complexes on target cells. Once bound to target cell pHLA, these bispecifics accumulate at the target cell-T cell interface, engage PD-1, and inhibit T cell signaling.

We further show that target cell-bound PD-1 agonists are effective inhibitors of T cell function. At picomolar concentrations, these bispecifics inhibit secretion of effector cytokines from both primary CD4⁺ and CD8⁺ T cells and impair autoreactive CD8⁺ T cell-mediated cytotoxicity. Importantly, these PD-1 agonists are unable to inhibit T cells when free in solution and not bound to target cells. Hence, these immune modulating bispecifics have the potential to deliver localized specific immune suppression while avoiding systemic immunosuppression. These features make them an attractive and novel platform to treat autoimmune diseases.

W66. Autoreactive B Cell and Follicular Helper/Regulatory T Cells Crosstalk in Pemphigus, a Paradigm of Autoantibody-mediated Diseases

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Pemphigus is a severe autoimmune bullous disease mediated by pathogenic autoantibodies that target the inter-keratinocytes adhesion proteins desmogleins 1 and 3. We showed that rituximab (anti-CD20) in combination with short-term oral corticosteroids therapy has better efficacy and safety than prednisone alone in moderate to severe forms of pemphigus. In addition, maintenance of rituximab infusions 12 and 18 months after the initial cycle of rituximab has demonstrated its interest in preventing long-term relapses and good tolerance. We report herein the effect of therapy on circulating self-reactive B cell populations.

We observed that, after initial depletion of pathogenic self-reactive B cells, post-rituximab reconstitution was associated with a modulation of the adaptive immune system as determined by transcriptomic and phenotypic analysis. Indeed, while not preventing the re-emergence of self-reactive B cells, they mainly consisted in naïve unswitched IgM+CD27⁻ self-reactive populations that were unable to secrete anti-desmoglein autoantibodies. Using a MHC tetramer presenting the immunodominant peptide of desmoglein 3, we are currently investigating the specific T cell response, i.e. self-reactive follicular helper T cells (Tfh) versus follicular regulatory T cells (Tfr). Data to date tend to show that, in the long term, rituximab induces a decrease in the frequency of self-reactive Tfh as compared to baseline and an increase in circulating self-reactive Tfr. Results of on-going experiments will be presented.

Our results indicate a role for the autoreactive B cell and Tfh crosstalk in pemphigus disease and suggest that autoantigen-specific Tfr could be involved in the long-term efficacy of rituximab.

W67. Identification of Novel Epitopes and Their Associated MHC-Restricted T-cell Clonotypes in the Periphery of Patients with Type 1 Diabetes

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Decoding the immune synapse will allow an in depth understanding of the T-cell repertoire in Type 1 Diabetes. This is essential in identifying the key drivers of diabetogenesis and pathogenesis, in addition, these insights will provide disease stratifying biomarkers and will open up new possibilities to develop novel targets for therapeutic intervention. Here we report how applying CIPHER™, a DNA-barcoded multimer-based single cell sequencing platform, allows for the interrogation of T-cells from peripheral blood and pancreatic tissue of high-risk individuals, T1D patients and healthy donors and enables the rapid discovery of biologically-relevant epitopes and TCRs. CD8⁺ T-cells are the predominant infiltrate observed in pancreatic islets and associated with beta cell destruction. To interrogate the immune repertoire of CD8⁺ lymphocytes we designed a HLA-A*02:01 CIPHER™ DNA-barcoded multimer library comprising 707 epitopes derived from 46 T1D-relevant genes and analyzed the samples by single cell sequencing. PBMC samples from T1D+ and healthy donors were screened with the CIPHER™ library and the discovered TCRs were cloned and shown to signal when stimulated with their cognate antigen through a NFAT reporter gene assay. Our technology allows us to detect T-cell-detected HLA-peptide pairs, associated TCRs and transcriptional profiles, all on an individual cell basis. We have discovered multitude of validated TCR-antigen pairs to previously reported as well as novel autoimmune epitopes. The epitopes seen by multiple T1D patients and recognized by T cells with memory or effector phenotypes will allow for the development of novel therapeutics to induce tolerance of autoreactive cells in patients with T1D.

W86. Neutrophil Extracellular Trap (NET) Formation in Patients with Vasculitis

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NET formation, extrusion of chromatin and granular components, is essential in host defense. NET formation has been linked to inflammation and autoimmunity, including anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV). Neutrophil infiltration is seen in aortic specimens of patients with Takayasu's arteritis (TAK) and affected arteries of giant cell arteritis (GCA), two large-vessel vasculitis (LVV). Whether NET formation occurs in LVV is not known.

Objective: To compare levels of NET formation across different types of vasculitis.

Methods: Plasma levels of NETs (neutrophil elastase (NE)-DNA complexes) were analyzed in healthy controls (n=30), and patients with AAV (granulomatosis with polyangiitis (GPA, n=196), microscopic polyangiitis (MPA, n=74)), TAK (n=66) and GCA (n=82), at time-point of remission or flare. Disease activity was assessed using physician global assessments (PGA).

Results: NE-DNA complexes were elevated in patients with GPA ($p < 0.001$), TAK ($p < 0.05$) and GCA ($p < 0.001$), but not MPA, at time-point of remission and flare, as compared to healthy controls. In patients with TAK, but none of the other conditions, elevated levels of NE-DNA complexes were seen at time-point of flare as compared to remission ($p < 0.05$), with NE-DNA levels correlating with PGA ($r=0.28$, $p < 0.05$). Levels of NETs did not correlate with markers of inflammation, as assessed by CRP.

Conclusion: NET formation, as assessed by NE-DNA complexes, is elevated in both small and large vessel vasculitis, suggesting a potential common disease link in several types of vasculitis. Further studies are needed to determine underlying factors mediating neutrophil activation in otherwise quiescent disease.

W133. CXCR5⁺ CD8⁺ Tfc Cell Function in Autoimmunity

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CXCR5⁺ CD8⁺ T cells are an emerging immune subset that arise during infections and cancer. Our lab has previously revealed this subset to expand in autoimmunity within IL-2KO mice. This expansion lead to increased B cell class-switching, plasma cell differentiation and survival while also demonstrating a dominant helper-like role with cytolytic potential. Previous studies have shown CXCR5⁺ CD8⁺ T cells to upregulate markers of exhaustion during chronic infection and cancer. Our lab has also demonstrated this phenomenon with a bulk RNAseq of IL2KO mice showing differences in inhibitory markers between CD4 and CD8 T follicular cells. Here we have begun investigating CXCR5⁺ CD8⁺ T cells in the context of systemic lupus erythematosus using the MRL/lpr model. We hypothesized that the CXCR5⁺ CD8⁺ population would expand as disease is exacerbated with increased exhaustion while producing cytokines that influence CD4 and CD8 T cell function. We show that this population expands as disease progresses with differences between male and female mice, typical of autoimmunity. Utilizing flow cytometry, inhibitory markers (CD160, PD-1, Tim3, CTLA4, Lag3) and effector molecules (IFN γ , IL-10 and IL-21) were seen to increase correlating with disease progression at 2, 4 and 6 months of age. These studies indicate that CXCR5⁺ CD8⁺ T cells may act as B cell regulators within germinal center reactions and have Th1-like function in the lymph node and spleens of MRL/lpr mice. We will continue to unravel CXCR5⁺ CD8⁺ T cell function and contribution to autoimmune disease progression.

W159. Epigenetic and Immunological Analysis as Mechanistic and Diagnostic Indicators in Tregopathies

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Pathogenic single-gene mutations causing quantitative and/or functional defects in regulatory T cells (Tregs) lead to “Tregopathies” as part of Primary Immune Regulatory Disorders. At the Stanford Center for Genetic Immune Diseases (CGID), we apply novel multiparametric assays to dissect the origin and evaluate the extent of the immunological abnormalities associated with autoimmunity and autoinflammation in these rare disorders. These studies will be instrumental for personalized treatment in patients with different mutations in the same or different gene and with highly variable clinical presentation. We have applied an epigenetic method to quantify Tregs in whole blood by measuring DNA demethylation of specific region of the *FOXP3* gene (TSDR). In addition, we use flow and mass cytometry by time-of-flight (CyTOF) panels to seize abnormalities along the Treg- effector T cells (Teff) axis including the FOXP3⁺ Tregs, type 1 T regulatory cells (Tr1), T helper (Th) 1, Th2, Th17 and T follicular helper (Tfh) cells. To complement the cellular evaluations, 76-plex luminex assay quantifies soluble protein levels in blood plasma, indicating changes in the immune-complex network. Currently, we have applied these technologies to patients with mutation in FOXP3, CD25, CTLA-4 and STAT genes. The approach will extend to patients with similar clinical manifestations but unknown causative genes, collected within a national study group part of the Primary Immune Deficiency Treatment Consortium. These studies will provide precise mapping of the immune cell types and pathways affected by different mutations and will open to new treatment options including cell and gene therapy.

W221. Reducing the Risk of Progressive Multifocal Leukoencephalopathy in Relapsing-remitting Multiple Sclerosis Patients Treated with Natalizumab

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Background. Natalizumab (NTZ) is a humanized anti- α 4-integrin (CD49d) monoclonal antibody that prevents leukocyte migration into the central nervous system (CNS). It is indicated for treatment of relapsing-remitting multiple sclerosis (RRMS). Despite its high effectiveness, its use is associated with risk of developing Progressive Multifocal Leukoencephalopathy (PML), a CNS infection due to JC virus (JCV).

NTZ administrated in extended interval dose (EID) has demonstrated to reduce the CD49d receptor occupancy levels (RO) without compromising the effectiveness of the treatment. Moreover, NTZ in EID of 300mg/6wks suggests lower risk of developing PML compared with the standard dose range (SD) of 300mg/4wks in patients with anti-JCV antibodies.

Objectives. To establish a scientific base to personalize NTZ dosage with the aim of reducing the risk of PML.

Methods. Ongoing transversal study in 30 RRMS patients under NTZ treatment (10 SD or EID patients with clinical or radiological activity, and 20 EID patients without clinical and/or radiological activity).

Peripheral blood samples were analysed by flow cytometry to determine CD49d RO in lymphocyte subpopulations together with CD49d, NTZ, CD29 and β 7-integrin, performed at two different timepoints separated by 3 NTZ administrations. At these same points, other biomarkers in serum will be analysed.

Results. After flow cytometry analysis of CD4 and CD8 T and B lymphocytes, preliminary results demonstrate that patients receiving EID have less NTZ bounded to CD49d. Interestingly, this reduction is not affecting the clinical and radiological effectiveness of NTZ treatment.

Conclusions. Our preliminary results suggest that EID reduces the percentage of CD49d RO maintaining the effectiveness of NTZ treatment.

Th19. IL-17 is Expressed in Insulin-containing Islets of Donors with Type 1 and Type 2 Diabetes

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Purpose: IL-17 is an important effector cytokine driving immune-mediated destruction in autoimmune diseases such as psoriasis. Blockade of the IL-17 pathway after the initiation of insulinitis was effective in delaying or preventing the onset of type 1 diabetes (T1D) in rodent models. Expression of IL-17 transcripts in islets from a donor with recent-onset T1D has been reported, however, studies regarding IL-17 protein expression are lacking. We aimed to study whether IL-17 is being expressed in the islets of diabetic donors.

Methods: We stained human pancreatic tissues from non-diabetic (n=5), auto-antibody positive (aab+) (n=5), T1D (n=6) and T2D (n=5) donors for IL-17, Insulin, and Glucagon, and for CD45 in selected cases. High resolution images were acquired with Zeiss laser scanning confocal microscope LSM780 and analyzed with Zen blue 2.3 software.

Results: We observed a strong cytoplasmic staining for IL-17 in many insulin-containing islets of donors with T1D and T2D, accounting for an average of $7.8 \pm 8.4\%$ and $14.9 \pm 16.8\%$ of total islet area, respectively. Both beta and

alpha cells were sources of IL-17, but CD45 was not a major source in those donors. Expression of IL-17 was reduced in islets of non-diabetic donors, aab+ donors and in insulin-deficient islets of donors with T1D.

Conclusion: Our finding that IL-17 is expressed in islets of donors with T1D or T2D is quite intriguing and warrants further mechanistic studies in human islets to understand the role of IL-17 in the context of metabolic and immune stress.

Th22. PD-1 Bispecific Antibodies for Localized Immunomodulation in Autoimmune and Inflammatory skin Diseases

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The PD-1 axis is an important regulatory pathway that suppresses autoimmune disease progression through both inhibition of the self-reacting T-cells as well as upregulation of T-regs. Agonism of PD-1 is an attractive approach to establish immune homeostasis in autoimmune diseases. Pandion Therapeutics is developing bispecific antibodies that combine a “tether moiety” that targets a tissue of choice with a PD-1 agonist. This novel process delivers the therapeutic molecules to the tissues (sites) of autoimmune and inflammatory diseases for a potentially improved tissue localized efficacy while mitigating the risks associated with systemic immunotherapies.

Here we report the engineering of PD-1 agonists with a skin-targeting tether that inhibits T cell activation in a tissue specific manner. These skin-tethered immune effectors were assessed for drug-like properties in biophysical assays and *in vitro* and *in vivo* assays for target binding, cellular activity and tissue specific-localization. Moreover, these bispecific antibodies were tested in preclinical models such as huPBMC-engrafted xeno-GVHD, vitiligo and humanized Imiquimod-induced psoriasis model. Strikingly, a selective accumulation of the bispecific antibodies to the skin was observed and correlated with a tether-dependent efficacy compared to a non-tether control.

We believe that this therapeutic approach has the potential to drive the resolution of cutaneous inflammation, providing an opportunity for developing new targeted therapies for autoimmune and inflammatory skin diseases.

Th23. Technical Validation and Utility of a DR0401 Tetramer Assay for Type 1 Diabetes: a Multi-center Study

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Validated biomarkers are needed for antigen-specific T cells in type 1 diabetes (T1D). Towards this end, we performed a technical validation of an HLA class II tetramer assay and evaluated its utility by characterizing CD4⁺ T cells in cross-sectional samples of subjects with T1D. An *ex vivo* tetramer assay was applied to enumerate and phenotype DRA-DRB1*04:01 (DR0401)-restricted T cells specific for multiple epitopes from GAD65, IGRP, preproinsulin, and ZnT8, and a positive control influenza epitope, in a single staining tube. The assay was applied to PBMCs from T1D subjects and a prepared clone spiked PBMC sample. Single center replicate testing was performed on blinded samples, followed by multi-center blinded testing at five laboratories using centralized reagents. All laboratories successfully identified tetramer-positive T cell populations specific for each epitope in the clone-spiked sample. As expected, influenza had the lowest %CV, while IGRP had the highest within and between centers. Only IGRP exceeded the target %CV value of 30. Precision testing of replicate T1D samples resulted in a mean %CV < 35 for all epitopes except ZnT8. Multi-center testing resulted in higher mean %CV values with ZnT8 being most variable. The assay was applied to a cross-sectional sample set, for which frequencies were lowest for IGRP and ZnT8, with some variability attributable to technical factors. Significant association was found between higher T cell frequencies and a shorter duration of T1D at draw. These results suggest that a multicolor class II tetramer assay is reproducible and useful and can be implemented in multiple laboratories.

Th27. Characterizing Skin Resident Memory T Cell Formation in Murine Cutaneous Lupus Erythematosus

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Cutaneous Lupus Erythematosus (CLE) is a spectrum of autoimmune connective tissue diseases that are characterized histopathologically by interface dermatitis and lupus band reaction. CLE's current treatment options are based on SLE treatments and include topical steroids, antimalarials, and other immunosuppressants. CLE patients exhibit flares that can be triggered by environmental stimuli such as UV light. Tissue-resident memory T cells (Trm) mediate flares in autoimmune skin disorders, though their specificities, functional molecules, and survival factors in CLE have not yet been described. We used a mouse model of CLE to examine the development of Trm skin lesions. In this model, OVA peptide-activated DO11CD4⁺ T cells were injected intravenously into sub-lethally irradiated TLR9KO li-TGO mice that express TGO transgene in the MHCII cells after being fed with Dox chow. Mice in this model developed IgM and IgG1 autoantibodies and exhibited cutaneous T cell accumulation that positively correlated with the severity of skin lesions. We found that some antigen-specific skin T cells in these mice expressed phenotypic markers consistent with Trm, which persisted after antigen withdrawal. Trm cells were enriched in the skin as compared to the draining lymph node and spleen. Disease scores also peaked more rapidly during flare induction than during primary disease, as do ANA titers. Preliminary analysis of human blister biopsies from CLE patients exhibited phenotypic markers of Trm (CD8⁺CD103⁺CD69⁺ T cells) in the skin. Based on our data, we hypothesize that targeting Trm in CLE may be a durable treatment strategy.

Th44. Expression of Pro-Resolution Genes by Type 1 Conventional Dendritic Cells Differentiates Uveitis Subtypes

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Background

Uveitis is a heterogeneous group of ocular inflammatory diseases, for which targeted immune therapies remain ineffective for a significant number of patients. Improved pathophysiologic understanding of the mechanisms that differ between disease subtypes are needed to develop a precision medicine approach to uveitis.

Methods

To identify molecular features that differentiate patients with two forms of uveitis, acute and chronic, we performed single cell RNA Sequencing (scRNA-Seq) on aqueous fluid and peripheral blood from patients with active uveitis.

Results

We identified several types of ocular myeloid cells in all patients, including type 1 conventional dendritic cells (DC1), which have not previously been described in ocular inflammation. DC1s were unique in their expression of a panel of anti-inflammatory, or pro-resolution genes, including *IDO*, *HAVCR2* (Tim-3) and *BTLA*, whereas the other myeloid cells expressed predominantly pro-inflammatory genes, suggesting that DC1s may promote the resolution of ocular inflammation. Additionally, DC1s from patients with acute uveitis expressed more anti-inflammatory genes than DC1s from patients with chronic uveitis, including *FALSG*, *RARG* and *ALOX15*, suggesting DC1s specifically promote the resolution of acute uveitis.

Conclusion

Unbiased transcriptional analysis of small volume ocular fluid biopsies can shed unprecedented insight into pathophysiologic mechanisms that distinguish uveitis subtypes. Taken together, these data suggest that DC1s play a unique role in promoting the resolution of ocular inflammation. Furthermore, expression of key immunoregulatory genes by these cells differentiates two clinically distinct forms of uveitis. Finally, DC1s may represent a diagnostic and therapeutic target for precision medicine in uveitis.

Th57. Lymphatic Dysfunction in Murine Lupus Photosensitivity

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The lymphatic system is composed of vessels which carry fluid, soluble molecules, and cells from peripheral tissue to draining lymph nodes. Photosensitivity, an exaggerated inflammatory response in response to ultraviolet radiation (UVR), is present in most patients with Systemic Lupus Erythematosus (SLE). Lymphatic dysfunction has been shown to induce photosensitivity in wild-type models, thus we hypothesized that lymphatic dysfunction could contribute to photosensitivity in SLE. We examined MRL/lpr lupus prone mice for lymphatic function by injecting Evan's Blue into the ear and measuring retention. Ear thickness and flow cytometric analysis were used to assess photosensitivity. MRL/lpr mice had greater Evan's blue retention compared to controls suggesting lupus prone mice have impaired lymphatic drainage. We then investigated the utility of improving lymphatic drainage using two approaches. First, we used manual lymphatic drainage (MLD) in the MRL/lpr mice. MLD

improved lymphatic drainage and reduced photosensitivity. Second, we induced a lupus phenotype in a novel mouse model with enhanced lymphatic function (inducible lymphatic endothelial cell specific PTEN KO) using topical imiquimod. PTEN KO mice had reduced photosensitivity and reduced systemic immune activation. This data suggests that lymphatic dysfunction contributes to photosensitivity in murine lupus and improving lymphatic flow, even with simple MLD, can ameliorate photosensitivity. Future studies will determine the etiology of lymphatic dysfunction in murine lupus and the mechanism of lessened photosensitivity with improved lymphatic drainage. If similar immune circuitry defects are present in patients with SLE, altering lymphatics could be a novel target for new therapeutics.

Th72. Autoreactive B Cells Escape Peripheral Checkpoint in Sjögren's Syndrome

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Although it is widely acknowledged that B cells play a central role in many autoimmune diseases (AID) including primary Sjögren's syndrome (pSS), a full understanding of their characteristics has not been elicited. This study aims at characterizing circulating autoantigen-specific B-cells in patients with pSS compared to healthy subjects.

We developed a new flow-cytometry method to detect circulating auto-reactive B cell based on the specificity of their B-cell receptor (BCR). Phenotype analysis showed that circulating B cells that reacted to SSA (SSA⁺ B cells) in patients were enriched in the memory B cell compartment compared with healthy controls. It suggests that in AID, these auto-reactive cells are able to differentiate into IgG isotype-switched cells and escape peripheral tolerance checkpoint but not in healthy subjects. Interestingly, Natural auto-reactive B cells present in healthy subjects, are able to secrete only IgM isotype autoantibodies upon *in vitro* stimulation but not IgG class switched antibodies. A genomic analysis of the antibody repertoire as well as a transcriptional profiling of these cells by single-cell RNA seq is ongoing to understand further the differences of these autoreactive B cells between healthy subjects and patients with AIDs.

Our results suggest that autoreactive B cells escape peripheral tolerance checkpoint and are able to differentiate into IgG isotype-switched cells in patients with AIDs but not in healthy subjects.

Th76. The Opposing Roles of MDSCs and pDCs in Male and Female Systemic Lupus Erythematosus

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Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by a heterogeneous constellation of symptoms involving the skin, joints, hematologic system, and kidneys. SLE, among other autoimmune diseases, disproportionately affects female patients compared to males. SLE patients are known to have altered IFN α production, which is largely produced by pDCs; cells thought to be partly responsible for SLE pathogenesis. Their function may be enhanced by estrogen. Oppositely, MDSCs are increased in SLE patients, and lupus-like

mouse models show that testosterone may affect MDSC numbers. We aimed to compare these cell subsets among male and female SLE patients. Clinical data and blood samples from patients with SLE as defined by SLICC criteria at the Cleveland Clinic were obtained in conjunction with the Cleveland Clinic Lupus Registry. We obtained levels of sex hormones, pDC and MDSC subsets, and IFN α stimulating genes (ISG) from serum and whole blood. Data was compared to healthy controls. SLE patients had lower levels of pDCs, and while overall MDSC levels were not different, the ratio of M-MDSCs to PMN-MDSCs was decreased compared to healthy controls in females only. Among SLE patients, we identified a negative relationship between %pDCs and M/PMN MDSC ratio. Furthermore, in SLE patients, %MDSCs were positively correlated with serum Estradiol. In conclusion, while pDCs and MDSCs appear altered in Lupus, preliminary data does not fully support sex as impacting the number of cells produced. However, studies are ongoing to observe functional alterations in these cell groups among males and females.

Th91. Effect of Citrullination on the Processing and Presentation of Rheumatoid Arthritis Autoantigens

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CD4⁺ T cells are implicated as drivers of autoimmunity to citrullinated proteins in rheumatoid arthritis due to the high-affinity, class-switched nature of anti-citrullinated protein antibodies (ACPAs) and the contribution of certain HLA-DR alleles to RA susceptibility. However, the effect of citrullination on MHC class II processing and presentation of RA autoantigens to CD4⁺ T cells remains unknown. Here we examine the hypothesis that citrullination impacts this process via destabilization of antigen folding and modification of protease cleavage. The native and citrullinated forms of several well-known RA autoantigens were digested *in vitro* by a cocktail of lysosomal cathepsins for proteolytic mapping or incubated with monocyte-derived dendritic cells (mo-DCs) in a natural antigen processing assay (NAPA). Peptides generated by digestion or presented by mo-DC HLA-DR molecules were identified by mass spectrometry. We found that the peptide repertoire was indeed altered by citrullination. Using proteolytic mapping, we detected changes in the overall cleavage pattern and observed an increase in the abundances of individual peptides following citrullination of fibrinogen and vimentin. Utilizing NAPA, we observed both creation of newly presented peptides and loss of presented peptides upon citrullination of fibrinogen. Strikingly, all peptides whose presentation was destroyed by citrullination contained a citrullination site. Together these results suggest that both protease cleavage and selection of peptides by HLA-DR are impacted by citrullination. Our data thus support the hypothesis that citrullination induces the presentation of a new peptide repertoire, which could then activate autoreactive T cells and drive the loss of immune tolerance to citrullinated autoantigens.

Th118. Expanded PD-1hi HLADR⁺ CD4 T Cells Correlate with Lung Disease Severity in Systemic Sclerosis

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Systemic sclerosis (SSc) is an autoimmune disorder characterized by microvascular disease and progressive tissue fibrosis. Interstitial lung disease (ILD) is the major cause of morbidity and mortality in SSc, yet there are few biomarkers to quantify the extent of pathologic immune activation. Initial flow cytometry of PBMC from SSc patients revealed an expansion of PD-1hi CD4⁺ T cells in SSc patients compared to healthy controls (p=.0017). To extend these observations, we immunophenotyped T cells from peripheral blood of an independent cohort of SSc patients (n=56) and healthy controls (n=18) using a 39-marker mass cytometry panel. Data was analyzed by

manual gating, dimensional reduction and clustering approaches. Our results showed that the frequency of a PD-1hi CXCR5- ICOS- HLA-DR+ CD4+ T cell population was significantly increased in SSc patients when compared to healthy controls (0.23% vs .115% p= .0022). The frequency of this subset was expanded in patients with SSc-ILD compared with SSc without ILD (p= .0002) and in patients with severe ILD when compared with mild ILD (p= .0193). The frequency of PD-1hi CXCR5- ICOS- HLA-DR+ CD4+ Tph cells showed an inverse association with forced vital capacity (FVC % predicted, r= -0.3676, p=.0053) and diffusing capacity for carbon monoxide (DLCO % predicted, r= -0.3391, p= .0106). Together, these results highlight a specific expanded CD4+ T cell population that is strongly associated with presence and severity of lung disease in SSc. Further studies will evaluate whether this marker can reliably predict the rates of lung function decline in SSc-ILD.

Th124. Non-class-switched, Clonally-expanded B Cells are Found Among Memory B Cells in Anti-tRNA Synthetase Syndrome Patients

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Anti-tRNA synthetase syndrome (ARS) is a rare, severe systemic autoimmune disease that can involve muscle, joint, lung, and skin. Diagnostic autoantibodies (e.g. Jo-1, PL7, and PL12) track with clinical manifestations. Without immunosuppression, disease progression leads to irreversible organ damage. To improve patient outcomes, a more complete understanding of the mechanisms that control autoreactive B cell immune tolerance escape and expansion in ARS is needed. We previously showed that Jo-1-binding B cells (JoBCs) are skewed towards a non-class-switched (IgM⁺) CD21^{lo} memory (CD27⁺) subset in Jo-1⁺ ARS patient peripheral blood. To define V gene usage and mutation among B cell receptors (BCRs) expressed by JoBCs, we immortalized primary B cells from Jo-1 ARS patients as hybridomas. BCR sequencing of Jo-1-binding hybridomas revealed highly mutated BCRs, including VH4-34, a V gene known to have germline cross-reactivity with commensal bacteria. Single-cell BCRseq/CITEseq technology identified expanded B cell clones that had a IgM⁺ memory phenotype in both Jo-1 ARS and non-Jo-1 ARS patients. In the Jo-1 ARS donor, clonally expanded B cells expressed VH4-34 and had fewer V gene mutations than clones expanded in a non-Jo-1 ARS donor, which expressed VH3-48. Clonally-expanded B cells were observed among plasmablasts in both donors which were highly mutated, but plasmablast expansion was more robust and primarily IgA-switched in the non-Jo-1 ARS patient, whereas expanded plasmablast clones were IgD⁺ in the Jo-1-ARS patient. These data suggest that memory entry occurs prior to class-switching, an event associated with plasmablast differentiation.

Th128. SQZ™ TAC Cell Therapy Platform Induces Antigen-Specific Tregs and Prevents Onset of Diabetes in Adoptive Transfer Models

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Most autoimmune disease therapeutics are focused on broad immunosuppression, which can result in adverse effects and increase the risk of infection and cancer. Therefore, antigen-specific therapies that suppress autoreactive cells without inducing systemic immunosuppression are needed. The Cell Squeeze® technology utilizes microfluidic cell squeezing to engineer cell function. To induce tolerance, red blood cells (RBCs) are engineered to encapsulate target antigens to generate tolerizing antigen carriers (TACs). Cell squeezing makes the SQZ™ TACs resemble senescent RBCs enabling them to be cleared by the physiological mechanism of RBC clearance known as eryptosis. Here we show that SQZ™ TACs are rapidly cleared from circulation by phagocytic cells in the spleen and liver where the antigen is processed and presented to antigen-specific T cells

in a tolerogenic fashion. SQZ™ TAC treatment in several accelerated models of T1D led to significant delay in onset of T1D disease incidence. Notably, SQZ™ TAC treatment significantly reduced the number and function of autoreactive CD4 and CD8 T cells, particularly in the pancreas. Mechanistically, SQZ™ TAC treatment led to reduced proinflammatory cytokine secretion by inducing deletion of antigen-specific T cells, impairing trafficking of pathogenic CD4 and CD8 T cells to the pancreas and increasing antigen-specific Tregs. Importantly, these Tregs were able to mediate bystander suppression. Altogether our results suggest that SQZ™ TACs are a versatile platform for induction of multimodal mechanisms of antigen-specific tolerance and enable treatment of complex autoimmune diseases.

Th131. High-Dimensional Analysis Reveals Abnormal B Cell Subsets Associated with T and Myeloid Cell Subsets in the Idiopathic Inflammatory Myopathies

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The idiopathic inflammatory myopathies (IIM) are a clinically heterogeneous group of conditions affecting the skin, muscle, joint, and lung in various combinations. The purpose of this study was to investigate the immunologic heterogeneity through detailed immunophenotyping of IIM patients and healthy controls. Peripheral blood mononuclear cells from seventeen patients with a clinical diagnosis of inflammatory myositis in the inpatient or outpatient setting and 18 healthy controls were collected between September 2017-September 2018. Immunophenotyping using mass cytometry by time of flight (CyTOF) was performed to simultaneously characterize B, T, and innate cell subsets. Data was analyzed using a combination of supervised biaxial gating and unsupervised algorithms including t-distributed stochastic neighbor embedding (tSNE), cluster identification, characterization, and regression (CITRUS), and marker enrichment modeling (MEM). Here, we identified two distinct immunophenotypes amongst IIM patients. In one phenotype, increased CD19⁺CXCR4hiCCR7hi cells correlated with increased CD3⁺CXCR4hiCD38hi ($r=0.62$, $p=0.009$) and CD14⁺CD16⁺CXCR4⁺CD38⁺HLADR⁺ ($r=0.61$, $p=0.01$) populations. In the second phenotype, increased CD19⁺CD21loCD11c⁺ correlated with an increased CD3⁺CD4⁺PD1⁺ ($r=0.60$, $p=0.01$) population. There are also shared immunologic features amongst IIM patients compared to healthy controls including decreased surface expression of RP105/CD180 on B cells (median mass intensity 39.9 ± 16.0 v. 60.9 ± 20.1 , $p=0.002$) and reduction of all circulating CD3⁺CXCR3⁺ subsets (2.7 ± 2.4 v. $9.6 \pm 8.1\%$ of all PBMCs, $p=0.0004$). This study demonstrates that IIM immunophenotypes are heterogeneous, but shared immunologic features are also present and could represent therapeutic targets in IIM. Future work is needed to correlate variable immunophenotypes to clinical manifestations and treatment responses.

Th132. RIPK3 (Receptor-Interacting Protein Kinase 3)-deficiency in Antigen-presenting Cells Dampens the Pro-inflammatory T Cell Response Following Antigen Presentation

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STUDY OBJECTIVE

We have previously shown that RIPK3 (receptor-interacting protein kinase 3)-deficient mice are protected from the development of an induced murine model of systemic lupus erythematosus (SLE). The development of SLE autoantibodies in this model is associated with the generation of an antigen-specific T cell response. We hypothesize that RIPK3-deficient antigen-presenting cells (APCs) are impaired in generation of a robust T cell response, thereby impacting induction of SLE in our murine model.

METHODS

We performed a complete cellular immunophenotyping on mice deficient in RIPK3, compared with wild type (WT) C57BL/6 mice. We also assessed the function of RIPK3-deficient APCs in an *in vitro* antigen presentation assay using ovalbumin (OVA) and OVA-specific OT-II T cells.

RESULTS

No major differences in immune cell composition and phenotype were identified between naïve RIPK3-deficient and WT mice. However, RIPK3-deficient bone marrow-derived dendritic cells (BMDCs) were found to produce lower levels of pro-inflammatory cytokines (interleukin [IL]-6 and tumor necrosis factor [TNF]- α) following lipopolysaccharide (LPS) stimulation. Moreover, presentation of OVA by RIPK3-deficient BMDCs resulted in a lower proportion of interferon (IFN)- γ producing OT-II T cells, compared to antigen presentation by WT BMDCs. In contrast, T cell proliferation was similar whether antigen was presented by RIPK3-deficient or WT BMDCs.

CONCLUSION

Our results suggest that RIPK3 deficiency in APCs impacts antigen presentation to T cells, identifying a possible mechanism by which RIPK3-deficient mice are protected from induction of SLE.

Th150. A Microparticle Strategy to Induce Tolerogenic Dendritic Cells for Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is a painful autoimmune disease characterized by joint inflammation leading to bone and cartilage destruction. There is currently no cure, and treatment options require lifelong management of immunosuppressive drugs. However, these drugs are accompanied by many adverse effects such as increased risk of infection, increased risk of cancer, as well as acute and chronic toxicities. The COVID19 pandemic has underscored a critical need to uncover alternative therapies that do not immunocompromise the patient. One promising approach is the administration of tolerogenic dendritic cell which is being explored in clinical trials to treat various autoimmune diseases. Building on this, we have rationally designed a novel poly lactic-co-glycolic acid-based microparticle system, termed REGvac, which induces *in vivo* generation of tDCs to promote antigen-specific tolerance towards RA. This is achieved through subcutaneous delivery of (i) a phagocytosable microparticle encapsulating RA-associated antigens (type II collagen and citrullinated fibrinogen) alongside a tolerizing factor (vitamin D3) and (ii) a non-phagocytosable microparticle to foster DC migration to a tolerogenic environment through sustained release of granulocyte-macrophage colony-stimulating factor (GM-CSF) and transforming growth factor- β 1 (TGF- β 1). Using a collagen- and fibrinogen-induced model of severe RA, we found that treatment with REGvac was able to induce RA remission and halt disease progression. Additionally, in REGvac-treated mice we observed a 2-fold increase in percentage of CD25⁺FOXP3⁺col-II tetramer⁺ Tregs of all CD4 cells within the popliteal lymph nodes, compared to treatment with methotrexate. These results demonstrate that REGvac induces antigen-specific Tregs to promote therapeutic effects and disease remission.

Th173. Investigating the Role of SH2B3 Allotypes in Type 1 Diabetes Pathogenesis

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Genome wide association studies have identified a single nucleotide polymorphism (SNP) *rs3184504* (R262W, C > T) within exon 3 of *SH2B3*, a gene associated with increased risk for Type 1 Diabetes (T1D). The *SH2B3* gene encodes LNK (lymphocyte adaptor protein), an adaptor protein expressed highly in hematopoietic and endothelial cells. Importantly, LNK serves as a potent negative regulator of inflammatory signaling in leukocytes and endothelial cells. Past studies on donor PBMCs suggest that the risk allotype, *SH2B3*^{262W}, contributes to T1D pathogenesis by failing to regulate inflammatory cytokine signaling. We hypothesize that *SH2B3*^{262W} will result in increased inflammatory events contributing to incidence and severity of T1D, with expression of the risk variant in interacting cells resulting in a positive additive effect on T1D development. Currently, using CRISPR/Cas9 technology involving high efficiency sgRNAs and homology directed repair templates for the risk (T) or common (C) alleles, we are developing induced pluripotent stem cells (iPSC) edited at *rs3184504*. iPSC from the same donors have also been differentiated into endothelial cells (iECs) and other innate immune cells to be used in studying the interaction of these cells in an isogenic system. Preliminary results validating the iECs indicate their functional similarity to HUVECs with the upregulation of adhesion molecules with TNF α treatment. Additionally, scRNAseq analysis from donor spleens in our repository indicates robust *SH2B3* expression in macrophages, neutrophils, B cell subsets, and dendritic cells respectively. Results from this study will contribute to our knowledge and understanding of how *SH2B3*^{262W} influences mechanistic disease progression and clinical outcomes.

Th176. T-cell Receptor Repertoire of Mice with Organ-specific Autoimmunity Resulting from a Partial Defect in T Cell Negative Selection and Dendritic Cell Enhancement

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Previously, we reported a new genetic model of spontaneous autoimmune disease in which mice with a hypomorphic mutation of *Aire* (*Aire*^{GW/+}) combined with deletion of *Lyn* develop autoimmune uveitis with 50% penetrance. Here we demonstrate that a key autoantigen in this model is interphotoreceptor retinoid-binding protein (IRBP) as *Aire*^{GW/+} *Lyn*^{-/-} *IRBP*^{-/-} mice failed to develop uveitis. The expansion of CD4⁺ T cells recognizing amino acids 271-290 ("P2") of IRBP correlated strongly with autoimmune disease in mice with intact IRBP. To study the role of these T cells, we did single-cell TCR sequencing for P2-specific CD4⁺ T cells isolated from eye-draining lymph nodes (LN) of *Aire*^{GW/+} *Lyn*^{-/-} mice with and without uveitis. Both mice showed expansion of these T cells in eye-draining LNs, but it was greater in mice with disease. Expansions of particular T cell clonotypes were more evident in LN of mice with disease, and even greater in the retinas of mice with disease. We reconstituted several P2-specific TCR clonotypes in a T cell hybridoma line and verified their specificity. One of the TCR clonotypes was expressed in developing thymocytes by retroviral transduction of bone marrow stem cells from *Rag2*^{-/-} mice and transplanted into WT or *Aire*^{GW/+} recipient mice. Remarkably, the small residual expression of IRBP in the thymus of *Aire*^{GW/+} mice was sufficient to induce substantial negative selection of thymocytes with this TCR clonotype. Thus, deficiency of negative selection of P2 tetramer-specific TCR promotes the development of uveitis in *Aire*^{GW/+} *Lyn*^{-/-} mice, even if negative selection is only partly compromised.

Th177. Relapsing Remitting Multiple Sclerosis Patients have an Altered Gut Fungal Microbiome

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Trillions of microbes such as bacteria, fungi, and phages are part of the normal human microbiome and have emerged as an important factor in the pathobiology of diseases, including multiple sclerosis (MS). Although gut bacterial dysbiosis has been extensively studied in MS, the significance of the fungal microbiome (mycobiome) is an understudied and neglected part of the intestinal microbiome in MS. The aim of this study was to characterize the gut mycobiome of patients with Relapsing-Remitting Multiple Sclerosis (RRMS) and compare it to healthy controls (HC) along with bacterial – fungal correlation and functional analysis of the fungi in the gut. We characterized and compared the mycobiome of 20 RRMS patients and 33 HC using Internal Transcribed Spacer 2 (ITS2) of fungi and compared mycobiome interactions with the bacterial microbiome using 16S sequencing. Our results indicated that there is mycobiome dysbiosis in RRMS patients with increased *Basidiomycota* and decreased *Ascomycota* at the phylum level with increased abundance of *Candida* and *Epicoccum* along with the decreased abundance of *Saccharomyces* at the genus level in RRMS compared with HC. We also observed bacterial dysbiosis together with increased ITS2/16S ratio, altered fungal and bacterial relationship, and altered fungal functional pathways in RRMS patients compared to HC. Taken together, our study demonstrates a shift in the fungal microbiome with associated changes in the bacterial microbiome in MS patients. Future studies utilizing larger datasets need to be performed to validate the findings from the study.

Th186. Disruption of the Signal Regulatory Protein Gamma (SIRPy) and CD47 Signaling Pathway in CD8⁺ T Cells Results in an Augmented Effector Phenotype

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Genetic polymorphisms associated with increased risk for T1D have been identified in the Signal Regulatory Protein Gamma (*SIRPG*). However, it remains unclear how SIRPy expression on CD8⁺ T cells and its binding partner CD47 control autoimmunity, and how risk variants alter immunity in T1D etiopathogenesis. We hypothesize that loss of SIRPy:CD47 signaling in CD8⁺ T cells would result in increased cell activation and cytotoxicity.

To test our hypothesis, we created *SIRPG* and *CD47* knockouts (KOs) in both Jurkat T cell lines and primary human CD8⁺ T cells using CRISPR-Cas9 gene-editing. KOs exhibited significantly increased expression of the activation markers, CD25 (*SIRPG*: 2.3-fold, $p \leq 0.01$; *CD47*: 1.4-fold, $p \leq 0.05$) and CD69 (*SIRPG*: 1.7-fold, $p \leq 0.01$; *CD47*: 2.2-fold, $p \leq 0.001$) and lower expression of the negative regulators CTLA-4 (*SIRPG*: 0.6-fold, $p \leq 0.001$; *CD47*: 0.5-fold, $p \leq 0.001$) and PD-1 (*SIRPG*: 0.6-fold, $p \leq 0.001$; *CD47*: 0.5-fold, $p \leq 0.001$) assessed by flow cytometry compared to non-edited controls. We identified alterations of memory T cell differentiation *in vitro* as KOs differentiated significantly more terminally differentiated effector memory cells (T_{EMRA}) (*SIRPG*: 3.5-fold, $p \leq 0.001$; *CD47*: 3.2-fold, $p \leq 0.01$). Lastly, we observed significantly (P -values: $p \leq 0.05$ – $p \leq 0.001$) higher expression of effector cytokines, including an approximately 2-fold increase of IL-2, TNF α , IFN γ , perforin, and granzyme B in KO cells.

These results demonstrate that loss of SIRPy:CD47 signaling may alter CD8⁺ T cell activation, effector differentiation and cytotoxic function. Studies are underway to assess the impact of risk variants at the *SIRPG*

locus on T cell phenotype and the potential targeting of the SIRPy:CD47 axis in modulating islet β -cell autoimmunity.

COVID-19

W65. Intravenous Immunoglobulin for Treatment of Severe Coronavirus Disease 19 (COVID-19) – A Literature Overview

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Rationale: COVID-19 was declared a global pandemic in March 2020. Previous experiences with SARS-Cov-2 showed that the main pathogenesis and pulmonary dysfunction lay in the overall cytokine dysregulation. Intravenous immunoglobulin (IVIg) has been recognized for its anti-inflammatory and immunomodulatory effects.

Methods: Based on evidence, clinicians have hypothesized that IVIg therapy may improve the prognosis of critically-ill COVID-19 patients. A literature search was performed using the search terms: Corona, COVID-19, IVIg, Immunoglobulin, Multisystem Inflammatory Syndrome in Children, MIS-C, and Kawasaki-like syndrome.

Results: 33 published reports were identified describing the beneficial effects of IVIg in treating COVID-19 and associated inflammatory syndromes (i.e., multisystem inflammatory syndrome in children [MIS-C]). A retrospective, multicenter cohort study that included 325 adult critical patients demonstrated early high-dose IVIg administration improves the prognosis of critical COVID-19 patients. An observational study conducted in 10 COVID-19 patients demonstrated short-term moderate-dose corticosteroid combined with high-dose IVIg effectively reversed severe, deteriorating COVID-19 patients who failed initial low-dose therapy. A randomized double-blind placebo-controlled trial among 59 patients with severe COVID-19 demonstrated that the administration of IVIg in patients who did not respond to initial treatment could improve the clinical outcome and significantly reduce mortality rate. Additionally, a prospective randomized study demonstrated that the use of IVIg reduced the rate of progression of respiratory failure required mechanical ventilation in COVID-19 patients.

Conclusion: It may be useful to consider high-dose IVIg at the time of initiation of respiratory distress to potentially promote satisfactory clinical recovery and reduce burden of care for COVID-19 patients.

W138. A Majority of Uninfected Adults Show Pre-existing Antibody Reactivity Against SARS-CoV-2

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Pre-existing cross-reactive cellular and humoral immunity to SARS-CoV-2 has been found in individuals in absence of prior viral exposure. However, while pre-existing CD4 T cell reactivity was better documented, the extent of pre-existing viral *antibody* reactivity has been difficult to quantify at the population level due to a lack of positive/negative thresholds.

Using an orthogonal antibody testing strategy combining a highly sensitive quantitative multiplex assay and a specific FDA-approved CLIA assay, we estimated that ~0.6% of 276 non-triaged adult participants from the

greater Vancouver area, Canada had a prior SARS-CoV-2 infection between May 17th and June 19th 2020. Based on pre-existing sero-reactivity thresholds defined in infants' sera in whom maternal antibodies have waned, more than 90% of the 273 non-SARS-CoV-2-exposed adults showed detectable antibody reactivity to its spike, receptor-binding domain (RBD) and N-terminal domain (NTD), or its nucleocapsid (N) proteins. This antibody reactivity was also observed with pre-pandemic sera, was evenly distributed across age and sex, partially correlated with reactivity to circulating coronaviruses and was partially outcompeted by soluble spike proteins from circulating coronaviruses. Peptide mapping on the viral proteome using a SPOT array assay revealed that this pre-existing antibody reactivity broadly localized to spike, its RBD and to conserved non-structural viral proteins, and the immunoreactivity profiles in individual samples varied markedly.

Most adults display pre-existing antibody cross-reactivity to SARS-CoV-2 antigens without symptomatic and PCR genetic test evidence of prior exposure to this virus. Further studies are required to understand how this reactivity impacts COVID-19 clinical severity and SARS-CoV-2 vaccine responses.

W146. Immunopathology in MIS-C: Elevated Alarmin and Cytotoxicity Signatures and Autoreactivity that Correlates with Severity

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Multisystem inflammatory syndrome in children (MIS-C) has emerged as a life-threatening post-infectious complication occurring unpredictably four to six weeks after mild or asymptomatic SARS-CoV-2 infection in predominantly healthy children across the world. We define immune abnormalities in MIS-C compared to adult COVID-19 and pediatric/adult healthy controls using single-cell RNA sequencing, flow cytometry, antigen receptor repertoire analysis, unbiased serum proteomics, and *in vitro* assays. Although we find no evidence of active infection, we discover elevated S100A-family alarmins in myeloid cells and enrichment of serum proteins that map to myeloid cells and pathways including cytokines, complement/coagulation, and fluid shear stress in MIS-C patients. Moreover, NK and CD8 T cell cytotoxicity genes are elevated, and plasmablasts harboring IgG1 and IgG3 are expanded. Furthermore, we detect elevated binding of serum IgG from severe MIS-C patients to activated human cardiac microvascular endothelial cells in culture. Thus, we define immunopathology features of MIS-C with implications for predicting and managing this syndrome in children and better understanding age-related control of the immune response.

W147. SARS-CoV-2 T Cell Specific Cellular Immunity in Pediatric Patients with Chilblain Lesions During the Pandemic

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Background

Clinical manifestations associated with SARS-CoV-2 in humans are mainly respiratory and gastrointestinal. However, several publications have described diverse skin manifestations appeared during the pandemic, although the causal association with SARS-CoV-2 has not yet been clearly demonstrated.

Aim

To evaluate antigen-specific lymphocyte proliferation against SARS-CoV-2 in pediatric patients with skin manifestations with negative PCR and negative serology against the virus.

Methodology

We prospectively collected clinical and immunologic data of 30 pediatric patients with acral cutaneous lesions during the months of March and April 2020. The SARS-CoV-2 PCR was negative in all cases and serological studies were positive in only one patient. We performed a dye-based proliferation assay to evaluate the Ag-specific T-cell response against SARS-CoV-2. We analyzed 16 patients with skin manifestations and used 7 patients with systemic COVID-19 as positive control and 7 non COVID-19 patients as negative controls. Leucocytes subpopulations were also analyzed by flow cytometry.

Results

13 out of 16 patients (81.3%) showed specific lymphocyte proliferation against SARS-CoV-2 peptides. In the systemic COVID-19 control group, 6 out of 7 patients (85.71%) resulted positive in the proliferation assay whereas in the negative COVID-19 group 2 out of 7 were positive (28.57%). Moreover, cutaneous lesions patients presented a characteristic leucocyte profile with low percentages of Tfh and Th2 and high levels of pDC and CD38⁺Th cells.

Conclusion

The evaluation of specific cellular immune response to SARS-CoV-2 is useful for the diagnostic of patients presenting non-classical COVID-19 symptoms with negative PCR and serology against SARS-CoV-2.

W153. Serum Profiling in Children Identifies Seropositive IgG Responses to Endemic Coronaviruses that Cross-protect Against SARS-CoV-2

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Purpose. The serum antibodies in individuals forms in response to infections, the microbiome, vaccinations and environmental exposures. The specificity of IgM and IgG antibody responses was compared among a cohort of children to identify associations between seropositive responses to SARS-CoV-2 relative to other infections and vaccines.

Methods. The serum IgM and IgG antibody reactivities in children seen prior to and during the COVID-19 pandemic was compared with microfluidic arrays containing 120 distinct antigens. Seropositivity to endemic RNA and DNA viruses, including SARS-CoV-2, was assessed. Neutralization assays were performed on individuals who had seropositive IgG responses to SARS-CoV-2. Comparisons were made among COVID-19 negative and positive individuals in relation to antibody responses to viruses, vaccines and autoantigens.

Results and Conclusion. Nearly 15% of children, seen prior to the COVID-19 pandemic, had seropositive IgG antibody responses that cross-reacted with the spike proteins from SARS-CoV-2 and several endemic coronavirus species including SARS-CoV, HCoV-NL63 and HCoV-HK1. These serum samples neutralized SARS-CoV-2, determined using a spike protein expressing pseudotyped lentivirus in a neutralization assay. Individuals with seropositive responses to the spike protein had elevated nfi levels to pneumococcal and MMR vaccines. Finally, a cohort of COVID-19 positive children exhibited higher IgG levels to various autoantigens. Our findings suggest some children are less susceptible to COVID-19 than adults due to their endemic coronavirus responses developed early in life.

Th17. Anti-SARS-CoV-2 Antibodies in Kidney Transplant Recipients

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Solid organ transplant recipients are at increased risk of severe outcomes with infection by SARS-CoV-2, the etiologic agent of COVID-19. Antibodies directed against the virus may be protective. A thorough characterization of anti-SARS-CoV2 immune globulin isotypes in organ transplant recipients has not been reported.

Using a semi-quantitative Luminex-based multiplex assay, we determined antibody levels from 48 SARS-CoV-2 PCR+ kidney transplant recipients. We measured total IgG, IgM, IgA and IgG subtypes of antibodies directed against 5 distinct viral epitopes including the viral nucleocapsid protein as well as multiple regions of the spike protein including the receptor binding domain.

We identified multiple patterns of antibody responses. Specifically, 5 subjects were seronegative and 29 subjects had IgM, IgG and IgA antibodies specific for multiple epitopes of SARS-CoV-2. The 14 remaining subjects displayed a mixture of immunoglobulin isotypes. Longitudinal samples from one subject demonstrated dynamic changes from IgM+IgG⁺ → IgM-IgG⁺. Utilizing the semi-quantitative aspect of the assay, we found that IgG antibodies to the full Spike and Spike S1 domains were present at a statistically reduced level compared to immunocompetent controls while those directed against the Spike S2 domain were statistically higher. Interestingly, 77% of these subjects had detectable IgA directed against combinations of spike and/or nucleocapsid specificity. IgG subtype analysis and correlation between antibody expression patterns with clinical severity is under investigation.

Overall, these studies indicate that solid organ transplant recipients have the capacity to mount a dynamic antibody response to SARS-CoV-2 infection and that this response differs from immunocompetent individuals.

Th49. High Throughput Single Cell Antibody Discovery Method to Identify Anti-SARS-CoV-2 Antibodies

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Multi-analyte single cell technologies have potential to greatly accelerate antibody discovery. Here we demonstrate anti-SARS-CoV-2 antibody discovery using the 10x Single Cell Immune Profiling Solution v2 with custom reagents and novel computational approaches. We labeled antigens of interest with unique reporter oligonucleotides captured via single cell sequencing alongside BCR sequences and transcriptomic profiles from each cell. Using our Barcode-Enabled Antigen Mapping (BEAM) method, we identified B cells from 100 million PBMCs of a convalescent COVID-19 donor that bound specifically to both wild-type and D614G SARS-CoV-2 spike proteins. Natively paired, heavy and light chain full-length sequencing data was obtained from each cell and spike-binding BCRs were assigned into clonal families. BEAM scores were generated for each antibody based on the detected antigen barcodes and properties of each antibody.

This method identified 240 highly-specific antibodies from a diverse set of nearly 200 clonal families using BEAM scores to down-select candidate antibodies. The whole process generated the candidate sequences within one week after sample processing. We further validate our approach by evaluating the antigen affinity of a subset of antibodies using surface plasmon resonance as well as their ability to disrupt an ACE2-spike protein interaction in a cellular assay. This method provides new insights into B cell biology at unprecedented resolution and a foundation to further scale and bring new efficiencies to the antibody discovery process.

Th56. Is There a Relationship Between ABO and Rh Blood Group with COVID-19 Risk of Infection and Complication in a Southern New Jersey Population?

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Background: During the COVID-19 pandemic, studies reported on the association of ABO blood groups with infection susceptibility and severity. There are studies that have linked blood group A to an increase in infection rate and severity. Our study aims to evaluate this association for ABO and Rh blood groups within a diverse Southern New Jersey population's healthcare system.

Methods: Retrospective review was performed on Cooper University Hospital's (CUH) COVID-19 positive patients treated from January to May 2020. Mann Whitney U, Chi Square, and Independent t tests compared blood group distribution between CUH's 451 COVID-19 positive population and CUH's 117,496 general population. We also determined the relationship between blood groups with mortality and ICU admission.

Results: CUH patient blood group distribution was: 32.4% A, 3.1% AB, 17.3% B, 47.2% O; 90% Rh+, 10% Rh-. No group demonstrated statistically significant increased infection risk, ICU admission, or mortality. However, male gender ($p < 0.001$), history of malignancy ($p = 0.003$), history of transfusion ($p = 0.010$), and elevated lactate dehydrogenase (LDH) ($p = 0.003$) and creatinine ($p = 0.001$) at time of COVID-19 diagnosis showed a significant link with mortality.

Conclusion: Our study did not indicate an association between ABO or Rh blood groups with COVID-19 susceptibility, morbidity, and mortality. However, we noted several other risk factors for mortality including male gender, history of malignancy and transfusion, and elevated LDH and creatinine, warranting further investigation.

Th65. CD4 T Cell-SARS-CoV-2-peptide-challenge-response in Individuals with Mild/Moderate COVID-19 Symptoms Alters with Age

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The intricacies of the adaptive-immune response to SARS-CoV-2 is of vital importance towards combatting the current pandemic in which we find ourselves. Those at most-risk are known to be the elderly and those with co-morbidities. The research performed within this collaborative project analyzes the link between age and the adaptive-immune response to SARS-CoV-2 peptides.

This research utilizes a cohort of over 900 individual's PBMCs collected in March-April 2020, taken from the citizens of a COVID-19 outbreak-spot in Heinsberg, Germany. All participants were non-hospitalized and were either non-infected, or recovered and were asymptomatic, or with mild/moderate COVID-19 symptoms from all ages.

One of the main findings of this project outline a direct link between age and the CD4 T cell response to SARS-CoV-2 peptide pools from the S1-region of the spike-protein of SARS-CoV-2, measured via intracellular-cytokine staining. Lesser links were discovered from peptide-pools generated from nucleocapsid or the S2-region of the spike protein. S1-CD4 T cell challenge responses yielded significantly stronger CD4 Th1 responses which were also more poly-functional.

The successful recovery of at-risk individuals and their adaptive-immune-response to SARS-CoV-2 can yield valuable information about the immune-fingerprint of these individuals and the areas of the SARS-CoV-2 protein which generate the most robust T cell responses. This furthermore can help to inform future vaccine-strategies which could be tailored to account for an aged immune system.

Understanding the pathophysiology of the inflammatory-response to SARS-CoV-2 is crucial if we are to combat COVID-19, which is likely to become endemic.

Th78. Targeting TMEM176B Controls Inflammasome-dependent T Cell Dysfunction in Coronavirus Infection

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The NLRP3 inflammasome/IL-1 β pathway has been shown to play a key immunopathogenic role in COVID-19. However, the mechanisms by which inflammasomes are controlled and lead to severe disease and death in coronavirus disease are largely unknown. We speculated that the NLRP3 inflammasome inhibitor, TMEM176B, may control coronavirus-induced pathogenesis.

Using a mouse coronavirus infection model (MHV-A59), we show that *Tmem176b* is a key host player that controls viral infection by inhibiting inflammasome activation. *In vivo*, *Tmem176b*^{-/-} mice showed worse survival

and increased viral load upon MHV infection as compared to WT and *Tmem176b*^{-/-}-Casp1^{-/-} animals. IL-1 β blockade significantly protected *Tmem176b*^{-/-} mice in a CD8-dependent manner. Accordingly, MHV-A59-infected *Tmem176b*^{-/-} animals had fewer total and MHV-A59-specific CD8⁺ T cells and decreased *in vivo* CD8-dependent cytotoxicity against MHV-A59 antigens. PD-1 blockade significantly improved the survival of MHV-A59-infected *Tmem176b*^{-/-} mice. We then found that the flavonoid isoquercetin enhanced *Tmem176b* activity in a dose-dependent manner and inhibited MHV-A59-induced inflammasome activation in mouse dendritic cells. *In vivo*, isoquercetin significantly diminished MHV-A59 viral load. TMEM176B expression was increased in peripheral blood monocytes from mild COVID-19 in comparison to severe patients. Isoquercetin inhibited severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-induced inflammasome activation in human monocytes *in vitro*. Moreover, isoquercetin hinders coronavirus-induced PD-1 expression *in vitro* in human CD4⁺ and CD8⁺ T cells. Thus, targeting TMEM176B with isoquercetin could control inflammasome activation and immune dysfunction triggered by coronavirus infection. Ongoing experiments are trying to determine whether isoquercetin can control SARS-CoV2 infection *in vivo*.

Th93. A Single Center Evaluation of Allergic Reactions in Healthcare Workers After the Pfizer-BioNTech COVID-19 Vaccine

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Objectives

To evaluate allergic reactions to the first dose of the Pfizer-BioNTech COVID-19 vaccine in healthcare workers (HCW) referred to Allergy and Immunology clinic at University of Miami/Jackson Memorial Hospital.

Methodology

Testing was performed per modified Banerji et al 2020 protocol. Each compound was tested at 2 sites simultaneously via skin prick and if negative, intradermally.

Results

Five patients were evaluated, four with a history of non-anaphylactic reactions. Reactions included palpitations, dyspnea, facial numbness, flushing, urticaria, and angioedema within 1 hour of the vaccine. Patient 1 (P1) and P2 had a negative test. P3 had positive skin prick to PEG330; and P4 to PEG400, methylprednisolone acetate (containing PEG3350), and methylprednisolone sodium succinate (control). P5 had a history of non-anaphylactic reactions to vaccines and medications but history of anaphylactic reaction to Pfizer vaccine, with positive Pevnar 13 intradermal testing. Testing complications included throat tightness in P4 after skin prick. Patients were provided counseling for future medication exposure.

Discussion

We describe results of skin prick and intradermal testing with vaccine excipients amongst HCWs receiving the Pfizer COVID-19 vaccination with three confirmed allergic reactions to PEG400, PEG3350, and polysorbate 80. While the Pfizer vaccine contains PEG2000, polysorbate 80 was tested given cross reactivity with PEG as it is added to other COVID-19 vaccines. As of January 21st our institution has administered 27,248 Pfizer vaccines with incidence of confirmed allergy of 0.01%.

Conclusion

Our findings add to the literature on identifying COVID-19 vaccine components that may lead to immediate allergic reactions.

Th99. Immunosuppression in Doctors Working with COVID-19 Patients

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COVID-19 infection is characterized by high contagiousness. Doctors working with COVID-19 patients receive a significant viral load. We compared the cytokine status of doctors working with COVID-19 patients (n=33) and healthy donors (n=31).

Analysis of 41 humoral factors in blood serum of doctors and donors using Milliplex cytometric kit showed the presence of three groups of proteins that significantly differ in concentration: factors with a concentration of 200-15,000 pg/mL (n=10); 10-100 pg/mL (n=6) and 0-10 pg/mL (n=15).

The first group included chemokines or growth factors that control blood homeostasis: Eotaxin, GRO, MDC, PDGF-AA, sCD40L, IL-4, IP10, MCP-1, RANTES, and VEGF. Significantly reduced level of GRO, sCD40L, IL-4, IP-10, MCP-1 and increased level of MDC and PDGF-AA was found in the blood of doctors. The second group included innate immunity defense against infection: G-CSF, IFN α 2, IL-1RA, IL-1a, TNF- α , MCP-3, EGF, and FGF-2. Of these, the growth factors G-CSF, EGF, and FGF-2 decreased significantly, apparently also due to the expense of vascular repair. Finally, the factors of the third group are mainly acute phase proteins-cytokines: GM-CSF, IFN- γ , IL-10, IL-12P40, IL-12P70, IL-13, IL-15, IL-2, IL-6, IL-7, IL-8, FLT-3L, TNF-b, MIP-1a, and MIP-1b. The level of acute phase factors in the blood serum of the doctors working with COVID-19 patients was normal.

Thus, it is shown that the immune system of the doctors is in the activated state at the level of the restoration of the vascular bed damaged by the virus while adaptive immunity it not activated.

Th100. Evaluation of Complement Pathway Activation Indicators in COVID19 patients

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Background: The immune-driven pathologies have been observed in severe cases of SARS-CoV infections with proven contribution of complement activation in its pathogenesis. It may be protective by favouring viral clearance, but over activation results in acute and chronic inflammation, tissue injury and the activation of coagulation. Thus, complement activation was evaluated to explore complement-mediated inflammation and coagulopathy.

Methods: Plasma samples of consented participants (n=40 cases; n=40 controls) were evaluated for C4b, C5b-9, IL-6, D-dimer and C1-Inhibitor by ELISA Kits. Statistical analyses were carried out using Stata, version 12 (Stata Corp., Texas, USA).

Results: Significantly increased levels of C4b, D- dimer, IL6, along with decreased levels of C1 INH were found in cases (mean age= 54.25) than in controls (mean age= 27.25). However, C5b-9 levels were not significantly raised in cases. The significantly higher levels of C4b in cases with a cut-off value of ≥ 121.5 with optimum sensitivity and specificity 77.5% and 85% respectively, suggest that C4b may be considered as an adjunct diagnostic marker in diagnosis of SARS-CoV2 infection. False negative results, by any diagnostic test can be more consequential, as it sets the infected persons free to infect others. In such cases, C4b evaluation may be of great help in detecting infection in cases which have been falsely labelled as negative and can be instrumental in limiting the spread of infection.

Conclusion: Our study proposes C4b as an important candidate diagnostic marker to eliminate false negative cases.

Th103. Immunomodulatory Capacity of Human Placental Mesenchymal Stem Cells on Covid-19 Infected Patients

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INTRODUCTION

Mesenchymal stem cells (MSCs) can immunomodulate and suppress T cell proliferation, activation and cytokine production.

OBJECTIVE

To assess the in-vitro immunomodulatory function of human placental MSCs on lymphocyte populations of COVID-19 patients with or without respiratory failure.

METHODOLOGY

- 1.- After discharge, peripheral blood lymphocytes from 20 admitted COVID-19 patients (5 moderate, 8 severe and 7critical) were stimulated with SARS-Cov2 S, N and M peptide pools.
- 2.- MSCs were isolated from a cesarean healthy donor woman.
- 3- Lymphocytes from patients were re-stimulated with SARS-Cov2 S, N and M peptide pools and co-cultured with previously characterized donor MSC.
- 4- Markers of activation of CD4 and CD8 patient's lymphocytes and supernatant cytokines and chemokines were measured

RESULTS

- SARS-Cov2 Th and CD8 cell responses were observed in 100% patients.
- Isolated MSCs expressed characteristic cell surface markers. Also, they were capable of self-renewal and differentiate into chondrocytes, osteoblasts and adipocytes.
- After co-culture, basal and after re-stimulation with S, N and M peptides production of IL2, IFN-gamma, TNF-alfa by memory-Covid T cells levels significantly decreased ($p < 0.01$ for every peptide pool). Moreover a significant decrease in IFN-induced chemokines was observed ($p < 0.01$, for all of them).

CONCLUSIONS

- *In vitro*, an important immunoregulatory and anti-inflammatory effect of MSC in SARS-CoV-2 T lymphocytes was observed. More studies are needed to confirm such effects before initiate *in vivo* clinical trials to evaluate security and efficacy of this therapy in severe Covid 19 patients.

Th119. Dynamic Host Response Determines Susceptibility to SARS-CoV-2 Infection

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The factors that determine susceptibility to SARS-CoV-2 upon exposure are not fully understood. We studied innate immune defenses against SARS-CoV-2 in the context of innate immunity induced by the virus itself or another virus. Using transcriptomics and biomarker-based tracking in serial patient samples, we first defined the airway innate response to SARS-CoV-2. In patients, SARS-CoV-2 initially replicated exponentially with a doubling time of ~6hr and triggered a dynamic interferon response, which rose and fell in parallel with viral load. Inhibiting innate immune signaling in organoid experiments showed that ISGs induced by SARS-CoV-2 could not prevent early viral replication but did somewhat curtail replication at low MOI. In contrast, heterologous induction of ISGs by prior infection with rhinovirus completely blocked SARS-CoV-2 replication. These results demonstrate dynamic airway innate immune responses in human subjects and show that exposure to another virus can protect against SARS-CoV-2 through heterologous induction of innate immunity. These results support a model in which an individual's recent exposures influence susceptibility to SARS-CoV-2 infection.

Th143. Localized Delayed Hypersensitivity Reactions to the COVID-19 Vaccine: A Case Series

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To combat the Coronavirus Disease-2019 (COVID-19) pandemic, two mRNA vaccines (Pfizer-BioNTech and Moderna) have received Emergency Use Authorization in the United States. Since authorization, patients have developed delayed localized cutaneous vaccine reactions that have been dubbed "COVID arm." We performed a retrospective case series to describe: 1. the course of localized cutaneous injection site reactions to COVID vaccine, 2. subsequent second dose reaction, and 3. reaction histopathology. We identified sixteen cases at a tertiary medical center in New Haven, Connecticut between January 20 - February 12, 2021. We collected age, gender, past medical history, clinical course, treatment (if any), clinical photographs, and histopathology. Thirteen of 16 cases were female (3 male) with age range 25-89 years. Localized cutaneous reactions developed near the injection site 2-12 days after Moderna COVID-19 vaccine and were pruritic, tender, edematous, pink plaques. No participant received the Pfizer-BioNTech vaccine. Biopsy demonstrated mild predominantly perivascular mixed infiltrate with lymphocytes and eosinophils, consistent with dermal hypersensitivity reaction. Most individuals who received the second vaccine dose developed a similar localized injection-site reaction with shorter post-vaccination latency period. No serious vaccine adverse events were reported. Our series demonstrates that the clinical and histopathologic findings of the localized injection site reactions to the Moderna COVID vaccine are a delayed-type hypersensitivity reaction. This reaction may occur sooner after second vaccine dose, but it is self-limited and is not associated with serious vaccine adverse effects. In contrast to immediate hypersensitivity reactions (e.g. anaphylaxis, urticaria), "COVID arm" is not a contraindication to subsequent vaccination.

Th170. Immune Phenotyping of SARS-CoV-2 Infection in Pregnant Women Admitted for Delivery in Hamilton, Ontario, Canada

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Rationale: We aim to identify soluble and cellular immunological profiles, in maternal peripheral and umbilical cord blood, related to COVID-19 infection during pregnancy.

Methods: Recruitment of obstetric patients with and without SARS-CoV-2 infection in pregnancy is ongoing. Participants complete questionnaires on risk behaviours, health history, and vaccination status. Maternal blood is collected upon admission and umbilical cord blood at delivery. Comprehensive immunophenotyping of circulating cell populations in maternal peripheral and umbilical cord blood are performed by flow cytometry. T cell responses are assessed by *in vitro* activation assays. Serum is stored for quantification of cytokine levels. Prior SARS-CoV-2 exposure or vaccination are confirmed by serology.

Results: To date, 23 patients have been enrolled. Four of 8 recruited patients with COVID-19 infection during pregnancy have delivered. Serologic analyses have confirmed evidence of prior SARS-CoV-2 exposure. Enumeration and immunophenotyping of T cells (naïve, memory, terminally differentiated, CD4⁺ and CD8⁺ T cells, and CD4⁺ Tregs and Th1/Th2/Th17 subsets), B cells, NK cells, monocytes (classical, intermediate, non-classical), myeloid progenitors, neutrophils, and eosinophils are being done. Preliminary data suggest that CD4⁺ and CD8⁺ T cells from pregnant women recovered from COVID-19 show increased activation after polyclonal stimulation, compared to cells from non-pregnant recovered women. CD4⁺ T cells from COVID-19-recovered women demonstrate similarly elevated activation responses to SARS-CoV-2 spike, glycoprotein membrane, and nucleocapsid peptides.

Conclusions: Understanding cellular and soluble immunity to COVID-19 infection will help inform the management of COVID-19 infection in pregnancy and can help in the development of counselling decisions regarding vaccination during pregnancy.

Th172. SARS-CoV-2 Reinfection in a Cohort of Healthcare Workers with Different Risk of Exposure to COVID-19 Patients

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A higher rate of SARS-CoV-2 reinfection has been observed after waning of natural immunity. Healthcare workers are at high risk of developing COVID-19. The aim of this study was to explore if a higher exposure to COVID-19 patients is associated with a higher immune response and/or a different risk of reinfection.

We conducted a retrospective cohort study of 3,722 healthcare workers at a two-center hospital, with 1 year follow-up. The cohort was divided according to the risk of exposure to patients with COVID-19 (high vs. low). COVID-19 cases in staff were identified by molecular testing in all suspected cases and close contacts. Viral load and antibody levels were obtained. SARS-CoV-2 genomic sequencing was performed for viable samples.

We identified 1,066 (28.6%) workers with confirmed COVID-19 from which 21 (1.97%) had a second episode. The incidence of COVID-19 was higher in the high-risk group. The incidence of possible reinfections showed no difference. Possible reinfection cases had a lower viral load in the first episode compared with the rest of the positive staff and showed a low rate of seroconversion (25.0%). The second episode was associated with a higher viral load compared to the first episode in the staff with reinfections and a higher rate of seroconversion (90.5%). Only a pair of samples from one individual was viable for genomic sequencing and confirmed reinfection with a

different strain of SARS-CoV-2. These findings suggest that reinfections occur in individuals with a lower immune response after the first episode.

Th175. Cytokine Milieu in COVID-19 and Therapeutical Intervention of Oral Low Dose Cytokine Therapy

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For a virus to survive in the body it needs to switch off the cytokine signalling pathways of innate immune responses. The virus then triggers deleterious immune responses which drives inflammation and produces tissue damage.

In the initial stage of COVID-19 infection interferon lambda protects the epithelial cells from viral invasion. Being an anti-inflammatory cytokine, it prevents the trigger for a cytokine storm. If the body fails in stopping viral entry with lambda, then interferon beta blocks viral entry into adjacent healthy cells. If that fails, then NK cells are used to kill off the infected cells using IL-15. If NK cells also fail, then the job goes to the adaptive immune system consisting of TH1 cells which enable macrophages to secrete interferon gamma and IL-12. The virus blocks TH1 activity and induces TH2 activity by increasing IL-4, this not only inhibits viral die off but also induces fibrotic healing. The failed TH1 cell activity blocks IGG2a antibody formation and enables the virus to spread. The viral infection then spreads into the alveoli which causes cell death, this then drives the innate immune response with a release of TNF α , IL-1 and IL-6 from the neutrophils and macrophages. As the neutrophils build up they cause a depletion of lymphocytes; this is the cytokine storm which causes further damage and shock leading to CVS and kidney failure.

We propose low dose cytokine combination of interferon lambda, gamma, beta, IL-12, IL-15 and lactoferrin to help treat mild to moderate COVID-19 infections.

Immuno-engineering and Cellular Therapies

W18. Cellular Immune Phenotypes Associated with Antisense Oligonucleotide-associated Thrombocytopenia in Non-human Primates

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Antisense oligonucleotides (ASOs) are a new class of single-stranded DNA based drugs that hold great therapeutic potential. Preclinical and clinical studies have reported events of ASO sequence-specific lowering of platelet counts (PLT), the incidence and severity of which varied among sequences, studies, and species. The underlying cause of a rarer, sporadic thrombocytopenia (TCP), associated with severe decreases in PLTs, points to immune-mediated TCP. Mauritian monkeys tend to experience a higher incidence of ASO-induced TCP compared to other Asian monkeys. Therefore, in this pilot study, we used a mass cytometry-based intracellular cytokine staining assay, to evaluate the immune-phenotypic and functional changes in cryopreserved PBMCs, collected over 8 timepoints of ASO therapy (ISIS 405879) from 12 Cambodian and 12 Mauritian monkeys (9 treated and 3 controls). Unsupervised clustering was performed across markers used for cell type identification in the pooled dataset, followed by unsupervised comparison at each timepoint and then longitudinal analysis. Major immune cell types showed differential abundance between the two groups prior to start of ASO therapy. These included IFN γ and TNF producing polyfunctional effector T cells (CD4⁺ and CD8⁺) which were lower and MIP1b producing monocytes and DCs which were higher, in the Mauritian monkeys. Immune populations also changed as a result

of this treatment, wherein IL-17 and GM-CSF producing T cells and IgM producing B cells increased markedly in Mauritian monkeys. Identification of these differentially abundant immune cell subsets in sensitive monkeys could help decipher potential immune mechanisms contributing to severe TCP in phenotype 2.

W84. Lipid Nanoparticle-mediated mRNA Delivery for CAR T Cell Engineering

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Current chimeric antigen receptor (CAR) T cell engineering methods primarily manipulate cells via *ex vivo* viral vectors to induce permanent CAR expression, which can lead to severe adverse effects such as neurotoxicity and cytokine storm. Alternatively, mRNA has been explored as a promising strategy for inducing transient CAR expression in T cells to mitigate the adverse effects associated with viral vectors, but most commonly requires electroporation for mRNA delivery, which can be cytotoxic. Here, ionizable lipid nanoparticles (LNP) were engineered for CAR mRNA delivery to human T cells for applications in T cell engineering and immunotherapy. Two libraries composing a total of 24 LNPs were synthesized via microfluidic mixing and screened for luciferase mRNA delivery and toxicity in Jurkats—an immortalized human T cell line. Both libraries were orthogonally designed with respect to excipients including cholesterol, phospholipid, PEG, and ionizable lipid. Top-performing LNPs from these libraries were also shown to deliver CAR mRNA to primary human T cells and induce CAR expression at levels equivalent to electroporation, but with substantially reduced T cell cytotoxicity. Furthermore, when compared in an *in vitro* co-culture assay to assess therapeutic efficacy, CAR T cells generated with LNPs, electroporation, and viral vectors were all shown to elicit potent cancer-killing activity. These results demonstrate the ability of LNPs to deliver mRNA to primary human T cells to induce functional protein expression and indicate the impact of excipients on LNP-mediated T cell transfection.

W117. Receptor Engineered TRuC Tregs Maintain a Regulatory and Suppressive Phenotype in Murine Hemophilia A Model

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The most serious complication to exogenous factor VIII (FVIII) replacement therapy in hemophilia A remains the development of inhibitory antibodies. Antigen specific Tregs have the potential to induce tolerance to FVIII therapy. We engineered Tregs using T cell receptor fusion constructs (TRuCs) generated by complexing FVIII specific scFv to the N-terminus of murine TCR ϵ . We confirmed TCR dependent surface expression of TRuCs using a FVIII binding assay, where co-transduction of FVIII-TRuC with TCR α /b transported TRuC to the plasma membrane in a TCR deficient murine T cell line. Furthermore, co-transduction of HEK-293 cells with CD3 and TCR α /b confirmed the requirement for an intact TCR-CD3 complex for TRuC surface expression. TRuCs recapitulate TCR based signaling in an MHC-independent manner and *in vitro* FVIII stimulation led to upregulation of CD69, Ki67, CD28, FoxP3 and CTLA4. Dampened signaling downstream of TCR/CD28 (pAKT S473, pERK and pS6) and low-level cytokine release (IL-2, IL-4, IL-17, IL-10 and IFN γ) confirmed maintenance of Treg phenotype. FVIII stimulation *in vivo* did not result in a loss of lineage stability in transduced Tregs, as TRuC Tregs retained FoxP3 expression (92.75 \pm 0.3%). Naïve BALB/cF8e16^{-/-} mice receiving weekly IV injections of 1.5 IU BDD-FVIII following adoptive transfer of 5 \times 10⁵ TRuC Tregs were more effective at suppressing inhibitor formation. TRuC group did not develop detectable inhibitors (0.23 \pm 0.23 BU/mL) at 4 weeks compared to control group (69.42 \pm 33.99 BU/mL) or those receiving polyclonal Treg therapy (24.35 \pm 19.19). This study underlines the potential of engineered TRuC Tregs to suppress anti-drug antibody formation against a soluble therapeutic protein.

W119. Improved Non-viral CAR-reprogramming of Conventional and Regulatory T Cells Using CRISPR-Cas and Double-stranded DNA

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Chimeric Antigen Receptor (CAR) redirected T cells are a potent treatment option for certain hematological malignancies. For many solid tumor entities, CAR T cell therapy is under development. Furthermore, CAR reprogramming of regulatory T cells (Tregs) is studied for the treatment of allogeneic immune responses in transplantation medicine.

Recently, site-specific insertion of CARs into the T cell receptor (TCR) alpha constant (*TRAC*) locus using gene editing and adeno-associated viruses was shown to generate CAR T cells with improved functionality over their retrovirally transduced counterparts. However, the development of viruses for gene transfer is complex and associated with extensive costs at early clinical stages.

Here, we provide an economical, virus-free method for efficient CAR insertion into the *TRAC* locus of primary human T cells via CRISPR-Cas mediated homology-directed repair (HDR). Previous studies by Roth *et al* and Schober *et al* showed moderate transgene integration efficiencies ranging from 5 % (for a 2.1 kb insert) to 15 % (for a 1.5 kb insert). By optimization of electroporation conditions and by pharmacological modulation of DNA sensing and repair pathways, we developed a stable protocol for highly efficient gene editing with TCR-to-CAR replacement rates exceeding 50 %. Resulting TCR-deficient CAR T cells show antigen-specific cytotoxicity and cytokine production *in vitro*. Furthermore, this protocol was also successfully adapted for the efficient generation of CAR Tregs.

Thus, we provide a GMP-compatible non-viral platform technology that lays the foundation for clinical trials and fast-track generation of novel CAR T cells applicable for autologous or allogeneic off-the-shelf use.

W139. Engineered Type 1 Regulatory T Cells have a Cytotoxic Profile and Kill Myeloid Cells

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T regulatory cells play a key role in modulating innate and adaptive immunity. The IL-10 secreting, FOXP3⁺ type 1 regulatory T cells (Tr1) develop in the periphery, secrete high levels of IL-10 and TGFβ1, intermediate levels of IFN-γ and low IL-4. Tr1 cells primarily exert their suppressive function via secretion of IL-10 and granzymeB-mediated killing of myeloid cells. There is significant interest in harnessing the suppressive potential of Tr1 cells for cell-based treatment in autoimmune, inflammatory and transplant-related disorders. However, to date, Tr1 cell isolation and *in vitro* expansion have been limiting their use as cell therapeutics. To overcome this, we generated a cell therapy product by engineering human CD4⁺ T cells to overexpress *IL10*. CD4^{IL-10} have functional properties comparable to Tr1. We performed RNA sequencing on CD4^{IL-10} and control CD4^{GFP} cells to investigate transcriptional differences between the two cell types and identify genes responsible for the acquired regulatory function. We showed that overexpression of *IL10* drastically upregulated cytotoxicity-related genes in CD4^{IL-10} without changing the typical Tr1-like cytokine profile of these cells. As previously reported, high granzymeB

expression was observed in CD4^{IL-10} compared to control. Additionally, four novel molecules, CD244, KLRD1, KLRC1 and FASLG contributing to CD4^{IL-10} cytotoxicity were identified. We also confirmed the killing of CD4^{IL-10} against myeloid cells and a set of 40 primary pediatric AML blasts. In conclusion, CD4^{IL-10} are cytotoxic cells that can kill majority of AML blasts. Thus, CD4^{IL-10} is a novel cell therapy product to control undesired effector T-cell responses and mediate antitumor activity.

W149. Immunomodulatory Hydrogels for Bone Regeneration in Response to Bisphosphonate-related Osteonecrosis of the Jaw

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Bisphosphonate-related osteonecrosis of the jaw (BRONJ) is a chronic inflammatory disease resulting in bone necrosis. Mesenchymal stromal cells (MSCs) are a promising cell-based therapy for bone regeneration, yet concurrent differentiation and secretome production is difficult to maintain. Upon implantation, MSCs upregulate bioactive factor production to modulate the inflammatory environment, potentially at the cost of directly contributing to tissue repair, yet it is unknown if the controlled delivery of similar biomolecules may allow MSCs to undergo osteogenic differentiation. Here, we aim to use microparticles within hydrogels to deliver local, instructive factors and interrogate MSC differentiation in inflammatory culture conditions relevant to BRONJ.

To interrogate the effects of chronic levels of inflammation on osteogenic differentiation, MSCs were treated with 50 pg/mL of TNF α for 21 days. Results indicate that this inflammatory environment does not affect MSC osteogenesis. Furthermore, we used a Design of Experiments (DOE) approach to determine the combination of immunomodulatory factors (IMFs), specifically IL-10, IL-4, and PGE₂, that maximize osteogenic differentiation. For IMF delivery, PLGA microparticles (MPs) were loaded into gelatin methacrylate hydrogels at 0, 1, 2.5, 5, and 10 mg/mL to determine their effects on hydrogel mechanical properties. MP density did not affect gel storage modulus. These findings inform future studies which include treatment of macrophages and MSCs with IMF-loaded MPs to determine their effect on tissue regeneration and immune modulation. This work aims to demonstrate that IMF presentation affects osteogenic potential of MSCs under inflammatory conditions and will provide a strategy to regenerate bone in patients with BRONJ.

W150. THOR-809: A CD25-biased PEG-IL-2 Engineered for Autoimmune Therapy Using a Semi-synthetic Organism

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Regulatory T cells (Tregs) are critical in the maintenance of immune homeostasis. Treg dysfunction is associated with autoimmune (AI) and inflammatory-related diseases. One promising therapeutic strategy for treatment of AI disorders via Treg stimulation is the cytokine interleukin-2 (IL-2). While IL-2 therapy has shown efficacy in various autoimmune disorders, poor pharmacokinetics and pleiotropic immune responses have limited its widespread therapeutic utility. Here we employed a microbial production technology that leverages a six-letter semi-synthetic DNA code to engineer a PEGylated IL-2 with enhanced pharmacokinetics, specificity for Tregs, and reduced activation of CD8⁺ T effector and NK cells. A library of recombinant IL-2 molecules was constructed with genetically encoded non-natural amino acids at sites peripheral to the IL-2 receptor beta and gamma interfaces. Site-specific pegylation of these compounds at the non-natural amino acid and screening identified THOR-809, a pegylated IL-2 variant that retained high affinity for IL-2 receptor alpha but was attenuated for

binding IL-2 receptor beta. Administration of THOR-809 in mice resulted in the expansion and activation of Tregs with minimal effects on CD8⁺ T or NK cells. In cynomolgus monkeys, THOR-809 administration resulted in the expansion of peripheral Tregs and induction of markers of Treg suppressive function without significant expansion of CD8⁺ T or NK cells. Finally, THOR-809 administration in mice suppressed the delayed-type hypersensitivity (DTH) response to the keyhole limpet hemocyanin (KLH) challenge. Together, these results demonstrate that THOR-809 is a potent and specific Treg activator and support continued development of this compound for the treatment of autoimmune disease.

Th16. Preclinical Safety and Efficacy Validation of CD4LVFOXP3 Cells as an Innovative Treg-like Cell-based Gene Therapy for IPEX Syndrome

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FOXP3 is an essential transcription factor for regulatory T cell (Treg) function, and a key regulator of immune tolerance. Genetic or acquired defects in Treg play a key role in immune mediated diseases including Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) syndrome. We have optimized a protocol for the generation of Treg-like cells from CD4⁺ T cells of healthy donors or IPEX patients, by lentiviral-mediated gene transfer of FOXP3(CD4^{LVFOXP3}) which acquire stable functional regulatory properties. The CD4^{LVFOXP3}, despite being generated from effector T cells, are phenotypically stable in the presence of rapamycin, which is a widely used treatment for IPEX syndrome. Indeed, CD4^{LVFOXP3} show similar stability to that of expanded Tregs in the presence of rapamycin. Furthermore, autologous CD4^{LVFOXP3} generated in GMP grade conditions are functionally stable after cryopreservation and showed dose-dependent xeno-GvHD protection. In vivo studies also showed that CD4^{LVFOXP3} do not inhibit response to pathogens or tumor clearance.

To facilitate pre-clinical safety and efficacy assessments, we have developed a humanized-mouse model in which the FOXP3 gene is knocked-out (KO) using CRISPR/Cas9 in human HSPCs, then transplanted into immunodeficient mice. The use of multiple sgRNAs targeting the FOXP3 locus significantly improved targeting when compared to a single sgRNA. The immune-deficient mice transplanted with FOXP3-KO HSPCs developed lymphoproliferation 10-12 weeks after transplant, which was controlled by CD4^{LVFOXP3}.

These data complete the IND-enabling studies supporting the clinical use of CD4^{LVFOXP3} in a Phase 1 trial to treat IPEX syndrome and future clinical use in other immune-mediated diseases caused by insufficient or dysfunctional FOXP3⁺Tregs.

Th28. An Immobilized DL-4- and TNF α -based Culture System to Generate High Numbers of Non-modified or Genetically Modified Immunotherapeutic Human T-lymphoid Progenitors

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Several obstacles to the production, expansion and genetic modification of immunotherapeutic T-cells *in vitro* have been restricting the widespread use of T-cell immunotherapy. In the context of hematopoietic stem cell transplantation (HSCT), delayed naïve T-cell recovery contributes to poor outcome. A novel approach to overcome the major limitations of both T-cell immunotherapy and HSCT would be to transplant human T-lymphoid progenitors (HTLPs) allowing the reconstitution of a fully functional naïve T-cell pool in the patient's thymus. However, it is challenging to produce HTLPs in high numbers to meet the clinical needs. Here, we found that adding tumor necrosis factor alpha (TNF α) to an immobilized notch delta-like ligand 4 (DL-4)-based culture system led to the generation of a large number of non-modified or genetically modified HTLPs from either cord blood (CB) or mobilized peripheral blood (mPB) HSPCs through accelerated T-cell differentiation, enhanced HTLP cell cycling and survival. We also demonstrated that the HTLPs exposed to TNF α , whether genetically modified or not, differentiated rapidly and efficiently into T-cells *in vitro* and *in vivo* in NSG mice. This study provides a clinically suitable cell culture platform for the generation of high numbers of clinically potent non-modified or genetically modified HTLPs for accelerating immune recovery after HSCT and T-cell based immunotherapy (including CAR T-cell therapy).

Th63. Treatment of Immune-Mediated Diseases with Tolerogenic Acellular Artificial Antigen-presenting Cells that Provide IL-2 and TGF-beta will Generate Protective CD4 and CD8 Foxp3⁺ Regulatory T Cells

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Regulatory T cells require IL-2, TGF- β and continuous TCR stimulation for induction and maintenance. Previously, we have reported that NPs loaded with IL-2 and TGF- β can induce CD4 and CD8 Foxp3⁺ Tregs in mice that can protect them from a lupus-like syndrome. Here we report that PLGA nanoparticles (NPs) coated with anti-CD2 or anti-CD3 and encapsulated with only IL-2 can function as tolerogenic artificial antigen-presenting cells (aAPCs) that target CD4 and CD8 cells *in vivo* and will provide both IL-2 and TGF- β in the local environment to generate protective Foxp3⁺ Tregs. In mice, these aAPCs induced Tregs that prevented a lupus-like syndrome. Moreover, anti-CD2 coated NPs induced TGF- β producing NK cells that critically supported CD4 and CD8 Tregs induction and function. Elimination of these NK cells not only abolished the protective activity of the NPs, but also exacerbated lupus glomerulonephritis. Purified NK cells from mice treated with anti-CD2 coated aAPCs *in vivo* protected mice from lupus-like renal disease in a TGF- β dependent manner. In humans anti-CD3 (Fab')₂ coated aAPC loaded with IL-2 only increased CD4 and CD8 Tregs in NOD/SCID immunodeficient mice that protected them against a human anti-mouse graft-versus-host disease. These studies highlight the protective effects of IL-2 and TGF- β in immune-related disorders and suggest acellular aAPCs that provide TGF- β without being encapsulated will be both efficacious and safe. Moreover, the TGF- β producing NK cells induced with anti-CD2 coated aAPCs containing IL-2 appear essential in supporting the maintenance of Tregs needed to predominate and control pathologic immune cells.

Th74. Development of a Xeno-free and Serum-free Serum Replacement for T and NK Cell Culture

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Cancer immunotherapy has become one of the most promising fields in the treatment of oncologic related disorders. It takes advantage of the body's own immune defenses, by harnessing the power of T cells and NK cells. Expansion and/or differentiation of cells with the right therapeutic profiles, in a consistent and safe manner,

requires the use of defined cell culture reagents. One major cause of variability is serum, due to its undefined composition. Serum is a complex mixture of hundreds of components, that may vary in concentration with the gender, age, diet, health, and genetics of the donor. Here we present the development strategy of a novel xeno-free, serum-free formulation optimized to support T and NK cell culture. After identifying the essential cell property parameters, and establishing appropriate cell culture assays, we initiated formulation optimization. We were able to identify the critical serum components required for immune cell culture. The presence and amount of such components influence cell expansion, viability and cell characteristics. We ultimately developed a serum replacement formulation, that consistently outperforms human AB serum, while bringing the benefits of being fully defined. In addition, we have gained a detailed understanding of the impact of each critical component, and how best to tailor the formulation to any desired cell profile that can benefit the immunotherapy field.

Th218. Monobody-based CAR T Cells as a New Alternative for Solid Cancer Therapy

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The therapeutic drawback of solid tumors originates from their heterogeneity and immunosuppressive tumor microenvironment (TME). In this regard, solid cancers are supposed to be more strategically approached by CAR T therapy than hematologic malignancies. Solid tumor-targeting CAR T should aim multiple targets and be able to efficiently infiltrate into immunosuppressive TME. Classical CAR T cells constructed with tumor antigen recognizing scFv from monoclonal antibodies fused to signaling moieties from T cell receptor and co-stimulatory molecules, which may not allow accommodating multiple functional components in the CAR construct. Here, we investigated whether the monobody derived from the fibronectin type III domain (10 kDa) could replace scFv (27 kDa), which may enable 2 or 3 specific monobody cloning in the place of 1 scFv. In the present study, we constructed a monobody-based CAR T recognizing the Ephrin receptor A2 (EphA2) via lentiviral transduction system. We tested cytotoxicity of 2 different second-generation CAR T cells, harboring CD28 or 41BB signaling domains, against EphA2-expressing PC3 cancer cells using 3D spheroid culture and NSG mouse xenograft models. mEphA2 cells fast infiltrated into 3D spheroid cores and efficiently killed target cells by inducing apoptosis. mEphA2 CAR T cells efficaciously suppressed PC3 tumor growth in NSG mice, which coincided with significant expansion and maintenance of input CAR T cells. These results obviously testify that monobodies is capable of replacing scFv in the construction of multicistronic and or multifunctional CAR T cells aiming multiple targets in heterogenous cancer cells in immunosuppressive TME.

Immuno-genetics

W137. De lineating Human CD8⁺ Regulatory T Cells Heterogeneity

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One major goal in transplantation is to develop a novel specific and non-toxic anti-rejection immunotherapies. Strategies based on regulatory T cells (Tregs) are promising to prevent graft rejection. Our group has shown that rat and human CD8⁺CD45RC^{low/-} Tregs display significant suppressive function. The team has also shown that cell therapy using human CD8⁺CD45RC^{low/-} Tregs was efficient to prevent graft rejection and GVHD in humanized NSG mice models. However, the heterogeneity of the CD8⁺CD45RC^{low/-} Tregs population is important from a phenotypic point of view.

We sorted CD8⁺CD45RC^{low/-} Tregs from blood of healthy volunteers and sequenced their transcriptomes by single cell RNA sequencing methods. To our knowledge those are the first single cell RNA-seq datasets of human CD8⁺ Tregs. The cell transcriptomes analysis highlighted the heterogeneity inside the population with the identification of 4 distinct clusters with a specific signature. We identified one cluster of particular interest based on its immune and regulatory gene signature. However, other genes such as TGFβ were expressed by all clusters inside CD8⁺CD45RC^{low/-} T cells. We then focused our analysis on genes that encode for membrane proteins that have never been used for CD8⁺ Tregs isolation yet such as TNFR2 and ITGB1. We confirmed their specific expression at protein level on human PBMC from healthy volunteers by a subset of CD8⁺CD45RC^{low/-} T cells. We also deeper analyzed membrane markers by performing a screening of CD molecules on surface of CD8⁺ Tregs to identify new specific membrane markers for isolating the strongest CD8⁺ regulatory T cells.

Th73. Genetic Polymorphism and Deficiency of Human Complement C4B in Health and Disease

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Human complement C4 is one of the most diverse innate immune effectors. Deciphering the molecular basis of polymorphism and genetic deficiency of its isotypes C4A and C4B would help understand their roles in the pathogenesis of autoimmune and inflammatory disorders. We identified subjects with the fastest migrating C4B allotype, B7 (HC74), and deficiency of C4B protein caused by genetic mutations in a young Caucasian (MS630), an Asian family (E133) with anti-NMDA receptor encephalitis, and a European family (E94) with multiplex lupus. Those C4B genes were amplified and sequenced. The novel polymorphism for C4B7 is a G→A transition leading to R729Q at the anaphylatoxin-like region. *NciI* RFLP helped identify six more subjects with this polymorphism. In subject MS630, a C-nucleotide deletion at codon-755 led to frameshifts and a stop at codon-767 in his single C4B gene. In subject E133 with encephalitis, the mutant C4B gene had a G→A transition at a tryptophan codon leading to a nonsense stop, W660x. The mutant C4B gene was present in a haplotype with HLA-DRB1*04:06, B*15:27 and A*11:01. In the multiplex family with two SLE-related mortality and low C4 levels, the culprit haplotype was HLA-A30, B18 and DR7 that segregated with two linked, short, defective C4B genes with identical mutations at the donor splice site of intron-28. Identical C4B mutations with the same HLA-haplotypes were found in two other multiplex families with severe lupus nephritis. Annotation of sequence results uncovers a new hotspot of variations proximal to cleavage sites for processing and activation of complement C4.

Immuno-oncology

W2. Nuclear and Cytoplasmic Expression of Total and Phosphorylated GLI1/GLI3 in Prostatic Cancer with Different Gleason Scores

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Background: Prostate cancer (PCa), an androgen-dependent malignancy, is the second leading cancer-related among men worldwide. The most common histopathological grading system is Gleason scoring, which specifies the degree of malignancy. A key factor in PCa development is the activation of signaling pathways implicated in cell differentiation and growth control, such as Hedgehog-GLI. Its aberrant activation contributes to cancer by enhancing survival, stemness, metastasis, and angiogenesis. GLI proteins are transcriptional effectors of this pathway; GLI1 is considered an activator, while GLI3 a repressor. Nuclear expression is associated with the induction of target genes involved in carcinogenesis. Their expression has not been evaluated in different Gleason degrees in prostate tissues.

Aim: To identify the nuclear and cytoplasmic expression of GLI1 and GLI3 in PCa samples with different Gleason scores.

Methods: Immunohistochemistry assays were performed with antibodies directed to GLI1 and GLI3 (total and phosphorylated) in PCa samples with different Gleason scores.

Results: Nucleus and cytoplasm expression were analyzed. A high expression of GLI1 in the nucleus and cytoplasm was observed in all Gleason scores. Unlike nuclear expression, the vast majority of the cancer tissue expressed cytoplasmic p-GLI1 regardless of the Gleason score. Finally, nuclear GLI3 was highly expressed in Gleason score of 8/9; however, p-GLI3 nuclear expression was mainly in high Gleason scores.

Conclusion: An important expression of nuclear GLI3/p-GLI3 was observed in high-grade PCa samples and nuclear pGLI3 expression in contrast to pGLI1 was elevated, which may indicate that pGLI3 could potentially have a role in processes related to aggressive PCa.

W9. Engineered Variant Domain Fusion Proteins Provide Checkpoint Inhibition and Tumor Antigen-Dependent CD28 Costimulation Resulting in Potent Anti-Tumor Immunity

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Checkpoint inhibition (CPI) has been shown to be an effective anti-tumor therapy, but CPI alone is frequently insufficient to control tumor growth, and costimulatory signals may also be required to produce significant anti-tumor responses. PD1/PDL1 are established CPI targets and TMIGD2 is an inhibitory receptor expressed on T cells that is engaged by its cognate ligand HHLA2 on tumor cells, leading to inhibition of T cell responses.

Variants of CD86 with increased CD28 affinity were engineered using our directed evolution platform. PD1 and TMIGD2 variants were also engineered for increased affinity to PDL1 and HHLA2, respectively. Fusion proteins were generated including either PD1 or TMIGD2 domains, an effectorless Fc, and an engineered CD86 domain to generate proteins to provide target-dependent costimulation (TDC).

PD1-CD86 and TMIGD2-CD86 TDC proteins enhanced T cell costimulation in multiple in vitro T cell response assays, and costimulation was dependent on target cell lines expressing PDL1 or HHLA2, respectively. Both TDC proteins enhanced anti-tumor responses in vivo in a syngeneic MC38 implantation model only when MC38 cells expressed human PDL1 or HHLA2, respectively. Both also enhanced antitumor responses in a humanized tumor implantation system when the tumor cell line expressed the corresponding target antigen but failed to control tumor growth when tumor cells lacked expression of the target protein.

Tumor antigen-specific antitumor therapy can be achieved with fusion proteins that combine engineered CPI and CD28 costimulatory domains. Such novel biologics may provide promising approaches to enhancing the efficacy of CPI monotherapies and to address checkpoint inhibitor-resistant tumors.

W21. Single Cell Proteogenomic Analysis Elucidates Treatment Options and Identifies a Potential Therapeutic Escape Mechanism in a Multiple Myeloma Patient

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Multiple Myeloma is characterized by a series of relapses due to the evolution of tumor clones. Upon relapse, it is often not clear which therapy should be used next nor how many distinct clones may exist to be treated. In order to identify possible treatment options for MM patients following relapse, we used the enhanced single cell analysis with protein expression (ESCAPE) RNA-Seq platform to simultaneously measure cell surface protein expression and total mRNA at the single cell level. Combining ESCAPE RNA-Seq with automated cell annotations using MapCell, we were able to identify the tumor and the normal cells within longitudinal samples taken from the same MM patient. Subsequent analysis identified discordance between the expression levels of the protein and RNA of a therapeutic target, showing the importance of measuring both protein and RNA in tumor samples and hinting at an escape mechanism evolved by those cells. We were also able to make therapeutic suggestions based upon these data. Going forward, single cell proteogenomic analysis should become a standard tool for understanding blood cancers and stratifying patient populations for more precise treatments.

W22. Unleashing Inflammasome Activation Through TMEM176B Blockade Triggers Th17-dependent Tumor Immunity in the Context of PD-1 Blockade

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The role played by inflammation in tumor immunotherapy and T cell exhaustion remains controversial. Blocking the cation channel TMEM176B, an NLRP3 inflammasome inhibitor strongly expressed in conventional type 2 DCs (cDC2), induces inflammasome and CD8-dependent tumor immunity leading to reinforced anti-PD-1 therapy. Here we wished at understanding the mechanisms by which unleashing inflammasome activation improves anti-PD-1 therapy. We observed that cDC2 purified from the tumor-draining lymph nodes of EG7 tumors-bearing mice and treated with Boritinib (a potent TMEM176B inhibitor) enhanced expression of IL-17A in tumor-specific CD4⁺ T cells as compared to cDC2 from untreated animals. Similar results were observed with cDC2 from *Tmem176b*^{-/-} animals as compared to WT and *Tmem176b*^{-/-}*Casp1*^{-/-} mice. Boritinib, improved survival of 5555 melanoma-bearing mice treated with anti-PD-1 antibodies in comparison to monotherapies in an *Il17a*-dependent manner. Preliminary results showed that Boritinib plus anti-PD-1 therapy diminished the relative number of intratumoral progenitor exhausted and terminally exhausted CD8⁺ T cells while increasing transitory exhausted cells in comparison to PD-1 blockade alone. This effect of Boritinib on exhausted CD8⁺ T cells was lost in *Il17a*^{-/-} mice. Accordingly, adoptive cell transfer (ACT) of tumor-specific Th17 cells improved the anti-tumor efficacy of PD-1 blockade while enhancing CD8-dependent *in vivo* cytotoxicity against tumoral antigens. We finally observed that intratumoral inflammasome and Th17 gene signatures were associated with clinical responses in anti-PD-1-treated melanoma patients whereas inflammasome and Th17 genes significantly

correlated with exhaustion markers. Our results suggest that an inflammasome/Th17 axis may regulate CD8⁺ T cell exhaustion in anti-PD-1 therapy.

W58. Alphataxin, a Small-molecule Drug that Elevates Tumor-infiltrating CD4⁺ T Cells, in Combination with Anti-PD-1 Therapy, Suppresses Murine Renal Cancer and Metastasis

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By promoting the cytotoxic function of CD8⁺ T cells, immune checkpoint inhibitor therapy, e.g. programmed cell death protein-1 (PD-1), effectively inhibits tumor growth in renal cell carcinoma. Yet, as many as 87% of cancer patients do not respond to immune checkpoint therapy. The function of cytotoxic CD8⁺ T cells integrally relies on CD4⁺ T helper cells. Remarkably, despite advances in immunotherapy, there are no pharmaceutical treatments that increase circulating CD4⁺ T cell counts. Nor has there been much attention given to tumor-infiltrating CD4⁺ T cells. The orally available small-molecule drug Alphataxin targets a pathway that regulates the number of circulating CD4⁺ T cells. We aimed to examine how Alphataxin affected tumor growth in a murine model of renal cell carcinoma. Alphataxin, in combination with anti-PD-1 antibody, significantly elevated the ratio of circulating and tumor-infiltrating CD4⁺/CD8⁺ T cells. In one study, following orthotopic implantation of syngeneic renal cell carcinoma, combination treatment resulted in 100% regression of tumor growth. Moreover, in mice implanted orthotopically with one log more tumor cells, doubling Alphataxin dose in combination treatment led to 100% regression in one-third of mice and 81% suppression of tumor growth in the remaining two-thirds of mice. Lung metastasis was evident in all mice except combination-treated mice. Orally available Alphataxin, the first and only drug developed to increase CD4⁺ T cells, in combination with anti-PD-1, is a powerful therapeutic method that provides long-term remission in renal cell carcinoma and potentially other T cell-responsive cancers by increasing the number of CD4⁺ tumor-infiltrating T cells.

W61. Cancer Cell Autonomous TLR9 Activation During Mitophagy Dictates Efficacy of Chemoimmunotherapy in Cold Tumors

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Association of chemotherapy with anti PD-1/PD-L1 mAb became the standard of care for patients bearing a lung non small cell lung cancer (NSCLC). However, many patients with poorly immune infiltrated tumor-also called-cold tumor- fail to gain benefit from this therapy. Using histological cold tumors resistant to immune checkpoint inhibitors, i.e., a transplantable and carcinogen induced lung adenocarcinoma, we observed that cisplatin pemetrexed (CDDP/PEM) induce stigmata of immunogenic cell death but remain unable to synergize with anti PD-L1 mAb because of absence of T cells recruitment. Using drug screening we showed that adding MEK inhibitor with CDDP/PEM trigger CXCL10 production, T cells recruitment and restore antitumor efficacy of anti PD-L1 mAb. CDDP/PEM plus MEK Inhibitor promote optineurin dependent mitophagy and TLR9 activation by mitochondrial DNA thus resulting in CXCL10 production. Invalidation of TLR9 or autophagy/mitophagy process abort the antitumor efficacy of CDDP/PEM plus MEK Inhibitor and anti PD-L1 therapy. In human with NSCLC and melanoma, high optineurin and CXCL10 expression are associated with better response to checkpoint inhibitors. Thus our results underline a new role of TLR9 and mitophagy as a new target to switch cold into hot tumors.

W68. Transplant Patient with Metastatic Cancer Responds to Cemiplimab After Failing a Different Anti PD-1 Immunotherapy

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Cemiplimab and pembrolizumab are anti-Programmed Death-1 (PD-1) therapies approved for patients with recurrent and/or metastatic cutaneous squamous cell carcinoma (CSCC) that progress despite treatment with surgery and radiation. Their efficacy and safety in solid-organ-transplant recipients (SOTR) is not well-studied. The PD-1 axis plays a role in preserving graft tolerance. The T cells produced following administration of PD-1 inhibitors act against both tumor antigens and donor alloantigens, leaving patients with significant risk of allograft rejection. We report a case of a renal transplant patient who developed metastatic CSCC following multiple surgeries, three cycles of adjuvant radiotherapy, and minimization of immunosuppression. He was treated with pembrolizumab and achieved a complete response. However, the patient developed immune checkpoint inhibitor (ICI)-induced allograft rejection requiring pembrolizumab discontinuation. His allograft was salvaged following IVIg and steroids. Off therapy, the patient developed recurrent disease. He was rechallenged on pembrolizumab and treated with a dynamic prednisone dosing schedule (40mg the day prior to infusion, 20mg daily for 5 days, and 7.5mg daily dose) to mitigate the chance of worsening renal rejection. Kidney function remained stable, however he failed pembrolizumab rechallenge. He was trialed on carboplatin and cetuximab and developed further progression. Subsequently, he was started on cemiplimab with the same peri-infusional prednisone schedule and achieved a partial response. His creatinine remains stable. This case illustrates the ability to salvage ICI-induced renal rejection and response to cemiplimab following failure of pembrolizumab. Currently, a trial for renal and stem cell transplant patients treated with cemiplimab and peri-infusional prednisone is underway.

W85. Mantis Viewer: Software for Analysis of Multiplexed Tissue Imaging for Cancer Immunotherapy

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At the Parker Institute for Cancer Immunotherapy (PICI) we have built a platform to help scientists better analyze their multiplexed tissue imaging data. The application is called Mantis (<https://mantis.parkerici.org/>). Mantis was built in response to a problem that we were experiencing when analyzing data from our clinical trials: imaging technology has improved rapidly over the last decade, but the analysis software has not kept up. Mantis is a desktop application that has been built specifically to visualize and analyze images generated using multiplexed imaging technologies such as Vectra, MIBI, CODEX, IMC, and more. . Multiplexed imaging is a technique that is used to visualize the location and quantity of proteins in tissue samples. In the field of cancer immunotherapy, this type of data is uniquely suited to understand the Tumor Microenvironment, enabling scientists to determine the type and quantity of immune cells present and to analyze their spatial relationship to tumor cells. This insight into the TME is crucial for learning about mechanisms of response and resistance to Immunotherapy. Mantis makes use of segmentation data that identifies cells in an image to allow users to generate plots and populations much like one would with flow cytometry, and then visualize these populations on the image. Overall, Mantis has dramatically improved the process by which our researchers analyze microscopy data, enabling discoveries like novel characterizations of the immune infiltration in Metastatic Pancreatic Cancer.

W88. Multicellular Immune Hubs and Their Organization in MMRd and MMRp Colorectal Cancer

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Immune responses to cancer are highly variable, with mismatch repair-deficient (MMRd) tumors exhibiting more anti-tumor immunity than mismatch repair-proficient (MMRp) tumors. To understand the rules governing these varied responses, we transcriptionally profiled 371,223 cells from colorectal tumors and adjacent normal tissues of 28 MMRp and 34 MMRd patients. Analysis of 88 cell subsets and their 204 associated gene expression programs revealed extensive transcriptional and spatial remodeling across tumors. To discover hubs of interacting malignant and immune cells, we identified expression programs in different cell types that co-varied across patient tumors and used spatial profiling to localize coordinated programs. We discovered a myeloid cell-attracting hub at the tumor-luminal interface associated with tissue damage, and an MMRd-enriched immune hub within the tumor, with activated T cells together with malignant and myeloid cells expressing T-cell-attracting chemokines. By identifying interacting cellular programs, we thus reveal the logic underlying spatially organized immune-malignant cell networks.

W92. Characterization of Immune Checkpoint Inhibitor-associated Arthritis in Electronic Health Record Data

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Immune checkpoint inhibitors (ICIs) are an important pillar of cancer therapy with demonstrated efficacy in a variety of malignancies. However, they are also associated with immune-related adverse events (irAEs) that affect a number of organ systems with varying severity, inhibiting patient quality of life and in some cases the ability to continue immunotherapy. Here, we characterize ICI-associated arthritis in electronic health record (EHR) data. Thirty-one patients with new ICI-associated arthritis were chart abstracted from a cohort of all patients who received checkpoint therapy for cancer (n=2550) in a single-center retrospective study. Joint involvement tended to be diffuse; 28 cases involved large joints and 18 involved small joints. 18 patients were evaluated by rheumatology. 10 of 12 patients tested were rheumatoid factor negative. 11 of 11 patients tested were anti-cyclic citrullinated peptide negative. 5 of 8 patients tested were anti-nuclear antibody negative. 24 patients were treated with steroids. 8 patients were also treated with hydroxychloroquine or joint injections. The majority of patients underwent anti-PD-1/PD-L1 immunotherapy (n=29). 14 patients received anti-CTLA-4 immunotherapy, 13 in combination with anti-PD-1. Significantly, immunotherapy was held or discontinued due to the arthritis in 9 of 31 cases. Findings in this cohort are consistent with previously reported ICI-associated arthritis in the literature (variable patterns of joint involvement, predominantly seronegative, and primarily treated with steroids) and distinct from typical inflammatory arthritides. Cognizant of the barrier that ICI-associated arthritis poses to immunotherapy, future steps are to further explore these cases to inform earlier identification of ICI-associated arthritis and risk factors.

W106. Generating Macrophage-induced Anti-tumor Immunity in Ovarian Cancer with Mannosylated Nanoparticles

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Tumor-associated macrophages (TAMs), the most prevalent immune cell in most tumors, exhibit phenotypic plasticity, making them a desirable target for immune modulation. We have previously identified the nuclear factor-kappaB pathway as a target for altering macrophage phenotype via delivery of small interfering RNA (siRNA) against the inhibitor of NF-kappaB alpha (IκBα). The limitations of delivering free siRNA led to our development of a polymeric nanoparticle (NP) capable of loading siRNA and decorated with mannose (MnNPs) to target CD206 on M2-like TAMs.

MnNPs were used to treat two mouse models of ovarian cancer. In an aggressive TBR5 model, we showed targeted delivery to macrophages in tumors and the ascites, fluid build-up associated with ovarian cancer. Treatment with MnNPs loaded with IκBα siRNA (IκBα-MnNP) resulted in significant decreases in ascites volume and tumor weight. qRT-PCR analysis of cells in the ascites and tumors revealed significant increases in expression of pro-inflammatory markers. Immunofluorescence staining of tumor sections showed an increase in CD8 T cell infiltration in the IκBα-MnNP treated mice, confirming that classical macrophage activation can “prime” tumors for T cell infiltration. The slow-developing ID8 tumor model was used to examine treatment of late-stage disease. In this model, the changes in tumor weight were not as pronounced, but the IκBα-MnNP treatment did significantly reduce ascites accumulation, providing some therapeutic benefit even in advanced disease. Despite not significantly affecting tumor weight, histological analysis of tumor sections revealed indications of tumor cell death in the treatment group suggesting that additional optimization may provide greater benefit.

Th58. Adding Plinabulin (Plin) to Pegfilgrastim (Peg) Reverses the Immune-suppressive Potential of Peg While Offering Superior Prevention of Chemotherapy-induced Neutropenia (CIN) Versus Peg Alone

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Introduction

Peg, a long-acting G-CSF is standard of care for the prevention of CIN. Plin, is an anti-cancer agent that also prevents CIN independent from G-CSF. Plin combined with Peg provides superior CIN prevention: Grade 4 Neutropenia frequency was 68 % vs 86% (p=0.0015) with Plin+Peg versus Peg alone (Blayney ASCO 2021). Peg produces de-novo immature neutrophils (bands, promyelocytes/myelocytes), which have similarity to granulocytic myeloid derived suppressor cells (gMDSCs) (Pillay CellMolLifeSci 2013), known to promote metastatic growth by reverting EMT/CSC phenotype and tumor cell proliferation (Ouzonova Nature 2016). We evaluated the effects of adding Plin to Peg on Peg-induced immature neutrophil (N) generation.

Methods

In the Phase 3 PROTECTIVE-2 CIN study BPI-2358-106 (NCT03294577), Breast Cancer patients were randomized to Plin+Peg (n=111) or Peg (n=110). The pre-defined endpoint of frequency of N Bands, Promyelocytes/Myelocytes was evaluated by central laboratory (Covance) through Day (D)0 to D15 of cycle 1.

Results

Frequency of N Bands was significantly higher with Peg vs Plin+Peg (p=0.0003), and started to increase on D8 through D15 with Peg alone, but remained fairly flat with Plin+Peg. The difference in N Band frequency was significant on D9 (p=0.004), D10 (p< 0.00001), D11 (p< 0.00001), D12 (p< 0.02) favoring Plin+Peg. A similar pattern occurred with Promyelocytes/Myelocytes, with significantly higher frequency on D10 (p< 0.00001) and D11 (p=0.0018) for Peg vs Plin+Peg.

Conclusion

Plin prevented Peg-induced production of de-novo immature Neutrophils. Combining Plin with Peg not only has superior CIN prevention, but also reverses the immune suppressive potential with Peg alone.

Th96. Epigenetic Mechanisms of Colorectal Cancer Tumor Promotion by *Parvimonas micra*, An Opportunistic Pathogenic Bacterium of the Oral Cavity

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Colorectal cancer (CRC) is among the most common malignancies worldwide with high mortality rate. Environmental factors including chemical agents and food materials are known to contribute to the risk and consequences of CRC development. The human gut microbiome is comprised of trillions of microorganisms, which form a dynamic interface with the environment. Microbiota play a critical role in the maintenance of the gut tissue, in the maturation of body defense system, metabolism and absorption of nutrients. However, certain bacteria have potential carcinogenic and inflammatory properties that become evident when there is damage into gut tissue or when these bacteria outgrow other microorganisms. Amongst these a particular type of bacteria that originates from the oral cavity, *Parvimonas* was highly associated with changes of function of genes that are causatively involved in CRC. By using *in vitro* human microbiome reconstituted mice, we demonstrated that *Parvimonas micra* potentiates epigenetic modifications in several genes of colon epithelial cells. Most importantly, the changes into Wnt/ b-catenin pathway related genes that initiates CRC was observed. We also detected increased tumor load and altered DNA methylation in circulating blood lymphocytes of mice with genetic predisposition of CRC. Taken together, we hypothesized that oral commensal bacteria exemplified as *Parvimonas micra* promote CRC by introducing DNA methylation that activate pro-inflammatory and tumor promoting pathways in the gut tissue and in tumor infiltrating immune cells. The implications of this hypothesis go beyond curiosity, as these are fundamental mechanisms of disease that can be targeted to prevent and cure cancer.

Th102. Long-term Immune Monitoring in Non-small Cell Lung Cancer Patients Treated with PD-1/PD-L1 Axis Blocking mAbs

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Immune checkpoint inhibitors are one of the main immunotherapies used to treat non-small cell lung carcinoma (NSCLC), by blocking inhibitory pathways of the immune response, such as the PD-1/PD-L1 axis, to reinvigorate the ongoing immune-directed attack of the tumour. These immunotherapies have proven to be effective and safer than chemotherapy and have shown to both increase the survival rate of many cancer types, and expand the median survival of responsive patients. However, there is a need of biomarkers to determine which patients might benefit the most to these therapies, as current biomarkers, such as PD-L1 tumour expression, are not as accurate as desired.

We hypothesize that peripheral blood lymphocytes are directly affected by the treatment and can be used as biomarkers of treatment response. Hence, we evaluated the effects that PD-1/PD-L1 axis blocking immunotherapies induce in peripheral blood T-cell subsets of NSCLC patients by flow cytometry during 12-month follow-up.

Among the main findings we encountered a significant decrease during disease progression of Treg and CD4⁺ EM Th1/Th17 subsets, as well as in CD4⁺ T-cell subsets at different activation stages. We also found a significant decrease during treatment of peripheral activated CD8⁺ T-cells. Most strikingly, within the worst prognosis group based on PD-L1 tumour expression, we obtained preliminary data on significantly different values of peripheral CD4⁺ T-cell subsets depending on the outcome.

Based on these results, the study of peripheral blood lymphocytes might entail a potential source of biomarkers to select those patients that benefit the most to PD-1/PD-L1 blocking immunotherapies

Th104. TNFR2 Blockade Decreases Immunosuppression in a Mouse Model of PDAC

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Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive cancer, highly resistant to standard chemotherapy treatment and immunotherapy. One main feature of PDAC stroma is the infiltration of immunosuppressive regulatory T cells (Tregs) which have been shown to correlate with survival and tumor progression in PDAC patients. Tregs highly express TNF α receptor 2 and TNFR2⁺ Treg cells display strong Tcell suppressive capacities in different types of human tumors and mice models of cancer. However, the role of TNFR2 in PDAC is still unknown. We hypothesized that TNFR2 inhibition could reverse the balance of effector and regulatory T cells in PDAC tumors and trigger an efficient anti-tumoral response.

Results: anti-TNFR2 mAb treatment of an orthotopic mouse model of PDAC significantly impaired tumor growth. By flow cytometry, we showed that TNFR2 inhibition induced a significant decrease of Treg tumor infiltrated cells associated with an increase of CD4⁺/ Foxp3⁺ cell frequency. Importantly, CD8⁺ cell proportion was increased in both tumor and draining lymph nodes, thus resulting in an improved CD8/Treg ratio. Immunohistology staining confirmed higher CD8 infiltration in anti-TNFR2 treated tumors compared to control. Unexpectedly, TNFR2 blockade did not show any impact on myeloid and myeloid-derived suppressor cells proportion despite these cells highly express TNFR2.

Conclusion: These results highlight TNFR2 as a promising target to overcome Treg immunosuppression and increase effector T cell infiltration and activation in PDAC tumors, and allow the development of new combined therapeutic strategy.

Th112. Homeostatic Response Regulates Tumor Growth

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Genetic and epigenetic regulation predetermines tumor growth rates. The aim of this study was to compare the role of factors regulating tissue homeostasis on the rate of tumor growth and tumor cell selection. To this end stable GFP expressing cell lines CT26-eGFP and Pan02-eGFP were generated and 0.5x10E6 cells s.c. inoculated into genetically compatible BALB/c or C57BL/6 mice accordingly. CT26-eGFP induced tumors were characterized by high unlimited growth in vivo producing 200-500 mm³ tumors within 2-3 weeks while Pan02-eGFP tumors never reached 150 mm³ during a month. Histology demonstrated that Pan02-eGFP tumors were highly fibrotic. Analysis of fluorescent cells in the tumors showed that all Pan02-eGFP cells lost GFP expression while were able to grow in vitro after tumor homogenates transfer. Contrary to it CT26-eGFP preserved GFP expression on the basic level. Analysis of fibrinogen isoforms, tissue cytokines, metalloproteinase 12 and its inhibitors TIMP as well as inflammatory cytokines IL-6 and IL-10 gene expression analyzed by quantitative PCR demonstrated that the expression of all these genes was much higher in Pan02-eGFP in a comparison with CT26-eGFP (Table).

Table. Delta Ct Pan02-eGFP to CT26-eGFP

Fibrinogen		Metalloproteinases		Tissue cytokines		Immune cytokines	
Alpha	91	MMP-12	1,8	Interleukin-25	26	Interleukin-6	5,4
Beta	30	TIMP-1	5	Interleukin-33	10,3	Interleukin-10	7,8
Gamma	67	TIMP-2	4	Thymis stromal lymphopo etin	7		

Consequently, activation of tissue homeostatic response limits tumor growth, stimulates tumor fibrosis and orchestrates enhanced tumor cell selection.

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Th140. Immunogenetic Variation Involved in Natural Killer Cell Education and NK Cell Infiltration are Associated with Outcome in Non-small Cell Lung Cancer Patients Treated with Immune Checkpoint Blockade

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Natural Killer (NK) cells are important contributors to antitumor immune responses. Besides NK cell abundance in the tumor and diverse tumor immune evasion strategies targeting NK cells, the immunogenetic composition of patients' genomes is considered to be an important determinant of NK cell effectiveness.

We inferred HLA allelic variation and KIR gene presence using germline whole-genome sequencing data from 1,395 patients across three atezolizumab (anti-PD-L1) clinical trials (IMpower130, IMpower131, IMpower150) in non-small cell lung cancer (NSCLC). Patients treated with atezolizumab who carried at least one copy of both *KIR2DL3* and its ligand *HLA-C1* had longer overall survival (OS) compared to patients without this NK-cell educating interaction (N=955, HR=0.71, p=0.0002). A similar trend was observed for the interaction of *KIR3DL1* and *HLA-Bw4* (HR=0.84, p=0.04). No significant associations could be found in the control arms of the trials

(N=440). With regard to NK cell abundance, we showed a significant association of high (above-median) NK cell infiltration with longer OS (N=619, HR=0.75, p=0.01), using a gene signature derived from RNA-sequencing data (Cursons et al. CIR. 2019). Again, no significant association was found in the control arms (N=288). Furthermore, we identified *HLA-C1* carrier status (no KIR data available) to be an indicator of clinical benefit in a published dataset of melanoma and NSCLC patients (N = 1,535, HR = 0.74, p = 0.01, data from Chowell et al. Science. 2018).

In summary, our results suggest a significant role for both NK cell genotypes and degree of infiltration in patient responses to anti-PD-L1 cancer immunotherapy.

Th145. TGFb Modifies Local Tumor Growth and Control of Invasiveness Within the Tumor Microenvironment

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In BALB/c CD200R1KO mice cure of local and metastatic growth of EMT6 cells followed surgical resection of localized tumor and immunization with irradiated cells and CpG. Wild-type (WT) animals develop pulmonary and liver metastases within 20 days of surgery. No cure occurred with poorly immunogenic 4THM breast cancer, and tumor growth increased in CD200R1KO vs WT mice. We used two-phase cultures where bone marrow mesenchymal stromal cells (BMMSCs)-in some cases with DLN-T lymphocytes from tumor-immunized mice-were cultured in collagen gels, with tumor cells seeded in medium above this gel. Tumor invasion from the liquid to the gel layer was measured by enumerating tumor cells in the gel layer after collagenase digestion and culture at limiting dilution. Cytokine levels of the digest were analyzed by ELISA.

BMMSCs from WT and CD200R1KO mice augmented seeding/growth of EMT6 and 4THM tumor cells into the collagen matrix. Production of TGFb, IL-6 and IL-17 was seen in gel and liquid phases in cultures with DLN cells. IL-6 and IL-17 alone (no DLN) in the gel matrix increased invasion of tumor cells. Inclusion of DLN cells from EMT6 immune or 4THM immune mice in collagen gels modified tumor invasion. Increased 4THM tumor invasion occurred with CD200RKO-DLN, while DLN of EMT6 immune mice attenuated EMT6 tumor invasion, despite IL-6/IL-17 in the gel layer. Anti-TGFb in the gel layer reversed the modulation of EMT6 invasion by immune DLN cells, with no effect on invasion of 4THM.

Conclusion: Multiple factors modulate tumor invasion, including micro-environmental stromal elements and cytokines.

Th167. Cutaneous T-cell Lymphoma and Canine Epitheliotropic Lymphoma: A Comparative Analysis

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Cutaneous T-cell lymphoma (CTCL) is a rare type of skin cancer involving T lymphocytes in the skin. Canine epitheliotropic lymphoma (EL) is a spontaneous cutaneous lymphoma in dogs also arising from the T lymphocytes in the skin and mucosa. Many studies have identified immune genes, pathways and cells that drive

the pathogenesis of CTCL, including interleukins, chemokines, cell cycle control/oncogenes, and other leukocytes. Data suggest that similar processes are involved in the pathogenesis of EL in canines. Here, we present case studies of 6 canines with EL which occurred spontaneously in client-owned companion dogs. We performed comparative transcriptomics studies on 160 genes from lesional skin biopsies from these cases and from cases of 5 healthy canines, in order to identify any significant differences that may reflect oncogenesis and immunopathogenesis. We further sought to determine if the oncogenic processes of EL and CTCL are conserved across humans and canines by comparing our Nanostring data to previously published datasets. Similar chemokine profiles were observed in dog EL and human CTCL, and we are performing ongoing analyses to validate potential biomarkers and drivers of disease. Future studies exploring the oncogenesis of spontaneous malignancies in companion animals will expand our understanding of these disorders, and will be useful in developing targeted therapies, repurposing drugs for veterinary and human medicine, and predicting disease prognosis and treatment response.

Th178. Role of Chronic Stress on Anti-tumor T-cell Responses in Ovarian Cancer

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A cancer diagnosis can increase stress hormones and lead to altered psychological states, such as chronic stress. Work from our team suggests that chronic stress promotes an increased inflammatory response. Preliminary data show an altered CD4⁺/CD8⁺ T-cell ratio and a heterogeneous expression of exhaustion markers in advanced disease. Therefore, we hypothesized that chronic stress results in loss of effector T-cell responses and increased exhaustion markers. Here, we measured T-cell activity markers, cytokine, and cortisol levels from ovarian cancer ascites. We also utilized pre-clinical mouse models of ovarian cancer to determine if chronic stress affects T-cell effector functions and tumor progression. Results showed a significant increase in pro-inflammatory cytokines: Eotaxin, IL-6, and IL-7, in chemo-resistant and recurring tumors. Also, IL-7 was significantly associated with high cortisol levels in patients, while anti-tumor cytokines IP-10 and IFN-gamma were significantly downregulated. Epinephrine treatment of CD8⁺ T-cells isolated from ascites led to decreased co-expression of CD38 and Granzyme B, suggesting a role for stress hormones in T-cell activity suppression. Furthermore, daily restraint stress led to significant induction of tumor growth in ID8 and IG10 ovarian tumor-bearing mice. In sum, our data suggest that stress hormones are associated with the upregulation of pro-inflammatory cytokines, downregulation of anti-tumor cytokines, and decreased CD8⁺ T-cell function that can lead to reduced immunotherapy efficacy and tumor progression. Future experiments will measure stress hormone metabolites, metanephrine, and normetanephrine, and their role in pro-inflammatory responses and PD-1 inhibition targets in ascites from ovarian cancer patients.

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Infectious Diseases

W132. A Recombinant BCG Vaccine is Safe and Immunogenic in Neonatal Calves and Reduces the Clinical Disease Caused by the Respiratory Syncytial Virus

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The human respiratory syncytial virus (hRSV) is the leading agent of acute lower respiratory tract infections in children. To date, licensed vaccines to prevent hRSV are not available. A recombinant BCG vaccine expressing the Nucleoprotein of hRSV (rBCG-N-hRSV) protects mice against hRSV infection, eliciting humoral and cellular immune protection. Further, this vaccine was recently shown to be safe and immunogenic in human adult volunteers. To investigate the safety and immunogenicity of this vaccine in a neonatal model of RSV infection, we conducted two independent experiments using the neonatal calf model. **Methods:** Newborn, colostrum-replete Holstein calves were either vaccinated with rBCG-N-hRSV, WT-BCG, or left unvaccinated, and then challenged with bovine RSV strain 375 via aerosol route. **Results:** Vaccination with rBCG-N-hRSV was safe and well tolerated, with neither systemic adverse effects nor evidence of vaccine-enhanced disease following bRSV challenge. Administration of rBCG-N-hRSV in calves increased virus-specific IgA and virus-neutralization activity in nasal fluid and increased the proliferation of virus- and BCG-specific CD4⁺ and CD8⁺ T cells in PMBCs and lymph nodes at 7dpi. Further, rBCG-N-hRSV vaccinated calves developed reduced clinical disease in comparison to unvaccinated control calves, although neither pathology nor viral burden were significantly reduced in the lungs. Interestingly, we also observed reduced clinical disease in WT vaccinated calves. **Discussion:** These results suggest that the rBCG-N-hRSV vaccine is safe and immunogenic in neonatal calves. These data from a newborn animal model supports the rBCG-N-hRSV vaccine as candidate for infant immunization against hRSV.

Th5. Preservation of Lymphocyte Functional Fitness in Perinatally Infected HIV+ Pediatric Patients with Sub-optimal Viral Control

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Background: Host-pathogen dynamics associated with HIV infection are quite distinct in children and adults. In this study we interrogated the functional fitness of the immune response in two US based cohorts of perinatally infected HIV+ patients with early anti-retroviral therapy (ART) initiation but divergent patterns of virologic control. We hypothesized that sub-optimal viral control in perinatally-infected HIV+ patients on ART will compromise their immune functional fitness

Methods: The immune responses in six study subjects in each of the two HIV+ cohorts were benchmarked against the responses measured in eleven age-range matched, uninfected healthy control subjects. Lymphocyte responses were examined by intra-cellular cytokine secretion, degranulation assays as well as phosflow. A subset of these data were further queried by automated dimensionality reduction and clustering algorithms. Finally, we evaluated the humoral immune responses to four childhood vaccines in all three cohorts.

Results: Our results demonstrated that contrary to expectations pediatric HIV+ patients with sub-optimal viral control displayed no significant deficits in immune functional fitness. In fact, the patients that displayed better virologic control lacked functional Gag-specific T cell responses and compared to healthy controls they displayed signaling deficits and an enrichment of mitogen-stimulated CD3 negative and positive lymphocyte clusters with suppressed cytokine secretion.

Conclusions: These results highlight the immune resilience in HIV+ children on ART with sub-optimal viral control. With respect to HIV+ children on ART with superior viral control, our data indicate that this cohort might potentially benefit from vaccines that could selectively stimulate HIV (including low-fitness variants) specific-CD8 T cells.

Th18. Immune Dysregulation in Myalgic Encephalomyelitis/Chronic Fatigue (ME/CFS) and Long COVID-19 Syndromes: CD8 T-cell Over Activation and Exhaustion, Increased CD4⁺CD8⁺ T-cells and Aberrant Cytokines

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Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a complex disorder affecting numerous organ systems and biological processes. ME/CFS may be preceded by infection, and the chronic manifestation of illness may represent an altered host response to infection, or an inability to resolve inflammation. Here, we hypothesize that in ME/CFS an aberrant response to an immunological trigger like infection may result in a dysregulated immune system, leading to immunosuppression. Long COVID-19 syndrome patients have symptoms similar to ME/CFS. In both patient groups we observed altered expression of exhaustion markers like CTLA4 and 2B4, decrease in CD8 T-cell number, and function, particularly IFN γ /TNF production. The long COVID-19 patients had evidence of sustained activation of both T-cell populations with increased CD38 and HLA-DR. This was associated with a compensatory increased frequency of activated CD4⁺CD8⁺ T-cells. In chronic ME/CFS donors both T-cell populations were spontaneously producing cytokines, subdividing into two types: (1) FoxP3⁺ cells producing IL9 (female donors), (2) IL17-producing cells (male donors). The long COVID-19 patients showed the type 1 aberrant cytokine profile. These results are consistent with immune dysregulation with over activation and exhaustion of CD8 T-cells as observed either in chronic viral infections or tumor environments. The observed exhaustion was associated with a compensatory increase in activated CD4⁺CD8⁺ that make unusual cytokines known to interact with the nervous system. These findings identify potential biomarkers and mechanisms driving the immunopathogenesis of ME/CFS leading to future therapies and suggest long COVID-19 may cause ME/CFS. (Funding: Ramsay Award, Solve ME/CFS Initiative; NIH R01AI159314).

Th45. Epigenetic Regulation of Macrophage Priming During *Staphylococcus aureus* Infection

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Staphylococcus aureus is the leading cause of skin and skin structure infection (SSSI), a primary portal of entry for invasive infection. Patients with SA SSSI have a high 1-year recurrence. The goal of this project is to determine epigenetic mechanisms of protective memory against SA SSSI. We have shown innate memory, specifically macrophages (Mf), protects mice against SA SSSI evidenced by decreased bacterial burden in skin and distal organs. Priming potentiated the opsonophagocytic killing of SA by bone-marrow derived Mf (BMDM) in vitro, and their adoptive transfer into naïve skin afforded protective efficacy in vivo. Here, we investigated epigenetic mechanisms of anti-SA efficacy in murine BMDMs. DNA from naïve (uninfected) or primed (SA SSSI) were analyzed for differential methylation regions (DMR) using reduced representation bisulfite sequencing (RRBS). Present findings indicate the protective memory afforded by BMDM was mediated by epigenetic modifications in immune-related genetic regions. Primed BMDM exhibited significantly different DMRs as compared to naïve BMDM. Specifically, 268 DMRs occurred in distal upstream regulatory regions to known genes while 443 DMRs occurred within gene coding regions. Pathway analyses revealed DMRs predominant in genes integral to macrophage function, such as antigen presentation, phagocytosis, cytokine signaling and oxidative killing. These findings reveal epigenetic mechanisms of macrophage innate memory against recurrent MRSA infection. Functional testing of these genes in response to SA infection is needed to confirm their protective role. These insights may provide new targets for vaccine and immunotherapeutic development against MRSA.

Th53. Effect of N-terminal Region of Human Parvovirus B19 VP1 Unique Region on Cardiac Injuries in Naïve Mice

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Human parvovirus B19 (B19V) has been linked to cardiac disorders, and the immune response induced by B19V is considered to be an important pathogenic mechanism of myocarditis or Dilated cardiomyopathies (DCM). Recently, many studies have shown that the unique region of VP1 (VP1u) plays a role in inducing heart damage. However, the pathological function of B19V-VP1u in inducing cardiac injuries is still unclear. This study investigated the role of B19V-VP1u and different regions of B19V-VP1u in the induction of cardiac injuries in naïve mice. A significantly higher MMP-9/MMP-2 ratio and elevated expression of inflammatory cytokines, including IL-6 and IL-1b, were detected in the left ventricles of the mice injected with B19V-VP1u (1-227 aa), B19V-VP1u-A (1-60 aa), B19V-VP1u-B (61-129 aa), and B19V-VP1u-C (130-195 aa), accompanied by increased expression of p-ERK and p-P38. Notably, significantly lower expression levels of IL-6 and IL-1b, have observed in the left ventricles of the mice injected with B19V-VP1uD (196-227 aa). In addition, significantly increased amounts of ANP, H-FABP, and CKMB have detected in the left ventricles of the mice injected with B19V-VP1u, B19V-VP1u-A, and B19V-VP1u-B, as well as infiltrated lymphocytes. Significantly higher serum IL-1b, IL-6, TNF- α , and IFN- γ levels were also detected in the mice injected with B19V-VP1u, B19V-VP1u-A, and B19V-VP1u-B. These findings showed for the first time that the N-terminal region (1-129 aa) of B19V-VP1u induces cardiac injury markers and provides clues for understanding the possible functional regions within B19V-VP1u.

Th66. Activation of Monocytes by Dengue NS1 Antigen Leads to the Production of Secretory and Cytoplasmic Phospholipase A2 Enzymes

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Introduction & objective: Vascular leak is the hallmark of severe dengue infection. Platelet activating factor (PAF) and NS1 protein of the dengue virus (DENV), have shown to play a role in vascular leak. Since cytoplasmic (cPLA₂) and secretory phospholipase (sPLA₂) generate PAF, we sought to investigate if NS1 induced these enzymes from monocytes.

Methods: THP-1 cells and primary human monocytes (n=4) were co-cultured with varying concentrations of DENV1 NS1 protein and the activity of cPLA₂ and sPLA₂ were measured in cell lysates and culture supernatants respectively at different times points. Lipopolysaccharide (LPS) was used as a positive control and all experiments were done in triplicate and on two different occasions.

Results: The sPLA₂ activity gradually rose in the THP-1 culture supernatants and was highest at 24 hours, while cPLA₂ activity was highest at 12 hours and then gradually declined. At 24 hours, sPLA₂ levels were significantly higher in THP-1s (p=0.03) co-cultured with NS1 (500ng/ml) compared to the untreated wells. The cPLA₂ levels were also significantly higher (p=0.0002) at 12 hours in THP-1 cell lysate, compared to the untreated wells. The

activity of sPLA₂ was significantly higher ($p=0.0001$) in primary human monocytes at 6 hours when co-cultured with NS1 (500ng/ml).

Conclusion: Dengue NS1 appears to induce sPLA₂ and cPLA₂ activity in THP-1s and primary human monocytes and thereby possibly inducing the production of PAF, contributing to vascular leak.

Th67. Antibody and T Cell Responses to a Single Dose of the AZD1222/Covishield Vaccine in Previously SARS-CoV-2 Infected and Naïve Health Care Workers in Sri Lanka

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Background: To determine the immunogenicity of a single dose of the AZD1222/Covishield vaccine, we assessed the immunogenicity, among health care workers (HCW) in Sri Lanka.

Methods: SARS-CoV-2 antibodies was carried out in 607 naïve and 26 previously infected HCWs 28-32 days following a single dose of the vaccine. Haemagglutination test (HAT) for antibodies to the receptor-binding domain (RBD) of the wild type virus, B.1.1.7, B.1.351 and the surrogate neutralization assay (sVNT) was carried out in 69 naïve and 26 previously infected individuals. Spike protein (pools S1,S2) specific T cell responses were measured by ex-vivo ELISpot IFN γ assays in 76 individuals.

Findings: 92.9% of previously naïve HCWs seroconverted to a single dose of the vaccine, irrespective of age and gender; and ACE2 blocking antibodies were detected in 67/69 (97.1%) previously naïve vaccine recipients. Although high levels of antibodies were found to the RBD of the wild type virus, the titres for B.1.1.7 and B.1.351 were lower in previously naïve HCWs. Ex-vivo T cell responses were observed to S1 in 63.9% HCWs and S2 in 31.9%. The ACE2 blocking titres (sVNT) significantly increased ($p < 0.0001$) from a median of 54.1 to 97.9% of inhibition, in previously infected HCWs and antibodies to the RBD for the variants B.1.1.7 and B.1.351 also significantly increased. **Interpretation:** Single dose of AZD1222/Covishield vaccine was highly immunogenic in previously naïve individuals inducing antibody levels greater than following natural infection. In infected individuals, a single dose induced very high levels of ACE2 blocking antibodies and antibodies to RBDs of SARS-CoV-2 variants of concern.

Th115. Mucosal Parameters Predict the Clinical Outcome of Anal Intraepithelial Neoplasia in Men Living with HIV

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Anal HPV infection and anal intraepithelial neoplasia (AIN) are prevalent among men who have sex with men (MSM). We investigated the mucosal correlates of AIN clinical outcome in HIV-positive, ART-treated MSM. For each participant, anal biopsies were collected from both AIN-free and AIN-confirmed tissue sites at baseline, with follow up 6-12 months later. Mucosal T lymphocyte activation (HLA-DR and CD38 co-expression) was defined by flow cytometry. Blood HPV-specific T cell responses were assayed by ELISpot, and anal microbiome by bacterial 16S sequencing. Statistical analysis used SPSS, and LEfSe analysis the Galaxy online platform.

Natural AIN regression occurred in 11/21 participants. Regression was associated with baseline intra-lesional activation of T cells (CD8⁺ p=0.006; CD4⁺ p=0.013), with no difference seen in T cells derived from AIN-free tissues (CD8⁺ p=0.673; CD4⁺ p=0.778). AIN tissue-specific T cell activation resolved after AIN regression (p=0.018) while the level of activation in AIN-free tissue remained unchanged (p=0.612). Anorectal swab cytokine levels were similar in regressors and non-regressors (IL-10 p=0.696; IFN- γ p=1.000), suggesting that inflammation associated with natural AIN regression is highly tissue localized. PBMCs from regressors demonstrated much stronger IFN- γ ELISpot responses to HPV peptides (E6 p=0.001; E7 p=0.002), and blood HPV-specific responses correlated strongly with intra-lesional T cell activation (ρ =0.622; p=0.008). Non-regressors had more anti-inflammatory bacteria in the anorectal mucosa, and the relative abundance of these bacteria negatively correlated with intra-lesional T cell activation (CD8⁺ ρ =-0.567, p=0.011; CD4⁺ ρ =-0.646, p=0.004), suggesting a potential role for the anorectal microbiome in modulating AIN immune clearance.

Th156. Evaluating the Immunogenicity and Vaccine Efficacy of Three Variants of a Human Filariasis Vaccine Candidate, rBmHAXT

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During the manufacturing process of a multivalent fusion protein vaccine (rBmHAXT) for Lymphatic Filariasis (LF), we observed significant protein loss due to protein aggregation. To overcome this, we synthesized three variants of rBmHAXT, (1) variant with key cysteine mutations (Δ Cys), (2) variant with linker sequences within the rBmHAXT sequence (GS), and (3) a variant with cysteine mutation and addition of linker sequences (Δ Cys+GS). In this study, we evaluated the immunogenicity and vaccine efficacy of these variants to select the best vaccine formulation for cGMP manufacturing. We immunized groups of five (5) Balb/c mice subcutaneously with 25 μ g of the respective antigen variant along with 1 μ g of GLA plus alum adjuvant. Three doses were given at 15 days interval. Control animals received adjuvant only. Following three vaccinations, all animals developed high titer of anti-rBmHAXT IgG, IgG1, IgG2, and IgG3 serum antibodies thereby confirming that all three variants are highly immunogenic in mice. To determine the vaccine efficacy, we challenged all vaccinated animals with infective larvae (L3) of *Brugia malayi*. Our results showed that vaccination with the Δ Cys variant gave 97% protection, which was comparable to the level of protection (90%) conferred by the rBmHAXT parent vaccine. Immunization with GS and Δ Cys+GS variant also gave significant protection compared to the adjuvant control group, however, was not as high as those in the Δ Cys immunized mice. Overall, our studies demonstrated that the rBmHAXT variants are highly immunogenic and retained the high vaccine efficacy as the parent rBmHAXT vaccine. Studies supported by R44 AI140708-02.

Inflammatory Diseases

Staphylococcus Enterotoxin Beta Alone Can Generate Robust IL-13 and IFN- γ Production from Circulating T Cells of Adult Atopic Dermatitis Patients

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Background

Atopic dermatitis (AD) is a chronic inflammatory skin disorder dominated by TH2 cytokines. There are few adequate models to study AD, and these do not effectively recapitulate disease heterogeneity. We developed a human *in vitro* model for generating robust type 2 cells. Here, we demonstrate how exposure to Staphylococcus enterotoxin B (SEB) alone can lead to expansion of type 2 cells from AD blood.

Methods

PBMCs isolated from adult AD patients were cultured for 7 days in the presence of *S. aureus*-derived superantigen (SEB) +/- thymic stromal lymphopoietin (TSLP) to replicate the AD inflammatory cutaneous environment. Cellular immunophenotyping was performed on harvested cells to detect canonical TH2 markers as well as intracellular cytokine production.

Results

Exposure to TSLP+SEB sustains high numbers of AD patient memory CD4⁺ CD45RO⁺ T cells *in vitro* compared to control or SEB alone; CD4⁺ T cells expressed high levels of activation (CD69, HLA-DR) and costimulatory (ICOS, OX40) markers and Th2-transcription factors (GATA3, pSTAT6). Discrete populations of IL-13⁺ IFN- γ ⁺ and IL-13⁺ IFN- γ ⁻ cells were found along with a small number of double-positive cells. CD8⁺ T cells produced higher levels of IFN- γ compared to CD4⁺ T cells. Notably, SEB alone was also sufficient to generate these Th1 and Th2 cytokines from patient blood.

Conclusion

Our *in vitro* AD model allows for the robust expansion of circulating IL-13⁺ Th2 cells from AD patient blood, thought to be key to disease pathogenesis, and facilitates investigation of various skin-derived AD-associated factors such as *S.aureus*-derived SEB.

W30. MATISSE: A Novel Method Allowing Single-cell Imaging Mass Cytometry Analysis of Immune, Stromal and Epithelial Cells in Heterogeneous Patient Tissues

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Imaging mass cytometry (IMC) has emerged as a powerful method for visualizing and assessing cellular heterogeneity in tissues; however, resolving individual cells within the tissue sample, i.e. single cell segmentation, remains challenging. In tissue of patients with inflammatory bowel disease (IBD), which contains severely inflamed and dysplastic tissue, it was impossible to adequately identify and analyze cells in dense regions and the epithelial layer. To address this problem, we developed MATISSE, a method that combines fluorescence microscopy and multiplex IMC to achieve improved segmentation over the current state-of-the-art, thus enabling more complete identification of epithelial cells, stromal cells, and infiltrating immune cells in

colorectal tissue sections. Together, MATISSE allows detailed multiparameter visualization and single cell analysis of the inflammatory processes and interplay between epithelium, fibroblasts, and immune cells in IBD tissue. Currently, we are applying MATISSE to a cohort of IBD patients at high risk for development of colitis associated cancer, to identify risk factors for cancer initiation.

W57. Soluble BTLA is Induced Following Targeting of BTLA *In-vivo* by ANB032, a Novel BTLA/HVEM Modulator Therapeutic Antibody for the Treatment of Autoimmune Disease

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B and T lymphocyte attenuator (BTLA) is an immune checkpoint molecule that contributes to the regulation of T cell, B cell and dendritic cell function. The role of BTLA in autoimmune disease and cancer has been demonstrated. Studies have described soluble checkpoint molecules, including BTLA, in serum or in tumors of diseased patients that correlates negatively with disease outcome, suggesting immune suppression.

We previously disclosed ANB032, a novel anti-BTLA monoclonal antibody that modulates BTLA signaling. In cynomolgus monkeys, we observed elevated levels of soluble BTLA (sBTLA) in the serum that correlated to ANB032 exposure and reduced BTLA expression on T and B cells. The discovered sBTLA represents the intact extracellular portion of BTLA.

ANB032 was highly efficacious in a human PBMC-NOD-*scid* IL2 γ^{null} (NSG) graft versus host disease model. ANB032 led to reduction of BTLA expression on human T cells and human sBTLA detection in the serum. Flow cytometric analysis of human T cells demonstrated that ANB032 dose-dependently reduced pathogenic T cell activation and expansion.

We found that BTLA is a substrate of cleavage by PR3, suggesting that BTLA is naturally shed by proteolytic cleavage. We propose a mechanism whereby enzymatic cleavage of BTLA is enhanced by monoclonal antibody targeting, which may play a role in the anti-inflammatory therapeutic effect of ANB032.

W62. Dissecting the Regulatory Immune Landscape of Adipose Tissues by Mass Cytometry After Vertical Sleeve Gastrectomy in Nonhuman Primates (NHPs)

Amar Singh¹, Julia Nugent¹, Naoya Sato¹, Parthasarathy Rangarajan², Ravi Masuria², Anna Tran², Laura Hocum-Stone³, Melanie Graham⁴, Sayeed Ikramuddin¹, Bernhard Hering¹ and Sabarinathan Ramachandran¹

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Immune alterations associated with vertical sleeve gastrectomy (VSG), the most common bariatric surgery procedure, remain largely undefined. The immune landscape of peripheral blood lymphocytes (PBLs), visceral and subcutaneous adipose tissues (VAT/SAT) collected pre-and post-VSG in a spontaneously obese NHP model were assessed using a primatized custom 36 antibody CyTOF panel. Unsupervised clustering analysis (tSNE/FlowSOM) was performed to dissect the complex phenotypic heterogeneity of myeloid cells (MCs:CD3-CD20-lym) and Treg cells (Tregs:CD4+CD25+CD127-lym). Of the twelve distinct cellular clusters (C) of MC, VSG-induces significant reduction in activated (C1) and resting (C2) MCs in both AT compartments. In VAT only, we observed a substantial increase in MCs showing phenotypic signatures of MDSCs (C3, ~1.5-fold), anti-

inflammatory cells (C9, ~7-fold), and DC-10(C10, 2-fold). Of the six phenotypically distinct clusters of Tregs, resting (C1), and highly activated (C3, C4) Tregs were substantially increased in VAT (~47- and 3-fold), SAT (1.1- and 1.5-fold) and PBLs (0.65-, >2-fold) after VSG. Activated nonmigratory, resting and memory Tregs (Clusters 2, 5 and 6) remain unchanged in PBLs and decreased in AT post VSG. Clusters 3 and 4 enriched with PPAR γ ^{hi} FoxP3^{hi} Tregs demonstrated increased expression of CD11c/CD49b/CD69⁺ along with variable expression levels of CD11b/CD103/CD107a/Tbet/HLA-DR, suggesting the expansion of highly activated tissue-resident Tregs after VSG. These observations demonstrate that VSG induces heterogeneous subsets of MCs and Tregs with distinct phenotypes in AT. Via serial sampling of portal/peripheral blood and VAT/SAT, our preclinical model facilitates the interrogation of VSG-induced immunological alterations and the identification and generation of novel therapeutic targets.

W63. Comprehensive Immune Survey of the Visceral Adipose Tissue (VAT) by Mass Cytometry After Vertical Sleeve Gastrectomy (VSG) in Nonhuman Primates (NHPs)

Amar Singh¹, Julia Nugent¹, Ravi Masuria², Anna Tran², Naoya Sato¹, Parthasarathy Rangarajan², Laura Hocum-Stone³, Melanie Graham⁴, Sayeed Ikramuddin¹, Bernhard Hering¹ and Sabarinathan Ramachandran¹

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Obesity is associated with chronic low-grade inflammation and increased risk for life-threatening diseases such as diabetes and cancer. VAT accumulation is strongly linked to metabolic syndrome in humans. While it's widely acknowledged that bariatric surgery is the most effective therapy for the treatment of obesity and metabolic syndrome, it remains largely undetermined whether immune mechanisms contribute to these benefits. Laparoscopic VSG was performed in spontaneously obese NHPs. Peripheral blood lymphocytes (PBLs) and VAT collected pre-/post-VSG were assayed for frequency and phenotype of regulatory and effector immunocytes using a customized 36 antibody NHP CyTOF panel. VSG induced an overall regulatory phenotype in VAT with an increase in anti-inflammatory macrophages (ratio M2/M1, >6-fold), DC-10 (>1.5-fold) and B10 cells (>2-fold) in VAT. The number of Tregs (3-fold) and Tr1 (2-fold) cells substantially increased. Notably, after VSG VAT Treg cells displayed a highly activated phenotype (LAG-3⁺CD69⁺Ki67⁺), PPAR γ ⁺ Tregs expanded (3-fold), and serum IL-33 increased, along with >2-fold reduction of activated (HLA-DR⁺CD69⁺) monocytes, DC cells as well as activated (HLA-DR⁺CD38⁺) and proliferating (Ki67⁺) T and B cells. VSG promotes a robust anti-inflammatory and suppressive regulatory environment in VAT dominated by highly activated Tregs and anti-inflammatory macrophages. The elevated peripheral IL-33 level and VAT-PPAR γ ⁺ Tregs indicate a possible role of the IL-33/ST-2/PPAR γ axis in the differentiation of these tissue Tregs. This exploratory data in a translational preclinical model provides support for subsequent studies designed to deeply investigate the unique mechanisms of metabolic surgery that can be targeted to improve outcomes of patients with obesity.

W71. The Ratio of Interleukin-6 to Transforming Growth Factor-beta is Associated with Obesity and Systemic Inflammation in Children

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Childhood obesity is a risk factor for chronic conditions such as type 2 diabetes. Interleukin (IL)-6 is elevated in inflammatory disorders, including obesity-associated inflammation, and is associated with insulin resistance. Transforming growth factor (TGF)- β is an anti-inflammatory cytokine, however, elevated levels occur in patients with metabolic syndrome. The co-presence of IL-6 and TGF- β can lead to a pro-inflammatory state. Thus, IL-6/TGF- β balance might be important in immunometabolic homeostasis. Here, we measured the IL-6 to TGF- β ratio in children and assessed its association with metabolic and inflammatory biomarkers. A total of 77 children (aged 5-16 years) with body mass index (BMI) percentiles in the normal-weight ($n=13$), over-weight ($n=2$) and obese ($n=62$) categories were included. Plasma levels of biomarkers were quantified using ELISAs. The IL-6 to TGF- β ratio was directly correlated with BMI percentile ($r=0.44$, $P=0.01$, $n=33$), BMI Z-score ($r=0.42$, $P=0.01$, $n=33$), waist circumference ($r=0.46$, $P=0.03$, $n=22$), body fat (%) ($r=0.55$, $P=0.0015$, $n=30$), and fat to muscle ratio ($r=0.72$, $P=0.0001$, $n=23$), but inversely correlated with muscle (%) ($r=-0.73$, $P=0.0001$, $n=23$). IL-6 to TGF- β ratio was directly correlated with leptin ($r=0.4$, $P=0.015$, $n=36$) but inversely correlated with total cholesterol ($r=-0.47$, $P=0.011$, $n=28$) and low-density lipoprotein ($r=-0.44$, $P=0.025$, $n=26$). IL-6 to TGF- β ratio was directly correlated with C-reactive protein ($r=0.5$, $P=0.0075$, $n=27$), calprotectin ($r=0.36$, $P=0.029$, $n=36$), and monocyte chemoattractant protein-1 ($r=0.41$, $P=0.012$, $n=36$). In conclusion, IL-6 to TGF- β ratio is correlated with metabolic and inflammatory biomarkers, and thus, an imbalance of these cytokines might be an important factor in metabolic disease development.

W79. Increased Frequency of CD4⁺PD1⁺ICOS⁺ T Cells in IBD Tissue

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Crohn's Disease (CD) and Ulcerative Colitis (UC) are heterogeneous diseases of the gastrointestinal tract with poorly characterized disease pathogenesis. In order to better understand the disease pathophysiology and identify cell subsets of interest/biomarkers, matched peripheral blood and Lamina Propria Leukocytes (LPLs) were obtained from Crohn's Disease ($n=31$), Ulcerative Colitis ($n=17$) and non-IBD controls ($n=17$) from tissue resections of the gastrointestinal tract. In addition, normal adjacent tissues (NAT) with matched diseased tissues were acquired for CD ($n=11$) and UC ($n=3$) donors. Phenotyping of these samples was performed using high parameter flow cytometry panels. Analysis shows that the percentage of CD4⁺PD1⁺ICOS⁺ cells are significantly elevated in the peripheral blood and Lamina Propria (LP) of CD and UC patients vs non-IBD controls, and in the disease tissue compared to NAT samples. The phenotype of these cells suggest they could belong to a T follicular helper or T peripheral helper subset, which have been associated with autoimmunity. However, these cells show negative correlations with the percentage of Switched Memory B cells (CD19⁺IgD⁺CD27⁺) and plasmablasts (CD19⁺CD38⁺CD27⁺CD20⁻) in both CD and UC LPLs. Furthermore, the percentage of CD4⁺PD1⁺ICOS⁺ cells is negatively correlated with the percentage of IFN γ ⁺ and TNF α ⁺ CD4 memory (CD4⁺CD45RO⁺) T cells in CD LPLs. Finally, these CD4⁺PD1⁺ICOS⁺ cells show positive correlations with the percentage of Tregs in both CD and UC LPLs. In order to further characterize this T cell subset and understand better their significance in disease, high parameter flow cytometry and functional assays are in progress.

W111. Development of a Human Skin Commensal Microbe for Bacteriotherapy of Atopic Dermatitis and Use in a First-in-human Randomized Clinical Trial

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Staphylococcus aureus frequently colonizes patients with atopic dermatitis (AD) and exacerbates disease by promoting inflammation and barrier disruption. However, treatment with antibiotics does not typically improve this disease. In this study, we examined if topically applied bacteria selected from healthy human skin can inhibit the detrimental functions of *S. aureus* and assess the safety of this approach in human subjects with AD. In mice, topical application of *Staphylococcus hominis* strain A9 (ShA9) selectively killed *S. aureus*, expressed an autoinducing peptide (AIP) that inhibited *S. aureus* *psma* expression, improved skin barrier function and decreased T_H2-mediated inflammation. To assess safety a first-in-human, double-blinded, randomized trial was conducted for one week on 54 *S. aureus* positive AD subjects randomized 2:1 ShA9 to vehicle. The results showed subjects treated with ShA9 had a lower frequency of any adverse events ($P=0.044$) and a decrease in live *S. aureus* ($P < 0.001$) compared to vehicle. The presence of mRNA for AIP on subjects treated with ShA9 correlated with decreased *psma* mRNA on the lesional skin ($P < 0.001$). Clinical improvement correlated with a decrease in either *S. aureus* or *psma* mRNA expression in all subjects. 21 of 36 subjects with *S. aureus* directly killed by ShA9 demonstrated clinical improvement with decreased local eczema inflammation severity (EASI) ($P=0.043$) and systemic disease severity (SCORAD) ($P=0.047$). These observations illustrate how bacteriotherapy with a multifunctional human commensal microbe can benefit AD and has a low risk of short-term adverse events.

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W131. Development of pH-Sensitive Microgels for Oral Delivery of Filarial Cystatin for the Treatment of Inflammatory Bowel Disease

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Ulcerative colitis is a type of inflammatory bowel disease (IBD) that affects around 6.8 million people globally. We have previously reported that, intraperitoneal administration of recombinant cystatin from filarial parasite *Brugia malayi* (rBmaCys), alleviated the clinical and pathological symptoms of colitis in mice. Since the ultimate goal is to develop cystatin for human use, intraperitoneal administration is not a suitable route. Hence, in this study, we developed pH-sensitive microgel-encapsulated rBmaCys for oral delivery targeting to the colon. Microgels were prepared by dropping 300µg of rBmaCys protein containing 2% sodium alginate to ice-cold 2% calcium chloride solution. The average size of rBmaCys-microgels were 296.87µm. The protein loading capacity of microgels was estimated to be 34.36%. The in-vitro protein release study in physiological gastrointestinal pH showed maximum 40% of protein release at simulated colonic pH7.4 after 24 hours. The protein release in stimulated intestinal fluid at pH6.8 was 32.56%, whereas just 12% protein was released at the simulated stomach pH of 1.2. The swollen size of rBmaCys-microgels was highest (average 1013.11 µm) at the colonic pH. Whereas, intra-gastric administration of FITC-labeled microgels into mice showed maximum fluorescence in the terminal small intestine advancing to the caecum and colon. Together, our findings suggest that this strategy of using pH-responsive oral microgels can be effectively used for the treatment of IBD using rBmaCys. This approach can be further developed for the delivery of microgel loaded biotherapeutic molecules. Study supported by Blazer Foundation of Rockford.

W148. Lymphocyte Subpopulations Analysis in Psoriatic Patients Treated with Biological Drugs

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Background

Psoriasis is an autoimmune disease characterized by scaly and itchy skin lesions. In recent years the emergence of new biological drugs has revolutionized its treatment especially in the most severe cases of the disease. These new drugs work by blocking the major cytokines involved in the inflammatory response of psoriasis, thereby achieving a drastic reduction in lesions and improving patients' quality of life.

Aim

To analyze changes in T-cell subsets in peripheral blood of patients with psoriasis treated with biological drugs.

Methods

Using flow cytometry, we evaluated effector and regulatory T cells subpopulations in fresh peripheral blood. We analyzed patients with psoriasis treated with Adalimumab (anti-TNF α , N=20), Ustekinumab (anti-IL23, N=26) Secukinumab (anti-IL17, N=9), patients with active psoriasis without biological treatment (N=14) and controls without psoriasis (N=21).

Results

Patients with active psoriasis presented a lymphocyte profile characterized by decreased levels of Th1 central memory (CM), Th2 CM, and regulatory T cells with respect to treated patients and controls without psoriasis. Whereas patients treated with Adalimumab and Ustekinumab showed T-cell profiles similar to controls without psoriasis.

Moreover, patients receiving Secukinumab had a lymphocyte profile similar to controls but with a significant decrease of the number of Th17 effector memory (EM) and Th1 / Th17EM subpopulations.

Conclusion

These findings provide an overview of the effects of the biological treatments over the lymphocyte subsets and can serve as a starting point for the selection of the most appropriate drug and for the evaluation of the risk of opportunistic infectious diseases in psoriatic patients.

W160. Inflammatory Adipocytes and Macrophages in the Visceral Adipose Tissue of Obese Human Subjects Suppress the Development of Treg Cells

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Adipose tissue (AT) inflammation results from multiple interactions among adipocytes and immune cells. Mounting evidence indicates that obesity is associated with the induction of inflammatory macrophages and adipocytes with altered expression of MHC class II, PPAR γ , HIF-1 α , ADAM17 and adipokines. Conversely, tissue Tregs are critical to the regulation of VAT inflammation and maintenance of a lean phenotype. An *in vitro* co-culture system was utilized to explore the role of adipocytes/macrophages in Treg generation. Adipocytes and macrophages isolated from visceral adipose tissue (VAT) biopsies (n=5, obese human subjects) were co-cultured for 5 days with conventional human CD4⁺ T cells (n=2) treated with lithocholic acid (LCA) or Treg differentiation factor (TRDF: TGF β , IL-10, retinoic acid). The frequency of Tregs (CD4⁺FoxP3⁺) was analyzed by flow cytometry. VAT adipocytes from obese subjects significantly inhibited Treg generation induced by LCA up to 51% (25.4 \pm 13.2%) and Treg generation induced by TRDF up to 46% (23.9 \pm 10.2%). The inhibition of LCA- or TRDF-induced Treg generation was more prominent in the presence of VAT macrophages. The addition of VAT macrophages to co-cultures suppressed LCA-induced Treg generation by up to 48% (36.9 \pm 6.8%) and TRDF-induced Treg generation was inhibited up to 42% (27.5 \pm 6.5%). Our *in vitro* coculture studies suggest that adipocyte/macrophage-T cell crosstalk significantly modulates the immune microenvironment in the VAT through regulation of tissue Treg development. Identifying the antigen(s) and the pathways through which adipocytes/macrophages interact with the immune cells in the AT microenvironment can identify new potential therapeutic targets to specifically block obesity-induced adipose inflammation.

Th69. An Adoptive Platelet Transfer Assay to Study Platelet-mediated Immune Regulation in NASH Mice

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Immune modulation by platelets has recently been described in the progression of nonalcoholic steatohepatitis (NASH), an important liver cancer risk factor. However, their mechanistic impact on immune regulation is largely unknown. Here, we created an experimental framework to directly explore platelet-dependent immune regulation by combining adoptive platelet transfer with diphtheria toxic (DT)-induced platelet depletion in PF4-DTR mice in the setting of NASH.

Mice were fed with methionine/choline deficient diet to induce NASH. Platelets were isolated from blood of healthy or NASH mice, and labeled with fluorescent dye, CFSE. Aggregation of CFSE-labeled platelets was overcome with the addition of platelet degranulation inhibitors, citrate dextrose, PGI₂, PGE₁ and apyrase, during CFSE labeling and centrifugation. PF4-DTR mice were given subcutaneous DT toxin injection to deplete endogenous platelets. CFSE-labeled platelets were transferred into recipient mice by tail vein injection. Circulation kinetics of endogenous (CD41⁺CFSE⁻) or transferred platelets (CD41⁺CFSE⁺) were assessed by flow cytometry.

Donor platelets from NASH mice demonstrated a significantly shorter half life compared to those from healthy mice when transferred into healthy recipient mice. In the context of NASH, DT toxin injection was able to cause a complete depletion of endogenous platelets for at least 10 days. Single transfer of platelets can restore and maintain >50% of original platelet levels for two days after DT treatment of PF4-DTR mice with NASH.

This protocol serves as a framework to study the immune regulatory capacity of platelets in NASH. In addition, this protocol can be expanded beyond NASH and has much broader applications.

Th81. Vixarelimab, An Anti-OSMR-beta Fully Human Monoclonal Antibody, Inhibits OSM-induced Activation of Intestinal Fibroblasts in TNF-refractory Ulcerative Colitis

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Oncostatin M (OSM) and associated type 2 receptor chain, OSMR-beta, are elevated in inflammatory bowel disease (IBD). High levels of these proteins have been linked to increased disease severity and resistance to anti-TNF-alpha therapy. OSM can activate intestinal fibroblasts and promote intestinal inflammation. We demonstrate, in human intestinal fibroblasts, that OSM can synergize with TNF-alpha or IL-1-beta, which are both overexpressed in IBD, and significantly induce expression of fibroblast activation proteins such as ICAM-1, MCP-1 and PDPN. Vixarelimab inhibits OSM-mediated upregulation of these proteins and this inhibition is further enhanced with anti-TNF-alpha or anti-IL1R. Moreover, lipopolysaccharide stimulation of human monocytes induced expression of OSM and other proinflammatory cytokines. Subsequent activation of fibroblasts with the conditioned medium was significantly inhibited by vixarelimab alone or in combination with anti-IL1R. In addition, we performed ex vivo tissue explants studies with biopsies isolated from TNF-refractory ulcerative colitis patients. We stimulated those cultures with either recombinant OSM or lipopolysaccharide to induce endogenous OSM secretion from immune cells and observed marked increases in multiple transcripts including ICAM-1, PDPN, MCP-1 and SOCS3. Consistent with our in vitro observations, vixarelimab markedly inhibited the upregulation of these transcripts. We confirmed in the biopsies used for these explant cultures as well as in dissociated cells from ulcerative colitis patients, an enhanced level of fibroblasts in disease compared with healthy controls. Together, these data suggest that vixarelimab may represent an approach for treating TNF-refractory ulcerative colitis patients with high levels of circulating OSM, by inhibiting OSM-mediated fibroblast activation.

Th116. Spirulina Stimulates TNF α and IFN γ Production via Toll-like Receptor-4 Activation of Classical Monocytes and Monocyte-derived Dendritic Cells in Dermatomyositis Patients *in vitro*

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The herbal supplement Spirulina has previously been shown to stimulate TNF α and IFN γ production in Dermatomyositis (DM) patients *in vitro* predominantly via TLR4 and TBK1 activation. Using Flow cytometry, we investigated Spirulina's immunostimulatory effects at the cellular level. Of the 8 cell lineages evaluated (CD4, CD8, CD11c, CD123, CD11c⁺CD14⁺ Monocyte-derived Dendritic cells (moDCs), CD14⁺⁺CD16⁻ Classical Monocytes (CMs), CD14⁺⁺CD16⁺ Intermediate Monocytes, and CD14⁺CD16⁺⁺Non-Classical Monocytes), 0.3 mg/ml of Spirulina had the greatest effect on moDC and CM production of TNF α or IFN γ ($p < 0.05$) when examining effects on median fluorescent intensity (MFI) and percent of cell type or total population secreting TNF α or IFN γ . With stimulation at 0, 0.3, and 1 mg/mL of Spirulina, the percent of moDCs secreting IFN γ increased from a mean (SEM) of 1.01% to 96.40% and 96.90% (1.80) ($p < 0.0001$), respectively, and MFI increased similarly ($n = 3$, $p < 0.01$). The mean percent of CMs secreting IFN γ also increased ($p < 0.0001$), and pre-treatment with TLR4 inhibitor suppressed CM activation ($p < 0.05$). Moreover, the MFI of CMs secreting IFN γ increased significantly ($p < 0.005$). TLR4 or TBK1 inhibition decreased MFI for both moDCs and CMs ($p < 0.05$ and $p < 0.001$, respectively). TNF α ⁺ moDCs increased from 1.14% of total moDCs with no stimulation to 49.10% (12.4) at 0.3 mg/mL Spirulina ($p < 0.05$). TLR4 and TBK1 inhibition suppressed the percentage of Spirulina-induced moDCs secreting TNF α ($p < 0.05$); TLR4 inhibition trended towards significance in CMs ($p = 0.053$). These data demonstrate that Spirulina induces CM and moDC activation of inflammatory cytokines in DM, likely via TLR4 and TBK1 activation.

Th157. Evaluating the Therapeutic Potential of Filarial Parasite-derived Cystatin in a Mouse Model of Atopic Dermatitis

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Brugia malayi recombinant cystatin (rBmaCys) has therapeutic efficacy in colitis and arthritis. In this study, we evaluated if rBmaCys has therapeutic activity in a 2,4-dinitrochlorobenzene (DNCB) induced Atopic dermatitis (AD) model in mice. AD was induced in Balb/c mice (n=5) by applying 100µL of DNCB in 3:1 (v/v) acetone: olive oil mixture, on to the shaved dorsal skin. Skin was sensitized with three doses of DNCB (0.5% on day 1 and 0.1% on days 7 and 10). We prepared a topical formulation of rBmaCys by mixing 15µg of rBmaCys into 60g of aquaphor and 40gm of petroleum gel. Animals were treated for 5 consecutive days starting on day 14 after starting DNCB treatment. Control group of animals with AD were treated with aquaphor only. Another group of animals with AD were treated with 1% hydrocortisone as a positive control. Clinical parameters of AD, histopathological changes in the skin tissues and immunological parameters were evaluated for comparison. The clinical severity and symptoms of AD like itch, erythema/hemorrhage, edema, excoriation/erosion, and scaling/dryness was reduced in mice topically treated with rBmaCys-ointment. All the parameters were compared with aquaphor and hydrocortisone controls. Histological analysis of skin tissue samples revealed that rBmaCys treatment reduced epidermal hyperplasia, dermal edema, and infiltration of the inflammatory cells. Serum IgE levels did not show any significant differences after rBmaCys treatment. In conclusion, results from our studies demonstrate potential biotherapeutic activity against AD. Studies supported by Blazer Foundation of Rockford.

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W56. ADAP Regulates Multiple Aspects of Invariant Natural Killer T Cell Homeostasis and Function

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Invariant natural killer T cells (iNKTs) are innate T lymphocytes that are critical in modulating immune response in cancer, infection and inflammation. However, the mechanisms that regulate iNKT cell development and function are not fully known. In the current study, we demonstrate that adhesion and degranulation promoting adaptor protein (ADAP) is dispensable for iNKT cell thymic development but is required for their peripheral maintenance. Strikingly, ADAP-deficient (*Adap*^{-/-}) mice have significantly reduced iNKTs in the periphery that is associated with decreased surface expression of the critical homing receptor (CXCR6), homeostatic proliferation and increased apoptosis. Consistently, *Adap*^{-/-} iNKTs have markedly reduced expression of pro-survival factors (PLZF, ICOS, GATA-3 and Id2) but increased levels of pro-apoptotic molecules (Bax, Bad and Bim). Functionally, *Adap*^{-/-} iNKTs have significantly reduced cytokine production and bystander immune cell activation as well as poor anti-tumor response, both *in vitro* and *in vivo*. In agreement with these findings, ADAP-silenced human iNKTs have remarkably impaired cytokine production and cytotoxicity against leukemia targets *in vitro*. Mechanistically, preformed transcripts for interleukin (IL)-4 and IFN-γ as well as several microRNAs are reduced in *Adap*^{-/-} iNKTs. Additionally, these cells exhibit reduced capacity to form conjugates and have diminished actin polymerization at the immunological synapse. Collectively, our findings establish a novel and pivotal role for ADAP in iNKT cell homeostasis and function.

W95. The Role of Coagulation Protease-mediated Signalling in Renal Fibrosis

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Introduction:

Chronic kidney disease (CKD) is increasing in prevalence. Although it has numerous causes, all result from progressive replacement of specialised nephrons and kidney microvasculature by fibrosis, as myofibroblasts deposit excess extracellular matrix (ECM). I hypothesise that thrombin-mediated signalling via Protease-activated receptors on myofibroblast precursors plays a critical role in disease progression.

Methods:

Transgenic mice expressing membrane-tethered anticoagulants on specific cells, or control wild-type (WT) mice were treated with aristolochic acid (AA) to induce acute kidney injury (AKI) with subsequent renal fibrosis prior to culling. Renal fibrosis was quantified by picrosirius red-staining, quantification of collagen content, and rt-qPCR for ECM components. In addition, two strains of WT mice received IV injections of a novel, membrane-tethered direct anti-thrombin (PTL060) with each injection of AA.

Results:

Expression of anticoagulants on vascular cells changed myeloid cell recruitment during AKI (Ly6CHi, iNOS⁺ cells reduced; Ly6CLo, CD206⁺ cells increased), but did not influence degree of AKI or development of progressive fibrosis. Only the strain expressing anticoagulants on myofibroblast precursors showed reduced cortical fibrosis. PTL060 did not influence degree of AKI, but changed myeloid recruitment and inhibited cortical fibrosis.

Discussion:

These data suggest that thrombin plays two independent roles during AKI, altering the pattern of myeloid cell recruitment, and preventing progressive fibrosis, most likely by influencing a myofibroblast pre-cursor. This ongoing work is expected to yield novel insights, with potential translational potential, into the molecular and cellular basis of progressive CKD.

W125. Lactic Acid Suppresses MRGPRX2 Mediated Mast Cell Responses

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MAS related G-protein coupled receptor X2 (MRGPRX2) is a G-protein coupled receptor (GPCR) expressed in human mast cells that has been implicated to play an important role in causing pseudo-allergic reactions as well exacerbating inflammation during asthma and other allergic diseases. Lactic acid, a byproduct of glucose metabolism, is abundantly present in inflamed tissues and has been shown to regulate functions of several immune cells. Because the endogenous ligands for MRGPRX2 (substance P and LL-37) are elevated during pathologic conditions such as cancer and asthma, and given that lactic acid levels are also enhanced in these patients, we explored the role of lactic acid in regulating mast cells response via MRGPRX2 and MrgprB2, the mouse orthologue of the human receptor. We found that lactic acid suppressed both the early (Ca²⁺ mobilization and degranulation) and late (chemokine/cytokine release) phases of mast cell activation; this data was confirmed in LAD2, human skin

and mouse peritoneal mast cells. In LAD2 cells, the reduction in degranulation and chemokine/cytokine production mediated by lactic acid was dependent on the pH. In agreement with our *in vitro* studies, lactic acid also reduced passive systemic anaphylaxis to compound 48/80 (a known MRGPRX2/MrgprB2 ligand) and skin inflammation in a mouse model of rosacea that is dependent on MRGPRB2 expression on skin mast cells. Our data thus suggest that lactic acid may serve to inhibit mast cell-mediated inflammation during asthma and reduce immune response during cancer by affecting mast cell activation through MRGPRX2.

W130. MyD88-dependent Toll-like Receptor Signaling Triggers Trained Immunity Via Macrophage Metabolic Reprogramming

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Opportunistic infections are a major threat to public health, and new prevention strategies are needed. Immunomodulation aimed at boosting immune function has become an attractive strategy to meet this critical need. Harnessing the potential of trained immunity, whereby innate leukocytes maintain 'memory' of prior exposure to a microbial product resulting in a more robust response to subsequent infection, is one promising approach. We have shown that Toll-like receptor (TLR) 4 activation triggers this phenomenon and protects against infections through reprogramming of macrophage metabolism and antimicrobial functions. TLR4 uniquely signals through both MyD88- and TRIF-dependent pathways; the relative contribution of these pathways in trained immunity is unknown. We hypothesized that TLR-mediated infection resistance is driven by macrophage metabolic and functional reprogramming via MyD88. Mice were treated with the MyD88-selective agonist CpG, TRIF-selective Poly I:C, or dual activating monophosphoryl lipid A (MPLA) prior to systemic infection with *Staphylococcus aureus*. Treatment with CpG and MPLA, but not Poly I:C, improved survival and bacterial clearance. MyD88-mediated survival benefit was lost in macrophage-depleted mice, whereas mice that were administered MPLA- or CpG-treated bone marrow-derived macrophages (BMDMs) prior to infection were protected. Metabolism, mitochondrial content, phagocytosis, and respiratory burst were assessed after agonist treatment in BMDMs from wild type (WT), MyD88 knockout (KO), and TRIF KO mice. MPLA and CpG augmented metabolism and antimicrobial functions in WT and TRIF KO BMDMs, but not in MyD88 KO BMDMs, while Poly I:C had no effect. These data suggest MyD88 signaling is important in TLR-mediated trained immunity.

W135. DNASE2-mediated Degradation in Homeostatic Control of Chemotherapy-induced Cytosolic DNA Load

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Chemotherapy causes DNA damage and impacts immune processes. High therapeutic doses kill cancer cells, and yet we found that survivor cells display an altered phenotype of elevated pro-inflammatory genes and increased cytosolic DNA accumulation that distinguish them from normal cells. At low doses with limited cytotoxicity, acute treatment of human cancer cells A549 and HeLa using cytarabine (or ara-C, a nucleoside analog) or doxorubicin (a topoisomerase II inhibitor) caused DNA damage as marked by g-H2AX using flow cytometry and immunofluorescence. We also observed increased cytosolic DNA load that triggered the DNA sensing STING pathway and upregulated type I interferon genes IFN β , MX1 and CXCL10, in a dose-dependent manner. Ara-C or doxorubicin treatment could increase the expression of stress-induced NK activating ligand

MICA—potentially increasing immune recognition for NK cytolytic action. Indeed, co-culture of drug-treated cells with NK-92 MI cells at fixed effector: target ratios showed clear reduction of live cells percentage compared with untreated cells, as assessed by propidium iodide exclusion. Interestingly, there was a concomitant increase in the acidic lysosomal endonuclease DNASE2A expression as examined by immunoblot and DNASE2A enzymatic activity by a plasmid nicking assay. DNASE2A preferentially degrades double-stranded DNA, and plays a key homeostatic role in clearing damaged nuclear DNA in the cytosol via autophagic transport to avoid inflammation. We therefore hypothesize that by inhibiting the activity of DNASE2A or intervening in the autophagic transport of intrinsic DNA load in cancer cells, we can achieve optimal innate immune responses at reduced chemotherapeutic doses to benefit tumor therapy.

Th10. The Negative mTOR Regulator DDIT4L Limits Innate Immune Activation in Neonatal Monocytes

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Neonates are at a disproportionately high risk for infection in early life, particularly those who are born prematurely. Innate immune response to pathogens are attenuated in an inverse gestational age-dependent manner in neonatal myeloid cells. We previously showed upregulation of the negative mTOR regulator, DDIT4L and reduced mTORC1 regulated pathways, including glycolysis and protein translation, in preterm monocytes. We hypothesize that DDIT4L negatively regulates innate immune responsiveness in fetal myeloid cells.

We found that DDIT4L expression increased in an inverse gestational age-dependent manner upon LPS stimulation, and that metabolic limiting conditions such as 2-DG (to block glycolysis) or CoCl₂ (to simulate hypoxia) further upregulated DDIT4L expression in neonatal, but not in adult monocytes. Functionally, preterm monocytes showed low spare respiratory capacity compared to adult or full-term monocytes, suggesting more limited cellular bioenergetics, despite equivalent mitochondrial mass.

Using a doxycycline-inducible DDIT4L lentivirus in monocytic U937 cells, we show that DDIT4L overexpression reduced phosphorylation of mTORC1 (Ser2448) and its target ribosomal protein S6 (Ser235/Ser236) and decreased LPS-stimulated IL-8 production. DDIT4L overexpression also reduced cell size and proliferation, without affecting cell viability.

Lastly, we found that reduced DDIT4L gene expression in lung macrophages from preterm infants was associated with increased pro-inflammatory cytokine transcript levels and more severe inflammation-related chronic lung disease. Together, these data suggest that DDIT4L protects myeloid cells against immune over-reactivity in the fetal environment. Intriguingly, lack of expression of this developmental mTOR regulator may also exacerbate inflammatory pathologies in preterm infants during the neonatal period.

Th52. TIM-3 Modulate Human Macrophage Pro-inflammatory Phenotype

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TIM-3 is highly expressed on myeloid cells in both human and mice and is involved in the regulation of immune responses. Decreases in TIM-3 expression on T cells has been observed in multiple human autoimmune diseases, including psoriasis, rheumatoid arthritis, type 1 diabetes and multiple sclerosis. In contrast, *in vivo* blockade of TIM-

3 enhances antitumor immunity and suppresses tumor progression in both mice models and in human clinical trials. While the role of TIM-3 has been extensively investigated in T cells, its role in human myeloid cells is unknown. Here, we studied the effect of TIM-3 knock-down in human myeloid cells. Upon shRNA transduction, we differentiated monocytes into macrophages and delineated their phenotype. We profiled their transcriptome by RNA-sequencing, and we studied them functionally, through analysis of cytokine secretion and T-cell stimulatory ability. TIM-3 loss induces an activated myeloid phenotype, which we hypothesize is linked to autoimmunity.

Th87. JAK but not NFκB Mediates Prolonged Endothelial Cell Activation After Cytokine Stimulation

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We aimed to determine the dynamics and mechanisms of resolution of endothelial cell (EC) inflammation after cytokine withdrawal, and identify possible mediators of chronic vascular inflammation.

Primary human aortic EC and HMEC-1 were treated with TNFα (20ng/mL) or IFNγ (200U/mL) continuously or primed for 3hr followed by 3-45 hours in normal medium. mRNA and protein expression was measured at 1-48hr.

Continuous TNFα induced a wide array of chemokines and leukocyte adhesion molecules E-selectin, ICAM-1, VCAM-1, CD164 and BST2. IFNγ upregulated ICAM-1, BST2, IP-10, I-TAC and MIG. Unlike TNFα, IFNγ caused a late phase response, with mRNA appearing after 6hr and protein at 18-48hr. Most TNFα-mediated genes were NFκB dependent, while JAK inhibition suppressed IFNγ-induced ICAM-1, BST2 and chemokine expression.

When EC were primed for 3hr with TNFα, E-selectin and VCAM-1 decayed to near baseline by 24hr later. TNFα-primed EC did not produce chemokines after 6hr and NFκB genes promptly declined. However, 3hr exposure to IFNγ triggered late induction of BST2, ICAM-1, IP-10 and I-TAC, to the same level as continuous IFNγ (18-48hr). Similarly, JAK1, STAT1, STAT2 and IRF expression was prolonged through 24hr. Inhibition of new transcription with cycloheximide or impairment of JAK activity with ruxolitinib or JAK I inhibitor in the IFNγ-withdrawal period prevented later upregulation.

in summary, in endothelium, NFκB-mediated activation rapidly contracts; while JAK/STAT events are sustained, independent of cytokine presence, and continue to drive new transcription of leukocyte recruitment proteins. Thus JAK inhibition could be a novel therapeutic avenue to blunt chronic vascular inflammation.

Th137. TRIM21 as a Regulator of UVB-driven IFN Responses in Lupus

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Patients with systemic lupus erythematosus (SLE) experience photosensitivity, with exposure to ultraviolet light (UV) driving lupus flares, although the mechanism(s) linking UVB exposure to systemic effects are unclear. Our previous work has shown that TRIM21, an autoantigen in SLE, functions as a negative regulator on the pathways driving IFN expression. Here we explore that TRIM21 regulates systemic inflammation following UVB exposure. WT (C57BL/6) and TRIM21 KO mice were irradiated with UVB (100mJ/cm²) consecutively for 1 and 3 weeks. In UVB exposed areas in both sets of mice, erythema, inflammatory cell infiltrates and induction of type I IFN developed. After UVB exposure in TRIM21^{-/-} mice, we observed splenomegaly, enhanced expression of IFN-stimulated genes (ISG) genes in the blood and spleen and higher expression of Siglec1, IFN inducible protein, on

Ly6Chi inflammatory monocytes in the spleen and blood cells. Inflammatory cytokines and chemokines were also detected significantly higher in serum of TRIM21 KO after UVB exposure, all suggesting that loss of TRIM21 in mice results in enhanced IFN-driven responses systemically, mimicking the increase in disease activity at the systemic level seen in SLE patients following UVB exposure. Mechanistically, we found enhanced UVB and cGAMP induced IFN β expression and higher the level of DDX41, cytosolic DNA sensor, in TRIM21 $^{-/-}$ BMDMs. In translating these results to human disease, we found in whole blood from patients with cutaneous involvement, TRIM21 mRNA levels are significantly decreased. Taken together our results indicate that TRIM21 protects UVB induced inflammation in response to DNA sensing in SLE.

Th144. Maternal Asthma Risk in Relation to Infant and Early Life Hematopoietic Progenitor Cell Profiling: Evidence for *in utero* Innate Immune Memory

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Maternal allergy and asthma status is a recognized risk factor for the future incidence of allergic diseases in the offspring. We sought to identify whether this association is discernable in the receptor expression profiles of CD34 $^{+}$ hematopoietic progenitor cell (HPC) of the infant at birth and in early life.

For this pilot study, ten participants were randomly selected from a larger cohort of pregnant women and their offspring living in Fresno, CA, an area of consistently elevated air pollution. We collected and ficolled cord blood (CB) and one-year-old peripheral blood (PB) from the same individual and stored PBMCs in liquid nitrogen. Flow cytometry was used to analyze CD34 $^{+}$ HPC expression of the following key immune receptors: GM-CSFR, IL3R, IL5R, IL6R, IL17R, ST2, TLR2, TLR4 and TSLPR. We tested whether infants' CB and 1-year PB HPC exhibited a differential receptor profile depending on the asthma status of the mother.

There was a significantly lower proportion of TLR2 $^{+}$ HPC in the 1-year PB of infants born to asthmatic mothers ($p=0.001$), and a trend towards lower proportion of ST2 $^{+}$ HPC ($p=0.051$)

and GM-CSFR $^{+}$ HPC ($p=0.064$) in the cord blood.

We show that maternal asthma is associated with cellular changes in infants' HPC at age 1, indicating fetal immune priming. A lower circulating HPC carrying TLR2 may lead to impaired immune response to infections. Whether these changes are due to maternal or fetal genetics, or maternal *in utero* exposures such as from air pollution, asthma exacerbations or medication intake remains to be determined.

Th146. Neutrophil Extracellular Trap Formation Enhances Macrophage Killing of Bacterial Pathogens

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Professional phagocytes form the backbone of the innate immune response and are equipped with an arsenal of antimicrobial processes. While many of these processes have been explored in isolation, how immune cells cooperatively combat bacterial pathogens at the site of infection is unclear. The proteins forming calprotectin, S100A8 and S100A9, are the most abundant cytosolic proteins in neutrophils and important components of the innate immune response. S100A9-deficient (A9 $^{-/-}$) mice are protected from *Staphylococcus aureus* infection with

lower bacterial burdens in the heart. We have uncovered an intracellular function for S100A9, where A9^{-/-} neutrophils produce more mitochondrial superoxide, thereby promoting the terminal formation of neutrophil extracellular traps (suicidal NETosis). This establishes intracellular S100A9 as a critical molecular rheostat in neutrophil function. Increased suicidal NETosis does not heighten neutrophil killing of *S. aureus* in isolation, but rather augments macrophage (Mφ) killing. More specifically, NET formation enhances Mφ antibacterial activity by facilitating phagocytosis of *S. aureus* by Mφ and transferring neutrophil-specific antimicrobial peptides to Mφs. Finally, similar results are observed in response to other phylogenetically distinct bacterial pathogens implicating this as an immune defense mechanism that broadly enhances antibacterial activity. These results demonstrate that achieving full bactericidal activity through NET formation requires Mφs, and that accelerated and more robust suicidal NETosis makes neutrophils, especially when A9^{-/-}, adept at increasing antibacterial activity.

Th155. Transcriptional Profiles of CD14⁺ Cells *in situ* in Melanoma Reveal Plasticity, Localization Dependent Function and Specific T Cell Interactions

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Myeloid cells play a major role in tumors and include cells with different functions that can be grossly summarized as: (1)Antigen capture for presentation (dendritic cells) or for degradation (macrophages); (2)Tissue repair (macrophages) and (3)effector function (mast cells, monocytes, granulocytes). To study myeloid cells' functional status in intact tumor microenvironments, we established a comprehensive approach for cellular and molecular analysis. Polychromatic immunofluorescence and histocytometry showed that: (1)CD14⁺ cells represent the majority of the tumor immune infiltrate, (2)most of infiltrating T cells are in direct contact with CD14⁺ cells rather than melanoma cells, (3)distribution of CD14⁺ cells show two distinct patterns: CD14⁺ cells within cancer nest (intratumoral) in close interactions with melanoma cells and loaded with melanoma protein; CD14⁺ cells surrounding cancer nest (stromal) do not show melanoma protein cargo. Using laser capture micro-dissection, we harvested CD3⁺ T cells and CD14⁺ cells based on their tissue location and melanoma protein load for downstream analysis. Transcriptional profiling showed that CD14⁺ cells clustered according to their tissue localization (intratumoral/stromal), while T cells did not. Computational analysis revealed distinct signatures associated with different inflammatory and metabolic pathways in intratumoral and stromal CD14⁺ cells. Thus, transcriptome differentiates functional status of CD14⁺ cells related to their localization within tumor. Further the intra/stromal CD14⁺ signature clusters TCGA patients with significantly better long-term survival, which in metastatic melanoma was linked with dendritic cells signature in the stroma. Finally, combining our CD3⁺ and CD14⁺ LCM data we identified localization specific pairs of receptor/ligand interactions between myeloid and T cells.

Th158. Neutrophil Maturation and Responses to Infectious Pathogens are Regulated by Microbiota-induced Pcyox11

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We found that the proteomic signatures of neutrophils derived from germ free (GF) and specific pathogen free (SPF) mice were significantly different at baseline and during infection, demonstrating significant plasticity. To identify molecular pathways, we set-up an *in vitro* system where neutrophil progenitors were transduced with lenti-guides to knock-down key microbiota-driven gene targets. We knocked out 19 candidates and tested their killing of *P. aeruginosa*. Excitingly, few of the targets demonstrated a significant decrease in the neutrophil bactericidal capacities. We chose to study the properties of PCYOX11 KO cell lines as no prior function has been associated with PCYOX11. This gene encodes a potential prenyl cysteine oxidase as the PCYOX11 protein shares high degree of amino acid homology with Pcyox1, an enzyme that catabolizes prenylated proteins in the cell. Analysis of the *in vitro* maturation of the PCYOX11 KO myeloid cell lines revealed that the generated PCYOX11 KO neutrophils had significantly lower viability and matured faster than the WT neutrophils. Consistently, PCYOX11 heterozygote mice showed changes in myeloid differentiation and altered responses to infection with *P. aeruginosa*.

In conclusion, neutrophil responses, although innately determined, are adapted and molded by the commensal presence.

Th164. Microbiota-derived Indole Derivatives Regulate Key Cellular Processes Involved in Arthritis Pathogenesis

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Microbiota-derived metabolites are implicated in numerous diseases. However, little is known about how two indole derivatives, indole-3-aldehyde (IAld) and indole-3-acetic acid (I3AA), influence the pathological processes involved in rheumatoid arthritis, which has a global prevalence of 0.3-1% according to the WHO. For this purpose, we comparatively analyzed these two metabolites using established cell-based models of innate inflammation, angiogenesis, and osteoclastogenesis. Although these indole derivatives are structurally similar, their bioactivities were profoundly different. IAld but not I3AA, inhibited TLR2- and TLR4-induced pro-inflammatory cytokines (IL-1 β and IL-6) in RAW 264.7 (RAW) murine macrophages. IAld also exhibited pro-angiogenic activity; whereas, I3AA showed anti-angiogenic activity on human endothelial cells (HUVEC). Additionally, the differentiation of RAW cells into osteoclasts with receptor activator of NF- κ B ligand was drastically enhanced in the presence of IAld at as low as 12.5 μ M, but I3AA had no effect on osteoclastogenesis. Dependence of these outcomes on the aryl hydrocarbon receptor (AhR), was tested using CH-223191, a strong AhR inhibitor, because IAld and I3AA are putative AhR ligands. AhR-inhibition mitigated the anti-angiogenic activity of I3AA but failed to affect any of the IAld-mediated effects. Furthermore, in LPS-treated RAW cells, the alteration of NF- κ B and MAPK pathway activation by IAld and I3AA was likely not responsible for their anti-inflammatory activity. Our findings suggest that changes in the relative bioavailability of these indole derivatives may differentially impact the progression of arthritis, and possibly that of other diseases that share the above-mentioned cellular processes [T32 (A1095190)].

Mucosal Immunology

W38. Mucosal Immune Response to a Novel Viral Vectored Candidate MERS Vaccine and Inactivated MERS-CoV Antigens in Human Nasopharynx, and Anti-MERS-CoV Specific IgG Antibodies in Convalescent MERS Sera

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Background: Currently, there is no licensed MERS-CoV vaccine. Viral vector-based MERS-CoV vaccine expressing surface Spike protein is a promising vaccine candidate, and has been shown to induce both humoral and cell-mediated immune responses in animal models. Human nasopharynx-associated lymphoid tissues (NALT) are known to be induction site for immunity against upper respiratory tract infection. Intranasal vaccination is an effective strategy against respiratory infection. There is currently limited data in mucosal immunity in NALT to MERS-CoV vaccines.

Objectives: Our research aimed to evaluate mucosal immunity to a novel Chimpanzee adenovirus vectored vaccine expressing MERS-CoV Spike protein (ChAdOx1-MERS-CoV vaccine) and inactivated MERS-CoV antigens in NALT from both children and adults. Anti-MERS-CoV specific IgG antibody levels in convalescent MERS patient serum samples were also analysed.

Methods: Adenotonsillar mononuclear cells (MNC) were isolated and co-cultured with the ChAdOx1-MERS-CoV vaccine, Heat Inactivated or Irradiated-MERS-CoV antigens. An ELISA assay was used to measure anti-MERS-CoV specific IgG antibody production in adenotonsillar MNC culture supernatants as well as from serum samples taken from convalescent MERS patients.

Results and Conclusion: Significant mucosal antibody responses in NALT were detected from both children ($p < 0.001$, $n=20$) and adults ($p < 0.001$, $n=13$), following stimulation by inactivated MERS-CoV antigens and ChAdOx1-MERS-CoV vaccine. Mucosal immunisation with adenovirus-vectored MERS-CoV vaccine may be a promising vaccination strategy against MERS-CoV infection. High level of anti-MERS-CoV IgG antibodies detected in convalescent MERS-CoV patient serum samples ($p < 0.001$, $n=23$) may suggest the development of protective humoral immunity following MERS-CoV infection in humans.

W91. IL-18 Signaling is Required for Helios⁺Foxp3⁺ TREG Cell-mediated Control of TH1 and TH17 Immune Responses in Viral and Parasitic Infectious Disease

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The adaptive immune response engaged in the course of infectious disease is highly specialized and geared to facilitate a quick return to tissue function. Foxp3⁺ regulatory T cells, a subset of CD4⁺ T cells, are particularly involved in the suppression of immune responses and the promotion of mucosal homeostasis. To do so, T_{REG} cells undergo tissue-specific adaptations that enable them, notably, to sense alarmins of the IL-1 family. In this report, we investigated IL-18, a known promoter of T_H1 immune responses, whose role on T_{REG} cells remains largely unknown. During *Influenza*, *L. major* and *C. neoformans* murine infection models, we observed that a majority of tissue-localized T_{REG} cells express IL-18R1 and T_H1-like characteristics. *In vitro*, IL-12 is specifically required for the expression of IL-18R1 and T_H1-like characteristics in T_{REG} cells. Using tamoxifen-inducible T_{REG}-specific IL18R deficient mice (Foxp3^{ERT2-CRE} IL18R^{flxed}), we show that IL-18, while not required for the T_H1-like differentiation, migration or proliferation of T_{REG} cells, is determinant for these cells to control T_H17, not T_H1 or T_H2 responses. We observed increased pathophysiology during *Influenza*, reduced skin inflammation during *L. major* and lower fungal burden during *C. neoformans*, suggesting a unique mechanism by which IL18R1⁺ T_{REG} cells influence the outcome of disease. Collectively, our results show that IL-18 favors T_H1-like T_{REG} cells that restrict T_H17 immune responses specifically. Understanding how T_{REG} cells modulate their adaptation to local tissue environments and promote the specialization of the immune response is key towards developing targeted therapies against both infectious and non-infectious diseases.

Th108. Single Cell Analysis of NEC Mucosa Reveals Inflammatory CD16⁺ CD163⁺ Monocytes Traffic to Sites of Inflammation

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Necrotizing enterocolitis (NEC) is a devastating complication of prematurity. Advances in diagnosis and treatment have been limited and current therapy is non-specific. In-depth single-cell analysis of the small intestine can identify specific phenotypes of NEC and therapeutic targets. Samples from initial surgery for NEC (sNEC, n=12, gestational age (GA) 23-39 weeks, wks) were compared to neonates with non-immune congenital anomalies (Neonatal n=4, GA 31-33 wks) and discarded fetal intestinal tissue (n=3, GA 16-20 wks). Single-cell RNA sequencing (scRNAseq) coupled with suspension (CyTOF) and imaging (IMC) mass cytometry was performed.

Abnormalities in neutrophil and monocyte populations were noted. We differentiate sNEC into three distinct phenotypes based on the type of neutrophilic infiltrate observed. In ~40% of NEC cases, aged neutrophils with increased expression of CD11b and CD38 were abundant. In another ~40% of NEC cases, neutrophils had enhanced expression of CD56 and CXCR3 consistent with newly recruited neutrophils. In the third group with ~20% of cases, immature (cKIT⁺) neutrophils were abundant. A subtype of monocytes/Mφ expressing CD16 and CD163 were enriched in NEC and located adjacent to blood vessels. scRNAseq analysis showed that CD16⁺CD163⁺ monocytes/Mφ were inflammatory and transcribe inflammatory genes: TREM1, IL1a, and IL1b, IL8, and calprotectin. Gene set enrichment analysis identified pathways involved in chemotaxis, migration, phagocytosis, toll-like receptor activation, reactive oxygen species, and cytokine signaling.

In summary, we described three distinct NEC phenotypes and identified a novel subtype of inflammatory monocyte/Mφ that can serve as a potential biomarker and therapeutic target in NEC.

Neurologic Autoimmune Diseases

W36. Skewing of the B Cell Receptor Repertoire in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome

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Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a chronic, intractable disorder, characterized by profound fatigue, post-exertional malaise, sleep disorders, cognitive impairment, pain, autonomic dysfunction among others. ME/CFS is often triggered by an infectious episode and associated with an aberrant immune system. Recent pandemic revealed that many patients suffered from long haul COVID-19, which is characterized by serious fatigue or other symptoms similar to ME/CFS. Here we report that ME/CFS is a disorder characterized by skewed B cell receptor gene usage. By applying a next-generation sequencing to determine the clone-based IGHV/IGHD/IGHJ repertoires, we revealed a biased usage of several IGHV genes in peripheral blood B cells from ME/CFS patients. Results of receiver operating characteristic (ROC) analysis further indicated a possibility of distinguishing patients from healthy controls, based on the skewed B cell repertoire. Meanwhile, B cell clones using IGHV3-30 and IGHV3-30-3 genes were more frequent in patients with an obvious infection-related episode at onset, correlated to expression levels of interferon response genes in plasmablasts. In addition, we have confirmed upregulation of various anti-autonomic autoantibodies such as anti- β_1 adrenergic receptor antibody in patients. Collectively, these results imply that B cell responses in ME/CFS are directed against an infectious agents or priming antigens induced before disease onset. This approach will help to understand the pathogenesis of long-haul COVID-19.

W136. T Cell and Myeloid Cell Subsets as Key Players During Multiple Sclerosis (MS) Disease Onset: A Cerebrospinal Fluid Transcriptomic Study

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Cerebrospinal fluid (CSF) lymphocytes are important contributors to MS disease activity. Due to their fragile nature and low cell counts, have limited prior studies, particularly in cryopreserved sample. Specific lymphocyte subsets triggering, and propagating CNS-compartmentalized inflammation remain unknown. Using single-cell RNA sequencing (sc-RNA-seq), we characterized the profile of CSF lymphocytes in MS patients (13 untreated, 6 ocrelizumab-treated), compared to 8 other neuroinflammatory diseases (ONID). We also tested a CSF cell cryopreservation protocol adapted for sc-RNA-seq analysis. All samples were analyzed immediately after lumbar puncture (fresh), except 6 samples with cryopreserved replicates.

After removing doublets, RBCs, and low-quality cells, we analyzed 64,106 cells. Top level clustering revealed 86% T-cells, 10% myeloid cells, 2% NK-cells, 1% B-cells, 0.5% plasma-cells/plasmablasts, 0.5% plasmacytoid-DC.

We identified 13 distinct CSF-cell clusters: 1 ab-T cell (including 24 subclusters), 4 other T-cell (including: NKT, dg-T), 5 myeloid-cells (including: microglia-like, monocytic, myeloid DC, granulocytes), NK, plasmacytoid DC, B-cells and plasmablasts clusters. The two microglia-like cells subtypes (3% of cells) were amongst the 4 myeloid subtypes reduced in the newly diagnosed RRMS group compared to ONID. Further, 3 CD4⁺ and 1 CD8⁺ memory T-cell subsets increased in frequency in RRMS, highlighting potential differences during early stages of MS. The

ocrelizumab group, displayed increased myeloid-cells frequency, similar CD4⁺ and CD8⁺ T-cell patterns to the ONID group, while B-cells reconstituted after 6 months post-infusion. All clusters were conserved in the cryopreserved samples, indicating a successful cryopreservation. Our data revealed novel early CSF population shifts in MS, that may shed light on new cellular mechanisms.

W145. Examination of Stability and Progression Profiles in CIS Using RNA-seq Single-cell Analysis in Peripheral Blood Mononuclear Cells

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This study uses single-cell mRNA analyses to identify immune cell profiles in Clinically Isolated Syndrome (CIS) patients associated with higher risk of MS relapse or new central nervous system (CNS) disease activity on brain MRI. Peripheral blood mononuclear cells were collected at study entry and six months from six CIS participants in the ITN020AI STAyCIS atorvastatin trial. Only three of the six participants met the primary endpoint defined as ≥ 3 new T2 lesions on MRI or an MS relapse within 12 months. RNA from about 10,000 PBMC per sample were identified at the single-cell level using the 10X Genomics Chromium platform and analyzed using Cell Ranger and Seurat R software. 31 clusters of differentially expressed genes were assigned as specific cell types; baseline frequencies of clusters enriched >2-fold for macrophage, NK, NKT, CD4⁺ T cell, and B cell markers were associated with disparate outcomes at six months. Several clusters of B cells, CD4⁺ and CD8⁺ T cells were expanded from baseline at six months in participants with CNS disease activity at that time, while no notable changes were observed in those with stable disease. Our results show varied frequencies of innate and adaptive leukocyte populations in participants with disparate clinical outcomes, suggesting a predictive biomarker for aggressive early intervention. Expanded clusters of CD4⁺ and CD8⁺ T cells and B cells in blood at the time of disease activity may identify biomarkers leading to acute demyelination. Follow-up studies will incorporate protein data to better define cell subtypes associated with disparate outcomes.

Th3. Changes Induced in Lymphocyte Subpopulations by Dimethyl Fumarate Treatment in Multiple Sclerosis Could Identify "NEDA" Patients

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Background

Optimal response to dimethyl-fumarate (DMF) in multiple sclerosis (MS) is mediated by a shift to an antiinflammatory and immunoregulatory profile. In a preliminary study of 22 MS patients followed 12 months, we observed that, at 3 months of treatment, patients with "No evidence of disease activity (NEDA)" had a decrease in the Th1-like Th17 effector memory (EM) subpopulation.

Objective

To analyze long-term effect of DMF on lymphocyte subpopulations and its relationship with disease's activity.

Methods

Longitudinal prospective study in MS patients undergoing DMF treatment. A panel of T and B lymphocytes in

peripheral blood was analyzed by flow cytometry. Patients with a complete follow-up of more than 1 year are classified as: NEDA, MEDA (minimal clinical or radiological activity) or EDA.

Results

48 patients have been analyzed. After a 2.66 (1-5) years of follow-up. Changes induced: increase of naïve subsets in T [CD4 and CD8] and B-cells, decrease of central memory (CM) and EM T-cells, and memory B-cells; remained stable in the long-term, being more prominent in NEDA patients. In these, we found lower percentages of Th1 CM and EM pre-treatment, and of Th1, Th17 and Th1-like Th17 CM and Th1 and Th17 EM during the first 12 months. MEDA patients appear to behave like EDA patients in changes in Th1/Th17/Th1-like Th17 subsets.

Conclusions

Changes induced by DMF on the lymphocyte subpopulations remain stable over time. NEDA patients have an immunophenotype that seems to identify them. Immunomonitoring detects biological effect of the treatments.

Th34. Transcription Cofactor GRIP1 Differentially Affects Myeloid Cell-driven Neuroinflammation and Response to IFN β Therapy

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Multiple sclerosis (MS) is an autoimmune demyelinating disease affecting the Central Nervous System (CNS) whose etiology is still unknown. Growing evidence show that Macrophages (M Φ) and microglia (MG) are critical in the pathophysiology of Experimental Autoimmune Encephalitis (EAE), the mouse model of MS. Type I interferon beta (IFN β) and Glucocorticoids (GCs) are front line treatments for a subset of MS patients and disrupting each pathway in mice aggravates EAE. Interestingly, Glucocorticoid Receptor-Interacting Protein (GRIP)1, has emerged as a common player in both GC and type I IFN signaling cascade. Given that GRIP1 cooperates with GC and type I IFN pathways, the premise of this study was to evaluate the role of GRIP1 in neuroinflammation and in driving therapy in MS.

Surprisingly, myeloid-specific depletion of GRIP1 in our cKO mice dramatically reduced EAE severity compared to their control counterparts specifically during the neuroinflammatory phase of the disease. Transcriptome analysis of M Φ /MG isolated from spinal cords of these mice, at the bulk and single-cell level presented a lower inflammatory signature and persistence of homeostatic MG signature. These analyses also revealed that GRIP1-depleted myeloid cells failed to activate IFN pathway compared to control. Lastly, IFN β administration to GRIP1 myeloid-depleted mice did not improve EAE like it did in control mice which proved that GRIP1 is required to mediate its effect. All these findings led us to reveal an unexpected and versatile role of GRIP1 as it transcriptionally facilitates neuroinflammation in M Φ and MG, while it mediates the IFN β therapy in mice during EAE.

Th109. Pathogenic Th17 Cells in Autoimmune Myasthenia Gravis Exhibit a Pro-inflammatory Transcriptomic Signature that Drive Disease Pathogenesis

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Introduction: Myasthenia gravis (MG) is a chronic autoimmune disease of neuromuscular junction with accumulating immunological evidence suggesting a critical role for Th17 cells in disease pathogenesis.

Pathogenic Th17 are a subset of Th17 cells that co-produce IFN- γ and IL-17, and implicated as a cause of autoimmune disease. Notably, our group demonstrated an association of disease severity in MG with pathogenic Th17 frequencies. However, the molecular cues and signatures that segregate the differentiation of pathogenic and non-pathogenic Th17 cells in MG is undefined.

Methods: To explore gene signatures associated with pathogenic and non-pathogenic Th17 cells, we performed single-cell RNA sequencing (scRNA-seq) analysis on PBMCs from 4 MG patients with known pathogenic Th17 cells, as validated by flow cytometry. PBMCs were differentiated under Th17 polarization condition and activated (OX40⁺CD25⁺) CD4 T cells were flow-sorted prior to scRNA-seq.

Results: Using scRNA-seq, we were able to identify and compare single cells with four cytokine profiles: IFN- γ +IL-17⁺, IFN- γ +IL-17⁻, IFN- γ -IL-17⁺, and IFN- γ -IL-17⁻ producing CD4 T cells. In comparing pathogenic Th17 cells with the other three cytokine groups, the pathogenic Th17 cells demonstrated significant upregulation of genes associated with cytotoxicity (GZMK, GZMA), inflammation (IL-9, IL-22, IL-26), and chemotaxis (XCL2, CCL1, CCL5). Additionally, pathogenic Th17 cells exhibited higher levels of activation and proliferative capacity.

Conclusions: Our early investigation into pathogenic Th17 cells in MG revealed a pro-inflammatory gene signature compared to non-pathogenic Th17 cells. This observation is in line with our earlier work demonstrating an association between MG disease severity and frequencies of pathogenic Th17 cells in circulation.

Th148. Enhanced Influenza A H1N1 T Cell Epitope Recognition and Cross-reactivity to Protein-O-mannosyltransferase 1 in Pandemrix-associated Narcolepsy Type 1

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Narcolepsy type 1 (NT1) is a chronic neurological disorder having a strong association with HLA-DQB1*0602, thereby suggesting an immunological origin. Increased risk of NT1 has been reported among children or adolescents vaccinated with AS03 adjuvant-supplemented pandemic H1N1 influenza A vaccine, Pandemrix. Here we show that pediatric Pandemrix-associated NT1 patients have enhanced T-cell immunity against the viral epitopes, neuraminidase 175-189 (NA₁₇₅₋₁₈₉) and nucleoprotein 214-228 (NP₂₁₄₋₂₂₈), but also respond to a NA₁₇₅₋₁₈₉-mimic, brain self-epitope, protein-O-mannosyltransferase 1 (POMT1₆₇₅₋₆₈₉). A pathogenic role of influenza virus-specific T-cells and T-cell cross-reactivity in NT1 are supported by the up-regulation of IFN- γ , perforin 1 and granzyme B, and by the converging selection of T-cell receptor TRAV10/TRAJ17 and TRAV10/TRAJ24 clonotypes, in response to stimulation either with peptide NA₁₇₅₋₁₈₉ or POMT1₆₇₅₋₆₈₉. Moreover, anti-POMT1 serum autoantibodies are increased in Pandemrix-vaccinated children or adolescents. These results thus identify POMT1 as a potential autoantigen recognized by T- and B-cells in NT1.

Stem Cell and Organ Transplantation

W15. Immunologic Endotypes of Ischemia-reperfusion Injury in Human Liver Transplantation

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Ischemia-reperfusion injury (IRI) is a major risk factor for allograft rejection in orthotopic liver transplantation (OLT), involving complex interactions between innate and adaptive immune systems. However, no clinical therapeutics or patient-specific diagnostics are currently available. Therefore, we sought to identify associations between inflammatory endotypes and clinical presentations in OLT-IRI. We enrolled 164 OLT recipients in our IRB-approved study, and investigated evolving patient immune status in blood and tissue samples obtained pre-, intra- and post-transplant. We collected clinical parameters from medical and surgical records and examined whether there were any associations between immunologic endotype and clinical features. Our studies suggest four clinically actionable human endotypes associated with biopsy-proven IRI centered on the involvement of specific DAMP-PRR signaling pathways: 1) disulfide-HMGB1/TLR4/TNF α , 2) cfDNA/HMGB1/RAGE/TLR9, 3) ssRNA/TLR7 and 4) PGN/NOD2. High-risk IRI endotypes are associated with increased myeloid activation/infiltration, circulating cytokines and de novo HLA donor-specific antibodies, as well as poorer outcomes, including ACR, AMR and death. Low-risk IRI endotypes have increased tolerance induction in myeloid and T cells leading to improved outcomes and extended allograft/patient survival. Importantly, we identified female Hispanics with NASH as primarily having high-risk IRI endotype 1. Additionally, high-risk IRI endotype 2 is seen in patients with non-active HCC at the time of transplant, whereas those with active HCC frequently have low-risk IRI endotypes 3 or 4. Accurate immunologic endotyping of patients according to their specific pathogenesis and clinical presentation will allow more precise and personalized therapeutic strategies to reduce the influence of IRI in OLT.

W19. Transplantation and CMV Enhance CD8 T Cell Aging

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Cytomegalovirus (CMV) infection is a significant source of immune remodeling with age. After primary infection, CMV establishes latency and periodically reactivates, driving expansion of CMV-specific CD8 T cells over time, in a process called memory inflation. Inflated T cells have an altered phenotype associated with aging, including shortened telomeres, terminal differentiation state and altered cytokine production. Recent studies have suggested that memory inflation may be accelerated after solid organ transplantation. We hypothesized that transplant recipients with accelerated inflation have concomitant enhancement of CD8 T cell aging. We found a trend towards decreased telomere length in the first year post-transplant. T cells expressing age-associated marker CD57 and with a terminally differentiated phenotype (CD45RA⁺ CCR7⁻ CD27⁻) expanded post-transplant specifically in CMV seropositive recipients. RNA sequencing found that the terminally differentiated population was enriched for clonally expanded cells. Overall, these findings demonstrate enhancement of an aging phenotype in the context of CMV and transplantation. Further study is needed to identify the impact of this aging phenotype on T cell function and patient health.

W23. Immune Profiling of Migrating and Graft-associated $\gamma\delta$ T Cells after Human Intestinal Transplantation Reveals Unique Innate and Adaptive Features

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Innate- and adaptive-like features of human $\gamma\delta$ T cells are associated with different TCR repertoires, defined as $V\gamma9^+\delta2^+$ and non- $V\gamma9\delta2$, respectively. We performed phenotypic and clonal tracking of donor- and recipient-derived $\gamma\delta$ T cells in blood, intestinal grafts and bone marrow (BM) after human intestinal transplantation (ITx). We previously demonstrated that donor T cell macrochimerism (peak level $\geq 4\%$ in blood) is associated with less rejection. We now observed that increased $\gamma\delta$ T cell blood chimerism was present in patients with macrochimerism. Remarkably, donor $\gamma\delta$ T cells were also detected in recipient BM 105-357 days after ITx. Single-cell profiling of BM-infiltrating donor $\gamma\delta$ T cells revealed dominant $V\delta2$ clonotypes with cytotoxic effector T cell (Teff) phenotypes that might contribute to graft-versus-host responses. "Public" clonotypes of BM-infiltrating donor $\gamma\delta$ T cells were detectable in three patients tested and were shared by pre-transplant repertoires across donors and tissues. Many of these clones are $V\gamma9^+\delta2^+$ with zero N-additions that likely to originate from fetal liver and cord blood. However, these "public" dominant clones were not present in healthy control BMs, suggesting a transplant-induced migration pattern. In contrast to $\alpha\beta$ T cells, the turnover dynamics of $\gamma\delta$ T cells in the graft showed a stronger association with donor age than with the status of macrochimerism. Graft-repopulating recipient $\gamma\delta$ T cells showed Teff phenotypes early post-transplant and gradually developed into cytotoxic resident-memory T cells with "private" $V\delta1$ clonotypes. $\gamma\delta$ T cells may modulate immune responses to influence chimerism and graft rejection after ITx.

W64. Sequential Analysis of Renal Allograft Rejection at Single Cell Resolution

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BACKGROUND: Corticosteroids (CCS) and anti-lymphocyte globulin have been used to treat renal allograft rejection for decades, yet little is known about their effects on graft infiltrating immune cells. Similarly, the molecular understanding of rejection biology occurring under maintenance immunosuppression (tacrolimus or new biologics) at the single cell level is lacking. Here, we defined the transcriptomic profiles of individual kidney tissue and infiltrating immune cells at the time of rejection and after anti-rejection therapy (RejTx).

METHODS: Renal allograft recipients enrolled in a randomized clinical trial of iscalimab, an anti-CD40 mAb, were followed longitudinally during rejection. Renal allograft biopsies were collected serially - at initial rejection and after RejTx. 5' single cell RNA sequencing (scRNAseq) was performed with linked TCR sequencing and clonal analyses.

RESULTS: One patient with a Banff 1B rejection underwent RejTx (CCS, tacrolimus, MMF). Rejection failed to resolve across individual therapies and was characterized by scRNAseq analysis across 4 consecutive, serial biopsies. Inflammatory signals in multiple cell types in the graft were observed. Further analysis of immune cells showed transcriptomic changes consistent with changes in RejTx, but incomplete suppression of inflammation. Additionally, several expanded CD8⁺ T cell clones persisted across treatment episodes but were eventually displaced by a new set of expanded clones, despite ongoing, refractory rejection.

CONCLUSIONS: Refractory rejection under iscalimab is associated with 1) differential and incomplete suppression of inflammatory transcripts in immune and non-immune cells, and 2) failure to eliminate dominant TCR clones, with 3) an eventual emergence of new CD8⁺ TCR clones in the allograft.

W69. HMGB1-TLR4 and HMGB1-TLR9 Axes Differentially Polarize Macrophages to Distinct Phenotypes and Functions in Human Orthotopic Liver Transplantation Ischemia-reperfusion Injury

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Human hepatocellular damage caused by ischemia reperfusion injury (IRI) generates the disulfide form of HMGB1 (diS-HMGB1) that elicits myeloid cell activation and proinflammatory cytokine production. The role of the HMGB1-TLR4 and HMGB1-RAGE/TLR9 axes in mediating proinflammatory macrophage polarization is not well understood. We hypothesize that IRI-stressed human orthotopic liver transplants (OLT) release endogenous diS-HMGB1 that bind to TLR4 and TLR9/RAGE to induce pro-inflammatory macrophages that promote post-transplant alloreactivity. To test this hypothesis, human peripheral blood monocytes were stimulated with diS-HMGB1 alone or with a TLR4 inhibitor (TLR4i), TLR9 inhibitor (TLR9i), TLR4i+TLR9i, or HMGB1 neutralizing antibody (αHMGB1). Macrophage immunophenotypes and ROS production were analyzed by flow cytometry on Day 5. αHMGB1 treatment decreased macrophage CD86 and HLA-DR, increased PD-L1, and decreased ROS production compared to diS-HMGB1 alone. Blockade with TLR4i alone trended toward decreased CD86 and HLA-DR, TLR9i alone significantly decreased CD86 and HLA-DR, and combined TLR4i+TLR9i synergistically decreased CD86 and HLA-DR, approaching levels observed with αHMGB1 treatment. TLR4i had no impact on ROS production whereas TLR9i decreased ROS production to levels observed with αHMGB1 treatment. We conclude that diS-HMGB1 increases the antigen presentation capacity (CD86, HLA-DR) of macrophages via TLR4 and TLR9, whereas ROS production is solely regulated by the HMGB1-TLR9 axis. This study reveals that diS-HMGB1 has pleiotropic effects on macrophage phenotype and function depending upon interaction with TLR4 or TLR9. These results also imply neutralization of HMGB1, rather than TLR4/9 inhibition, during OLT is a potential treatment option for decreasing the diS-HMGB1-dependent post-OLT inflammation seen in IRI+ patients.

W120. Harnessing Innate Lymphoid Cells to Prevent Graft-versus-host Disease

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Graft-versus-host disease (GvHD) is a major obstacle limiting the success of hematopoietic stem cell transplantation (HSCT) for the treatment of haematological malignancies. While 70% of acute GvHD patients respond to current treatments involving broad immunosuppression, fewer than 30% of non-responders survive past a year, highlighting the need for more effective therapies. Innate lymphoid cells (ILCs) are a family of lymphocytes that rapidly respond to environmental signals and orchestrate downstream immune responses. While ILCs can promote inflammation, subsets of ILCs limit inflammatory immune responses to maintain immune and tissue homeostasis. In mice, ILC2s and ILC3s can inhibit autologous and allogeneic T cell responses. Post HSCT, increased circulating ILC2s and ILC3s are associated with reduced incidence of aGvHD, and mouse studies suggest that adoptive transfer of ILC2s may protect from GvHD. To assess if human ILC2s and ILC3s had the potential to directly limit GVHD, we developed methods to isolate and expand human ILC2s and ILC3s from blood that maintain expression of signature cytokines and transcription factors. Interestingly, both ILC2s and ILC3s expanded using our approach produce high levels of the regulatory cytokine IL-10 and the tissue repair factor amphiregulin. In xenograft GvHD studies, adoptive transfer of ILC2s and ILC3s reduced xenograft GvHD severity and prolonged survival of NOD-*scid* IL2Rγ^{null} mice. *In vitro* studies demonstrated human ILC2s and ILC3s could directly regulate allogeneic T cell cytokine production and proliferation, respectively. Our findings support that harnessing ILC2s and ILC3s may have applications in therapies aimed at preventing harmful allogeneic immune responses.

W134. Lentiviral Gene Therapy for Inherited Purine Nucleoside Phosphorylase Immune Deficiency

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Background: Purine nucleoside phosphorylase (PNP) deficiency causes T cell lymphopenia in patients, manifesting as increased susceptibility to infection, autoimmunity, malignancy, and developmental delays. We hypothesized PNP deficiency can be corrected by transplanting autologous hematopoietic stem cells transduced with a lentiviral vector containing the human PNP gene (LentiPNP).

Objectives: We will optimize the use of LentiPNP in vivo and assess its capacity to prevent premature death in PNP-deficient (PNP^{-/-}) mice. We will establish the safety of LentiPNP in vivo, and its ability to correct the immune abnormalities of PNP^{-/-} mice.

Methods: Lineage negative cells (lin⁻) were isolated from bone marrow of PNP^{-/-} mice, followed by an 18-hour cytokine stimulation, 24-hour transduction with the LentiPNP construct, and nine days of culture in MethocultTM 3434. For transplant, mice were received 500cGy or 700cGy total body irradiation (TBI) and transplanted lin⁻ cells from a healthy PNP^{+/-} donor or PBS.

Results: LentiPNP transduction significantly increased PNP expression in PNP^{-/-} lin⁻ cells grown in vitro, and increased PNP enzyme activity relative to the dose of LentiPNP. The numbers of blood, monocyte and granulocyte colony forming units (CFUs) generated from transduced and non-transduced cells were similar, indicating transduction did not jeopardize growth potential. Additionally, transplanting irradiated PNP^{-/-} mice with PNP^{+/-} lin⁻ cells normalized neutrophil and lymphocyte counts 42 days post-transplant.

Conclusions: This study demonstrates that LentiPNP can potentially correct for the lack of enzyme activity in PNP^{-/-} mice. Prolonged observation following transplant will ensure long-term engraftment and overall health of the mice before advancing to human trials.

W168. Donor-specific Anti-HLA Alloantibody in Kidney Transplant Recipients with COVID-19 Exhibit Different Immunoglobulin Class and Subclass Profiles Compared with Anti-SARS-CoV-2 Antibodies

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Both anti-HLA and anti-SARS-CoV-2 antibodies target protein antigens. The aim of the present study was to compare the class and subclass profile of such antibodies in a cohort of kidney transplant patients.

We detected and identified anti-HLA and anti-SARS-CoV-2 antibody in 48 kidney transplant recipients who were hospitalized for SARS-CoV-2 PCR⁺ infection. Anti-HLA antibodies were detected by single-antigen bead Luminex assay, with IgG class, and IgG1-2-3-4 subclass secondary antibody. Anti-SARS-CoV-2 antibodies were also detected by Luminex assay directed against 5 distinct viral epitopes including the nucleocapsid protein as well as multiple regions of the spike protein. The secondary antibodies addressed total IgG, IgG1-2-3-4, IgM and IgA class/subclasses.

The antibody profile included 12/48 cases of donor-specific anti-HLA antibodies, and 43/48 cases with anti-SARS-CoV2 antibodies. The majority of HLA-specific antibodies targeted HLA-DQ, with a dominant IgG class and an IgG1+IgG2+IgG3 subclass prevalence. However, anti-SARS-CoV2 antibody profile was characterized by increased prevalence of IgM (38/43, 79%) and IgA (41/42, 85%), and a lower prevalence of IgG2.

Overall, these data suggests that kidney transplant recipients with Covid-19 exhibit a humoral immune response both to donor-HLA and SARS-CoV-2. Although both are protein antigens, the allo-immune response has a high-IgG/low IgA pattern, while the Covid-19 antibody profile includes high IgA. Additional follow-up is needed to determine if the increased IgA is a consistent marker of anti-SARS-CoV-2 antibody response.

Th15. Urinary Metabolomic Profiling from Spontaneous Tolerant Kidney Transplanted Recipients is Enriched in Tryptophane-derived Metabolites

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Background: Spontaneous operational tolerance is the holy grail in solid organ transplantation. In kidney transplantation, spontaneous tolerance is characterized by a specific and active regulatory mechanisms. Previous reports suggest that the urinary compartment can reflect local inflammatory condition of the graft.

Objective: we hypothesized that spontaneous tolerant kidney transplanted recipients (KTR) would have a specific urinary metabolomic profile linked to immune regulatory mechanisms.

Patients & methods: We performed metabolomic profiling on urine samples from healthy volunteers, stable KTR under standard and minimal immunosuppression and spontaneous tolerant KTR using liquid chromatography in tandem with mass spectrometry (HPLC/MS). Supervised and unsupervised multivariate computational analysis were used to highlight urinary metabolomic profile and metabolite identification thanks to workflow4metabolomic platform.

Results: Urinary metabolome was composed of approximately 2700 metabolites ranging from from highly polar to apolar. Raw unsupervised clustering allowed to separate healthy volunteers and tolerant KTR from others. We identify a specific urinary metabolomic signature in spontaneous KTR of twelve leading ions which was mainly driven by kynurenic acid, a tryptophan-derived metabolite independent of immunosuppressive drugs, serum creatinine and gender. Moreover, we could identify that kynurenine and tryptamine pathways were also up-regulated in tolerant KTR.

Discussion/conclusion: Kynurenic acid and tryptamine enrichment allowed to identify putative mechanisms involved in spontaneous operational tolerance such as IDO expression, GRP35 and AhR signaling and microbiota-derived tryptophane metabolites such as indole alkaloids. Further studies are needed to better decipher the immune mechanisms at play in a long-term therapeutic perspective.

Th29. Time Dependent Blood Eosinophilia Count Correlate with Worse Immunological Outcomes in Kidney Transplant Recipients

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Background: There are growing evidences that type 2 immunity effectors (immunoglobuline E, eosinophils, mast-cell/basophils) play a role in auto-immune disorders but also in solid organ transplantation. The aim of this study was to evaluate the impact of blood eosinophil count (BCEo) during follow-up of kidney transplanted recipients with a stable graft function at 3-months on immunological outcome.

Material and methods: We performed a survival analysis (cause specific Cox model) between the occurrence of immunologic events (transplant rejection and/or the appearance of *de novo*DSA) and time dependent variation of BCEo adjusted on CNI and oral steroid at each measurement on a retrospective cohort of 1013 kidney transplanted patients in which common causes of increase in BCEo were excluded.

Results: Our data showed that BCEo > 0,3G/L was associated with a 2-time higher risk of immunological event and 3-time higher risk of rejection during follow-up independently of immunosuppressive regimen after 3 months post-transplantation.

Conclusion and clinical implication: Our data revealed that BCEo > 0,3G/L threshold could be an interesting and routine biological maker to monitor for immunological outcome along with other routine parameters in kidney transplantation at steady state after eliminating common causes of BCEo increase (PTLD, allergy/atopy, parasitic infections, drug induced hypersensitivity). These observation thus clearly open new perspectives and directions. Not only they clearly suggest that eosinophil count may be of clinical relevance when detected in blood as surrogate markers of rejection but they also raise the question of the involvement of eosinophils and type 2 immunity in kidney rejection.

Th62. Exosomes of MSCs Target M2-type Macrophages to Promote TGF- β -Dependent Microvascular Stabilization and Functional Recovery in Spinal Cord Injury

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Intravenous (IV) infusion of mesenchymal stromal cells (MSC) stabilizes the blood-spinal cord barrier and improves functional neurological recovery after spinal cord injury (SCI). However, the MSCs do not traffic to the injury site, but rather lodge primarily in the lungs for a few days. Here, groups of 8 young Sprague Dawley rats received 2×10^6 MSCs, their derived exosomes (MSC-exos, 2×10^{10}) in single dose or dividing that dose over 3 days starting on the 10th day following severe contusive SCI, or vehicle control. Locomotion, cell targeting, expression of TGF- β , TGF- β -R, vascular proteins and permeability were determined at 3, 7 and 14 days after treatments. MSC-exos released in vivo by infused MSCs, distribute systemically. At the injury site specifically associate with M2-type-macrophages. A single MSC infusion caused significant improvement in locomotor recovery, fractionated dosing of MSC-exos over 3-days was required for similar effects as infusion of the MSCs. Both MSCs and fractionated MSC-exos increased numbers and differentiation of M2-type-Macs expressing TGF- β , with accompanying expression of TGF- β receptors and tight junction proteins in the neighboring microvascular, resulting in reduction in blood spinal cord barrier vascular permeability. SUMMARY: In SCI, IV infused MSCs release MSC-exos in vivo over time that target lesional M2-Macs to increase their TGF- β production and further induce TGF- β signaling pathways in the local vasculature to mediate therapeutic effects, likely by the M2-Macs release of locally active secondary exosomes transferring miRNAs that induce TGF- β pathways in the neighboring micro vasculature to promote healing.

Th64. Inhibition of Inflammation by Tocilizumab and Etanercept in Nonhuman Primate Immune Cells

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The pro-inflammatory cytokines IL-6 and TNF- α synergistically inhibit allograft acceptance in murine models. Anticytokine therapy with the anti-IL-6R antibody tocilizumab and/or the soluble TNF receptor etanercept have been used off label to promote human allograft survival. These cytokine inhibitors could contribute to the induction of immune tolerance to allografts in translational nonhuman primate (NHP) models; however, their efficacy in NHPs was unproven. Accordingly, this study aims to investigate the ability of tocilizumab and etanercept to inhibit inflammatory responses in cynomolgus macaques. Inflammatory signaling in cynomolgus macaques peripheral blood mononuclear cells following inflammatory stimuli in the presence or absence tocilizumab and/or etanercept and the inflammatory signaling was analyzed using multiparametric phosphoflow cytometry. The effects of tocilizumab and etanercept were determined by analyzing the expression levels of activated intercellular signaling intermediates pSTAT3 and pNF- κ B. All data are expressed as percentage suppression in pSTAT3 and pNF- κ B expression levels against inflammatory stimuli. Tocilizumab suppressed 45% of IL-6-induced pSTAT3 expression in CD4⁺ T cells. Etanercept suppressed 56% of TNF- α -induced pNF- κ B expression in CD4⁺ T cells. Tocilizumab suppressed 56% and etanercept 15% of LPS-induced pSTAT3 expression in CD4⁺ T cells, respectively. The combined use of tocilizumab and etanercept suppressed 68% of LPS-induced pSTAT3 expression in CD4⁺ T cells. Our preliminary results suggest that the approved and clinically administered cytokine inhibitors tocilizumab and etanercept are effective in inhibiting inflammatory signaling in T cells in cynomolgus macaques, thereby supporting the investigation of their role as contributors to transplant acceptance and transplantation tolerance in translational models in nonhuman primates.

Th77. Generation of Human Germinal Centers Allowing Functional B Cell Maturation in Immunodeficient Mice

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Background: Germinal centers (GC) are the place for B cells' maturation and activation after antigenic stimulation. In the context of organ transplantation, this humoral response represents the main organ transplantation outcome determinant due to donor-specific antibodies (DSA) production. The current immunosuppressive strategy poorly controls humoral response after transplantation.

Methodology: To study the role of immunosuppressive strategies on human GC' formation we created a model that allows the generation of human GC' cells in vivo by injection of human blood cells directly into the spleen of immunodeficient mice (NOD / SCID / IL-2R γ - / -). The risk of xenograft-versus-host reaction was minimized by CD8⁺ depletion before injection. GC generation using blood from a healthy donor already vaccinated against hepatitis B allowed us to trigger a specific memory response after antigenic rechallenge (engerix vaccination) detectable through anti-Hbs antibodies secretion.

Results: GC characterization was done by flow cytometry and histology, by evaluating the frequency of B cells (CD19⁺), memory B cells (CD27hiCD38hi), plasmocytes (CD138⁺), and follicular T helper cells (CD4⁺ICOS⁺PD1⁺). Belatacept (CTLA4-Ig) administration at day 0 prevented the generation of GC. A delayed injection (day 12) preserved the apparition of GC, but significantly impaired their quantity. These morphologic changes were associating with a significant decrease in the level of human immunoglobulins (IgG, A, E, M) measured in mice' sera for both timings of injection.

Conclusion: Our model enables the study of immunosuppressive treatments' effects on GC' generation and DSA' production. Belatacept treatment impaired plasmocytes function and quantity as in treated transplanted patients.

Systems Immunology

W74. Defining Maximally Powered Single-cell RNA-seq Studies With scPOST

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As advances in single-cell technologies enable the unbiased assay of thousands of cells simultaneously, human disease studies are shifting to case-control study designs that seek to define differences between diseased and healthy conditions. A key goal in such studies is to identify shifts in cell state frequencies between conditions, such as an expansion of a cell state in diseased samples. These studies require precious clinical samples and costly technologies; therefore, it is critical to employ study design principles that maximize power to detect shifts in cell state frequencies. Here, we present single-cell Power Simulation Tool (scPOST), a method that enables users to estimate their power under different study designs. To approximate the specific experimental and clinical scenarios being investigated, scPOST takes prototype (public or pilot) single-cell data as input and generates *in silico* single-cell datasets for power analyses. We use scPOST to perform power analyses on three independent single-cell datasets that span diverse experimental conditions: a 21-sample rheumatoid arthritis dataset (5,265 cells) from synovial tissue, a 259-sample tuberculosis progression dataset (496,517 memory T cells) from peripheral blood mononuclear cells (PBMCs), and a 30-sample ulcerative colitis dataset (235,229 cells) from intestinal biopsies. Over thousands of simulations, we consistently observe that power to detect cell state frequency shifts is maximized by balanced sample structures (similar number of cases/controls), larger numbers of independent clinical samples, and reduced batch effects.

W83. B Cell Abnormalities in a Patient with Gaucher Disease Type 1

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Background: Gaucher disease (GD) is an inborn error of metabolism caused by mutations in the *GBA* gene resulting in the lysosomal accumulation of glucocerebroside. Enzyme replacement therapy (ERT) is the standard of care. Patients with GD are susceptible to frequent infections and gammopathies; however, the underlying immunological abnormalities have not been fully studied.

Methods: We report the pre-and post-ERT immunological evaluation of a 14-year-old male with compound heterozygote GD type 1 presenting with thrombocytopenia, neutropenia, and splenomegaly. Flow cytometry was used for detailed T, B, and NK cell immunophenotyping. The concentrations of immunoglobulins and specific IgG titers to protein and polysaccharide antigens were also measured.

Results: Before ERT, all subtypes of memory B cells were absent and only naïve and immature CD21^{low} cells were present. T and NK cell numbers, immunoglobulins, and specific antibody titres were all within reference ranges for age. After 1.5 years of ERT, subtypes of memory B cells are now present but lower than reference,

while the rest of the immunological investigations remain normal. The patient has not experienced significant infections, and the other manifestations of the disease have resolved.

Conclusions: Untreated GD seems to cause defects in B cell maturation into memory cells. The exact pathophysiology is not fully known, but others have described the possible involvement of sphingolipid metabolites in immature B cell apoptosis signalling. ERT for 1.5 years has only mildly initiated the memory B-cell differentiation process in our patient, and future immunological evaluations will be warranted.

W122. Identification of Disease-associated Cell States from Single-cell Data Using Co-abundant Transcriptional Neighborhoods

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Single-cell profiling is an essential tool for characterizing immune cells from blood and tissue in the context of human disease. As investigators generate datasets with 10s-100s of samples, there is a growing need to use cross-sample comparisons to better identify precise cell states associated with sample attributes like disease status or treatment outcome. Current statistical approaches typically map cells to a specific transcriptional structure—e.g., clusters or a transcriptional trajectory—and examine sample differences through that lens. By contrast, we present a method to identify cell populations of interest with more flexibility and granularity: covarying neighborhood analysis (CNA). CNA determines the cell abundance from each individual in many small regions across transcriptional space, termed neighborhoods. Using principal components analysis on the samples-by-neighborhoods matrix, CNA identifies neighborhood groups that covary in abundance and are likely to share function or regulation. The resulting principal components characterize dominant axes of inter-sample variation and are used for rigorous association testing by modeling sample attributes as a function of abundance in these covarying neighborhood groups. CNA is highly relevant to immune cells, for which cell subtypes exist along spectra of states that are closely related. In simulation, CNA is more powerful and better able to precisely identify associated cell populations than cluster-based approaches. In published datasets, CNA captures a Notch activation signature in inflammatory arthritis, substantially redefines monocyte populations expanded in sepsis, and identifies a previously-undiscovered cytotoxic CD4⁺ memory T cell state expanded among tuberculosis patients who rapidly progress to active symptoms.

W151. immuneML: An Ecosystem for Machine Learning Analysis of Adaptive Immune Receptor Repertoires

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Adaptive immune receptor repertoires (AIRR) are key targets for biomedical research as they record past and ongoing adaptive immune responses. The capacity of machine learning (ML) to learn complex discriminative sequence patterns renders it an ideal approach for AIRR diagnostic and therapeutic discovery. To date, widespread adoption of AIRR ML has been inhibited by a lack of reproducibility, transparency, and extendibility. immuneML addresses these concerns by implementing each step of the AIRR ML process in an extendable, open-source, AIRR-compliant ecosystem that is based on fully specified and shareable workflows. To facilitate

widespread user adoption, we provide beginner-friendly default workflows and a Galaxy interface to construct and execute custom workflows online. We demonstrate the broad applicability of immuneML by (i) replicating a large-scale study on immune state prediction, (ii) developing, integrating, and applying a new method for antigen specificity prediction, and (iii) showcasing streamlined AIRR ML interpretability-focused benchmarking.

Th61. Allele-specific Expression Changes Dynamically During T Cell Activation in HLA and Other Autoimmune Loci

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Genetic variants that predispose us to autoimmune diseases are over-represented in regulatory elements specific for memory CD4⁺ T cells. Hence, in order to understand the genetic mechanisms of autoimmunity, it is essential to study how genetic variants influence gene regulation in different T cell physiological states. In this study (Gutierrez-Arcelus, Baglenko et. al, Nature Genetics 2020) we analyzed allele-specific expression as a readout of genetic regulatory effects, and characterized how these regulatory effects are dynamically modulated during CD4⁺ memory T cell activation. We performed high depth RNA-seq in 24 healthy individuals at 8 different time points during stimulation with anti-CD3/CD28 beads. We uncovered that dynamic allele-specific expression during CD4 memory T cell activation is widespread and four-fold enriched in autoimmune disease loci. We discovered that the major autoimmune risk gene *HLA-DQB1* has 3 distinct regulatory haplotypes with expression profiles that could come from 3 different genes. We showed with CRISPR-Cas9 the causal variant driving the late-activation up-regulation of *HLA-DQB1* at mRNA and protein levels. This study highlights that (1) in order to understand genetic mechanisms of autoimmune disease we need to ascertain multiple cell states, and (2) HLA genes are not only highly variable in their protein sequences, but also in their levels of expression, which could play a role in modulating disease risk.

Th151. Identification of Non-invasive Serum Biomarkers to Classify Ulcerative Colitis and Crohn's Disease Subjects from Healthy Controls

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For inflammatory bowel disease (IBD), there is a need for blood-based biomarkers to monitor disease severity, disease progression and differentiate patients that would benefit from specific treatment regimens. We performed an exploratory analysis of serum protein markers in baseline samples collected from two Eldelumab Clinical Phase2 Studies in UC and CD (NCT01294410 and NCT01466374), vs. non-IBD controls to determine markers that could differ in disease states.

Using the high throughput Somalogics SomaScan Platform, we measured 5284 proteins in serum collected from 88 UC, 120 CD subjects, along with 78 normal healthy volunteers. Gene expression data from colon biopsies from these subjects was also generated.

Using a linear regression model, compared to control, 107 and 160 differentially expressed proteins were identified in UC and CD, respectively, at 5% false discovery rate with a fold change > 1.5. The proteins upregulated are associated with inflammation and immune functions while the downregulated proteins were associated with metabolism and colonic epithelial functions in both UC and CD. Some of inflammatory biomarkers identified in serum tracked with disease severity and also demonstrated concordant with mRNA expression in colon biopsies.

In parallel, an adaptive Least Absolute Shrinkage and Selection Operator (LASSO) based model with ~40 proteins were able to identify UC serum from controls with 87.5% accuracy.

The proteomics signatures developed may provide insights into disease pathology, classify subjects based on disease severity and monitor therapeutic responses.

Th152. A Single-tube, 44-marker CyTOF Assay to Assess Antigen-specific Immunity in Whole Blood Human Samples with Data Analysis Solution

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Monitoring the immune response in the setting of infectious disease and cancer is critical to assess disease status and targets of immune therapy. CyTOF® mass cytometry enables multiplex cellular phenotyping with more than 50 markers, making it ideal for comprehensive immune profiling. CyTOF technology utilizes antibodies tagged with unique monoisotopic metals, resulting in distinct signals that provide a high-resolution multiparametric landscape of a single cell. The Maxpar® Direct™ Immune Profiling Assay™ is a pre-titrated, dried-down, 30-marker antibody cocktail for immune profiling of human whole blood and PBMC by CyTOF. Paired with Maxpar Pathsetter™ software, stained samples are automatically resolved into 37 immune populations including major lineages and their subsets. In this study, we expanded the 30-marker assay to a 44-marker panel including exhaustion markers such as PD-1 and CTLA-4, co-stimulation markers 4-1BB and ICOS, and intracellular cytoplasmic markers IFN-γ, TNF-α, IL-2, perforin and granzyme B to assess cellular function in PMA/ionomycin-stimulated whole blood cultures. We modified the existing Maxpar Pathsetter model to automate the analysis of the expanded panel and report on additional functional parameters such as T cell exhaustion and cytokine production. Next, we applied this panel to whole blood stimulated with CMV peptides to investigate antigen-specific immune responses in a viral infection model in concert with in-depth phenotypic assessment. Collectively, we demonstrate the flexibility of the Maxpar Direct Immune Profiling Assay to incorporate additional surface and intracellular markers to study antigen-specific immunity in the context of whole blood immune profiling.

Th169. Formulating a Gene Signature for Diagnosis of Autoimmune and Infectious Diseases

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When patients with an underlying autoimmune condition like JIA or lupus report life-threatening symptoms, physicians need to quickly determine whether these symptoms are caused by an acute infection or a complication of their autoimmune condition. As immunosuppressive drugs are harmful to someone undergoing

an infection, accurate and timely diagnosis is critical. In recent years, host-response-based diagnostics have shown promise in accurately and non-invasively diagnosing a number of infectious and autoimmune diseases.

Here, we collected and curated blood transcriptome profiles of 14,587 patients from 42 countries across 122 independent datasets and sorted them into Infectious, Autoimmune, and Healthy categories. This data represents the biological, clinical, and technical heterogeneity present in real-world patient populations. Using a novel statistical framework, we created two signatures from this data: one to differentiate patients with autoimmune or infectious diseases from healthy individuals and another to differentiate between patients with autoimmune or infectious diseases. Both signatures achieve an area under the receiver operating characteristics curve (AUROC) of over 0.87 on completely independent datasets. Because our training and testing data included heterogeneity across many factors, these gene signatures can be utilized in diverse clinical populations. Furthermore, these signatures can aid physicians across a broad range of clinical scenarios, where existing diagnostics are invasive, expensive, or non-specific.