Abstract #: F1

EGFP-talin1 knock-in mice reveal talin1 recruitment and distribution during neutrophil rolling and migration

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Neutrophils express β2 integrins, mainly LFA-1 and Mac-1, which mediate neutrophil slow rolling, arrest, spreading and transmigration. For these functions, β2 integrins must be partially or fully activated. Talin1 tightly regulates β2 integrin activation by binding to the cytoplasmic tail of β2 integrins. However, the timing of talin1 recruitment and its distribution under physiological conditions remains poorly understood. To address this, we generated EGFP-talin1 knock-in mice, enabling real-time visualization of talin1 behavior during integrin activation. Our data demonstrate that EGFP-talin1 is robustly expressed in neutrophils and functions effectively without altering integrin expression levels. Using TIRF microscopy, we observed that Talin1 is recruited to the plasma membrane during neutrophil rolling and is extensively recruited when neutrophil arrest. Using intravital microscopy, we observed the dynamics of talin1 in migrating neutrophils within venules of cremaster muscle. Talin1 predominantly localized to the uropod (rear) and leading edge (front) during the early stages of migration, with its distribution progressively shifting to the leading edge during migration. These EGFP-talin1 knock-in mice provide a powerful tool for studying the mechanisms of integrin activation and its role in neutrophil migration under physiological conditions.

Abstract #: F2

Three-step transition from Tregs to exTregs

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Atherosclerosis is a chronic inflammatory disease in which regulatory T cells (Tregs) play a critical role in suppressing disease progression. However, atherogenic conditions can destabilize Tregs, leading to the loss of the lineage-defining transcription factor Foxp3 and the emergence of exTregs. The mechanisms underlying this transition remain unclear. Using Foxp3$^{eGFP\text{-}cre\text{-}ERT2}$ ROSA26$^{fl\text{-}STOP\text{-}fl\ tdTomato}$ *Apoe*$^{-/-}$ lineage-tracing mice, we identified a stepwise process in which Tregs convert into exTregs during atherosclerosis. First, eGFP+ tdTomato^{low} Tregs upregulate tdTomato expression. Second, these cells lose CD25 expression, marking the initial shift from an activated Treg state to an effector-like phenotype. Third, they downregulate Foxp3 and eGFP, fully acquiring an exTreg identity. IL-2 and TCR stimulation promote the transition of Tregs into the intermediate state, while proinflammatory cytokines such as IL-6 drive their further conversion into fully developed exTregs. IL17A+ Tregs appeared transiently in eGFP+ tdTomato^{hi} cells and did not differentiate into exTregs. Many eGFP- tdTomato^{hi} exTregs expressed IFN-γ and TNF-α. In conclusion, our findings define three steps in the Treg to exTreg transition: (1) loss of CD25, (2) expression of IL17A, (3) loss of FoxP3 and expression of IFN-γ. These insights provide a foundation for elucidating the molecular mechanisms governing Treg instability in atherosclerosis.

Words:200

Abstract #: F3

Title

The ubiquitin ligase Cul4b drives B cell proliferation and antiviral antibody responses

Authors

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High affinity neutralizing antibodies (Abs) are essential for immune protection against Influenza A virus (IAV) infection. Affinity maturation occurs in germinal centers (GC), where activated B cells undergo proliferation, somatic hypermutation, and selection. This requires high levels of DNA damage while maintaining a rapid proliferation rate. Cullin 4b (Cul4b) is an E3 ubiquitin ligase found in most cells that regulates cell cycle maintenance and responses to DNA damage. Cul4b serves as a docking protein for the Cullin RING Ligase 4 (CRL4) complex, which ubiquitinates substrate proteins for degradation. Because proliferation and DDR are essential for humoral immunity, we hypothesize that Cul4b also functions in GC B cells. To test this, we infected mice lacking Cul4b in B cells (KO) and wild-type (WT) controls with a mouse adapted H1N1 Influenza A virus (IAV), PR8. We measured GC B cells and IAV-specific serum Abs. We found these to be dramatically reduced in Cul4b KO mice. We also tested if B cells need Cul4b for clonal expansion by stimulating WT and KO B cells to proliferate in vitro in the presence of ultraviolet (UV) induced DNA damage. We found Cul4b promotes B cell proliferation after UV exposure. Understanding how Cul4b promotes B cell clonal expansion in the face of DNA damage will identify new targets to transiently amplify this pathway. This may be used to improve vaccines by encouraging Ab responses or can be inhibited to prevent tumor progression in cancer.

Abstract #: F4

Programmed Cell Death Protein 1 (PD-1) Regulates Metabolic Activity of Regulatory T cells in Ischemia-Induced Acute Kidney Injury

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Immune checkpoint inhibitors (ICPI) therapy, targeting PD-1 and its ligands PD-L1 and PD-L2, have revolutionized cancer care. However, ICPI use is linked to immune-related adverse events, including nephropathy, which manifests as acute kidney injury (AKI). AKI, a condition marked by a rapid decline in kidney function, is often caused by ischemia-reperfusion injury (IRI). If unresolved, AKI can progress to chronic kidney disease, renal failure, and death. Regulatory T cells (Tregs) are known to mitigate IRI-induced damage, and their protective function is dependent on PD-1 signaling. Treg stability and function rely on cellular metabolism, with a distinct preference for oxidative phosphorylation (OXPHOS) over glycolysis. Foxp3 expression alters T cell metabolism by suppressing glycolysis and promoting OXPHOS, while effector molecules like CTLA-4 and PD-1 further regulate these metabolic pathways. However, the role of PD-1 in controlling OXPHOS in Tregs remains unexplored. We demonstrate that global PD-1 KO mice get more severe loss of renal function and injury, when subjected to IRI. PD-1-deficient Tregs lose their protective ability against AKI despite retaining in vitro suppressive activity and Foxp3 expression. Transcriptomic analysis reveals altered expression of genes linked to function, proliferation, and mitochondrial metabolism in PD-1 KO Tregs. Furthermore, PD-1 KO Tregs exhibit reduced mitochondrial mass, membrane potential, biogenesis, and dynamics, leading to impaired mitochondrial fitness. These metabolic defects may stem from morphological alterations in mitochondria, highlighting PD-1's critical role in Treg-mediated nephroprotection. Our findings reveal the metabolic vulnerability of PD-1-deficient Tregs and underscore the importance of PD-1 signaling in Treg-mediated kidney protection.

Abstract #: F5

Caspase-8 expression in CD8+ T cells promotes pathogen restriction in the brain during *Toxoplasma gondii* infection

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Cell death is an integral restriction mechanism of intracellular pathogens. In the brain, however, death of long-lived cells can have detrimental effects on cognition, behavior, and survival. We have previously reported extensive cell death in the brain during infection with the intracellular parasite, *Toxoplasma gondii.* Here we focus on caspase-8, a regulator of extrinsic apoptosis, to help elucidate a cell-type-specific role of *Casp8* as a mechanism of pathogen control. We find that *Casp8*$^{-/-}$*Ripk3*$^{-/-}$ mice have an eight-fold increase in brain parasite burden compared to control mice and succumb to the infection within six weeks, regardless of a stronger Th1 immune response. To identify parasite interacted cells in the brain, we infected control and *Casp8*$^{-/-}$*Ripk3*$^{-/-}$ mice on a cre-reporter background with a cre-secreting parasite. As previously reported, primarily neurons report cre activity in C57BL/6 mice while in *Casp8*$^{-/-}$*Ripk3*$^{-/-}$ mice we found neurons, astrocytes, and CD8^{+} T cells had interacted with the parasite. Testing the role of *Casp8* in these individual cell types we found that *Casp8* deficiency in neurons and astrocytes did not impact the control of infection in the brain. In *CD8α*^{Cre}*Casp8*$^{fl/fl}$*Ripk3*$^{-/-Ai6}$ mice we found elevated levels of parasite in the brain despite comparable Th1 immune responses. Additionally, we identified infected CD8^{+} T cells suggesting a mechanism of parasite survival in the absence of *Casp8*. It has been well-characterized that the role of CD8^{+} T cells in restricting *T. gondii* is dependent on cytokine production and cytolytic killing of infected cells. Our data demonstrates that caspase-8 is an additional mechanism by which CD8^{+} T cells restrict parasite in the brain and mediate resistance to infection.

Abstract #: F6

The Role of CNS-Resident Antigen Presentation in *Toxoplasma gondii* Infection

Sydney A. Labuzan[1], Michael A. Kovacs[1], Anne E. Schuster[1], Lydia A. Sibley[1], Isaac W. Babcock[1], Maureen N. Cowan[1], Abigail G. Kelly[1], Tajie H. Harris[1]

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T cells are critical for controlling infection, but how their function is shaped in the brain remains unclear. *Toxoplasma gondii* infection is a useful model for studying neuroinflammation, with parasite burden serving as a marker of immune dysfunction. During infection, T cells and myeloid cells cross the BBB and infiltrate the brain. We observe that microglia and astrocytes upregulate MHCI, potentially presenting *T. gondii* antigen to locally reactivate CD8+ T cells. Using *B2m* deletion to prevent MHCI expression, we investigated how antigen presentation by astrocytes and microglia regulates CD8+ T cell function during *T. gondii* infection. We generated Cx3Cr1-CreERT2 x Rosa26Ai6 x $B2m^{fl/fl}$ and Gfap-cre x $B2m^{fl/fl}$ mice. At chronic infection (6wpi), we assessed IFNγ, TNFα, and iNOS by flow cytometry, along with parasite burden. In astrocytic B2m knockouts, we found enhanced CD4+ T cell cytokine production but no changes to CD8+ T cell cytokine production, increased iNOS+ myeloid cells, and elevated parasite burden—suggesting astrocytes influence CD8+ T cell cytotoxicity via MHCI. We plan to assess Granzyme B expression to further investigate. In contrast, microglial MHCI deletion had no impact, possibly due to compensation by infiltrating myeloid cells presenting to CD8+ T cells.

Abstract #: F7

Title: HLA-E Restricted RL9 and KF11-specific CD8+ T cells Detected in Acutely HIV-Acquired People with HIV Using Novel Multimer Synthesis Protocol

Authors: Kevin Maroney[1], Michael Rose[1], Ann Duerr[2], Anju Bansal[1], Paul Goepfert[1]
[1]University of Alabama at Birmingham
[2]University of Washington

Abstract:

Background

HLA-E restricted CD8+ T cells have been underexplored in HIV research, with only two reported epitopes, both also restricted by classical HLA-I alleles. We postulated that the apparent rarity of these cells might be due to many HLA-E binding peptides exhibiting low affinity.

Methods

We assessed the HLA-E stabilization potential of 180 predicted T cell epitope (PTE) 15-mers derived from HIV-1 Gag in HLA-E01:01 and HLA-E01:03 cell lines. Using the high-affinity RL9 epitope, we developed tetramers with a refined peptide exchange and multimer staining protocol. To enhance the detection of low-affinity interactions, we created high-valency HLA-E KF11 dextramers.

Results

Abstract #: F7

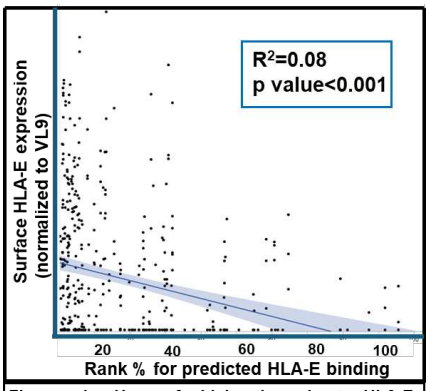

Figure 1. Use of high throughput HLA-E stabilization assay can identify potential candidate HIV peptides predicted to bind HLA-E. Correlation plot data is shown for Gag peptides for their binding to HLA-E*01:03. Scatter plot of 3 replicates each of HLA-E stabilization for 180 predicted T cell epitope (PTE) peptides (15 mers) normalized between no peptide (0% VL9) and A*02-VL9 (100% VL9). Normalized %VL9 α-E*01-APC (3D12 clone) stabilization over no peptide values post overnight 26°C pulse are shown on Y-axis against the rank percentile for the highest predicted binding epitope (9-14-mer) among all epitopes predicted to bind to E*03 through Net-MHC on X axis. Inverse correlation (lower percentile number indicates tighter binding) is represented as a line.

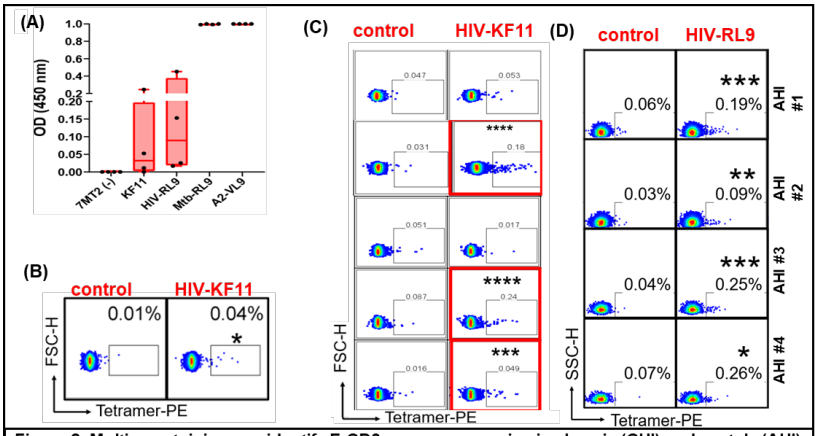

Figure 2: Multimer staining can identify E-CD8 responses ex-vivo in chronic (CHI) and acutely (AHI) HIV-acquired PWH. (A) Barplot of peptide exchange rate of indicated peptides for HLA-E*01:01 monomer preloaded with VMAPJTLVL peptide (7MT2) in an ELISA assay. Data for KF11 and RL9 is shown in relation to positive controls (Mtb-RL9 and A2-VL9 peptides). **(B)** KF11 loaded monomer was tetramerized and used to ex-vivo detect E-CD8s in an elite controller. **(C)** KF11 loaded monomer was dextramerized and the sensitivity and specificity tested in the same elite controller as in **(B)** in relation to a person living without HIV (PWO). Significance (* and **** are $p < 0.05$ and 0.0001 respectively) based on Fischer's exact T test between counts of parent and child response gate between indicated condition and control. **(D)** Tetramer staining of RL9 specific E-CD8 responses ex-vivo in people with acute HIV acquisition (AHI). Data is shown for all 4 tetramer+ HLA-A*02 individuals. Significance based on Fischer's exact T test between HIV-RL9 and the negative control is shown (*, **, *** are p-values of < 0.05, 0.01, 0.001 respectively).

Over 10 peptides stabilized HLA-E, though typically at lower levels than RL9 (Figure 1). The predicted HLA-E binder Rank% of the top internal predicted epitope within each 15-mer correlated significantly negatively with HLA-E stabilization results but with low reliability. This highlights the need to screen HIV-1 epitopes for HLA-E binding in unbiased assays. RL9 tetramers identified responses in 4 of 19 participants (26.7%) during acute HIV acquisition, notably including the only PWH with undetectable viral load off ART (Figure 2). High-valency HLA-E KF11 dextramers detected low-affinity interactions, identifying HLA-E restricted KF11-specific CD8+ T cells in chronically infected PWH that tetramers missed. These findings show that HLA-E restricted CD8+ T cell responses are primed early during HIV-1 acquisition and are more prevalent than previously recognized.

Conclusions

Our work underscores the previously underappreciated prevalence and functional importance of HIV-specific HLA-E restricted CD8+ T cells.

Funding Sources: R01 AI162168 , T32 AI7051-47 , P30 AI027767 , UAB Research Development Funds

Short Autobiography:

Born in Irvine, California, I earned a Bachelor's in Genetics and a Master's in Biotechnology. In Dr. Ilhem Messaoudi's lab, I studied macaque transcriptional responses to Ebola isolates. Now, in Drs. Paul Goepfert and Anju Bansal's lab, I study HLA-E restricted immune responses to HIV-1 for vaccines.

Abstract #: F8

Regulatory maturation of cDC1 accelerates effector CD8 T cell contraction after severe influenza A virus infection.

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XCR1+ dendritic cells (cDC1) are essential to start CD8 T cell responses against acute Influenza A virus (IAV) infection. In the recovery phase, once the infection is controlled, residual viral antigens sustain cytokine production by CD8 T cells and prolong lung damage, the role of cDC1 in regulating effector CD8 T cells during this time is unclear. To study the role of cDC1 in the regulation of CD8 T cells we infected mice with IAV and followed the kinetics of NP-specific CD8 T cells and mature cDC1, we found that IAV infection triggers two rounds of cDC1 maturation. The first round occurs during the acute infection and is followed by recruitment of effector CD8 T cells while the second round occurs in the recovery phase and is followed by contraction of NP+ CD8 T cells. To compare the function of these two rounds of mature cDC1 we performed adoptive transfers into Batf3$^{-/-}$ mice and *in vitro* cultures with OT-I cells and evaluated the capacity of CD8 T cells to produce IFNγ and TNFα. We found that mature cDC1 from the recovery phase inhibit cytokine production in CD8 T cells. Next, we did scRNA seq on mature cDC1 from naïve mice (homeostatic maturation), infected mice and recovering mice. Our data showed that cDC1 maturation is driven by TNFα signaling and IFNγ signaling in naïve and infected mice respectively. In recovering mice, mature cDC1 are enriched in genes associated with efferocytosis and immunoregulation. Finally, to evaluate the capacity of cDC1 to acquire apoptotic cells we infected green-fluorescent mice 8 weeks after reconstitution with congenic BM. We found that in the recovery phase, cDC1 are more efferocytic than in naïve and infected mice. Overall, our data shows that mature regulatory cDC1 control CD8 T cell responses after IAV infection.

Abstract #: F9

Cellular and molecular heterogeneity of lung tissue-resident memory T cells in protective immunity

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Lung tissue-resident memory T cells (TRMs) are essential defenders against respiratory reinfection, although the mechanisms regarding their heterogeneity and protective activities remain incompletely understood. The difficulties in manipulating polyclonal endogenous TRMs hinder exploring their natural responses. Although monoclonal TCR transgenic T cells are used, the diverse antigen repertoire during infection raises concerns about the ability of monoclonal TRMs to accurately mirror natural TRM responses against different antigens. By analyzing lung TRM scRNA-seq data from influenza infected mice, we observed two endogenous polyclonal TRM populations: one with effector T cells characteristics (TRM-EL); and the other upregulates conventional TRM features (TRM-Con). Our data suggested epitope-specificity of the two populations, with over 90% of nucleoprotein specific TRMs showing TRM-EL features, while TRM-Con predominantly reacts to polymerase acid. To explore the mechanisms controlling heterogeneity, we utilized the influenza that has lower antigen dose. We found that the low antigen led to diminished TRM-EL cell counts and identifications, suggesting that initial antigen dose determine TRM heterogeneity. Functionally, TRM-EL exhibits fast expansion and recall responses during reinfection compared to TRM-Con, indicating rapid protective potential of TRM-EL. Lastly, the reinfection challenge suggested that only the intact TRM-EL is required for efficient heterologous protection against influenza reinvasion. Taken together, our results provide mechanistic insight into the fate decision and functional heterogeneity of TRMs, with implications for enhancing protection against respiratory infection.

Abstract #: F10

Characterizing the role of astrocytic and microglial IL-33R signaling in neuroinflammation in Experimental Autoimmune Encephalomyelitis

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IL-33R (ST2), a member of the IL-1 receptor family, is expressed by microglia and astrocytes within the central nervous system (CNS). Notably, IL-33R expression is upregulated in postmortem brain tissue from individuals with multiple sclerosis, suggesting a potential role in the pathogenesis of neuroinflammatory diseases. Prior studies using global knockout models have demonstrated that IL-33R deletion exacerbates experimental autoimmune encephalomyelitis (EAE) in C57BL/6 mice and induces disease in normally resistant BALB/c mice, implying a neuroprotective role for IL-33R signaling. However, the cell type-specific contributions of IL-33R in glial cells remain unclear. We hypothesized that IL-33R signaling in microglia and astrocytes serves a neuroprotective function by regulating inflammatory processes during EAE in C57BL/6 mice. To test this, our lab generated tamoxifen-inducible conditional knockout mice targeting IL-33R in microglia (via the *Cx3cr1*-CreER promoter) and in astrocytes (via the *Aldh1l1*-CreER promoter), as well as conditional knockouts under the *GFAP-Cre* promoter. Unexpectedly, our data indicate that selective deletion of IL-33R in microglia or astrocytes does not significantly affect EAE disease severity or onset, nor does it alter immune cell composition in the CNS or periphery. These findings suggest that while global IL-33R signaling is important in modulating EAE pathogenesis, the specific contribution of microglial and astrocytic IL-33R in C57BL/6 mice might be limited or dispensable. Further studies are currently being conducted using the JHMV model of CNS demyelination to more comprehensively define the broader role of IL-33R.

Abstract #: F11

Cardiometabolic Disease Expands Basophil Heterogeneity and Promotes Pro-inflammatory Endothelial Interactions

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Cardiometabolic diseases (CMD) significantly contribute to global mortality. Elevated levels of reactive oxygen species, e.g. hydrogen peroxide (H_2O_2) promote a dysfunctional, pro-inflammatory endothelial phenotype. Our data indicate H_2O_2 production enhances basophil proliferation and activation. Traditionally associated with allergic reactions, basophils are emerging as critical mediators in CMD-related vascular inflammation. Circulating basophils can activate endothelial cells via cytokine secretion and degranulation, leading to increased expression of adhesion molecules. We hypothesize oxidative stress exacerbates endothelial dysfunction via expansion and activation of basophils**.** Using *in vitro* murine bone marrow cultures, we found modulation of H_2O_2 levels leads to differences in basophil proliferation and gene signatures related to inflammatory response pathways. Through single cell RNA-sequencing of basophils isolated from mice placed on a normal chow or high fat, high sucrose (HFHS) diet meant to mimic conditions of human CMDs, we have observed an expansion in basophil heterogeneity, activation signatures, and endothelial interacting genes in the HFHS diet fed mice. With ongoing studies, we are characterizing phenotypes of activated basophils using spectral flow cytometry, and ultimately, we will investigate the functional effects of removing basophils from circulation in diseased conditions with specific, diphtheria toxin-mediated depletion.

Abstract #: F12

PSGL-1 excludes HIV Env from virion surface through spatial hindrance involving structural folding of the decameric repeats (DR)

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[2]George Mason University, School of Systems Biology, Manassas, VA,

[3]National Cancer Institute, HIV Dynamics and Replication Program, Frederick, MD

P-selectin glycoprotein ligand-1 (PSGL-1), a mucin-like surface glycoprotein, is primarily expressed on lymphoid and myeloid cells. PSGL-1 has recently been identified as an HIV restriction factor, blocking HIV infectivity mainly through virion incorporation that sterically hinders virion attachment to target cells. PSGL-1 also inhibits HIV Env incorporation into virions. However, the molecular mechanisms of PSGL-1-mediated Env exclusion remained unclear. Here, we investigated the role of PSGL-1's extracellular (EC) and intracellular (IC) domains in Env exclusion. We demonstrate that both EC and IC are important for Env exclusion; when EC was deleted, PSGL-1 completely lost its ability to inhibit Env incorporation, whereas when IC was deleted, PSGL-1 partially lost this activity. In addition, when the decameric repeats (DRs) were deleted from EC, PSGL-1 also lost its ability to inhibit Env incorporation. Sequential DR deletion mutagenesis further demonstrated that a minimum of 9 DRs is necessary for Env exclusion. Molecular modeling of the DR structure revealed that PSGL-1 mutants with 7 or fewer DRs pose as an extended "rod-like" structure, whereas those with 9 or more DRs collapse into a "coil-like" structure that can spatially exclude Env. Our studies suggest a model in which Env exclusion involves Gag-mediated PSGL-1 targeting to the virion assembly site where DR-mediated spatial exclusion blocks Env incorporation.

Abstract #: F13

Single-cell multiomics resolves ETP heterogeneity and identifies Tcf1 and Lef1 as pre-thymic initiators of the ETP fate

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Bone marrow-derived multipotent hematopoietic progenitors seed the thymus and give rise to early thymic progenitors (ETPs), the earliest recognizable thymocytes with T-lineage potential. However, the factors that govern ETP formation are insufficiently defined. Using scRNA-seq and scATAC-seq, we dissected the heterogeneity of transcriptomic and chromatin accessibility (ChrAcc) landscapes of ETPs harbored in DN1 thymocytes. Tcf1$^-$ ETPs exhibited higher proliferative capacity and retained stronger non-T lineage potential, while Tcf1$^+$ ETPs were immediate, more robust precursors to T-lineage-specified DN2 thymocytes. Whereas Tcf1 alone was dispensable for generating ETPs, pre-thymic ablation of both Tcf1 and its homologue, Lef1, severely impaired ETP formation *in vivo*. Single-cell multiomic analysis of Tcf1/Lef1-deficient DN1 thymocytes revealed that Tcf1 and Lef1 exerted essential roles in transcription activation of *Notch1* or Notch pathway effector molecules including *Hes1* and *Hhex*, while concurrently suppressing gene programs leading to B-cell and myeloid lineage diversion. Furthermore, temporally controlled acute deletion in emergent ETPs *ex vivo* demonstrated critical requirements for Tcf1 and Lef1 in maintaining ETP lineage stability. These findings thus resolved the functional redundancy of Tcf1 and Lef1 and elucidated their pivotal roles as pre-thymic initiators of the ETP fate and intrathymic gatekeepers of ETP identity and T-lineage potential.

Abstract #: F14

Title: Characterizing the Role of TLR2-/- Eosinophils in Protection Against *Clostridioides difficile* Infection

Authors: Mahta Gooya[1], Farha Naz[1], William A. Petri, Jr[1].
Affiliation: 1. Division of Infectious Diseases and International Health, University of Virginia School of Medicine, Charlottesville, United States of America

Clostridioides difficile, a gram-positive, anaerobic, spore-forming bacterium, is the major cause of antibiotic-associated infectious colitis and is responsible for nearly 500,000 infections per year in the U.S., leading to diarrhea, pseudomembranous colitis, and, in severe cases, mortality. Eosinophils (Eos), innate type 2 granulocytes, are primarily known for their multifaceted roles in helminth infection and allergy. However, emerging evidence suggests that Eos also contribute to antimicrobial immunity, including protection against *C. difficile* infection (CDI). Our previous studies have demonstrated that increasing Eos levels via IL-25 injection or adoptive transfer of Toll-like receptor 2 (TLR2)−/− Eos enhances protection against CDI. Notably, Eos with functional TLR2 signaling do not confer protection, and TLR2 is required for *C. difficile*-mediated Eos depletion. We hypothesize that Eos degranulation is regulated by TLR2 signaling and crucially affects gut epithelial barrier integrity in CDI. We further hypothesize that IL-25 signaling in Eos increases expression of immunomodulatory (PD-L1, IL1Ra, TGF-β) and tissue repair factors (ECM components, MMPs), negatively regulating deleterious Th17 responses and bolstering epithelial barrier integrity. To elucidate mechanisms underlying Eos-mediated protection and TLR2/IL-25 modulation, we will employ bulk RNA sequencing, immunofluorescence microscopy, spectral flow cytometry, *in vitro* immunoassays, and *in vivo* genetic approaches. We aim to elucidate Eos function in CDI and characterize TLR2- and IL-25-dependent pathways influencing their protective capacity, offering insights into potential therapeutic strategies and early disease pathogenesis.

Abstract #: F15

A nutrient deficient, humanized diet drives Immunoglobulin A overproduction and small intestinal bacterial overgrowth in mice

Claire Williams [1,2], Yadeliz A. Serrano Matos[1,2], Lindsey Bihuniak[2], Caroline Danielski[1,2], Carrie Cowardin [1,2]

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Undernutrition and growth stunting present a major global health challenge, with broad impacts on immunity in stunted individuals. This condition is multifactorial and arises due to a combination of poor diet, infection, and disruption of the intestinal microbiota. Immunoglobulin A (IgA) is a key regulator of the microbiota, and alterations in IgA production and microbial targeting have been demonstrated in undernourished children. However, due to the multifactorial nature of the disease, the mechanistic basis for this increase in IgA is unclear. To test the specific effects of a malnourished diet on intestinal IgA responses, we fed young SPF mice a protein-and-micronutrient-deficient diet modeled after a malnourished human diet (named “Malawi-8” or “M8” diet). After 4 weeks of diet treatment, M8-fed mice had elevated small intestine luminal and serum IgA and higher proportions of intestinal IgA+ plasma cells relative to controls. These mice exhibit other hallmarks of intestinal inflammation including increased infiltration of immune cells into the small intestine lamina propria and more TH17 cells producing proinflammatory IL-17a, suggesting a microbiota-directed immune response. Indeed, M8-fed mice also exhibit bacterial overgrowth in the distal small intestine and have altered microbiota composition compared to controls, driving the hypothesis that undernutrition promotes overgrowth of immunogenic microbes, which could contribute to intestinal inflammation in undernourished humans. Key future directions to understand the IgA-microbiota relationship during undernutrition include identifying microbes of interest and key microbial features that drive increased IgA and inflammation during undernutrition.

Abstract #: F16

Spatially Resolved Analysis of T and B Cell Interactions in Merkel Cell Carcinoma

Authors: Gabriel Falcao Alencar, Haroldo Rodriguez, Tomas Bencomo, Paul Nghiem, Justin Taylor

Merkel Cell Carcinoma (MCC) is a rare and aggressive skin cancer with a high mortality rate. Effective treatment options are limited, highlighting the need to understand the underlying biology of MCC to develop better therapeutic strategies. Our recent work studying B cells and T cells within MCC tumors has indicated that patients with strong oncoprotein-specific B cell responses predicts long-term control of MCC. However, it remains unclear whether the association with improved outcomes is directly related to B cells involvement in tumor control or if it is due to an overall better immune response while not directly aiding in the control itself. To further explore this predictive association, we have begun to use spatial transcriptomics in order to profile interactions between T cells and B cells in the tumor microenvironment. Preliminary results indicate that patients with B cells closely interacting with both $CD4^+$ and $CD8^+$ T cells have higher survival rates, whereas interactions involving B cells and either $CD4^+$ or $CD8^+$ T cells alone do not correlate with improved survival. These findings suggest that the triad of B cells, $CD4^+$ T cells, and $CD8^+$ T cells is crucial for higher survival rates in MCC patients. Ongoing studies are focused on comparing survival rates and treatment responses to the immune landscape within patient tumors to further elucidate the role of these cellular interactions in MCC. Additionally, future studies will address whether B cells can directly present oncoprotein to $CD8^+$ T cells to enhance tumor killing or if tumor control is mediated indirectly through $CD4^+$ T cells.

Abstract #: F17

Proteomic and gene expression profiling the tissue microenvironment of Large Granular Lymphocyte Leukemia

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T-large granular lymphocyte leukemia (T-LGLL) is characterized by a clonal expansion of cytotoxic CD8 T- cells. The pathogenesis of LGLL is not fully understood, and with no FDA approved therapies, there is a need for a deeper understanding of the disease's molecular complexities and impact on the tissue microenvironment. It has been previously shown that T-LGLL cells accumulate and cluster in patient tissues including bone marrow and spleen. We confirmed via IHC staining for T-cell markers (CD3, CD8) that T-LGLL cells accumulate and form distinct linear arrays in intrasinusoidal spaces in patient tissues vs. healthy donors. In addition to these histopathological findings, we have generated single-cell RNA sequencing data from peripheral blood mononuclear cells from T-LGLL patients, which revealed the upregulation of genes related to cell adhesion pathways. To quantify cell-cell communication networks, we used LIgand receptor ANalysis frAmework (LIANA). These results were compared to healthy donors to identify shared and unique interactions between T-LGLL cells and normal counterpart CD8 TEMRA cells. Several genes related to cell adhesion pathways (PECAM and ANXA1) were uniquely expressed by T-LGLL cells. We hypothesize that the upregulation of these genes related to cell adhesion pathways as well as cell-cell interactions contribute to T-LGLL accumulation in tissues. Ongoing work will focus on spatial analysis of T-LGLL cell-cell interactions and transcriptional profiles in tissues utilizing single cell spatial transcriptomics technologies. Profiling the tissue microenvironment of T-LGLL will uncover biomarkers, pathogenic mechanisms, and novel therapeutic targets to disrupt pathways that promote LGLL pathogenesis.

Abstract #: F18

Role of IL-1β signaling in preventing the progression of primary amoebic meningoencephalitis

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Naegleria fowleri is the main cause of primary amoebic meningoencephalitis (PAM), a fatal condition. Infection generally occurs by inhalation of contaminated warm freshwater. Once *Naegleria fowleri* comes into contact with the olfactory epithelium, it migrates along the axons of olfactory sensory neurons and reaches the olfactory bulb. Its presence in the brain induces an extensive inflammation with infiltration of monocytes and neutrophils. To this day, there is still no effective cure against PAM.

To develop effective treatments against PAM, it would be necessary to better understand the underlying pathology and the components of the host immune response that could prevent amoeba progression in the brain and/or reduce excessive immune activity.

This study shows that neutrophils and IL-1β are instrumental in counteracting *Naegleria fowleri* infection. More precisely, that is IL-1β signaling within the non-hematopoietic compartment that slows PAM progress. It plays an important role in efficient recruitment of inflammatory immune cells inside the infected brain, however, it does not directly slow the final leukocyte transmigration process. Ongoing experiments seek to link IL-1β signaling on the endothelial compartment to localized expression of adhesion molecules as ICAM-1, ICAM-2 or VCAM-1 enabling firm leukocyte adhesion and subsequent diapedesis, as well as “protective” BBB breakdown.

Abstract #: F19

Title: **Identifying Common Human *STING* Genotype Associated with Alzheimer's Disease.**

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Abstract: STING is a key regulator of host defense, tissue inflammation, and anti-tumor immunity. Notably, the human *STING* gene has 5 common alleles that result in 25 possible common human *STING* genotypes. Currently, there is a lack of knowledge on the impact of these common *STING* genotypes on human health and disease. Here, we conduct the first-ever large-scale (~15,000 individuals) *STING* genotypes–phenotype analysis. This study uses data from The National Institute on Aging Genetics of Alzheimer's Disease (AD) Data Storage Site to analyze common human *STING* genotypes associations with AD. About 1 in 9 people aged 65 and older have AD. The analysis was done in Africans (control: 1,649; case: 1,314); non-Hispanic Whites (control: 5,916, cases: 2.356), early-onset AD (EOAD) in non-Hispanic Whites (controls: 743, cases: 1,671), and Hispanic (controls: 917, cases: 669). The odds ratio (OR), p value, and 95% interval were calculated. The analysis was adjusted for sex, APOE4 status.

Discovery: One *STING* genotype, found only in Africans, was identified with increased risk for AD (OD=**5.4418,** p-value= 0.0078). Another *STING* genotype was identified with reduced risk for AD in Africans (OD=**0.4017**, p-value=0.0385), and EOAD non-Hispanic Whites (OD=**0.624**, p-value=0.0375). Our findings reveal a role for common human *STING* genotypes in public health, paving the way for future mechanistic studies. The study is supported by the Gatorade Fund to L.J.

Abstract #: F20

Respiratory Mucosal Interferon Gene Response to Rhinovirus Infection in Children with Severe Asthma and Adenotonsillar Hypertrophy

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Background: Children with severe asthma (SA) commonly present with silent lung rhinovirus (RV) infection with granulocytic bronchoalveolitis.

Purpose: To examine nasal mucosal gene expression in response to RV in children with severe asthma compared to non-asthma controls.

Methods: We compared nasal mucosal gene expression differences with RV infection in children with SA (n=30) to children with adenotonsillar hypertrophy (ATH, n=21). All subjects underwent nasal wash and turbinate scraping. Pathogen analysis and qPCR were performed.

Results: Clinical type-2 markers and pathogen distributions were similar between groups, though SA had greater exposure to high-dose inhaled corticosteroids. In the absence of pathogens, nasal IFNA1 gene expression did not differ between groups. In children with SA, median IFNA1 expression was 2-fold greater with RV infection compared to pathogen free children, a greater difference than in the ATH group. SA also showed significantly greater expression of viral recognition genes MDA5 and RIG-I with RV infection. ATH showed similar, but non-significant, trends. Although CCL5 expression was higher with RV infection in both groups, its expression was only significant in the SA group. Additionally, CCL24 was upregulated with RV infection in SA but was lower in ATH.

Conclusions: Despite a higher burden of ICS treatment, nasal mucosal IFN gene expression differences in children with SA trend higher with RV infection compared to ATH. Whereas pathogen binding proteins go up as expected, we speculate silent RV bronchoalveolitis syndrome in SA may indicate downstream dysregulation of mitochondrial antiviral signaling (MAVS). RV infection in SA also promotes nascent mucosal type-2 inflammation genes.

Characters: 1,496

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Abstract #: F21

Oncolytic herpes simplex virus efficacy as a glioblastoma therapeutic is modulated by preexisting viral immunity

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Glioblastoma (GBM) is a common brain cancer with a devastating median patient survival of ~15 months. Effective therapies remain elusive, pointing to the urgent need for novel treatments. Oncolytic viruses (OV) offer hope in that they destroy infected tumor cells regardless of the cell's chemo- or radiation-resistance status while priming anti-tumor immune responses. OVs derived from herpes simplex virus (oHSV) are being used clinically to combat disease; early-phase clinical trials indicate marginal efficacy overall. However, recent observations indicate survival is doubled for HSV-1$^+$ GBM patients treated with oHSV (rQNestin34.5v2) compared to individuals with no preexisting immunity. Here we interrogate the immunological mechanisms driving this phenomenon using the syngeneic GL261N4 murine glioma model. To raise immunity to HSV-1, mice were immunized over two six-week intervals by intraperitoneal injection of HSV-1. Seropositivity was confirmed prior to tumor implantation and subsequent therapeutic intervention with oHSV. It was found that mice pre-immunized with HSV-1 exhibited an increased intratumoral recruitment of CD8$^+$ T cells, dendritic cells, and macrophages. Immune activation was corroborated by RNA sequencing analyses indicating upregulated pathways involving antigen processing, antigen presentation, and T cell receptor signaling in immunized mice. Unexpectedly, HSV-1 immunization decreased the median survival time in the GL261N4 model compared to HSV-1 naive animals. Due to these confounding observations, we are evaluating this phenomenon in additional murine tumor models and plan to investigate the possibility of oHSV seroconversion of HSV-1 naive animals.

Abstract #: F22

Respiratory viral infections prime accelerated lung cancer growth

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The COVID-19 pandemic has highlighted long-term health concerns beyond acute infection, yet its potential impact on cancer development and growth remains poorly understood. Here, we demonstrate that prior infection with SARS-CoV-2 or influenza virus promoted lung tumor progression by reprogramming the local immune landscape. Retrospective clinical analysis revealed that patients hospitalized with COVID-19 exhibited increased lung cancer incidence. Using multiple murine lung cancer models, we show that prior severe respiratory viral infections accelerated tumor growth and reduced survival. Mechanistically, prior viral pneumonia epigenetically remodeled the lung to establish a pro-tumor microenvironment, including the expansion of Siglec F^{hi} tumor-associated neutrophils, a transcriptionally reprogrammed, immunosuppressive population whose signature predicted poor prognosis in human lung adenocarcinoma. In parallel, epithelial compartments exhibited altered differentiation trajectories, with persistence of injury-associated alveolar intermediates positioned along tumorigenic lineages. We observe sustained chromatin remodeling at key cytokine loci in immune and structure cells, linking inflammatory memory to persistent immune suppression. Therapeutically, combined inhibition of neutrophil recruitment (CXCR2) and PD-L1 signaling restored $CD8^+$ T cell infiltration and suppressed tumor growth. Together, our findings establish a direct causal relationship between viral pneumonia, including COVID-19, and lung tumorigenesis, highlighting the urgent need to monitor survivors for elevated cancer risk and to develop targeted interventions and therapies aimed at preventing potential cancer bursts in COVID-19 convalescents.

Abstract #: F23

The IL-33/ST2 signaling axis regulates pulmonary type 2 immunity in SARS-CoV-2 infection and enhances COVID-19 disease severity

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Immunopathogenesis drives both severe acute COVID-19 and postacute sequelae of COVID-19 (PASC). Therefore, dissecting the immune response to SARS-CoV-2, the causative agent of COVID-19, is critical to understand disease pathogenesis and identify pathways targetable by therapeutic intervention. Our lab previously found a link between the type 2 cytokine IL-13 and COVID-19 severity, prompting us to investigate drivers of type 2 immunity in COVID-19. The alarmin cytokine IL-33 is a potent inducer of type 2 immunity, as its receptor ST2 is expressed by type 2 cells including type 2 innate lymphoid cells (ILC2s). We therefore sought to investigate the role of IL-33/ST2 signaling in mouse-adapted SARS-CoV-2 MA10 (MA10) infection, a mouse model of COVID-19. In MA10 infection, $ST2^{-/-}$ mice had improved survival as compared to wild-type mice. In a complementary pharmacologic approach, IL-33/ST2 signaling was inhibited using HpBARI_Hom2, a helminth-derived protein that blocks IL-33 signaling. In MA10 infection, HpBARI_Hom2-treated mice had improved survival and reduced pulmonary damage as compared to inert control-treated mice. Interestingly, HpBARI_Hom2 treatment did not reduce viral titers, suggesting an immunopathogenic role for IL-33/ST2 signaling independent of viral control. In MA10 infection, HpBARI_Hom2-treated mice had reduced IL-5 in bronchoalveolar lavage fluid and reduced IL-13 expression by pulmonary ILC2s, suggesting activation of ILC2s may be a mechanism through which IL-33/ST2 signaling drives pathogenesis, a hypothesis that will be tested in future mechanistic studies. This work identifies the IL-33/ST2 axis as a potential therapeutic target in treatment of infection-induced pulmonary immunopathogenesis.

Abstract #: F24

AT2 Cells Sustain Lung CD8 TRM Survival *In Vitro*

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The normal regeneration process in the lungs involves the differentiation of alveolar type 2 (AT2) progenitor cells into alveolar type 1 (AT1) cells, which undergo a transitional state characterized by high keratin 8 (KRT8). In severe lung damage from acute respiratory infections, however, transitional KRT8^{hi} cells fail to fully differentiate due to inflammatory signals from the exuberant CD8 TRM activity. Our previous work has demonstrated that CD8 signaling stalls AT2 differentiation by secreting IFN-gamma to activate macrophages, which secrete IL-1 beta that hinders AT2 differentiation. This work evaluates whether the transitional KRT8^{hi} AT2 cells provide signals leading to further CD8 TRM accumulation, establishing a bidirectional feed-forward loop interaction between AT2 and CD8 TRMs. Our preliminary data from co-culture experiments between AT2 and CD8 TRMs from Flu-infected mice showed increased CD8 TRM viability compared to single cultured CD8 TRMs. We also observed a trend in increased cell viability of CD8 TRM cultures with 50% of AT2 supernatant, indicating that factors secreted by AT2 may be responsible for promoting CD8 survival *in vitro*. Building on these findings, in our next step, we seek to identify the specific signaling molecules secreted by AT2 cells responsible for the increased CD8 TRM survival *in vitro*. Overall, our research aims to better understand the signaling pathways involved in the excessive accumulation of CD8 T cells resident in the lungs. By studying those mechanisms, our work can contribute to the field by determining potential therapeutic targets for post-viral pulmonary dysplastic repair.

Abstract #: F25

Spatial Transcriptomics of the Tumor Microenvironment Identifies Long Term Immunotherapy Responders in MMR-proficient, p53mut Endometrial Cancers

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Endometrial cancer is one of the only malignancies with increasing incidence and mortality. Immunotherapy is standard of care in this disease, but its success is dependent on tumor biology. It is established that tumors with p53 mutations or considered mismatch repair proficient (pMMR) will have inferior responses to immunotherapy, however, there are patients even among these with extended responses, which is not predicted by current biomarkers. We hypothesize that spatial transcriptomics is a predictive biomarker of immunotherapy response. Our experimental design includes a cohort of fourteen pMMR, p53-mutated endometrial cancer tumors all treated with anti-PD1 with concurrent tyrosine kinase inhibitor at the time of recurrence. All tumors are from time of diagnosis. This cohort was well-matched for age, race, and initial disease stage. Progression free survival ranged from 3 to 61 months and long-term response was defined as progression free survival of greater than 12 months on immunotherapy. Utilizing the CosMx Spatial Molecular Imager, we analyzed the spatial transcriptomic profile of these tumors enabling an understanding of immune cell interactions and gene expression profiles. Our data reveals differences in immune cell infiltration and overall immune cell populations between long- and short-term responders. We identified 22 cell clusters with several clusters decreased or absent among short-term responders. These differences indicate the role of spatial data as biomarker to predict immunotherapy response in endometrial cancer. Future directions include validation of spatial biomarkers using a combination of immunohistochemistry and mass cytometry to assess cell populations and phenotypes.

Abstract #: F26

Identification of Alternative Splicing Events regulating Tregs and exTregs Phenotypes in Atherosclerosis

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Atherosclerosis is a chronic inflammatory disease of the arterial wall and remains the leading cause of global death. Regulatory T cells (Tregs) play a protective role, primarily via anti-inflammatory cytokines. However, some Tregs become exTregs, losing the immunosuppressive ability and thereby exacerbating atherosclerosis. Alternative splicing is a ubiquitous mechanism that enhances RNA and protein diversity by removing or skipping exon elements. It has been implicated in development, disease progression, cell differentiation and regulation of cell-type-specific functions. However, its involvement in the conversion between Tregs and exTregs during atherosclerosis remains unclear. To investigate this, we analyzed public bulk RNA-seq data from sorted human Tregs ($CD4^+CD25^+CD127^{low}$) and exTregs identified using recently discovered surface markers ($CD4^+CD16^+CD56^+$). We found a significant subset of coronary artery disease (CAD) GWAS risk genes (n = 89) and splicing-related genes (n = 61) were dysregulated between Treg and exTreg cells. Further splicing analysis revealed approximately 10,000 differential splicing events, including over 800 occurring in genes that showed no changes in expression. Notably, several key genes encoding transcription factors (e.g., *IRF1* and *SAFB2*), ligands (e.g., *CLEC2D*), receptors (e.g., *NCL*) and CAD risk genes (e.g., *AIDA*, *MARK3*, *MIA3*, *NFU1*, *PINX1*, *SCML4*, and *UBE3B*), exhibited significant alterative splicing changes independent of their host gene expression differences. In summary, our findings suggest that alterative splicing may contribute to Tregs and exTregs phenotype transition and atherosclerosis progression, providing a valuable resource for future functional studies.

Abstract #: F27

Therapeutic VEGFC treatment provides protection against traumatic brain injury-driven tauopathy pathogenesis

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Traumatic brain injury (TBI) is known to increase the risk of developing Alzheimer's disease and tauopathy. However, how TBI mechanistically contributes to these neurodegenerative sequelae remains poorly defined. Emerging evidence suggests that impaired brain lymphatic clearance of neurotoxic debris plays a key role in driving neurodegeneration. Here, we tested whether enhancing brain lymphatic drainage after TBI using the lymphangiogenic factor VEGFC can protect against TBI-induced tauopathy. We show that a single mild TBI exacerbates neuropathology, microgliosis, and neurodegeneration in the PS19 tauopathy mouse model. Furthermore, viral vector-mediated delivery of VEGFC to the meninges 24 hours post-TBI attenuated tau-mediated neurodegenerative disease pathogenesis and cognitive deficits. Single-nucleus RNA sequencing revealed that VEGFC treatment post-mTBI reduced a RORB+ excitatory neuron population, previously observed in human AD near tau pathology. These findings suggest that targeting brain lymphatic repair may be a therapeutic strategy to reduce long-term tauopathy risk following head trauma

Abstract #: F28

The Therapeutic Potential of Interferon Lambda in *Clostridioides difficile* Infection

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Type III interferons, or interferon lambdas (IFN-λ), serve as the crucial first line of defense at mucosal barriers. Traditionally linked to antiviral response, emerging evidence suggests that IFN-λ may also play significant roles in autoimmunity and bacterial infections. Previous studies have demonstrated that gut bacteria can induce the expression of IFN-λ receptors in germ-free mice (Van Winkle 2022), and that supplemental IFN-λ mitigates intestinal inflammation in mice with dextran sodium sulfate-induced colitis (Broggi 2017).

Clostridioides difficile (C. difficile) infection is the leading cause of hospital acquired infection in the U.S., resulting from microbiome disruption and associated with high rates of recurrence and mortality. Previous work has established that the promotion of epithelial cell repair and the production of anti-toxin B antibodies is protective during *C. difficile* disease progression. Given that IFN-λ is primarily produced by epithelial cells and has been shown to promote epithelial barrier function in gastrointestinal infection (Ferguson 2019), we aim to explore the effects of recombinant IFN-λ in a mouse model of *C. difficile.* Furthermore, as IFN-λ receptors are expressed on both dendritic cells and B cells, we hypothesize that IFN-λ may promote the humoral immune response to *C. difficile*. We intend to investigate the therapeutic potential of IFN-λ, focusing on epithelial repair and generation of adaptive immunity.

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Citations:

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Abstract #: F29

Unraveling the Role of Melanoma-Cognate CD4+ T cells in Enhancing Vaccine-Induced Memory CD8+ T Cell Development

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Abstract

Cancer vaccines offer a broadly implementable approach for improving therapeutic immunity to human cancers. Previously, we conducted a clinical trial using MHC-I or/and II restricted peptide vaccine against melanoma with or without cyclophosphamide as a pre-treatment in patients with stage IIB-IV of melanoma (Mel44; NCT00118274). Notably, patients who received 6MHP vaccine exhibited significantly improved long-term clinical outcomes compared to tetanus vaccine, despite an initially weaker CD8+ & CD4+ T cell responses detected by IFNγ ELIspot. Our murine study have demonstrated that vaccine induced melanoma-cognate CD4+ T cells were more effective than non-tumor specific CD4+ T cells at promoting memory CD8+ T cell recall function. CD4+ T cells, particularly Th1, are key sources of IFNγ in which is known to support the differentiation of tumor-specific effector CD8+ T cells during priming. The disconnection between the primary response and clinical outcome in Mel44 suggests advantages of tumor-cognate CD4+ T cells in promoting durable memory CD8+ T cell responses via less IFNγ. We hypothesize that 6MHP-specific CD4+ T cells are less Th1 differentiated and promote memory CD8+ T cell development by increasing memory precursors and/or enhance their recall capacity during priming. We will assess the phenotypic and subsequent functional differences of tetanus vs. 6MHP-specific CD4+ T cells, the quantity and quality of memory CD8+ T cells in patients vaccinated with different regimens, and whether these differences in CD4+ T cells contribute to the impact on memory CD8+ T cells. These mechanistic studies will unveil novel therapeutic potential for cancer vaccines targeting tumor-associated antigens against solid tumors.

Abstract #: F30

Development of a tolerogenic mRNA vaccine for organ transplantation

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Organ transplantation is a critical procedure for patients with terminal organ failure, yet long-term graft survival is hindered by antigen-specific immune responses to the donor graft. Current treatments use nonspecific immunosuppressive therapeutics to promote short-term graft survival; however, there remains a critical need to develop treatments that target donor-specific lymphocytes that drive long-term graft rejection. mRNA vaccines are one approach to generate pro-inflammatory antigen-specific immune responses. Here, we hypothesize that by limiting the pro-inflammatory properties of a mRNA vaccine, it can be used to express donor antigens in a non-inflammatory capacity and eliminate alloreactive T cells. To develop a non-inflammatory vaccine, we sought to assess the immunogenicity of two different lipid nanoparticle (LNP) mRNA formulations, CKK and L10, using bone-marrow derived dendritic cells (BMDCS). We measured BMDC cytokine secretion, activation marker expression, and ability to prime alloreactive CD8 T cells after LNP stimulation. Both LNPs elicited a type-I IFN response, but only CKK-stimulated BMDCs secreted IL-6 and IL-12. A similar trend was observed with activation marker expression and CD8 T cell priming. CKK-stimulated BMDCs had increased CD86 and MHC II expression compared to L10- stimulated BMDCs and were more efficient at driving alloreactive CD8 T cell proliferation and IFNγ secretion. We are currently analyzing bulk RNA-seq data from LNP-stimulated BMDCs to identify the innate immune pathways triggered by the mRNA vaccines. We anticipating that this approach will allow us to develop a vaccine with limited immunogenicity and facilitate their use in driving tolerance to donor grafts.

Abstract #: F31

Identifying regulators of CAR-T cell survival and adaptation to improve efficacy against solid tumors

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Chimeric antigen receptor T (CAR-T) cell therapy can lead to durable responses in patients with some hematologic malignancies yet remains largely ineffective in solid tumors. Impaired persistence of CAR-T cells and the immunosuppressive tumor microenvironment (TME) are important barriers to overcome to improve efficacy against solid tumors. To test how T cell mode of tumor recognition affects fate and persistence, we developed a tumor model to compare tumor-specific CD8 T cells expressing either a CAR (CAR-T) or a transgenic T cell receptor (TCR-T) side-by-side. While these cells had similar survival and proliferative capacity *in vitro*, CD8 CAR-T cells had a survival disadvantage *in vivo* compared to TCR-T cells in the same TME, suggesting that CAR expression reduced CD8 T cell fitness in the TME. To further probe regulators of CAR-T cell survival, we used an *in vivo* CRISPR screen of cell death-related genes. Deletion of *Il2ra* gave CAR-T cells a fitness disadvantage *in vitro* but those CAR-T cells ultimately had a fitness advantage *in vivo* in the TME and spleen. This may be due to attenuation of strong IL-2 signaling *in vitro*, suggesting that CAR-T cells become dependent on IL-2 during *in vitro* expansion and this leads to impaired survival upon transfer *in vivo* where IL-2 can be limiting. Indeed, CAR-transduced CD8 T cells exhibit selective impaired survival or proliferation *in vitro* under conditions of short-term IL-2 starvation, and this is not rescued by supplementation with additional survival cytokines such as IL-7 or IL-15. Future studies will investigate IL-2 signaling and metabolic dependencies of CAR-T cells, with the goal of identifying strategies to mitigate this dependency and improve CAR-T cell persistence and function *in vivo*.

Abstract #: F32

Unraveling the effects of genetic and pharmacological Complex I targeting on ovarian cancer microenvironment

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Inhibiting mitochondrial complex I (CI) represents a promising anticancer strategy by impairing cellular adaptation to hypoxia and reducing cancer progression. This still needs to be demonstrated in the context of High Grade Serous Ovarian Cancer (OC). We aim to demonstrate that genetic and pharmacologic targeting CI decreases tumorigenic potential of OC preclinical models and define the role of TME in activating compensatory mechanisms. We generated a functional CI knock out (KO) in OV90 cell lines and evaluated tumor growth in NOD/SCID mice. We showed that OV90 CI KO xenografts presented with lower proliferation index than WT tumor masses. HIF1 stabilization was absent in CI KO tumors, followed by decreased HIF1-targeted genes expression. Compared to WT, tumors lacking CI presented with less necrosis and higher number of vessels, which tended to be smaller than in control masses. Stromal abundance was observed in CI KO xenografts, as well as increased intratumoral macrophage numbers. Nevertheless, CI KO tumors continue to thrive, suggesting a compensatory role of TME in supporting tumor growth, whereby KO cancer cells may rely on protumorigenic immune cell functions. To determine whether targeting CI may affect the biology of TME cells, we investigated *in vitro* M1 versus M2 polarization of THP-1 monocyte model upon CI inhibitors treatment and tube formation of endothelial cells. In parallel, we demonstrated the antiproliferative activity of two CI inhibitors *in vitro* on OV90 cells and in patient-derived *ex vivo* ovarian cancer models. Ongoing experiments in immunocompetent mouse models will help define the potential role of immune activation pathway during targeting CI, in the direction of novel combinatorial therapeutic strategies.

Abstract #: F33

Irf1 attenuates inflammatory gene programs in B cells and alters pathogenic B cell subsets in autoimmunity and inflammaging.

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Interferon regulatory factor 1 (IRF1) is a transcriptional regulator of inflammation. Given the role of inflammation in autoimmunity, we hypothesized that IRF1 would impact the progression of systemic lupus erythematosus (SLE). Consistent with this, human SLE B cells expressed higher *IRF1* levels and showed increased chromatin accessibility at IRF1-binding sites. To study the role of IRF1 in SLE, we analyzed lupus-prone B6.Yaa.*Fcgr2b*$^{-/-}$ (YFc) mice that lacked *Irf1* in all cells (YFc.*Irf1*) or only in B cells (B-YFc.*Irf1*). YFc.*Irf1* mice had reduced Ab secreting cells (ASCs) and age-associated B cells (ABCs), decreased serum autoAbs, reduced glomerular IgG deposition and attenuated kidney damage. Similar results were seen in B-YFc.*Irf1* mice, suggesting a B cell intrinsic role. To test whether *Irf1* drives inflammation in B cells, we analyzed aged B6 mice lacking *Irf1* in all cells (B6.*Irf1*) or only in B cells (B-*Irf1*). Both models had reduced ASCs, ABCs and age-associated kidney pathology. To understand how IRF1 regulates B cells, we performed scRNA-seq analysis on B cells from aged B6.WT and B6.*Irf1* mice. *Irf1*$^{-/-}$ B cells were enriched for genes associated with the IFN response and exhaustion. Likewise, flow cytometric analysis showed higher levels of exhaustion markers expressed by *Irf1*$^{-/-}$ B cells, and these cells failed to proliferate and differentiate into ASCs *in vitro*. Thus, while IRF1 is known to promote inflammation in most immune cells, in B cells it unexpectedly suppresses inflammatory gene programs. We conclude that the sustained hyper-inflammatory signature seen in *Irf1*$^{-/-}$ B cells, drives these cells to exhaustion and prevents them from contributing to B cell-mediated pathogenic responses that are associated with SLE and aging.

Supported by grants from NIH (R01 AI110508 & R01AI153365)

Abstract #: F34

PD-1 signal in T cell exhaustion: defined and confused

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Abstract

SHP-1 (Src homology region 2 domain-containing phosphatase 1) is a well-known negative regulator of T cells, whereas its close homolog SHP-2 is the long-recognized main signaling mediator of the PD-1 inhibitory pathway. However, recent studies have challenged the requirement of SHP-2 in PD-1 signaling, and follow-up studies further questioned the alternative idea that SHP-1 may replace SHP-2 in its absence. In this study, we systematically investigate the role of SHP-1 alone or jointly with SHP-2 in $CD8^+$ T cells in a series of gene knockout mice. We show that although SHP-1 negatively regulates $CD8^+$ T cell effector function during acute lymphocytic choriomeningitis virus (LCMV) infection, it is dispensable for $CD8^+$ T cell exhaustion during chronic LCMV infection. Moreover, in contrast to the mortality of PD-1 knockout mice upon chronic LCMV infection, mice double deficient for SHP-1 and SHP-2 in $CD8^+$ T cells survived without immunopathology. Importantly, $CD8^+$ T cells lacking both phosphatases still differentiate into exhausted cells and respond to PD-1 blockade. Finally, we found that SHP-1 and SHP-2 suppressed effector $CD8^+$ T cell expansion at the early and late stages, respectively, during chronic LCMV infection.

Abstract #: F35

Single-cell transcriptomic profiling of immune response to *Haemonchus contortus* in resistant and susceptible sheep

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Infection with gastrointestinal nematode *Haemonchus contortus* in parasite-resistant St. Croix sheep (STC) is characterized by reduced worm burden and rapid immune activation. Conversely, parasite-susceptible Suffolk sheep (SUF) display delayed immune responses and higher parasite load. While Th2-type responses are implicated in resistance, the frequency and diversity of cell immune cell populations contributing to a resistance phenotype remain unclear. To investigate immune cell heterogeneity in relation to parasite resistance, peripheral blood mononuclear cells (PMBCs) were isolated from parasite naive and primed SUF and STC for single cell RNA-sequencing. Cell clustering and differential gene expression analysis was performed to identify gene sets associated with phenotypic differences between infection status and between breeds. Thus, this study aims to characterize immune cell populations in the context of helminth infection utilizing a model of parasite susceptibility and resistance. Identifying critical immune cell types and mapping the immune cell landscape associated with resistance will improve our understanding of host-parasite dynamics. Together, comprehensive characterization of immune cell populations will inform improved parasite control strategies in susceptible livestock.

Abstract #: F36

Mechanical ablation enhances acute activation of melanoma-specific T cells & tumor antigen acquisition by conventional dendritic cells in a trafficking independent manner.

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Here, we investigated whether liberated, passively drained tumor antigen or cell-associated antigen trafficked by conventional dendritic cells (cDCs) elicits activation of tumor-specific T-cells post mechanically ablative boiling histotripsy (BH). Using the B16F10-ZsGreen (ZsG) model, we were able to track tumor antigen dissemination. We impeded cDC migration by administering αCCR7 i.p. on days 9, 11, and prior to BH on day 13. $CD8^+$ T-cells from PMEL transgenics were enriched, stained with CellTrace Violet, and adoptively transferred 2 h prior to BH. Tumor-draining lymph nodes (TDLNs) were harvested 24 and 96 h post BH and processed for spectral flow cytometry analysis. We have previously published that BH induces an increase in ZsG^+ cDCs in TDLNs 24h post treatment. In these studies, we observed ZsG^+ cDCs and tumor antigen post BH overlapping with the TDLN lymphatics. Increased ZsG^+ cDCs is not associated with $CCR7^+$ cDCs – indicating passive drainage. Additionally, BH elevated the activation status of PMEL T-cells ($Thy1.1^+V\beta13^+$), which correlates with ZsG^+ cDC1s – cDCs that canonically cross-present antigen to $CD8^+$ T-cells. We still observed increased ZsG acquisition in peripheral/migratory cDCs and TDLN-resident cDCs post BH even when cDC trafficking was impeded. Blocking cDC migration did not impact BH-induced T-cell expansion. We have found that BH significantly enhances melanoma-specific T-cell activation and induces T-cell expansion in TDLNs. Tumor antigen is captured by TDLN-resident cDCs, which can elicit T-cell expansion independent of cDC migration from the tumor microenvironment. These results both define mechanisms of BH-induced anti-tumor immunity and inform the design of improved combinatorial therapies.

Abstract #: F37

The ubiquitin ligase Cul4b is essential for antibody-mediated protection against Influenza A virus infection

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Influenza A virus (IAV)-specific antibodies (Abs) are secreted by plasma cells (PC) after viral infection or vaccination. B cells undergo affinity maturation prior to PC differentiation in germinal centers (GC), enduring rounds of somatic hypermutation (SHM), proliferation, and selection to gain high affinity. This requires stringent regulation of DNA damage response and cell cycle. Cullin4b (Cul4b), the backbone of an E3 ubiquitin ligase complex, ubiquitylates proteins for degradation to regulate cell cycle and DNA damage. As both processes are integral to GC B cell biology, we hypothesized that Cul4b promotes protective Ab responses by supporting affinity maturation. To test this, we immunized B cell-specific Cul4b knockout (cKO) mice and wild-type littermate controls with an mRNA vaccine encoding hemagglutinin from PR8, a mouse adapted H1N1 IAV. In Cul4b cKO groups, we found reduced IAV-specific Ab titers, fewer GC B cells, and impaired protection after homologous challenge, suggesting Cul4b promotes optimal Ab titers and immune protection. We also show that Cul4b supports high affinity Ab production and cell cycle in antigen-specific GC B cells. We believe Cul4b supports antiviral immunity by regulating cell cycle in GC B cells during SHM to promote formation of high affinity neutralizing Abs. Cul4b is a novel facilitator of protective Ab responses to viruses and a potential target for vaccines.

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Abstract #: F38

Tnfr1 is critical for immune cell infiltration into the brain during _T. gondii_ infection.

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Toxoplasma gondii is a ubiquitous intracellular protozoan parasite that is well-controlled by a Th1 immune response in the periphery but chronically infects the brain. Prior work has established that tumor necrosis factor (TNF) and interferon gamma (IFN-γ) enable macrophages to kill *T. gondii in vitro*. TNF receptor 1 (*Tnfr1*) signaling induces iNOS, which is critical for parasite control. Work from our lab shows that at 15 days post infection (dpi), TNF-α neutralization decreases monocyte infiltration in the brain. Based on these results, we hypothesized that TNF-α signaling through *Tnfr1* is required not only to induce iNOS expression but to recruit myeloid cells into the brain. Indeed, flow cytometry revealed that at 12 dpi, *Tnfr1*$^{-/-}$ mice recruited fewer inflammatory monocytes to the brain than wildtype mice. As expected, the recruited monocytes also expressed less iNOS by flow cytometry and qPCR of brain tissue confirmed the decrease in iNOS expression. Unexpectedly, *Tnfr1*$^{-/-}$ mice also had less expression of CCL2 and CCR2, a chemokine and receptor important for monocyte infiltration, along with decreased adhesion molecule expression. During chronic infection (28 dpi), the myeloid cell deficit persisted. Based on these results, TNF-α signaling through *Tnfr1* is important for inflammatory monocytes to reach the brain which was previously unappreciated. Future studies will reveal which cell types respond to TNF-α to promote immunity in the brain.

Abstract #: F39

Intrinsic Metabolic and Immune Dysregulation of Intermediate Monocytes in Sarcoidosis

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Sarcoidosis is a granulomatous disease characterized by dysregulated innate immunity, with elevated blood monocyte levels as a prominent feature. However, the metabolic and immunoregulatory profiles of monocyte in sarcoidosis remain poorly understood. Monocytes are classified into classical (cMo), intermediate (iMo), and nonclassical (nMo) subsets, with elevated intermediate monocyte levels (iMo_CD14$^+$CD16$^+$) particularly implicated in sarcoidosis. We hypothesize that iMos exhibit intrinsic metabolic and immune dysregulation in sarcoidosis patients. Using single-cell RNA sequencing (scRNA-seq) of peripheral blood mononuclear cells (PBMCs) from sarcoidosis patients and healthy controls, we observed a ~2.5-fold increase in iMo proportions in patients (*adj.p-val*=3.83E-05), along with a reduction in nMo and dendritic cell (DC) populations. High-resolution clustering identified a novel iMo_Granzyme-A$^+$ (*adj.p-val*=1.145E-04) subcluster enriched in patients, characterized by downregulation of mitochondrial and protein transport pathways, suggesting a metabolically impaired inflammatory phenotype. Race-stratified analyses further revealed an expansion of iMo_L-Selectin$^+$ (*adj.p-val*=0) populations and an enriched interferon gene signature in Black patients, suggesting race-specific immunometabolic adaptations. Collectively, our findings highlight a functionally distinct, metabolically dysregulated iMo subset as a potential driver of sarcoidosis pathogenesis and underscore immunoregulatory differences that may contribute to racial disparities in disease severity and progression.

Abstract #: F40

Investigating the Effect of Focused Ultrasound on Tumor-Associated Macrophages

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Focused ultrasound (FUS) has emerged as a non-invasive cancer therapy that directly destroys tumors and modulates the tumor microenvironment (TME). FUS uses focused transducers to generate mechanical waves that concentrate energy at a targeted site, leading to localized tissue ablation. This ablation releases tumor antigens and damage-associated molecular pattern molecules (DAMPs), stimulating innate and adaptive immune responses. Although pre-clinical studies have shown that FUS can enhance anti-tumor immunity, its efficacy as a monotherapy remains limited. One possible explanation is the activation of immune-regulatory mechanisms, particularly those mediated by tumor-associated macrophages (TAMs), during post-ablation wound healing. To investigate the impact of FUS on TAMs, we generated CosMx spatial transcriptomic data from a breast cancer clinical trial specimen treated with FUS. Preliminary findings indicate an accumulation of $SPP1^+$ (secreted phosphoprotein 1) macrophages around FUS-ablated regions. Multiple scRNAseq data from literature identified SPP1/TREM2 (Triggering Receptor Expressed on Myeloid Cells-2) as a TAM marker linked to immunosuppression and poor prognosis in various human tumors. We hypothesize that FUS promotes the recruitment of suppressive $TREM2^+$ TAMs to ablated regions, potentially dampening responses by T and NK cells. In the EMT6 breast cancer mouse model, $TREM2^+$macrophages were present within the tumor and expressed ARG1, indicative of their suppressive phenotype. FUS alone appears to transiently affect TAMs. Future studies will focus on evaluating whether depleting or inhibiting TAMs in combination with FUS treatment can enhance and sustain robust anti-tumor immune responses.

Abstract #: F41

Immunodominant Apolipoprotein B epitopes targeted by human CD8$^+$ T cells

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Abstract

Atherosclerosis, contributing to ~one-third of all deaths worldwide, is a chronic inflammatory disorder with an autoimmune component. Previously, apolipoprotein B (APOB) epitope recognition by human CD4$^+$T cells and CD8$^+$T cell infiltration and oligoclonal expansion in human atherosclerotic lesions has been shown. We now show that human APOB harbors dominant HLA-I-restricted epitopes that trigger autoreactive CD8$^+$T responses. Here, we used an *in-silico* approach to predict HLA-A*02:01 restricted epitopes. Using NetMHCpanEL-4 tool, we selected top ~0.1% of ranked apolipoprotein B (APOB) peptides binding to HLA-A*02:01. Expansion-based restimulation assays of PBMCs from HLA-A*02:01$^+$ donors showed that APOB induced expression of the activation markers (4-1BB, in combination with CD69 or CD25) and effector memory phenotype in CD8$^+$T cells. Intracellular cytokine staining assay showed robust IFNg production co-expressed with other proinflammatory cytokines like TNFa and cytotoxic molecules such as Granzyme B and Perforin in CD8$^+$T cells upon APOB mesopool stimulation. Deconvolution of responses to single peptides using an IFNγ ELISpot assay revealed 5 APOB epitopes that elicited the highest response, and they mapped to 2 major antigenic regions in APOB that are predominant targets of CD8$^+$T cells in multiple HLA-A*02:01+ donors. Our findings provide evidence of atherosclerosis related autoantigen (APOB) specific circulating CD8$^+$T cells in healthy humans.

Abstract #: F42

Functional Profiling of CXCR3-Expressing Plasma Cells in Systemic Lupus Erythematosus.

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CXCR3 is a chemokine receptor involved in recruiting immune cells to sites of inflammation, infection, and tissue damage. CXCR3 expression on plasma cells (PCs) is hypothesized to facilitate migration to inflamed or infected tissues, thereby enhancing antibody production and pathogen clearance. However, in autoimmune diseases such as systemic lupus erythematosus (SLE), $CXCR3^+$ PCs may contribute to disease progression by localizing to inflammatory sites, producing pathogenic autoantibodies, secreting pro-inflammatory cytokines, and infiltrating tissues. Compared to healthy controls (HC), B cells from SLE patients exhibit greater heterogeneity and an expansion of $CXCR5^-CD11c^+$ B cells that express the transcription factor T-bet.

Using a two-step *in vitro* culture system, we generated PCs from six B cell subsets: activated naïve (aNav), double negative 2 (DN2), and effector switched memory (eSW)— cells with $CXCR5^-CD11c^+$ expression, as well as their $CXCR5^+CD11c^-$ counterparts—resting naïve (rNav), double negative 1 (DN1), and resting switched memory (rSW) cells. RNA sequencing of PCs derived from these subsets revealed increased CXCR3 expression in those originating from $CXCR5^-CD11c^+$ B cells, a finding confirmed at the protein level. Due to limited yield of PCs for functional assays, we employed a lentiviral (LV) vector to overexpress CXCR3 *in vitro* and successfully generated CXCR3-expressing PCs. These cells are currently being evaluated via calcium influx, migration, and invasion assays to assess CXCR3 function.

Our ongoing work aims to validate these preliminary findings using PCs derived from all six B cell subsets in SLE patients, with the ultimate goal of elucidating the role of $CXCR3^+$ PCs in autoimmune pathogenesis.

Characters (no spaces): 1496

Abstract #: F43

Introduction: Despite brain cancer being the leading cause of cancer-related death in children, our group pioneered a novel immunologic platform known as adoptive cellular therapy (ACT), which significantly increased overall survival in brain tumor models. We observed that ACT significantly depleted intra-tumoral myeloid-derived suppressor cells (MDSCs). From this, we hypothesized that ACT-mediated MDSC depletion in the tumor microenvironment (TME) is driven by cell migration mechanisms. **Methods**: C57BL/6J mice intracranially received KR158b-luciferase glioma and were treated with ACT. This platform consists of irradiation, hematopoietic stem cells, dendritic cells, and tumor-reactive lymphocytes. Leveraging single cell RNA sequencing in conjunction with digital spatial profiling of the TME, we identified changes to TME-derived chemokines after ACT. Lastly, trans-well migration assays were utilized to determine the mechanistic impact of these chemokines on MDSC migration after ACT. **Results**: RNA and protein levels of CCL12 were significantly increased in untreated gliomas. Single cell RNA sequencing analysis revealed that CCL12 was significantly expressed by tumor associated macrophages and validated using an enzyme-linked immunosorbent assay. High-plex spatial profiling analysis revealed that CCL12 was significantly decreased after ACT. Lastly, trans-well migration assays revealed that MDSC migration was significantly abrogated after neutralization of macrophage-derived CCL12. **Conclusions**: These results suggest that ACT reduces CCL12, which acts as an MDSC 'homing signal'. In sum, ACT's disruption of the CCL12-MDSC axis represents an important mechanism to overcome glioma immunosuppression.

Abstract #: F44

Plasma Lacritin as 15-mer N-104 Suppresses Pancreatic Lymph Node TCF7^{hi} Progenitor-like Autoimmune Cells and Independently Regenerates Islet β-Cells

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Stem-like TCF7^{hi} autoimmune progenitor cells in pancreatic lymph nodes (pLN) sustain destructive immunity in type 1 diabetes (T1D). No stem-like TCF7^{hi} inhibitors have been identified. Here, we serendipitously discover such an inhibitor as lacritin, a protein with polypharmacology originally thought to be eye-restricted but now known to be in plasma where it is diminished in human T1D. We injected young non-obese diabetic mice for 8 weeks with the 15-mer lacritin peptide N-104 versus another peptide from lacritin's inactive domain. ScRNAseq revealed N-104 suppression of pancreatic draining lymph node (pLN) TCF7^{hi} stem-like autoimmune progenitor T cells, accompanied by reduced CD80/CD86 expression, particularly CD86, on T cells. N-104 also reduced Th17 cells, B–T collaborative cells, F4/80^{+} dendritic cells, and macrophage-like B cells, while increasing the Treg-to-T cell ratio. Pro-inflammatory Zbtb16^{+} iNKT17 cells were depleted, whereas protective CD4^{+} iNKT cells expanded. Validating imaging mass cytometry of pancreatic islets from N-104 treated mice displayed reduced infiltration of CD3^{+}, CD4^{+}, and CD8^{+} T cells, CD80^{+} B cells, and CD56^{+} NK cells, supporting the immunomodulatory role of N-104. *In-vitro* mass cytometry of isolated pancreatic islet cell further suggested a islets regeneration role of N-104 in enhancing β-cell proliferation. 90% of N-104 treated mice survived for at least 10 months with normal glycemia.

Abstract #: F45

ST6Gal-I targets CD25 and TGF-βRI/II to control iTreg differentiation

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Regulatory CD4 T cells (Treg) are a subset of T cells that maintains homeostasis by suppressing immune responses via several mechanisms, notably the depletion of IL-2. Both thymic and peripheral induced Treg (iTreg) express CD25, the high affinity IL-2 receptor α chain, which these cells require for their maintenance and use to sequester IL-2 from effector T cells. iTreg differentiate from naïve CD4 T cells in the peripheral tissues following T cell receptor, IL-2, and TGF-β stimulation. CD25 is highly glycosylated and N-glycan branching can impact CD25 stabilization, IL-2 signaling, and CD4 T cell differentiation. Beta-galactoside α2,6 sialyltransferase 1 (ST6Gal-I) is a broadly expressed Golgi enzyme which catalyzes the attachment of terminal α2,6 sialic acids to cell surface N-glycans. iTreg express ST6Gal-I and are highly α2,6 sialylated, however it is not known if CD25 is sialylated by ST6Gal-I and if this regulates iTreg differentiation and/or function. We hypothesize that ST6Gal-I modifies CD25, inhibiting downstream signaling and iTreg differentiation. We find that in vitro FoxP3 induction and survival of iTreg are augmented in the absence of ST6Gal-I. This enhanced iTreg differentiation and survival is driven by increased sensitivity to TGF-β, working synergistically with IL-2. To determine mechanistically how ST6Gal-I regulates iTreg differentiation, we performed immunoprecipitation of α2,6 sialylated iTreg proteins and show that CD25, as well as TGF-βRI and TGF-bRII, are targets of ST6Gal-I. We are currently investigating the influence of ST6Gal-I sialylation on IL-2 and TGF-β signaling in iTreg. In conclusion, we find that ST6Gal-I sialylates both CD25 and TGF-βRI/II and dampens iTreg differentiation in vitro.

Abstract #: F46

Title: Investigating the role of avidity in Fab-dimerized glycan-reactive antibody function

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Main Text: Viral spike proteins mediate entry into susceptible host cells and can be decorated with glycans that facilitate immune evasion. The viral spike protein of HIV-1 is one of the most heavily glycosylated proteins observed in nature and is the sole target for neutralizing antibodies (Abs). Glycans themselves are poorly immunogenic, and typically glycan-specific Abs have inherently low affinity and do not provide lasting protection against HIV-1. Recently, we have elicited Abs via vaccination and SHIV infection of rhesus macaques that uniquely form I-shapes to increase glycan avidity and are referred to as Fab-dimerized glycan-reactive Abs (FDGs). The extent to which avidity impacts FDG function is unknown. Here we show that the increased avidity acquired by FDGs is critical for their function. Ab mutations to eradicate I-shape formation or alter glycan binding mechanisms abrogated neutralization of HIV-1 isolates. Alternatively, increasing avidity via IgG dimerization enhanced glycan binding and neutralization potency. Our results demonstrate two mechanisms Abs may use to improve their glycan reactivity. Designing glycan-focused vaccines to elicit FDG-like Abs may allow for protection against diverse pathogens.

Abstract #: F47

Recognition of Commensal Flagellins by Host Immunity

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Flagellin, the protein subunit of the bacterial flagellum, is highly surveilled by the immune system. In the healthy human gut, members of the Lachnospiraceae family are the primary producers of flagellin. These beneficial microbes evade the flagellin sensor Toll-like receptor 5 (TLR5) in part by encoding silent flagellins which fail to bind dimeric TLR5. This mechanism of immune escape is insufficient to prevent adaptive immune responses to these flagellins in individuals with inflammatory bowel disease (IBD) and other conditions marked by chronic gut inflammation. Ongoing work in the lab aims to identify how these immune responses develop and how flagellin diversity in the human gut impacts TLR5 signaling. This work builds upon our discovery of silent flagellins to lay a foundation for future studies investigating the role of commensal flagellins in the pathogenesis of IBD.

Abstract #: F48

Type I IFN receptor blockade alleviates liver fibrosis through macrophage-derived STAT3 signaling

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Liver macrophages play a role in the development of liver fibrosis progression via the regulation of inflammatory signaling. However, the precise mechanisms of macrophages contributing to liver fibrosis progression remain unclear. Using a preclinical model of CCl4-treated mice, we determined the composition of immune cells and the alteration of inflammatory gene expression. Our findings revealed a significant increase in liver macrophages, particularly those derived from infiltrating blood monocytes, in fibrotic mice. The expression levels of type I IFN signature genes such as *IFNα*, *IFNβ*, *ISG15*, *USP18*, *Ifi44*, *Ifit1*, *Ifit2*, *IRF3*, and *IRF7* were elevated in fibrotic mice. To determine the role of type I IFN signaling in liver fibrosis, we administered an IFNAR-1 antibody to block this pathway for 3 days prior to harvesting the liver. IFNAR-1 blockade reduced macrophage numbers compared to control mice and alleviated liver fibrosis in mice with increased hepatocyte proliferation and apoptosis. The ratio of P-STAT3/P-STAT1 in monocyte-derived macrophages was increased in the IFNAR-1 blockade group compared to fibrotic mice, and this was related to the appearance of M2 macrophage differentiation. single-cell RNA-seq analysis indicated that IFNAR blockade affected inflammatory pathways involved in hepatocyte regeneration and fibrosis prevention. Taken together, IFNAR-1 blockade alleviates liver fibrosis progression by modulating macrophage inflammatory responses. These results provide insights for developing anti-fibrotic therapies against type I IFN signaling.

Abstract #: F49

Deciphering the effect of short-term fasting on the antibacterial capacity of neutrophils during infection

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Fasting prior to surgical operations is necessary to prevent complications such as aspiration while under general anesthesia. However, fasting also skews metabolite availability, which may place additional burdens on the immune system and render the host more susceptible to nosocomial infections. Glucose is readily depleted in the fasted state and serves as the primary carbon source for neutrophils, the first line of defence against invading pathogens. We hypothesize that decreased glucose availability in fasting individuals metabolically impairs neutrophils and prevents the antimicrobial processes required to protect the host from infection. Herein, we identify that neutrophils isolated from fasting hosts have decreased glycolytic and mitochondrial metabolic capacity compared to postprandial. Furthermore, in response to *Staphylococcus aureus*, fasted neutrophils fail to elicit oxidative stress or release extracellular traps to the same magnitude as postprandial. Fasted neutrophils remained impaired even when exposed to glucose *ex vivo*, indicating functional changes in neutrophils that render them less capable of combating infection. Taken together, our findings underscore the critical impact of fasting on neutrophil function, suggesting that preoperative fasting protocols may need to be reevaluated to better support immune function and reduce the risk of postoperative infections.

Abstract #: F50

Human lung CD8$^+$ T_{RM}-derived interferon-γ orchestrates subset specific antiviral programming in airway epithelial cells

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In the pulmonary mucosa, CD8$^+$ tissue resident memory T cells (T_{RM}) act as sentinels that rapidly respond to respiratory viruses. While mouse models have demonstrated that T_{RM} mediate protection at barrier sites through cytokine production and direct cell lysis, our understanding in human lungs remains limited. We investigated the functionality of virus-specific CD8$^+$ T_{RM} from non-transplantable, healthy human lungs by first quantifying the frequency and phenotype of these cells against prevalent viruses. While CD8$^+$ T cells targeting respiratory specific viruses displayed a heightened tissue residency profile (CD69$^+$ CD103$^+$ CD49a$^+$), RNAseq revealed that Flu- and CMV-specific lung CD8 T cells are functionally and transcriptionally similar. Next, we investigated how virus-specific CD8$^+$ T cells impact cells in the local lung environment. Following antigen stimulation, we noted significant activation of both innate and epithelial cells, dependent on IFNγ. Examination of epithelial cell subsets highlighted that basal cells were more activated than ciliated cells. Upon stimulating lung CD8$^+$ T_{RM} with flu peptide +/- anti-IFNγ, we conducted scRNAseq on lung epithelial cells and observed that peptide stimulated CD8$^+$ T_{RM} robustly activated anti-viral genes in basal cells, and to a lesser degree in secretory cells. Using Air-Liquid Interface (ALI) cultures, we modeled the effects of IFNγ mediated epithelial cell activation on viral replication. Our findings show how crosstalk between CD8$^+$ T_{RM} and respiratory epithelium activates ISG pathways in epithelial cells, specifically basal cells, and by doing so significantly decreases the frequency of infection and overall viral load.

Abstract #: F51

Altered intestinal migration during systemic autoimmunity

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Intestinal immune cells migrate to extraintestinal tissues. Early in life, dendritic cell migration from the gut to the thymus leads to the development of microbiota-specific T cells. Remarkably, aging and microbiota depletion significantly alter intestinal traffic, and few studies suggest that intestinal migratory cells can exacerbate systemic disease. However, the role of intestinal migratory cells in lupus remains unknown. Systemic lupus erythematosus (lupus) is an autoimmune disease characterized by systemic inflammation due to an exaggerated immune response against self-antigens in multiple tissues. In lupus, intestinal dysbiosis is linked with dysregulation of mucosal and systemic innate and adaptive immune responses. Using TLR7-dependent mouse models of lupus in KIKGR photoconvertible mice, we could track immune cell migration from the gut to extraintestinal tissues. To assess this, we exposed the cecum of KIKGR mice to UV laser light (UV 405nm) and characterized photoconverted cells in tissues such as the mesenteric lymph nodes (MLN), spleen, kidneys, and thymus. Induction of lupus in these mice led to an increase in intestinal migration to the thymus and the spleen post laser exposure compared to control mice, with an increase of CD4 T cells in both thymus and spleen and an increase in dendritic cells in the spleen. Further, crossing KIKGR mice to TLR7.1 Tg lupus-prone mice helped us identify changes early in life in hosts predisposed to develop lupus. Ongoing experiments aim to clarify whether these populations of migrating cells contribute to lupus progression and whether the specific pathobionts influence intestinal migration.

Abstract #: F52

Unraveling the Role of CXCR3+ Monocytes in Tumor-Driven Myelopoiesis and Immunity

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CXCR3+ monocytes have been identified as correlating with GD2 CAR T-cell expansion in pediatric patients, suggesting a role in T-cell activation. We hypothesize that CXCR3+ monocytes support T-cell activation and function through direct interactions with T cells and presenting tumor-derived antigens. Additionally, we propose that CXCR3+ monocytes migrate from tumors to draining lymph nodes.

Using the B16F10 melanoma model, flow cytometry analysis revealed an expansion of CXCR3+ monocytes in response to tumor burden. These monocytes were enriched in tumor-draining lymph nodes but were absent in contralateral and healthy lymph nodes. To assess their interaction with tumor-derived antigens, we utilized Zs-Green+ B16F10 melanoma cells, allowing tracking of tumor-derived GFP within immune cells. A CXCR3+ monocyte population in tumor-draining lymph nodes exhibited a GFP signature, suggesting antigen uptake, and expressed elevated levels of MHC II and CD86, indicative of an activated antigen-presenting phenotype.

These findings suggest that CXCR3+ monocytes respond to inflammatory signals in the tumor microenvironment and migrate to draining lymph nodes, potentially engaging in T-cell activation. Future studies will investigate the mechanistic role of CXCR3+ monocyte-T cell interactions in antigen presentation and examine the lineage maturation of these monocytes from the bone marrow in response to tumors.

Abstract #: F53

Title: Neutrophil Responses to Type 3 Pneumococcus: The Impact of Strain Variability

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Streptococcus pneumoniae (pneumococcus) remains the leading cause of community-acquired bacterial pneumonia, claiming the lives of over 300,000 children under 5 years old worldwide every year despite the availability of antibiotics and vaccines. Serotype 3 pneumococcus accounts for a significant proportion of hospitalized pneumonia cases, underscoring the reduced efficacy of host responses and vaccine-mediated protection. To better understand the mechanisms underlying serotype 3 interactions with host responses, we characterized six clinical strains *in vitro*, *ex vivo*, and *in vivo*. Our data suggest strain-specific differences in susceptibility, lung bacterial titers, and neutrophil recruitment in mice, as well as variability in neutrophil-mediated killing and antibody-mediated opsonization, none of which correlated with clonal clade, capsular polysaccharide (CPS) thickness, or CPS amount. To define the contribution of CPS to the observed variability, we generated capsule-switch variants by replacing the serotype 3 CPS with a serotype 2 CPS. Lethality was improved beyond that of the wild-type serotypes 2 and 3, suggesting that non-CPS factors contribute to lethality. These findings highlight complex, strain-specific host-pathogen interactions that influence serotype 3 pneumococcal virulence.

Abstract #: F54

Molecular drivers of CD8 T cell exhaustion across cancer and chronic infection

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Although CD8 T cells are crucial to the antitumor immune response, persistent antigen stimulation drives T cell differentiation into a dysfunctional or exhausted cell state. To resolve the factors involved in the development and maintenance of exhaustion our lab performed paired scRNAseq and scATACseq on antigen specific CD8 T cells across 11 conditions, including acute and chronic infection, and 4 orthotopic mouse tumor models. Upon integration, we identified numerous transcription factors (TFs) predicted to influence gene expression and regulate specification. Various members of the NFkB TF family were enriched across CD8 T cell states. Although NFkB is of known importance to the immune response, the exact role in CD8 T cells responding to chronic antigen remains unclear. We employed genetic mouse models to deplete *Ikbkg* (encoding IKKγ), the master regulator of canonical NFkB signaling, in the tumor microenvironment and in chronic infection. We found that IKKγ maintains the long-term persistence of exhausted CD8 T cells in cancer and chronic infection. IKKγ may also enforce distinct gene expression programs as we detected discrete phenotypes in each disease setting in *Ikbkg* deficient CD8 T cells. Therapeutic modulating of the canonical NFkB pathway may present a promising path for effectively manipulating T cell differentiation and durability in chronic infection or cancer.

Abstract #: F55

Dysfunction of children's T follicular helper cells in the context of cryptosporidiosis

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Cryptosporidium (Crypto), an apicomplexan parasite which infects the small intestine, is a leading cause of diarrheal mortality in children under 5. In our cohort of Bangladeshi children, repeated infections occur before immunity develops by age 4. Due to the lack of effective treatments for infants, understanding the initial failure of the adaptive immune response is crucial for future vaccine development. We observed that T follicular helper (Tfh) cells, involved in B cell activation, show an exhaustion-like phenotype in 2-year-old Bangladeshi children – a pattern not observed in American children. In untreated PBMCs, we found increased activation of Tfh cells at age 2. These Tfh cells fail to further activate upon stimulation with PMA, correlating with peak Crypto infection rates. To investigate the role of Tfh cells in Crypto immunity, we assessed the functionality of memory Tfh cells using an antigen-induced marker assay. Crypto-specific Tfh cells were identified in PBMCs of children aged 5 and 6 by upregulation of general activation markers following stimulation with Crypto extract. Results indicated a correlation between Tfh cell activation and number of Crypto infections by age 5. Children who exhibited a robust memory response to stimulation had fewer than 2 infections by age 5, while those who had a poor memory response had up to 7 ($p=0.0079$). Additionally, children with a robust Tfh cell memory response tended to have high concentrations of Crypto-specific antibodies, whereas those with low concentrations did not ($p=0.0479$). These results suggest that highly functional memory Tfh cells are essential for effective antibody production and subsequent protection, highlighting their importance in immunity against Crypto.

Abstract #: F56

Risk Biomarkers of Chronic Graft-versus-host disease in Children aged 10 years or younger and Children/adults Older than 10 years

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Assessment of risk biomarkers of chronic GVHD (cGVHD) after allogeneic hematopoietic cell transplantation (HCT) in the pediatric age group is lacking. Thus, we conducted a prospective cohort that had accrued 318 patients at six centers: (129 children ≤10 years and 189 children/adults >10 years). Six plasma biomarkers [CXCL9, interleukin 1 receptor-like 1 (IL1RL1), regenerating-islet-derived-3-alpha (REG3α), matrix metallopeptidase-3 (MMP3), dickkopf-WNT signaling pathway inhibitor-3 (DKK3), and sCD163] were assessed at Day (D)100 post-HCT. We performed D100 landmark analyses for cGVHD, dichotomizing the cohort at ≤10 years and using each biomarker cutoff for high and low risk groups as identified via Youden's index. IL1RL1 (>22 ng/mL) is associated with future cGVHD in both groups: ≤10 years [Hazard Ratio (HR) Confidence Interval (CI): 2.71 (1.25, 5.85), p=0.01] and >10 years [HR (95% CI): 1.91 (1.16, 3.15), p=0.01]. REG3α (>68 ng/mL), MMP3 (>13 ng/mL), and DKK3 (>47 ng/mL) are associated with cGVHD only in patients >10 years [HR (95% CI): REG3α: 1.97 (1.18, 3.29), p=0.01; MMP3: 2.05 (1.24, 3.39), p=0.01; DKK3: 2.25 (1.29, 3.93), p=0.004]. After adjustment for significant covariates (race/ethnicity, malignant disease, graft, GVHD prophylaxis, and ATG), high IL1RL1, REG3α, MMP3, and DKK3 remained associated with cGVHD in patients >10 years and IL1RL1 remained associated with cGVHD in younger patients. A 5-biomarker panel (IL1RL1, REG3α, MMP3, DKK3, and sCD163) associated with TRM in both groups. We conclude biomarkers measured 3 months following HCT are susceptibility and/or prognostic for cGVHD and TRM in both children and adults allowing for additional stratification.

Abstract #: F57

IL-5 SIGNALING PROTECTS MICE FROM ACUTE LUNG INJURY THROUGH AN EOSINOPHIL INDEPENDENT MECHANISM

Christopher F. Pastore[1], Cara L. Hrusch[2], Kathleen A. M. Mills[2], Brennan D. Stadler[1], Jalen Austin[1], Tania E. Velez[1], Anne I. Sperling[1]

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Acute Lung Injury (ALI) has many different causes such as lung infections, sepsis, mechanical stress, and complications arising from major surgery. These converge with epithelial and/or endothelial barrier dysfunction in the lungs leading to edema and hypoxemic respiratory failure. Cytokines such as IL-1β, TNFα, and IL-6 which are proinflammatory can further damage the airway, whereas type 2 cytokines such as IL-4 and IL-13 have been implicated with tissue repair. Previous data in our lab show that IL-5, another type 2 cytokine and previously thought to not be involved in tissue repair, rescues mice from the bleomycin mouse model of ALI. Mice lacking IL-5Rα succumb to ALI and have more edema in the lung. Restoring IL-5Rα in the hematopoietic or non-hematopoietic compartment is sufficient to rescue from ALI-induced death. Furthermore, because IL-5Rα has been shown to be critically important for eosinophils, experiments were done demonstrating that depletion of eosinophils did not impact recovery from ALI, suggesting an eosinophil independent role for IL-5Rα in the immune compartment. To elucidate the role of lung structural cells, we discovered that proliferating epithelial cells express IL-5Rα and in an IL-5Rα deficient mouse there is a corresponding defect in epithelial proliferation. Our data shows that IL-5 signaling is beneficial for the recovery from ALI and could be utilized in a potential therapeutic strategy.

Abstract #: F58

IL-9 producing type 2 innate lymphoid cells in the regulation of acute lung injury after allogeneic hematopoietic stem cell transplantation (HSCT)

Xingjun Wu[1], Yunfeng Zong[1], Hua Jiang[1], Debjani Dutta[1], Claudia Szabo[1], Samantha Pazmino[1], Abdulraouf Ramadan[2], Mark Kaplan[2], Courtney Rowan[2], Huanmei Wu[2], Sophie Paczesny[1]

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HSCT is a therapy for cancers of the bone marrow but T cell reactivity to alloantigens in normal tissues induces graft versus host disease (GVHD) including of the lung. Our laboratory has shown that soluble (s)ST2, a decoy receptor for IL33, is increased 10-fold in patients with lung GVHD vs controls while the membrane-bound ST2 expressed on regulatory T cells (Tregs) and ILC2 cells is decreased. Using murine models of HSCT and IL33 intranasal treatment or systemic blockade of sST2, we showed decreased GVHD severity, increased plasma IL33 while IFNγ and TNFα decreased in treated mice vs controls. The treated mice showed better pulmonary function, reduced infiltrating donor $IFN\gamma^+CD4^+$ and $IFN\gamma^+CD8^+$ T cells, increased frequencies of donor Tregs, and increased frequency of $GATA3^+ST2^+$ ILC2 in the lung. However, the role of ILC2s in the lung following HSCT has not been explored. Here we show that IL9 specifically produced by ILC2s, but not other subsets, is required for their regulatory effects. RNA-seq analysis of sorted ILC2s from naïve GATA3 reporter mice treated with IL33 showed increased Il9 and pu1 transcripts in ILC2s which was validated in allogeneic mice treated with IL33 or ST2 blockade compared to vehicle. Using hematopoietic and nonhematopoietic IL9 KO vs WT chimeras we confirmed that recipient hematopoietic ILC2 are the source of IL9 production. Tregs proliferation and activation are decreased in IL9 KO vs WT chimera. Further, whole genome methylation sequencing on sorted Tregs from IL9 treated mice showed significant hypomethylation in the Foxp3 region vs untreated mice. Together, this suggests that inhibition of sST2 or administration of IL33 during HSCT induce IL9 secreting ILC2 that prevent lung GVHD.

Abstract #: F59

Probiotic-derived exopolysaccharide interferes with Th2 priming and inhibits allergic inflammation

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Allergic asthma is an increasing burden in industrialized countries, where treatments are limited to treating symptoms rather than root causes. In recent years, the use of inhaled probiotic cocktails has shown promise in preventing allergic asthma. However, the mechanisms by which such probiotics prevent sensitization remain to be elucidated. In this study, we focused on exopolysaccharide (EPS) from *B. subtilis*, a ubiquitous soil bacterium. We found that EPS treatment prior to sensitization with house dust mite (HDM) limited lung mucus hypersecretion and eosinophilia. Furthermore, EPS treatment inhibited accumulation of T_H2, but not T_H1 or T_H17 subsets. To determine whether EPS was affecting T_H2 cell priming by dendritic cells (DCs), we compared the composition and phenotype of DCs in the lung and lung-draining lymph node (LDLN) after EPS and HDM treatment. Surprisingly, pre-treatment with EPS induced DC activation, as measured by an increase in CD86, and a decrease in the ability to uptake HDM and migrate to the LDLN. Additionally, EPS-pretreated BMDCs were unable to induce antigen-specific T cell proliferation *in vitro*. Together, our data suggest that EPS inhibits allergic sensitization by inducing DC activation prior to HDM exposure, thereby leading to decreased T_H2 priming and allergic inflammation. Thus, we provide a potential mechanism by which inhalation of a single prebiotic molecule can prevent allergic sensitization.

Abstract #: F60

IL-9 producing type 2 innate lymphoid cells in the regulation of acute lung injury after allogeneic hematopoietic stem cell transplantation (HSCT)

Xingjun Wu[1], Yunfeng Zong[1], Hua Jiang[1], Debjani Dutta[1], Claudia Szabo[1], Samantha Pazmino[1], Abdulraouf Ramadan[2], Mark Kaplan[2], Courtney Rowan[2], Huanmei Wu[2], Sophie Paczesny[1]

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HSCT is a therapy for cancers of the bone marrow but T cell reactivity to alloantigens in normal tissues induces graft versus host disease (GVHD) including of the lung. Our laboratory has shown that soluble (s)ST2, a decoy receptor for IL33, is increased 10-fold in patients with lung GVHD vs controls while the membrane-bound ST2 expressed on regulatory T cells (Tregs) and ILC2 cells is decreased. Using murine models of HSCT and IL33 intranasal treatment or systemic blockade of sST2, we showed decreased GVHD severity, increased plasma IL33 while IFNγ and TNFα decreased in treated mice vs controls. The treated mice showed better pulmonary function, reduced infiltrating donor $IFN\gamma^+CD4^+$ and $IFN\gamma^+CD8^+$ T cells, increased frequencies of donor Tregs, and increased frequency of $GATA3^+ST2^+$ ILC2 in the lung. However, the role of ILC2s in the lung following HSCT has not been explored. Here we show that IL9 specifically produced by ILC2s, but not other subsets, is required for their regulatory effects. RNA-seq analysis of sorted ILC2s from naïve GATA3 reporter mice treated with IL33 showed increased Il9 and pu1 transcripts in ILC2s which was validated in allogeneic mice treated with IL33 or ST2 blockade compared to vehicle. Using hematopoietic and nonhematopoietic IL9 KO vs WT chimeras we confirmed that recipient hematopoietic ILC2 are the source of IL9 production. Tregs proliferation and activation are decreased in IL9 KO vs WT chimera. Further, whole genome methylation sequencing on sorted Tregs from IL9 treated mice showed significant hypomethylation in the Foxp3 region vs untreated mice. Together, this suggests that inhibition of sST2 or administration of IL33 during HSCT induce IL9 secreting ILC2 that prevent lung GVHD.

Abstract #: F61

Therapeutic CD40 and TLR3 agonism induces durable, T cell-dependent survival in a murine model of glioblastoma.

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Glioblastoma (GBM) is the most common and aggressive primary CNS malignancy, and current treatments are debilitating and ineffective, inevitably resulting in tumor recurrence due to diffusely spread cells. Although the immune system can mount effective responses against CNS antigens, the response to GBM is poor. Consistent with a severely immunosuppressive tumor microenvironment, intratumoral antigen presenting cells (APCs), particularly conventional type 1 dendritic cells, are immature and exhibit suboptimal tumor antigen trafficking to draining lymph nodes. We hypothesize that concurrent CD40 and TLR3 agonism improves the immunostimulatory capacity of APCs to drive T cell priming that ultimately controls GBM growth. Using a syngeneic, orthotopic CT2A-ZsGreen model, we treat mice with agonistic anti-CD40, polyI:C (TLR3 agonist), and anti-PD-1 or control IgG. Nearly half of treated mice eradicate tumors in a T cell-dependent manner, as evidenced by MRI and durable survival. These “long-term survivors” are protected from rechallenge, indicative of functional immune memory and consistent with the accumulation of tissue resident memory T cells within their brains. This work demonstrates the potential for CD40 and TLR agonism in improving anti-GBM T cell responses, particularly regarding the formation of functional immune memory that can protect patients from tumor recurrence. We are actively elucidating the changes in APC function that drive T cell priming and tumor control.

Abstract #: F62

Molecular architecture of CD8 T cell adaptation to tissue and tumor microenvironments

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ABSTRACT

Tumor infiltrating CD8 T lymphocytes (TIL) with a tissue-resident memory (Trm) phenotype exhibit potent anti-tumor function and associate with improved patient outcomes in many solid malignancies. However, the molecular programs governing the formation of Trm-like TIL remain ill-defined. Here, we performed paired single-cell RNA-sequencing and single-cell ATAC-sequencing of TCR-matched CD8 T cells in models of infection and cancer in mice. Enhancer-driven regulons assembled from multiomic profiling data revealed a role for KLF2 in repressing formation of $CD69^{+}CD103^{+}$ Trm-like TIL and limiting anti-tumor activity. Conversely, we found sustained BATF expression enhanced formation of $CD69^{+}CD103^{+}$ TIL, contingent upon downregulation of KLF2. Last, we found TGFβ signaling and CD103 were necessary for Trm-like TIL formation, but BATF overexpression was sufficient to drive formation of $CD69^{+}CD103^{+}$ TIL in TGFBR2-silenced cells. These findings reveal key mechanisms of Trm-like TIL differentiation and provide insights into the molecular circuitry controlling T cell adaptation in tumor microenvironments.

Abstract #: F63

Title: Targeted combination therapy to overcome adenosine-mediated immune suppression in ovarian cancer

Authors: Rossymar Rivera Colón[1,2,3], Arianna Gravatt[3], Yaning Li[1,2,3], Asmaa O. Mohammed[1,2,3], Kilyna Nguyen[1,2,3], Khondamir Imomnazarov[4], Joel Linden[5], & Kristin G. Anderson[1,2,3]

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Ovarian cancer is one of the leading causes of cancer deaths among women, with persistent poor survival rates underscoring the need for innovative treatments. Engineered T cell therapies expressing high-affinity tumor-specific T cell receptors (TCRs) have shown promise in preclinical solid tumor models. We previously demonstrated that TCR-engineered T cells targeting mesothelin significantly extend survival in an immune-competent mouse model of advanced ovarian cancer. However, the immunosuppressive tumor microenvironment (TME) contains elevated adenosine levels, which leads to T cell dysfunction via activation of the A_{2A} receptor ($A_{2A}R$). Notably, high adenosine levels correlate with poor prognosis in ovarian cancer patients, suggesting this is an important pathway to address to improve immunotherapy efficacy. While $A_{2A}R$ inhibitors are under clinical investigation, immunotherapies enhancing endogenous antitumor T cell responses have yielded disappointing results in ovarian cancer. We hypothesize that combining engineered T cells with $A_{2A}R$ blockade could overcome these limitations, enhancing antitumor efficacy and T cell persistence. Preliminary *in vitro* data confirm that a selective $A_{2A}R$ inhibitor restores engineered T cell function in the presence of adenosine. Moreover, we found that ovarian cancer cells also express $A_{2A}R$, and inhibition slowed tumor growth *in vitro*, suggesting this therapeutic combination may have enhanced antitumor efficacy over either treatment alone. Thus, this combination strategy has the potential to be effective for ovarian cancer and other solid tumors that share adenosine-mediated immune suppression.

Abstract #: F64

Chimeric anti-HLA antibody receptor engineered regulatory T cells (CHAR Tregs) suppress allogeneic HLA-specific B cells from pre-sensitized transplant recipients

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Abstract

Organ transplantation is a lifesaving procedure. However, many patients on the organ waiting list harbor B cells directed against allogeneic human leukocyte antigen (HLA) molecules, often HLA-A2, greatly decreasing their likelihood of receiving a compatible organ and requiring higher doses of immunosuppressive drugs. Current strategies to desensitize patients non-specifically target circulating antibodies and B cells, resulting in poor efficacy and complications. Regulatory T cells (Tregs) are a subset of immune cells dedicated to suppressing specific immune responses by interacting with both innate and adaptive immune cells. Here, we modified Tregs with a chimeric anti-HLA antibody receptor (CHAR) consisting of an extracellular HLA-A2 protein fused to a CD28-CD3zeta intracellular signaling domain, driving Treg activation upon recognition of anti-HLA-A2 alloreactive B cells. HLA-A2 CHAR Tregs were stimulated specifically by anti-HLA-A2 antibody-producing cells, as evidenced by preferential expansion and upregulation of activation markers. CHAR activation had no negative impact on Treg stability, as measured by FOXP3 and HELIOS expression. Interestingly, CHAR Tregs were not cytotoxic towards anti-HLA-A2 antibody-producing cells, in contrast with CHAR $CD4^+$ T effector cells. Importantly, CHAR Tregs significantly suppressed IgG antibody production by B cells from HLA-A2 sensitized patients in the presence of HLA-A2-expressing cells. Altogether, CHAR Tregs represent a new type of engineered Treg aimed at inhibiting alloreactive B cells, providing a targeted and safe therapeutic strategy to desensitize transplant recipients.

Abstract #: F65

P2RY12 REGULATES MICROGLIAL TRANSCRITPTIONAL IMMUNO-METABOLIC AND ACTIVATION STATES

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Microglia are the resident immune cells of the central nervous system (CNS) and play essential roles in maintaining homeostasis and mediating immune responses in disease. In this context, the purinergic receptor P2RY12 is enriched in homeostatic microglia and is frequently downregulated in pathological contexts. In our study, we compared the transcriptional profiles of P2RY12 knockout (KO) to wild-type (WT) microglia. Our results revealed significant upregulation of genes involved in immune signaling and metabolic regulation in KO microglia, including the key metabolic regulator *PGK1*, which plays a role in glycolysis and energy production. Furthermore, we examined the response of KO microglia to LPS-induced systemic inflammation. In WT microglia, LPS treatment upregulated key genes involved in the oxidative stress response. However, in KO microglia, these genes showed minimal induction, indicating a dysfunctional antioxidant response. This suggests that P2RY12 is required for mounting a robust antioxidant response and for the proper management of oxidative stress during microglial immune activation and highlight the critical role of P2RY12 in regulating microglial immune and metabolic responses under both homeostatic and inflammatory conditions, providing insights into its involvement in CNS pathophysiology.

Abstract #: F66

A Systems Approach to Modelling Maternal - Fetal Immune Transfer

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Maternal immunity shapes infant health via transplacental antibody transfer, influenced by biological and social factors, including body mass index (BMI), stress, and social determinants of health (SDoH). These factors may alter maternal humoral immunity as well as transfer efficiency, impacting neonatal immunity. IgG transport relies on neonatal Fc receptor (FcRn) and Fc gamma receptors (FcγRs), with syncytiotrophoblasts and endothelial cells co-expressing this receptor combinations. Little is known about patient - to - patient variability in antibody transfer and the specific mechanisms of Fc receptor - mediated transfer. As such, my overarching objective is to establish how cytokine profiles and SDoH inform antibody transfer and to uncover the mechanisms behind this process. I integrated *in vitro* Transwell assays with *ex vivo* analysis of maternal - cord pairs from a UVA pregnancy cohort to investigate factors regulating IgG transfer. As a result, maternal C - reactive protein (CRP) correlated with pregravid BMI ($r = 0.58$, $p < 0.001$) and IL-6 ($r = 0.57$, $p < 0.001$), suggesting a pro-inflammatory state linked to reduced IgG1-4 transfer. Additionally, distinct cytokine profiles were observed across gestation. FcγRIIb overexpression enhanced IgG1 transcytosis in endothelial cells, suggesting a role in transfer regulation. These findings indicate that maternal inflammation and FcγRs may shape antibody transfer and fetal immunity, informing future vaccine strategies. Upcoming work will further explore IgG isotype transfer and Fc-mediated mechanisms through placental trophoblast layer to refine maternal-fetal immune dynamics and neonatal health implications.

Abstract #: F67

AXL drives regulatory cDC1 maturation to control CD8$^+$ T cell contraction during severe influenza recovery

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Activation of XCR1+ conventional DCs by the recognition of apoptotic cells (AC) turns them into mature regulatory cDC1 (mregDC1). We showed that mregDC1 differentiate in the lungs of mice that recover from Influenza A (IAV) infection and that they protect the lungs from damage by dampening effector CD8$^+$ T cell function. However, the mechanisms inducing mregDC1 after IAV infection remain largely unexplored. To investigate this, we performed scRNA-seq on cDC1 from the lungs of naïve, infected, and recovering mice. We found that cDC1 from recovering lungs uniquely upregulates *Axl* and *Elmo1*, genes associated with recognition and engulfment of AC. To test the role of Axl in the maturation of cDC1 we generated mice with *Axl* conditionally deleted in cDCs (Zbtb46-Cre, zDC-Axl) and, using flow cytometry, we confirmed Axl deletion in cDC1 from zDC-Axl mice. To test AC capture *in vivo*, we irradiated mice expressing a green fluorescent protein (Kik-green) and reconstituted them with either WT bone marrow (BM) or zDC-Axl BM. After reconstitution, we infected the Kik-green mice and quantified cDC1 that acquired green fluorescence. Our data showed that zDC-Axl BM abrogated the acquisition of green fluorescence in cDC1 but not in macrophages. Next, we infected WT and zDC-Axl mice and sorted mature cDC1 from the recovering lungs (16dpi) and loaded the mature cDC1 with $OVA_{257-264}$ peptide to perform *in vivo* and *in vitro* experiments to evaluate their capacity to block cytokine production in activated OT-I cells. We found that both *in vivo* and *in vitro*, the mature cDC1 lacking Axl fail to reduce cytokine production in effector OT-I cells. Overall, our data show that Axl is required for the acquisition of AC and mregDC1 function during the recovery phase of IAV infection.

Abstract #: F68

Title: The first-trimester maternal microbiome is predictive of chronic inflammation and unfavorable birth outcomes in a Bangladeshi population

Authors: Rachel H Boone, Rebecca Carpenter, G Brett Moreau, Rashidul Haque, M. Shahjahan Siraj, S. Masum Billah, William A Petri Jr., and Gregory Madden.

Unfavorable birth outcomes, such as preterm birth, can have detrimental impacts on a child's prognosis and later development. This concern is greatest in low-middle income countries where pre-term birth rates are highest and there are less resources for post-natal care. There is a lack in the understanding of what maternal factors contribute to these outcomes. Using randomly selected mother-child dyads from the Tangail district of Bangladesh, potential causes of unfavorable birth outcomes were investigated. We found that elevated inflammatory cytokines (i.e. CRP and AGP) during the first trimester were correlated with preterm birth (OR = 2.23; 95% CI: 1.03, 5.16). Due to high levels of both enteric infections and antibiotic usage in the Bangladeshi population, we hypothesized that an unfavorable composition of the maternal microbiome may lead to the observed increase in inflammatory markers and unfavorable birth outcomes. We found that the presence of the enteric pathogen aEPEC was significantly associated with increased odds of elevated AGP (OR = 1.70; 95% CI: 1.05, 2.76) but not CRP. It was also associated with increased odds of preterm birth (OR = 2.36; 95% CI: 1.21, 4.57). Additionally, a random forest model using the amplicon sequence variants of the maternal fecal microbiomes could predict elevated AGP (AUC = 0.752) and small vulnerable newborns (defined as preterm, low birth weight, and/or small for gestational age, AUC = 0.667). These results suggest that an unfavorable composition of the maternal microbiome during the first trimester of pregnancy may lead to chronic inflammation (as indicated by elevated AGP), contributing to unfavorable birth outcomes.

Abstract #: F69

Acquired CAR-T therapy resistance in a pre-clinical model of pediatric rhabdomyosarcoma minimal residual disease

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Current treatments for relapsed/refractory or metastatic rhabdomyosarcoma (RMS) are ineffective. Chimeric Antigen Receptor (CAR)-T cell therapies for solid tumors face numerous obstacles with limited clinical success. Treatment success requires understanding the mechanisms driving clinical failure. Toward this goal, we developed two models of CAR-T-resistant RMS minimal residual disease (MRD) to evaluate the mechanisms driving acquired resistance. Treatment with either αvβ3- or HER2-CAR-Ts nearly eliminates orthotopic RMS tumors in NSG mice. However, antigen^{pos} MRD remains six weeks post-treatment, despite CAR-T persistence. At the time of MRD, αvβ3- and HER2-CAR-Ts rapidly control a tumor re-challenge in the opposite leg. However, neither the original CAR-Ts nor a second CAR-T dose eliminate MRD at the primary site, consistent with the development of acquired resistance. Unique resistance mechanisms, distinct from antigen modulation, emerge based on the CAR-T treatment. Continued investigations into these mechanisms will enable superior CAR-T design and delivery that prevent acquired tumor resistance.

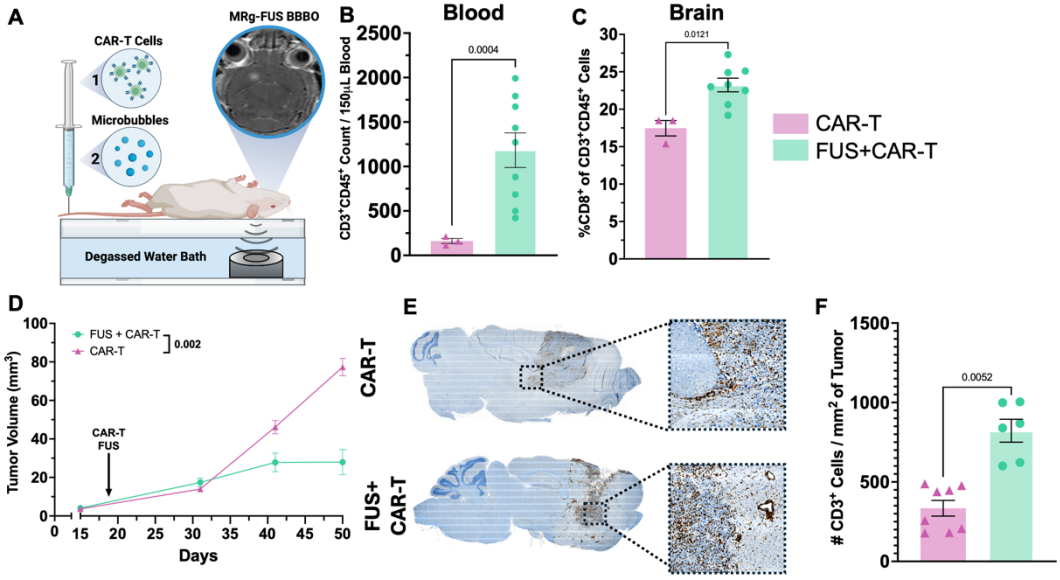
Abstract #: F70

Abstract Title: Focused Ultrasound-Mediated Blood Brain Barrier Opening Enhances Delivery and Efficacy of αvβ3-CAR-T Cells in a Breast Cancer Brain Metastasis Model

Authors: Thomas Sherlock[1], AeRyon Kim[2], Stefanyda Maslova[1], Andrew Thede[1], Dustin Cobb[3], Daniel Trey Lee[3], Natasha D Sheybani[1,2]

Affiliations: Biomedical Engineering, University of Virginia[1], Focused Ultrasound Cancer Immunotherapy Center[2], Pediatric Hematology/Oncology, University of Virginia[3]

Objectives: To investigate the impact of FUS-mediated blood brain barrier opening (BBBO) on $\alpha_v\beta_3$ CAR-T cell therapy in a pre-clinical breast cancer brain metastasis (BCBM) model.
Methods: NSG mice were intracranially inoculated with MDA-MB-468 cells to generate a xenograft model recapitulating key characteristics of BCBM. On day 18, $\alpha_v\beta_3$- or CD19-targeted CAR-T cells were administered intravenously and MR-guided FUS BBBO with microbubbles was performed (Fig 1A). Tumor outgrowth was monitored via serial contrast-enhanced MRI. CAR-T cells were enumerated in the blood and
brain via flow cytometry and spatially mapped with immunohistochemistry (IHC).
Results: Combination of FUS BBBO with $\alpha_v\beta_3$ CAR-T cells significantly constrained BCBM outgrowth compared to sham, CD19 CAR-T cell, and $\alpha_v\beta_3$ CAR-T cell monotherapy (Fig 1B). Absolute frequency of circulating CAR-T cells was significantly higher in the setting of FUS exposure (Fig 1C), as was proportion of CD8+ CAR-T cells in BCBM-bearing brains (Fig 1D). IHC staining in brains harvested at endpoint revealed a superlative elevation in tumor-localized CD3+ T-cell staining relative to controls (Fig 1E-F).
Conclusions: FUS BBBO combined with $\alpha_v\beta_3$ CAR-T cells offers a promising method for BCBM therapy. Future studies will aim to investigate involved mechanisms of interactivity between FUS and $\alpha_v\beta_3$ CAR-T cells, optimize acoustic parameters, and extend findings to other neuro-oncologic settings.



Abstract #: F71

Antagonism of Inflammation Signaling by NLRX1 During SARS-CoV-2 Infection

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Severe disease progression of SARS-CoV-2 infections is associated with an overactivation of the innate immune response which is responsible for inflammation and damage to host tissue. This unregulated production of cytokines is referred to as the cytokine storm. Preliminary data has shown NLRX1, a PRR involved in innate immunity, shown to regulate NF-kB and interferon signaling during influenza infection, is significantly downregulated in SARS-CoV-2 positive patients indicating NLRX1 is involved in modulation of the cytokine storm. We hypothesize that NLRX1 acts to antagonize inflammatory signaling to dampen the immune response and reduce damage to the host during SARS-CoV-2 infection. Using patient derived data, we found a positive correlation between upregulation of inflammatory cytokine expression and viral replication in SARS-CoV-2 positive patients. To further understand this phenotype, we use *Nlrx1*$^{-/-}$ mice and transduced human airway epithelial cells (Calu3). We observed a protective nature of NLRX1 through increases in viral titer and inflammation in airways of NLRX1 deficient mice. Furthermore, viral titer decreases in NLRX1 overexpressed Calu3 challenged with SARS-CoV-2 (MOI 0.1). Bone marrow derived macrophages from *Nlrx1*$^{-/-}$ mice show trending increases in expression of *Irf3, Ifnβ,* and *Ifnγ* compared to wildtype mice. Our data indicates that NLRX1 acts to decrease inflammatory signaling to prevent the cytokine storm and has a protective nature against SARS-CoV-2 infection. Examination of the differentiation of cytokine expression by NLRX1 could indicate a potential drug target.

Abstract #: F72

Improving Viability and Function of Ex Vivo Lymph Node Slice Cultures by Optimizing Flow and Oxygenation

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Lymph nodes (LNs) are highly organized and dynamic organs that constantly adapt to environmental changes, often initiating immunological responses. Ex vivo LN slices offer a valuable platform for studying immunological processes in short-term cultures; however, their functionality has shown to decline significantly after 48 hours, as characterized by reduced metabolic activity, decreased cytokine secretion, increased cell egress, decline in stromal health, and development of hypoxia in the tissue. Such factors limit their use in long-term applications, emphasizing the need for improved culturing methods. We hypothesized that controlled fluid flow, by mimicking biological shear stress and nutrient distribution of the lymphatic environment, will help maintain LN slice functionality in long-term culture.

To address this challenge, we developed a 3D-printed perfusion system to sustain LN slice functionality beyond 48 hours by simulating in vivo conditions. Our results confirmed that LN slices remained undamaged in shear markers VE-Cadherin and aSMA following overnight perfusion in the chip system. Further, we found that although cytokine secretion of CCL21 and CXCL13 remain unchanged, fluid flow reduced cell egress and BAFF secretion. Achieving long-term LN slice cultures would allow researchers to spatially map and monitor immune responses in LNs over time, significantly enhancing the study of complex immunological processes. This approach would also improve understanding of disease progression, immune aging, and therapeutic interventions.

Abstract #: F73

Mechanistic Insights from Computational Modeling of Maternal Immunization and Antibody Transfer

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Prenatal immunization leverages transplacental antibody transfer to confer neonatal immunity during early life. Rational design of prenatal vaccines has been impeded by gaps in the mechanistic understanding of maternal immunization response during pregnancy and its variability across populations. Multivariate linear modeling and dimensionality reduction were used to derive optimization parameters for an ordinary differential equations (ODE) mechanistic model of maternal IgG immunization response and transplacental antibody transfer. Using publicly available data, we developed a predictive model of tetanus, diphtheria, and pertussis (TDAP) immunization response in pregnant and non-pregnant women which uncovered differences in immune phenotype and subclass specific IgG response. Pregnant women had increased expression of naïve memory B cells, significantly higher concentrations of IgG3 pre-immunization, and increased levels of IgG1 across timepoints implicating differences in adaptive immune function during pregnancy. Dimensionality reduction identified BMI, gravidity, and previous immunization as mediators of response in pregnant women. Lastly, we observed heterogeneity of IgG3 half-life which when considered improved model performance. Data driven modeling uncovered biological variability and changes in immune function during pregnancy. The model resulting from these data can serve as a nuanced testbed for rational prenatal vaccine design.

Abstract #: F74

POU2F2 Regulates CD11c Expression and Abundance of Non-Classical Monocytes in Mouse Bone Marrow

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Non-classical monocytes (nMo; $CD16^+$ in humans, $CD115^+Ly6C^{lo}$ in mice) are long-lived cells involved in vascular surveillance and repair. Classical monocytes (cMo; $CD14^+$ in humans, $CD115^+Ly6C^{hi}$ in mice) serve as their precursors, but the precise mechanisms driving this transition remain unclear. Using single-cell RNA-seq and gene regulatory networks analysis (SCENIC package) of human PBMCs, we discovered increased expression and regulon activity of the transcription factor POU2F2 in nMo compared to cMo. While POU2F2 is known to regulate B cell proliferation via the PI3K/AKT/mTOR pathway, its role in monocytes is not well defined. To investigate this, we generated monocyte-specific Pou2f2 knockout mice (CX3CR1-Cre floxed-Pou2f2). We found that in peripheral blood, POU2F2 deficiency did not alter nMo numbers but significantly increased CD11c expression on nMo. Mass cytometry (CyTOF) analysis of bone marrow revealed a marked reduction in nMo abundance in Cre+ mice, along with elevated CD11c levels on nMo. These findings suggest that POU2F2 regulates both the abundance of nMo in the bone marrow and CD11c expression on these cells.

Abstract #: F75

Loss of germinal center formation impairs type 1 diabetes development independently of anti-insulin B lymphocyte expansion

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Germinal centers (GCs) form in secondary lymphoid tissues to promote T-B cell interactions and generate long-lived, high-affinity antibody responses. High affinity autoantibodies to insulin and islet autoantigens are used to predict and diagnose type 1 diabetes (T1D), implicating the GC response in disease pathogenesis. Our lab recently showed that CD4-driven deletion of the transcriptional repressor, Bcl6, impaired GC B cell and T follicular helper (Tfh) cell formation and spontaneous diabetes development in NOD mice. To further define the mechanism(s) mediating this protection, we tested the impact of CD4-driven *Bcl6* elimination on anti-insulin B cell activation, proliferation, and propagation of pro-inflammatory T cell responses in pancreatic islets. To achieve this, we used the NOD.VH125^{SD} BCR transgenic mouse model, in which a 1-2% population of anti-insulin B cells forms that caused accelerated diabetes development. Here, we show that CD4-driven loss of *Bcl6* in NOD.VH125^{SD} mice led to decreased upregulation of costimulatory molecules and proliferation by anti-insulin B cells in the pancreatic draining lymph nodes and pancreas, but not in other secondary lymphoid tissues (spleen, mesenteric lymph nodes). Tfh and GC B lymphocytes were reduced by loss of *Bcl6* in this model, as expected. Finally, CD4-driven loss of *Bcl6* in the NOD.VH125^{SD} model caused reduced lymphocytic infiltration of pancreatic islets (termed insulitis) and protected against diabetes. Overall, these results highlight the importance of CD4-driven Bcl6 expression to activate and license anti-insulin B lymphocytes as antigen-presenting cells, potentially in GCs. BCL6 inhibition thus holds promise as a more selective immunotherapy for T1D.

Abstract #: F76

Assessing susceptibility of cervical explant tissue to HIV-1 infection following masculinizing hormone therapy.

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Masculinizing hormonal therapy, an integral component to gender affirming care for transgender men (TGM), has immunomodulatory properties that may increase HIV acquisition risk. Testosterone activates both myeloid and lymphoid cells through androgen receptors (ARs), resulting in a shift in the cytokine milieu and a defective immune response. TNFα driven, chronic inflammation has been characterized for testosterone with concomitant dampened IFN-1 signaling. Dihydrotestosterone (DHT) has also been shown to promote M2 macrophage polarization in-vitro. Given the broad range of immunoregulatory effects of testosterone on innate and adaptive immune cells we hypothesized that it is likely that the immunological effects of testosterone will also manifest at sites of mucosal HIV-1 transmission, including the cervix of TGM receiving testosterone. The cervical mucosae are enriched with HIV-1 target cells inclusive of macrophages and CD4+ T cells that co-express ARs and therefore, potentially amenable to hormonal immunomodulation.

In an ongoing study, we investigate the impact of hormonal therapy on the phenotypes and HIV-1 susceptibility of cervical CD4+ T cells and macrophages, on an ex-vivo model of HIV infection using cervical tissue explants (CETs). Cervical mucosae obtained from TGM and cis gender women (CIS) undergoing medically indicated hysterectomies were sectioned into 2 mm^3 blocks. CETs were inoculated with subtype A and D transmitted founder (T/F) infectious molecular clones (IMCs) engineered to express secreted nano-luciferase (snLuc) and cultured on collagen rafts for 11 days. HIV infection kinetics were measured using snLuc. Immune cell populations and HIV infected cells were characterized using a multi-parameter flow cytometry panel: CD45, CD14, CD11c, CD3, TCR-αβ, CD4, CD8, CCR5, CXCR4, α4β7, CD45RA, CCR7, CD68, CD206, CD163, CD209, CD207, CD169, HLA-DR, CD80/86, CD38 including IFNγ, TNFα, and IL-10.

In preliminary findings, CD4+ T cells were of the effector memory phenotype in both TGM (95.08% ± 0.54%) and CIS CETs (87.52% ± 0.10%), although late activation marker (HLA-DR+CD38+) expression was higher in TGM (16.98% ± 10.2%) compared to CIS CETs (9.31% ± 4.74%). Consistent with this, we identified using the 26-color flow cytometry panel and automated clustering, the phenotype of HIV-1 infected CD4+ T cells as vastly CD4+ TEM cells. Although there were no statistical differences in the frequency of polarized macrophage states or Siglec-1+ macrophages, an “M1/M2” intermediate macrophage (Mi) phenotype predominated HIV-infected macrophages. Notably, both CD4+ TEM cells and Mi macrophages have been implicated in cellular viral reservoir formation and therefore deserving of further inquiry. While the co-expression of HIV co-receptors, CCR5 and CXCR4, was comparable between CIS and TGM CET derived cells, HIV replication kinetics were higher in TGM CETs compared to CIS CETs although without profound subtype differences. To further investigate other factors potentially contributing to the differed replication kinetics, we collected culture supernatants from uninfected, unstimulated CETs to assess “baseline” cytokine and chemokine profiles using Luminex. Our preliminary findings are suggestive of a more robust IFN-1 response in TGM compared to CIS CETs evidenced by higher secretion levels of IFNα. Other known pro-inflammatory immune mediators to HIV-1 acquisition risk, including the IFNγ induced protein (CXCL10) and IL-1α, were also elevated in TGM compared to CIS CETs. Taken together, our ongoing findings are

suggestive of a shift in activation and pro-inflammatory states in cervical tissue resident immune cells in response to in-vivo interactions consistent with testosterone administration. Further inquisition is needed, including epigenetic regulation, towards the betterment of hormone therapy outcomes.

Abstract #: F77

Targeting Anti-Tumor Phagocytosis to Treat Prostate Cancer

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Introduction: Immune therapies have limited success in metastatic castrate-resistant prostate cancer (mCRPC), largely due to immunosuppressive M2 tumor-associated macrophages (TAMs) in the tumor microenvironment (TME). Despite resistance, androgen deprivation therapy (ADT) remains the standard of care, and can induce immune permissiveness. We hypothesize that enhancing ADT-driven myeloid responses can overcome TAM-mediated immunosuppression and improve outcomes in advanced prostate cancer (PC).

Methods: Target identification was performed using bulk RNA-seq on AR-blockade (ARB) bone marrow-derived macrophages (BMDMs). Findings were validated in PC patient specimens using single-cell and bulk RNA-seq. In vitro and in vivo studies evaluated the anti-tumor efficacy of target modulators with ARB.

Results: NLRP3 and VPAC1 were highly upregulated in enzalutamide-treated M2-like BMDMs. Corroborating this, both proteins were enriched in TAMs of mCRPC and ADT-treated primary PC when compared to naive. Interestingly, ARB upregulated NLRP3 expression but not the inflammasome activity. Co-treatment with an NLRP3 agonist (NLRP3a), however, promoted cancer cell phagocytosis by the immunosuppressive M2. Further, ADT/NLRP3a co-treatment in an in vivo model of advanced PC resulted in significant tumor control, with tumor clearance in 55% of mice via TAM phagocytosis. On the other hand, VPAC1 antagonism enhanced Myc-CAP cell phagocytosis by M2-like macrophages, suggesting further synergy with ADT/NLRP3a.

Conclusion: VPAC1 and NLRP3 are androgen-regulated checkpoints in M2-like TAMs. Their modulation with ARB enhances phagocytosis and tumor control. We will further evaluate the anti-tumor efficacy of ARB/NLRP3a/VPAC1 antagonist co-treatment in murine models, which could provide a clinically attestable therapeutic strategy.

Abstract #: F78

Focused Ultrasound and αCD40 Drive Tumor Regression and Immunologic Memory via Innate and Adaptive Reprogramming in Breast Cancer

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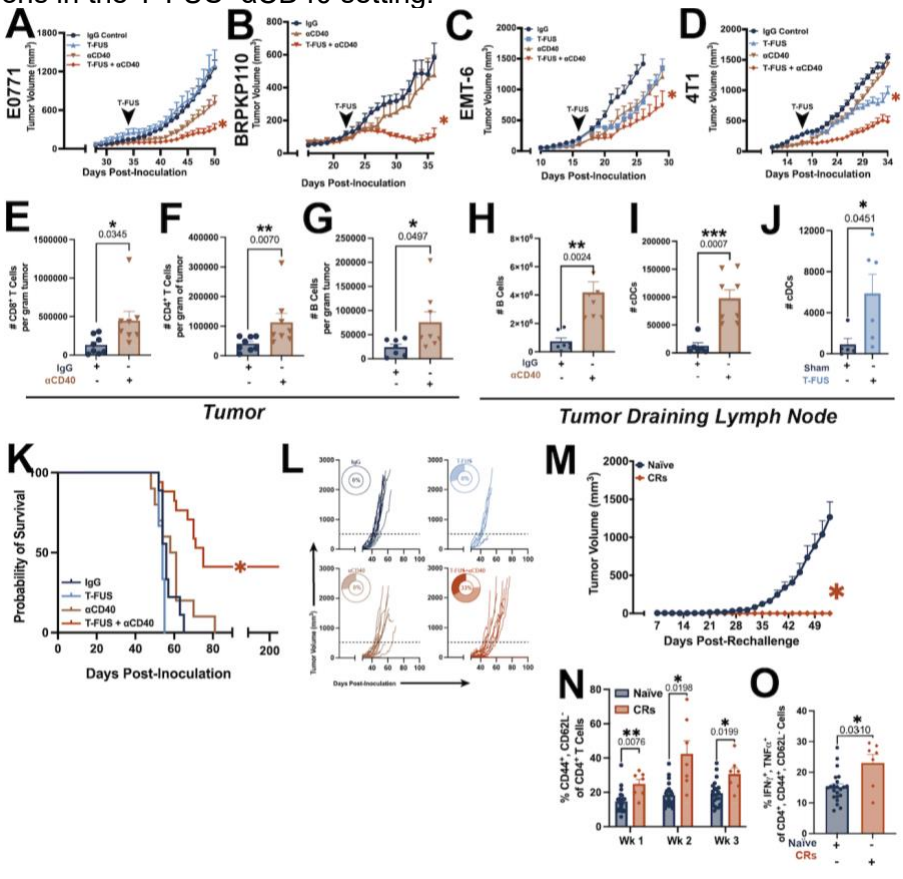
Objective: We aim to investigate mechanisms of innate and adaptive immunity driving complete responses to thermally ablative focused ultrasound (T-FUS) and αCD40 in breast cancer.

Methods: An αCD40 priming regimen was initiated one week prior to T-FUS in murine mammary carcinomas (E0771, BRPKP110, EMT-6, 4T1). Subtotal thermal ablation was performed with a custom ultrasound-guided FUS system. Tumor outgrowth and survival were monitored, yielding stratifications of progressive disease, stable disease, and complete response. Complete responders (CRs) underwent contralateral tumor rechallenge alongside age-matched, naïve wild-type controls. Multispectral flow cytometry was performed on tissue and serial blood samples.

Results: T-FUS+αCD40 elicited superlative growth control compared to controls (Fig.1A-D). αCD40 priming increased intratumoral CD4+, CD8+ T cell and B cell numbers (Fig. 1E-G). In the tumor-draining lymph node (TDLN), αCD40 monotherapy augmented representation of antigen-presenting cells, i.e. B cells & conventional dendritic cells (cDCs) (Fig.1H). Analogously, T-FUS monotherapy increased cDCs in TDLN (Fig.1J). T-FUS+αCD40 significantly prolonged survival of E0771-bearing mice, yielding a CR rate of 33% (Fig.1K-L). CRs remained protected upon rechallenge (Fig.1M) and displayed increased percentages of antigen-experienced $CD4$+ T cells ($CD4$+, $CD62L$−) (Fig.1N) and $IFN\gamma$+, $TNF\alpha$+ subset (Fig.1O) in circulation.

Conclusion: T-FUS+αCD40 offers a robust paradigm for murine breast tumor control – capable of conferring complete responses underscored by evidence of immunological memory. This study, utilizing a clinical ripe therapeutic platform, offers timely insights toward translation of T-FUS for breast immuno-oncology applications. Future studies will leverage serial imaging and blood biomarkers for forecasting responder/non-responder stratifications in the T-FUS+αCD40 setting.



Abstract #: F79

The Impact of mRNA-LNP Vaccine Regimen on the Expansion and Affinity Maturation of HIV-1 CD4 Binding Site-Specific B Cells in Rhesus Macaques

Authors: Harrison, Mitra[1,2], Yeh, Chen-Hao[2,3], Barr, Maggie[2,3], Parks, Rob[2,3], Eaton, Amanda[4], Mansouri, Katayoun[2,3], Montefiori, David C.[2,4], Edwards, Robert J.[2,3], Haynes, Barton F.[1,2,3], Saunders, Kevin O[1,2,4]

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Abstract: Recapitulating HIV-1 broadly neutralizing antibody (bnAb) development with sequential vaccination is a promising strategy to end the HIV-1/AIDS epidemic. Knowledge of the co-evolution between virus and bnAb lineages in persons living with HIV-1 has enabled the design of sequential immunogens that can engage bnAb precursor B cells and guide their evolution into broad neutralizers. Prototypical HIV-1 bnAbs targeting the CD4 binding site (CD4bs) can neutralize up to 90% of global HIV-1 isolates, but they exhibit high levels of somatic hypermutation. The optimal vaccine platform and regimen for inducing highly affinity matured CD4bs-specific neutralizing Abs (NAbs) is unknown. Here, we show that nucleoside-modified HIV-1 gp160-encoding mRNA-LNPs elicit potent and durable CD4bs-specific NAb responses in rhesus macaques that improve with longer intervals between sequential immunizations. Two-month intervals yielded a ten-fold increase in CD4bs-specific serum NAb titers at 8 months post-3rd immunization compared to one-month intervals. Durable CD4bs-specific NAbs remained detectable until at least 18 months post-4th immunization in the two-month interval group. Monoclonal antibodies isolated from memory B cells of the two-month interval group exhibit heterogenous CD4bs binding modes and neutralization signatures that are comparable to human CD4bs bnAbs. Our results demonstrate that longer intervals between sequential mRNA-LNP immunizations promote more potent, durable, and epitope-focused NAb responses. We anticipate that the CD4bs-specific B cell repertoires of the two-month interval group will contain higher affinity clones with greater levels of somatic hypermutation.

Abstract #: F80

The Potential Role of Alveolar Macrophage Peroxisomes in Treating Lung Fibrosis

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Idiopathic Pulmonary Fibrosis (IPF) is a progressive, chronic lung condition characterized by scarring of lung tissue. IPF has been around for decades, but there is a lack of effective treatments due to a limited understanding of the mechanisms causing fibrosis. We recently found that macrophage peroxisomes play a critical role during lung injury post-virus infection. We found that viral respiratory infections led to peroxisome remodeling due to interferons, especially interferon gamma, that altered peroxisome expression by decreasing their number and distribution within lung macrophages. Similarly, IPF lungs also undergo peroxisome remodeling. Our initial data examined IPF lungs and found that fibrotic regions were associated with a higher expression of KRT8 indicating fibrosis with a decreased expression of PEX14 and CD6, which mark peroxisome protein and macrophage respectively, when compared to healthier regions of the lung. Based on this, macrophage peroxisome dysfunction may lead to pulmonary fibrosis and less alveolar repair. Genetic models, histological and functional assays, and ex vivo co-culture systems will be used to prove whether macrophage peroxisome dysfunction could promote fibrosis and impair alveolar regeneration. In virally-infected mice, 4-PBA treatment led to lung recovery and a reduction of fibrosis. Focusing on peroxisome biogenesis may improve symptoms of IPF. Bleomycin is a toxin that will be used to induce fibrosis in mice, and pharmacological and genetic methods may be able to promote regeneration and reverse fibrosis. Focusing on lung macrophage peroxisome biogenesis may provide a therapy that potentially reverses the progression of chronic lung diseases like IPF.

Abstract #: F81

Abstract

Thymus independent artificial organoid predicts blocked T cell lineage fate of hematopoietic stem and progenitor cells under HIV/SIV infection condition

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Early thymocyte regeneration is critical to fuel T cell development in health and disease. Both, bone marrow and thymus play key roles establishing cellular network of early thymic precursors such as thymus seeding progenitor (TSP; $CD34^{+}CD7^{+/-}$), immature single positive (ISP; $CD4^{+}CD3^{-}$), and double positive (DP; $CD4^{+}CD8^{+}CD3^{-/+}$) thymocytes, essential for the emergence of T cell subsets. Studies suggest HIV/AIDS causing bone marrow and thymic dysplasia, however, the impact of the associated insult on the T cell lineage fate of hematopoietic stem and progenitor cells (HSPCs) remains elusive. Using an ex vivo artificial thymic organoid system and simian immunodeficiency virus (SIV)/nonhuman primate (NHP) model of HIV/AIDS, we investigated the T cell lineage fate of bone marrow-derived HSPCs. We show that SIV infection condition caused stepwise decline in thymocyte development

significantly impacting TSPs, ISPs and DPs regeneration under SIV infection. These thymic precursors progressively declined over the course of infection. The impact on HSPCs occurred as early as week 2 post infection. Interestingly, no decline in HSPC or thymocyte frequencies was observed in organoids cultured with SIV. Conversely, a direct impact of dual tropic HIV on thymocyte generation in organoids was observed pointing to a differential impact on thymopoiesis between HIV and SIV. A similar T cell lineage fate blockade was observed in HSPCs cultured with inflammatory cytokine. Together these results demonstrated an early blockade of T cell lineage fate of HSPCs under SIV infection condition likely due to host intrinsic factors. These findings have implications for T cell regenerative immunology and therapeutics under HIV/AIDS conditions.

Abstract #: F82

Abstract

Thymus independent artificial organoid supports complete thymopoiesis from nonhuman primate derived hematopoietic stem and progenitor cells

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Thymic output in health and disease, including HIV/AIDS, has been extensively studied using in-vivo and ex-vivo models. Attempt to recapitulate discrete stages of T cell developmental stages from thymic precursors have led to methods supporting ex-vivo T cell development, however their success has been limited. Recent advances using 3D culture systems have demonstrated improved T cell regenerative capabilities. However, their utility in nonhuman primate (NHP) system has not been tested. Here, we report development and characterization of a nonhuman primate (NHP)-based artificial thymic organoid (NHP-ATO) system capable of generating T cells from NHP hematopoietic stem and progenitor cells (HSPCs). The system supported the development of thymus-seeding progenitors ($CD34^+CD38^-CD7^+CD3^-CD4^-CD8^-$) and $CD38^+$ T cell subsets resembling recent thymic emigrants ($CD38^+CD3^+CD4^+CD8^-$ and $CD38^+CD3^+CD4^-CD8^+$) constituting naïve CD4 and CD8 T cell subsets. The early thymocyte populations, including immature single-positive ($CD4^-CD3^+$) and double-positive ($CD4^+CD8^+CD3^{-/+}$) thymocytes, were also recapitulated. Mature $CD4^+$ and $CD8^+$ T cells expressed functional TCRs and produced multiple cytokines upon stimulation. The cellular architecture and phenotypic diversity of NHP-ATO-derived thymocytes closely mirrored native thymic precursors, establishing this platform as a robust tool for investigating T cell ontogeny and evaluating therapeutic strategies using NHP models.

Abstract #: F83

Myeloid cell ferroptosis drives injury after hemorrhagic stroke

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Stroke is the fifth leading cause of death in the United States. Hemorrhagic stroke (HS), a subtype of stroke, is hallmarked by rupture of the vascular endothelium and lysis of red blood cells in the brain parenchyma resulting in increased inflammatory and oxidative stress. This invokes an immune response to the hematoma to protect neurons from inflammatory and oxidative damage. We hypothesized that myeloid ferroptosis, an oxidative form of cell death exacerbates injury during the glial and peripheral immune response to HS. To test this hypothesis, we sensitized myeloid cells to ferroptosis by knocking out glutathione peroxidase 4 (GPX4) which inhibits ferroptosis by reducing lipid hydroperoxides. We induced HS in *Cx3cr1*$^{ERT2\text{-cre}}$ *Gpx4*$^{fl/fl}$ with collagenase type IV and assessed mouse behavior by neurological deficit scoring and locomotion in an open field. We observed that GPX4 deficient mice exhibited exacerbated neurological deficit scoring after HS. To further support that myeloid cell ferroptosis contributes to injury after HS, we knocked out acyl-CoA synthetase long chain family, member 4 (ACSL4), a synthetase implicated as a ferroptosis sensitizer, in myeloid cells using *Cx3cr1*$^{ERT2\text{-cre}}$ *Acsl4*$^{fl/fl}$ and induced HS. We observed improved neurological deficit scoring in mice with ACSL4-deficient myeloid cells. Taken together, these data strongly suggest that myeloid cell ferroptosis is a pathological mechanism that drives secondary injury after HS and may contribute to detrimental outcomes in other neurotraumas.

Abstract #: F84

SHMT2 regulates Treg cell function and stability during mucosal inflammation

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Serine hydroxymethyltransferase 2 (SHMT2) is a mitochondrial enzyme essential for one-carbon metabolism, nucleotide biosynthesis, redox balance, and epigenetic regulation, making it critical for cell proliferation and survival. Previous studies have shown that SHMT2 is required for the expansion of effector $CD8^+$ T cells following infection. However, its role in $CD4^+$ T cell differentiation and immune regulation under inflammatory conditions remains unclear. Since germline deletion of SHMT2 in mice is embryonically lethal, we generated a T cell-specific SHMT2 conditional knockout (cKO) mouse (*Shmt2*$^{fl/fl}$CD4^{Cre}). SHMT2 deletion in T cells impaired Th1, Th2, and Th17 differentiation, while induced Treg development remained unaffected. To further investigate the role of SHMT2 in Tregs, we generated a Foxp3-restricted SHMT2 cKO (*Shmt2*$^{fl/fl}$Foxp3-YFP^{Cre}). Upon intranasal challenge with house dust mite extract, these mice exhibited elevated lung infiltration of eosinophils, neutrophils, $CD4^+$, and $CD8^+$ T cells, compared to wild-type (WT) mice. This was accompanied by increased production of Th2 and Th17 cytokines in the airways. Interestingly, *Shmt2*$^{fl/fl}$Foxp3-YFP^{Cre} mice had significantly more Treg cells than WT mice, in the inflamed lungs, suggesting that SHMT2 is dispensable for Treg development but is required for Treg suppressive function. Indeed, adoptive transfer of Treg cells from the *Shmt2*$^{fl/fl}$Foxp3-YFP^{Cre} mice failed to suppress colitis induced by naïve $CD4^+$ T cell transfer in *Rag*$^{-/-}$ mice. Collectively, these findings highlight a critical role for SHMT2 in regulating Tregs function and stability, and the therapeutic potential of targeting mitochondrial metabolic pathways in mucosal inflammatory diseases.

Keywords: Serine Hydroxymethyltransferase, Regulatory T cells, Lymphocyte fate commitment, Mucosal inflammation.

Abstract #: F85

Going With The Flow: Utilizing Autofluorescence to Maximize Flow Cytometry

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The validity of flow cytometry results depends on the ability to accurately distinguish between cells expressing specific surface or intracellular proteins and those that do not, based on fluorescent signals. All cells exhibit some degree of naturally occurring autofluorescence, which arises from endogenous biological structures. This intrinsic light emission can reduce signal resolution and complicate the identification of true positive signals within a sample. While every cell possesses some level of autofluorescence, the intensity varies depending on tissue origin and metabolic activity. Higher autofluorescence intensity makes it more challenging to detect specific fluorescent signals from labeled proteins, such as CD markers or intracellular targets.

In this study, we leveraged the sensitivity of full-spectrum flow cytometry to utilize inherent cellular autofluorescence as a tool for enhancing experimental design. Because distinguishing autofluorescence from true fluorophore signals is complex and depends on cell type and metabolic state, we developed a workflow to guide users in optimizing their flow cytometry experiments under diverse conditions. Samples from various digested and non-digested tissues, including aorta, adipose tissues, brain, gut, spleen, and bone marrow, were processed, stained for surface and intracellular proteins, and analyzed using a 4-laser (16V-14B-10YG-8R) Cytek Aurora with SpectroFlo software. Our results demonstrate that, although autofluorescence is an unavoidable aspect of flow cytometry, full-spectrum flow cytometry enables the effective management of cellular autofluorescence, thereby improving data resolution and the accurate detection of protein expression.

Abstract #: F86

Title: Oligodendrocyte Genomic Damage and Myelin Maintenance in Alzheimer's Disease

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Oligodendrocytes are a vital yet understudied cell type, especially in the case of neurodegeneration. This is despite their potential to mitigate neuronal degeneration through axonal structural support and metabolic supplementation, which may be pivotal in reinforcing neurons in times of cellular stress. Neuronal degeneration runs rampant in Alzheimer's Disease (AD), which is the most common form of dementia. Exhaustive research into AD pathogenesis has yet to yield safe and effective treatment. Even the most recent amyloid monoclonals do little to slow progression of disease and indeed any improvement seems to be clinically indiscernible. Additionally, these drugs carry a significant risk of vascular events. While amyloid-beta and tau accumulation and neuronal pathology are main hallmarks of AD, a wide cascade of cellular events are also present. Demyelination is a prominent pathological hallmark of AD and has even been shown to occur in the preclinical phase of disease. Studies have suggested that oligodendrocyte and myelin deficits precede tau and Aß accumulation, perhaps playing their own role in precipitating neurodegeneration and neuronal/axonal decline. The mechanisms of oligodendrocyte dysfunction in AD remain unknown. DNA damage has been increasingly linked to AD progression, and markers of DNA damage have been shown to be upregulated in oligodendrocytes in particular within both AD patients and animal models. This DNA damage can activate the innate immune response. DNA damage in oligodendrocytes and its ramifications could deprive neurons of crucial support, thereby accelerating degeneration. Here, I characterize DNA damage in oligodendrocytes and its downstream ramifications as well as general transcriptional changes in this cell type using spatial transcriptomics, immunostaining, novel DNA break mapping techniques, and other methodologies. I also investigate the downstream ramifications of this DNA damage on cellular stress and innate immune responses.

S83	Elizabeth Fink	University of Virginia	The Relationship Between Acute Respiratory Viral Infection, Chronic Inflammation, and Accelerated Aging
S84	Megan Yi	University of Virginia	Accelerated Aging as a Result of Viral Infection
S85	Nestor Prieto-Dominguez	The University of Alabama at Birmingham	Targeting Ifnγ-dependent Pros1 signaling to improve the macrophage anti-tumor response

Abstract #: S1

Distal inflammatory priming of normal mammary tissue by the gut microbiome drives breast tumor metastasis via metabolic dysregulation

Audrey Putelo, Simona Bajgai, Sree Kolli, Mitchell McGinty, Mika Poblete, Cara Hatzinger, Tzu-Yu Feng, and Melanie Rutkowski

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Hormone receptor-positive (HR+), HER2- is the most prevalent metastatic breast cancer subtype, constituting 73.1% of the metastatic disease population. Despite targeted therapies that have increased long-term survival, many patients develop and eventually succumb to metastatic disease. Though host-intrinsic factors that preferentially predispose certain individuals to metastatic disease are poorly defined, gut microbial health is increasingly recognized as a determinant of the metastatic potential of breast tumors. We demonstrated that gut commensal dysbiosis drives HR+ tumor dissemination via a CCL2/mast cell axis in the normal mammary tissue. Our present goal is to define how gut dysbiosis triggers tissue inflammation. Based on our data suggesting that primary bile acids are associated with dysbiosis-induced inflammation, metabolic dysregulation, and metastasis of HR+ tumor cells, we hypothesize that dysbiosis-induced upregulation of primary bile acids leads to systemic metabolic dysregulation, initiating cellular and/or molecular changes in normal mammary tissue that increase long-term susceptibility to metastatic disease. We propose that dysbiosis triggers a systemic metabolic shift that enhances the metastatic potential of HR+ tumors by shaping the immune landscape of the normal mammary tissue. These mechanistic studies will unveil novel therapeutic targets for the prevention of metastatic breast cancer that could be identified prior to tumor diagnosis. Considering the role of the gut commensal dysbiosis, modification of the microbiome, or targeting of commensal-associated metabolites emerges as a potential therapeutic to prevent or reduce the incidence of metastatic breast cancer.

Abstract #: S2

Title:

Radial Glial Cells as Source Antigen for Adoptive Cellular Therapy in Glioblastoma

Authors:

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Glioblastoma (GBM) is a lethal brain cancer with a five-year survival rate of 9.8%. Despite advances, mortality rates remain unchanged over the past two decades. Current immunotherapies are promising but often require tumor tissue, excluding patients with inoperable tumors or poor performance status. Tumor heterogeneity further complicates the identification of effective targets for immunotherapies.

Radial glial cells (RGCs) are an early neurodevelopmental progenitor that are typically only found in embryogenesis. They expressed some shared markers with glioma stem cells (GSC), and RGC-like cells have been found to contribute to GBM migration and heterogeneity. We hypothesized that RGCs harbor antigens shared with GBM tumors and that targeting these antigens through a renewable antigen library could improve upon adoptive cellular therapy (ACT) in preclinical GBM models.

Our ACT platform combines *ex vivo* expanded T cells, hematopoietic stem cells (HSCs), and dendritic cell (DC) vaccines electroporated with total tumor RNA (ttRNA) extracted from tumor sample. Using single-cell RNA sequencing, we identified immunogenic antigens shared between RGCs and GBM tumors, enabling construction of an enriched antigen cDNA library that could generate RGC RNA consistently. T cells expanded to target RGC antigens demonstrated specificity and released Type 1 cytokines in response to KR158B cells, a murine GBM cell line. When integrated into our ACT platform for murine GBM, these RGC T cells significantly improved survival, performing comparably to T cells targeting total tumor RNA (ttRNA). In summary, RGC antigens provide a renewable, tumor-independent strategy for ACT, confirming the value of RGC subpopulations in tumorgenicity and offering a method around the requirement for tumor extraction to generate RNA for immunotherapy.

Abstract #: S3

Investigating the role of NF-κB inducing kinase (NIK) in granulocyte plasticity associated with Hypereosinophilic Syndrome (HES).

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HES is an inflammatory disorder defined by high levels of eosinophils in the blood and tissues, resulting in organ damage and increased mortality. The mechanisms behind HES are not fully established, which poses challenges in diagnostics and treatment strategies. We have previously reported that loss of NIK, a key regulatory protein in the noncanonical NF-κB pathway, results in increased incidence of HES-like disease in mice, which has been linked to aberrations in Th2 cells and related cytokine production. We hypothesize that NIK also influences the development and education of eosinophils in the hematopoietic tissues independent of T-cell involvement. We have identified differences in metabolism, survival, and maturation in *Nik*$^{-/-}$ eosinophils when compared to wildtype eosinophils. We also investigated the emerging idea of granulocyte plasticity. Here we show the existence of subsets of “plastic” granulocytes expressing a mixture of eosinophil/neutrophil surface markers. We found that contrary to expectations, the predominant mature eosinophil subset in the bone marrow of *Nik*$^{-/-}$ mice expresses the classic neutrophil marker, Ly6G. We have also identified a population of cells in the spleen of *Nik*$^{-/-}$ mice that morphologically resemble neutrophils, but expresses the eosinophil marker IL-5Rα, which may represent a population of plastic neutrophils with the ability to phenotype switch to eosinophils upon exposure to IL-5, a key eosinophil cytokine. The existence of these plastic granulocytes and their ability to phenotype switch has only recently been reported in the literature and much is still unknown about these cells, including their potential role in the development and progression of eosinophilic diseases, such as HES.

Abstract #: S4

Investigating the role of TLR5 signaling in the failure of FLT3 ligand therapy for ovarian cancer

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Ovarian cancer is the most fatal gynecological malignancy with most patients developing therapy resistance and recurrence, highlighting the need for more effective therapies. Patients bearing ovarian tumors infiltrated with high frequencies of T cells experience significantly enhanced survival, suggesting the potential for immune therapies. Despite this, therapies such as PD-L1/PD-1 blockade are largely ineffective for ovarian cancer. Because cross presenting cDC1s are fundamental in orchestrating anti-tumor immunity and response to immune therapy, expansion of this immune subset via the DC growth factor fms-like tyrosine kinase receptor 3 ligand (FLT3L) is an attractive strategy for enhancing immune therapy response. Unfortunately, FLT3L therapy has been largely ineffective in clinical trials, with mechanisms leading to the failure unknown. Here, we demonstrate that toll like receptor 5 (TLR5) signaling, the only known ligand for which is bacterial flagellin, mediates failure of FLT3L therapy. Culture of bone marrow cells with FLT3L during chronic exposure to bacterial flagellin promoted differentiation of cDCs away from mature functional cDC1s towards immature monocytes and macrophages expressing high-levels of PD-L1 and having a reduced ability to activate CD8 T cells. Importantly, in vivo expansion of cDC1s using FLT3L therapy in combination with PD-L1 blockade achieved long-term survival in 80% of TLR5 KO mice bearing aggressive orthotopic ovarian tumors. No survival benefit was observed in wild-type mice. These data suggest that TLR5 signaling is orchestrating the failure of FLT3L therapy by driving accumulation of immunosuppressive myeloid cells at the expense of mature cross-presenting cDC1s.

Abstract #: S5

Title: Zika virus induces monocyte recruitment in the immunocompetent adult brain driving chronic inflammation

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Abstract

Zika virus (ZIKV) is a neurotropic pathogen linked to neuropathogenesis in adults, causing conditions such as Guillain-Barré syndrome (GBS) and fatal encephalitis. Intracranial injection of virus in immunocompromised mice have shown neuroinflammation and subsequent brain damage. However, the mechanisms underlying ZIKV-induced neuroinflammation in immunocompetent adult mice via peripheral infection remain unclear. To investigate this, we utilized a murine model of ZIKV infection via foot pad injection. Our findings reveal that acute ZIKV infection at 4 days post-infection (4 dpi) induces significant apoptosis and neuroinflammation in the adult brain, persisting up to 28 dpi. Notably, ZIKV infection triggers apoptosis in the hippocampus and cortex—key regions involved in memory—and induces early immune cell infiltration. Additionally, microglial activation occurs following infection at 7 dpi, with viral RNA detected in the brain. Bulk RNA sequencing of the hippocampus at 28 dpi further reveals the activation of inflammatory pathways, underscoring the prolonged neuroinflammatory response in the infected brain. Microglial activation is likely driven by infiltrating monocytes, as inhibiting monocyte recruitment reduced the expression of microglial activation genes. These results suggest that targeting monocyte-induced inflammatory mediators could be potential therapeutic interventions.

Abstract #: S6

Microglial cyclooxygenase-1 modulates cerebral capillary basal tone *in vivo*

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Myeloid cells, consisting of parenchymal-resident microglia and border associated macrophages (BAM), have been implicated in hypercapnia, but it is unknown which myeloid cell modulates which vessel type. In previous work, we documented that myeloid cells associate with the brain vasculature but did not distinguish their localization along the vascular tree. Here, using molecular approaches to distinguish microglia and perivascular macrophages, we establish that microglia are the only myeloid cells associating with capillaries which we refer to as capillary associated microglia (CAM). To determine if loss of CAM is sufficient to reduce capillary tone, we employed global and focal ablations and found significant reductions in capillary diameter and red blood cell flux, suggesting vasodilatory regulation by microglia. Cyclooxygenase-1 (COX1), an enzyme with known vasodilatory action, is predominantly expressed by microglia. To determine the necessity of microglial COX1 in regulating cerebral basal capillary tone *in vivo,* we performed genetic ablation of microglial COX1 and found a significant reduction in capillary flux and diameter. Together, this study reveals a novel role for microglial COX1 in maintaining basal capillary tone *in vivo* that may be relevant for targeting in disease.

Abstract #: S7

Conventional type 1 dendritic cells transfer mitochondria to CD8 T cells during antigen presentation.

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The adaptive immune response relies on communication between antigen-presenting cells (APCs) and T cells. APCs such as type 1 conventional dendritic cells (cDC1) present antigens to CD8+ T cells that clonally expand and differentiate to execute cytotoxic immune responses. This requires formation of an immune synapse allowing an exchange of information between cells. Recently, intercellular mitochondrial transfer (IMT) has been recognized as a form of cell-cell communication. CD8 T cells have been shown to uptake mitochondria (Mt.) from non-immune cells amplifying cytotoxicity and effector function. It is unknown if cDC1 can donate Mt. to CD8 T cells during antigen presentation, and if this form of IMT alters the function and differentiation of T cells. To address this, we generated mice with cDC1-specific Dendra2-labeled fluorescent Mt., (MitoDC). To examine how antigen-presentation regulates IMT, we used MitoDC mice and models of cell-derived antigen presentation using ovalbumin (OVA) containing apoptotic cells or OVA-expressing tumors. OVA-specific naïve CD8 T cells were transferred into host mice. 3-4 days later, we isolated cells from the spleen and lymph nodes of recipient mice and analyzed them by flow cytometry for antigen-dependent proliferation and expression of Dendra2. We found that transferred CD8 T cells received Dendra2-labeled Mt. from cDC1 in mice with OVA-expressing tumors and loaded apoptotic cell models. These findings support the hypothesis that antigen presenting cells donate respiring Mt. to T cells, potentially altering T cell metabolism to regulate anti-tumor immunity. Future studies will pinpoint if T cells receiving Mt. from cDC1s alter proliferation, differentiation, and cytotoxic function.

Abstract #: S8

Commensal dysbiosis mediates changes in mammary tissue mast cells and fibroblasts to promote HR$^+$ breast tumor dissemination

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Cancer is a leading cause of death among US women, with breast cancer accounting for 30% of new cases annually. While overall breast cancer survival rates have improved, metastatic cases remain a challenge. Most metastatic breast cancer patients are those diagnosed with the hormone receptor-positive (HR$^+$) Her2$^-$ subtype. However, there is a major gap in determining why certain patients are more susceptible to metastasis despite comparable initial diagnosis and treatments. This makes it imperative to identify modifiable host-intrinsic factors that promote metastatic spread. We have found that commensal dysbiosis, an inflammatory gut microbiome with low biodiversity, significantly increases HR$^+$ tumor dissemination and metastasis. Importantly, mast cells were identified to play a central role in this process. Our data suggest that dysbiosis is causing phenotypic changes in mast cells that drive fibroblast activation and subsequent tumor dissemination. It has been demonstrated that specific subsets of tumor-associated fibroblasts increase tumor growth and metastasis through immune suppression and fibrosis. However, the factors responsible for the generation of these pro-metastatic fibroblasts remain unknown. **We hypothesize that commensal dysbiosis reprograms mast cells to produce higher levels of pro-fibrotic growth factors to support differentiation of fibroblast subtypes that facilitate HR$^+$ tumor dissemination**. Through our findings, we aim to unravel the molecular mechanisms responsible for driving HR$^+$ tumor metastasis.

Abstract #: S9

Systemic-Prime + Mucosal-Boost Vaccination in Combination with Immune Checkpoint Blockade in Lung Tumor Bearing Mice

Sam Young, Audrey Eng, Jinyi Tang, Jie Sun

Immune checkpoint blockade (ICB) has revolutionized lung cancer treatment by reinvigorating dysfunctional anti-tumor T cell responses *in situ*. However, ICB efficacy is limited for many patients due to insufficient T cell presence within the tumor microenvironment. Expanding the current immunotherapeutic repertoire with strategies to enhance local anti-tumor T cell responses is crucial for overcoming this challenge. Focusing on lung anti-viral immunity, we have demonstrated that a systemic-prime + mucosal-boost vaccination strategy amplifies respiratory adaptive immune responses in healthy mice. Building on these findings, we hypothesize that engineering a systemic-prime + mucosal-boost vaccine strategy to therapeutically target lung tumor antigens will improve disease control. As a proof-of-concept, we have validated a systemic mRNA-prime + mucosal adenoviral-boost vaccine strategy targeting the tumor antigen, HELLO, expressed by the lung cancer cell line KP-HELLO. Vaccination of healthy mice results in robust HELLO-specific T cell activation in the lung, as assessed by *ex vivo* peptide stimulation. Moreover, we have initial indications that mucosal boosting in combination with ICB preferentially prolongs survival in mice bearing intratracheally established KP-HELLO tumors. Ongoing work seeks to further validate this finding before elucidating the cellular and molecular components responsible for mucosal-boost efficacy. Ultimately, we want to identify why selectively enhancing mucosal immunity is beneficial.

Abstract #: S10

CK2α Expression in B-cells Drives IgA$^+$ B-cell Infiltration into the Central Nervous System during Autoimmune Neuroinflammation

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Abstract

Protein Kinase 2 (CK2) is a tetrameric serine/threonine kinase comprising two catalytic (CK2α and/or CK2α′) and two regulatory subunits (CK2β). CK2 has been reported to influence immune responses. While CK2α's role in T-cells has been linked to experimental autoimmune encephalomyelitis (EAE), a model for Multiple Sclerosis, its specific function in B-cells during neuroinflammation is not well understood. We have shown that CK2α regulates B-cell development. In this study, we investigated the function of CK2α in B-cells using newly generated B-cell specific inducible CK2α deletion mice (C*snk2a1*$^{\Delta Cd20}$) in the myelin oligodendrocyte glycoprotein (MOG_{35-55})-induced active EAE model. Disease progression and inflammatory responses were analyzed at peak stage (day 16) and chronic stage (day 24) of EAE. Our findings revealed that *Csnk2a1*$^{\Delta Cd20}$ mice developed a distinct brain-targeted form of EAE and exacerbated classical EAE symptoms compared to *Cd20*-cre controls, accompanied by significantly lower IgA$^+$ B-cells in the central nervous system (CNS). These IgA$^+$ B-cells were the primary producers of IL-10, a key anti-inflammatory cytokine. Deletion of CK2α in B-cells impaired IL-10-producing IgA$^+$ B-cell recruitment into the CNS, which amplified neuroinflammation and exacerbated EAE clinical severity. Moreover, exogenous IL-10 administration partially inhibited EAE disease severity by promoting T regulatory cell infiltration into the brain. In conclusion, these findings underscore the

critical role of CK2α in B-cells in supporting the infiltration of IL-10-producing IgA^+ B-cells into the CNS, thereby modulating neuroinflammatory responses in EAE.

Abstract #: S11

Title: Gut-commensal dysbiosis modifies the lung environment to enhance breast cancer metastasis through a mast-cell dependent axis

The 5-year survival rate of metastatic breast cancer is 31%, however little is known as to what places patients at risk for metastatic disease. Here, we tested the hypothesis that gut commensal dysbiosis, an inflamed and unbalanced microbiome, is a host-intrinsic factor that enhances the severity of metastatic breast cancer. To test this, breast tumor cells were administered intravenously (IV) to mimic advanced metastatic disease. Mice with pre-established dysbiosis had significantly enhanced lung tumor burden and reduced survival. We next sought to define the cytokine and chemokine landscape in the lungs during commensal dysbiosis and primary tumor growth. We assessed chemokines and cytokines in the lungs of dysbiotic or non-dysbiotic mice prior to tumor initiation, and 12 days post-tumor initiation, a temporal point in which few to no micrometastasis are apparent in the lungs. Non-dysbiotic mice had a significant increase in IFNγ, suggesting an anti-tumor immune response that is not present in dysbiotic mice. Conversely, the lungs of tumor-bearing dysbiotic mice had increased IL-9, CXCL10, VEGF, and IL-13, indicating a pro-metastatic environment. The presence of IL-9 and IL-13 suggested that lung mast cells were the cellular mediator triggering modulation of the lung environment to favor breast metastasis to the lungs. To test whether mast cells influenced survival of dysbiotic mice with metastatic breast cancer, mice bearing IV tumors were treated with ketotifen, a mast cell stabilizer. Ketotifen significantly improved survival in dysbiotic mice. These data implicate the gut microbiota as a host-intrinsic factor that enhances the severity of metastatic breast cancer through activation or polarization of lung mast cells.

Abstract #: S12

Abstract title: Bubble therapy:Histotripsy for pancreatic tumor ablation modulates the tumor microenvironment and enhances systemic anti-tumor immunity in an *in-vivo* murine model.

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Pancreatic cancer remains a leading cause of cancer-related mortality, necessitating the need for innovative treatments. The study aims to evaluate the hypothesis that histotripsy ablation of pancreatic tumors reduces tumor burden, activates systemic immune responses, and induces an abscopal-like effect. Using a contralateral *in-vivo* study in C57BL/6J mice, we employed a customized 1 MHz, 8-element histotripsy transducer for partial tumor ablation (50–60%) to stimulate immune responses and transform the “cold” tumor microenvironment to hot. Treatment parameters included 250 Hz pulse repetition frequency and 250 pulses per focal point. Mice were analyzed at five time points (2 hours,1 day,7 days,14 days,21 days) for ablation zones, immune cell dynamics, and systemic transcriptomic/proteomic changes. The results show that Histotripsy significantly reduced tumor diameters in treated and untreated (contralateral) tumors compared to controls. Histological examination confirmed ablation zones. Flow cytometry analysis revealed significant changes in immune cell populations across treated and control groups. Transcriptomic analyses identified differentially expressed genes and pathways associated with systemic anti-tumor immune responses, while mass spectrometry highlighted antigens presented by antigen-presenting cells. Overall, the results indicate that histotripsy promotes a localized immune response, leading to immune system activation and substantial alterations in the tumor microenvironment. These systemic immunological changes present potential targets for adjuvant therapies, underscoring the promise of histotripsy as a therapeutic modality in pancreatic cancer treatment.

Abstract #: S13

Treg to cytotoxic exTreg transition in humans

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Under conditions of chronic unresolved inflammation characteristic of atherosclerosis, regulatory $CD4^+$ T cells (Tregs) become unstable and convert to cytotoxic exTregs. The mechanism driving this conversion in humans is unclear. Here we show unresolved endoplasmic reticulum (ER) stress as a key factor driving Treg instability. Human exTregs undergo ER stress and consequent mitochondrial dysfunction that remains unchecked due to defective mitophagy. Integrated stress response (ISR), a pathway that can trigger inflammatory signaling, is also upregulated in exTregs. exTregs are highly apoptotic and are more susceptible to stress-mediated cellular dysfunction due to their terminally differentiated state. Pro-atherosclerotic stressors such as oxLDL and interferon-γ induce ER stress, mitochondrial dysfunction and apoptosis of Tregs *in vitro*. We conclude that the maladaptive inflammatory environment triggers ER stress and mitochondrial dysfunction, contributing to Treg instability in atherosclerosis.

Abstract #: S14

CD2 costimulation improves efficacy of adoptive T cell therapy in solid tumors

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Adoptive T cell therapy (ACT) is a promising treatment option for patients with cancer. However, the success of ACT in patients with solid tumors has been underwhelming, and many cellular therapies fail to maintain long-term durability. Toll-like receptor (TLR) agonists can invigorate antitumor T cell responses when administered directly to patients, but these responses are short-lived and often coincide with toxicities. We previously repurposed the TLR9 agonist, CpG, commonly used in the clinic, during expansion of antigen-specific T cells for ACT. CpG fostered the expansion of potent $CD8^+$ T cells with a unique signature phenotype ($IL\text{-}2R\alpha^{high}ICOS^{high}CD39^{low}$) and potent antitumor activity. We discovered that CD2, a potent yet understudied costimulatory molecule, was one of the most significantly upregulated proteins in antitumor T cells activated with CpG. Blocking CD2 during expansion of antitumor T cells significantly impaired their ability to regress established melanoma. Of important clinical implications, we found that in patients with melanoma, CD2 is significantly upregulated on tumor infiltrating CD4 and CD8 T cells compared to normal donors and correlates with favorable outcomes in response to checkpoint inhibitors. Human tumor infiltrating CD4 and CD8 T cells display an a more stem-memory phenotype (TCF1+CD62L+) when stimulated with CD2/CD28/CD3 vs CD3/CD28. ICOS, CD25, and granzyme B expression are also increased on these human TILs when stimulated with CD2. Importantly, CD2 stimulation increases the lentiviral transduction efficiency and generates more human CAR T cells. In our preclinical work, murine CAR-T cells containing a CD2 signaling domain (CD2z) were more cytotoxic than CAR-T cells expressing 41BB or CD28 signaling domains. Our results demonstrate that activating the CD2 costimulatory pathway in preclinical and clinical studies results in more potent anti-tumor T cell, able to mediate regression of established melanoma and improves the generation human TIL and CAR-T cell quality. Our findings have immediate implications in the clinical treatment of advanced solid tumors with adoptive cell therapy.

Abstract #: S15

Dendritic cell dysfunction driven by hypoxia pathway activated DC tolerance as an immune evasion mechanism in adoptive cellular therapy

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Dendritic cells (DCs) are essential for initiating anti-tumor immunity. Our adoptive cellular therapy (ACT) significantly enhances DC and T cell infiltration into tumors, improving survival in murine brain tumor models. However, tumors develop immune escape mechanisms, limiting therapeutic efficacy. Here, we identify a mechanism of DC dysfunction driven by an enhanced hypoxia pathway in ACT-escaped gliomas. We found that ACT-escaped tumors retained adoptively transferred cytotoxic but non-exhausted T cells that failed to recognize antigen-shifted tumors. DCs from both untreated and ACT-escaped tumors exhibited impaired T cell activation. Transcriptomic analysis revealed downregulation of antigen-presentation genes and upregulation of tolerance-associated genes in ACT-escaped tumor DCs. Gene set enrichment analysis highlighted hypoxia pathway activation, and hypoxia induced DC tolerance genes via HIF1α, impairing T cell activation. Spatial transcriptomics confirmed a significant correlation between HIF1α and ARG1 in tumor-bearing brains. Moreover, increased immune infiltration in ACT-treated gliomas exacerbated hypoxia as supported by a highly correlated HIF1α and CD45 expression in ACT treated glioma bearing brain, further promoting DC dysfunction. Secretory factors from tumor-T cell interactions activated the hypoxia pathway and induced DC tolerance genes, including ARG2 which is independent of hypoxia inducement. In conclusion, our findings highlight an enhanced hypoxia pathway driven DC tolerance as a key driver of tumor escape in ACT-treated gliomas.

Abstract #: S16

Title: Deletion of Adult-Onset Asthma-associated Genetic Variants Increases *HDAC7* Expression.

Authors: Brennan D. Stadler, Donna M. Decker, Xiaoyuan Zhong, Funing Tian, Carole Ober, Nathan Schoettler, Anne I. Sperling

Asthma is the most prevalent chronic respiratory disease, and adult-onset asthma (AOA) is associated with more severe and persistent symptoms when compared with childhood-onset asthma. Genome wide association studies, reveal sets of single nucleotide polymorphisms (SNPs) at over 35 loci linked to AOA risk. Further genetic fine mapping of the 12q12.11 locus identified a credible set of 6 putatively causal SNPs near the *HDAC7* gene. HDAC7 is a class 2a histone deacetylase with known roles in mediating inflammatory diseases via deacetylation of histone and cytosolic proteins. We sought to further link HDAC7 and asthma by testing the hypothesis that SNPs in the HDAC7 gene locus modulate *HDAC7* expression. By in silico analysis, we identified that 3 of the SNPs were located in putative enhancer regions. To test whether these 3 SNPs could perturb gene expression, we transfected Raji and K562 cells with luciferase constructs containing the risk or non-risk allele for each SNP. No enhancer activity was found with any of the constructs, as luciferase activity was similar to our negative controls. Next, to test whether the deletion of all 3 SNPs influenced *HDAC7* expression, we used CRISPR Cas-9 mutagenesis to excise a region containing the three SNPs in Raji cells. Remarkably, qPCR showed a 2-fold increase in *HDAC7* gene expression in our CRISPR edited Raji cells ($p = 0.02$). Thus, these data show that the region including the 3 SNPs inhibits *HDAC7* expression. Future studies will focus on identifying how changes in HDAC7 expression may contribute to AOA risk.

Abstract #: S17

The decameric repeats (DRs) of PSGL-1 function as a basic antiviral unit in restricting HIV-1 infectivity

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PSGL-1(P-selectin glycoprotein ligand-1) is a dimeric, mucin-like surface glycoprotein that binds to selectins on endothelium to mediates leukocyte tethering and rolling for transmigration. Previous studies have identified PSGL-1 as an HIV restriction factor that blocks HIV infectivity through steric hindrance of particle attachment to target cells. A large part of PSGL-1's extracellular region consists of a mucin–like domain, the decameric repeats (DRs), which contains 14 to 16 DR tandems. Each DR consists of 10 consensus amino acids (-A-T/M-E-A-Q-T-T-X-P/L-A/T-) with numerous O-glycosylated threonines (30%) and prolines (10%). A hypothetic function of DR is to elongate and strengthen the protein backbone for selectin-binding. However, the role of DR in PSGL-1's antiviral activity remained largely unknown. In this study, we conducted DR deletion mutagenesis, deleting a single DR to all DRs, and quantified the impacts on PSGL-1's antiviral activities. Here, we report that the DR domain is necessary, and individual single DRs possess 3.8 to 18% antiviral activities. In addition, the basic DR activity is cumulative; increasing number of DRs is associated with increased antiviral activity. We also demonstrated that deleting the DR domain did not affect virion incorporation of PSGL-1 but diminished its ability of PSGL-1 to block virion attachment to target cells. Furthermore, we found that inserting the DR domain into the extracellular Ig-like domain of CD2 conferred anti-HIV activity to the chimeric CD2-DR molecules. These results suggest that DR functions as a basic antiviral unit in PSGL-1 restriction of HIV.

Abstract #: S18

Role of Myeloid cells in the development of Metabolic Associated Steatohepatitis (MASH) in animal model.

Wiktoria Szwalec

Mentor: Ira Schulman, PhD, Department of Pharmacology, UVA

Background: Metabolic Dysfunction-Associated Steatohepatitis (MASH) is an advanced form of Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD), characterized by liver inflammation, hepatocyte ballooning, and fibrosis, often progressing to cirrhosis or hepatocellular carcinoma. Nevertheless, the only FDA-approved treatment for MASH is effective in approximately 30% of patients. Macrophages, including Kupffer cells, infiltrating monocytes and neutrophils, play a crucial role in MASH pathogenesis by promoting inflammatory responses, fibrosis and exacerbate tissue damage. Liver X Receptor alpha (LXRα) is a transcription factor involved in lipid metabolism and immune regulation. The W441F mutation in the LXRα gene (*Nr1h3*) inhibits its transcriptional activity, leading to cholesterol accumulation in hepatocytes and driving the development of MASH in mice exposed to a high-fat, high-cholesterol (HFHC) diet. However, this loss of function can be reversed by treatment with the synthetic agonist T0901317, which restores LXRα activity and decreases all the phenotypes of MASH. Mouse models carrying this mutation provide valuable insights into the molecular mechanisms underlying MASH, particularly the role of macrophages in inflammation and fibrosis, which is poorly understood.

Objective: The aim of this study is to characterize the diversity of liver immune cell populations in MASH and the role of these cell populations in reversing MASH. Additionally, our studies will define changes in gene expression among different macrophage and neutrophil populations and evaluates the effects of LXR transcriptional activity treatment on their recruitment, activation, and inflammatory responses.

Methods: LXRα-W441F mutant mice were fed a high-fat, high-cholesterol diet for varying durations to assess immune cells infiltration dynamics. Mice were also treated with the LXRα agonist T0901317 for different time periods to evaluate its effect of reactivating LXR transcriptional activity on immune cell populations. Liver non-parenchymal cells (NPCs) were isolated and analyzed primarily by flow cytometry to characterize macrophage and neutrophil subsets. Single-cell RNA sequencing and PCR were used to assess gene expression changes, while immunohistofluorescence was performed to visualize immune cell distribution within the liver.

Results: Single-cell RNA sequencing of non-parenchymal cells (NPCs) from mice after four weeks of HFHC diet revealed distinct populations of macrophages, neutrophils, and other immune cells, with a higher abundance observed in mutant mice. Flow cytometry confirmed an increased presence of macrophages and neutrophils in mutant mice following the HFHC diet, while T0901317 treatment altered the proportions of these populations. Immunohistofluorescence staining is ongoing to further characterize the spatial distribution of immune cells in the liver.

Abstract #: S19

Glutaminase enhances anti-tumor activity of conventional type 1 dendritic cells in female mice

Authors: Teresa Tamborra-Walton[1,2,3], Kyung Eun Kim[1,2,3], Andrew Pahnke[1,2,3], Anton Zhelonkin[4], Riley Togher[4], Mary Philip[5], Denis A. Mogilenko[1,2,3,4]

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Sex-dependent differences in the tumor metabolic environment appear to contribute to dysregulated anti-tumor immune responses. It has been established that the tumor microenvironment (TME) has decreased levels of glutamine compared to normal tissues. Recently, it has been shown that female CD4 Th17 cells are more functionally reliant on glutaminase (GLS) expression than male cells, which correlates with increased inflammation. GLS is the mitochondrial metabolic enzyme that converts glutamine to glutamate. As females have better tumor control than males, we wanted to uncover whether antitumor immunity is also tied to glutamine metabolism through GLS expression in immune cells. Conventional type 1 dendritic cells (cDC1s) are antigen presenting cells that establish antitumor immunity due to their ability to cross-present tumor antigens to cytotoxic T cells. **We hypothesize that cDC1s can exhibit sex-specific differences of glutamine metabolism in the TME.** Using a genetic mouse model of liver cancer (ASTxAlb-Cre), we found that proportions of cDC1s expressing GLS protein were significantly higher in the tumor draining-lymph nodes of females than males, indicating that GLS expression may be sex-dependent in cDC1s. Using an implantable murine B16 melanoma model in wild-type (WT) and GLS knockout in cDC1s (KO) *Xcr1*-Cre;GLS$^{fl/fl}$ mice, we found that WT females developed smaller tumors than WT males. Interestingly, KO females had larger tumors faster tumor growth compared to WT females. These results indicate that female antitumor immunity correlates with and depends upon glutamine metabolism in cDC1s. Future studies are needed to understand how GLS activity and glutaminolysis regulate antitumor immunity mediated by cDC1s.

Abstract #: S20

Glutaminase is a novel immunometabolic regulator of dendritic cell functions

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Dendritic cells have been shown to utilize glutamine to enhance antigen presentation to T cells. However, the mechanisms of how metabolization of glutamine affects dendritic cell immune functions and controls disease progression remain unknown.

By employing dendritic cells specific GLS knockout (DC-GLS-KO) mouse model and a pharmacological inhibitor for GLS activity, we found that glutaminase (GLS), a rate-limiting enzyme converting glutamine into glutamate, in dendritic cells enhances antigen presentation to increase T cell proliferation and supports cytokine secretion *in vitro*. Interestingly, in the MC903-mediated atopic dermatitis model, DC-GLS-KO mice developed more profound skin inflammation compared to wild-type littermate mice. Surprisingly, we found that DC-GLS-KO mice had more IL-4 and IL-13, but not IL-5, producing CD4 T cells in ear-draining lymph nodes which are the key cell types in the pathogenesis of atopic dermatitis. To decipher the molecular mechanisms of how GLS in dendritic cells controls their antigen presentation to CD4 T cells, we treated rapamycin, the inhibitor for mTORC1, only to dendritic cells as previous literature showed that mTORC1 in dendritic cells restrains priming of CD8 T cells. Our preliminary data showed that inhibiting mTORC1 in GLS KO dendritic cells increased CD4 T cell proliferation, suggesting the potential role of mTORC1 in dendritic cells in selective antigen presentation and differentiation of CD4 T cells.

Collectively, our data suggest that GLS in dendritic cells is a novel immunometabolic regulator for controlling tolerogenic immune responses. We propose GLS as a new pharmacological target to modulate dendritic cell function in antigen-mediated inflammatory diseases.

Abstract #: S21

SGLT2 Inhibitors Modulate Inflammation by Altering Macrophage Metabolism and Decreasing Monocyte Platelet Aggregates

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Sodium-glucose cotransporter-2 inhibitors (SGLT2is) are diabetes drugs that reduce cardiometabolic diseases in patients with and without diabetes through unclear mechanisms. SGLT2is also reduce inflammation in animals and *in vitro*. We hypothesized that SGLT2is reduce inflammation in humans as a mechanism of cardiometabolic benefit.

In a pilot, we treated eight women with obesity and pre-diabetes with the SGLT2i empagliflozin (25 mg/day) and collected subcutaneous adipose and blood at baseline, 2 and 12 weeks of treatment. We performed single-cell RNA sequencing and computational metabolic modeling on immune cells.

Baseline characteristics include age 61±6.1yrs, weight 93±16.9kg, and BMI 35.6±4kg/m². Empagliflozin upregulated genes involved in mitochondrial metabolism and glutathione synthesis, important in the oxidative stress response, in lipid-associated macrophages (LAMs; $p<0.001$). We also found that empagliflozin reduced monocyte-platelet aggregates (MPAs) in blood (-2.9±2.1% [-5.0, -0.8], p=0.018 at 2 weeks; -4.1±2.1 [-6.2, -1.9], p=0.002] at 12 weeks). MPAs are key mediators of vascular inflammation in cardiovascular disease.

In summary, we found that empagliflozin increases expression of genes associated with mitochondrial metabolism and oxidative stress in adipose LAMs and decreases circulating MPAs. These changes may contribute to the cardiometabolic benefits seen with these drugs. Future studies will validate above findings in an ongoing randomized trial.

Abstract #: S22

IL-33/ST2 Signaling Regulates Treg Mitochondrial Metabolism and Lipid Utilization to Promote Renal Repair

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Regulatory T cells (Tregs) are pivotal in modulating immune responses and promoting tissue repair, yet the metabolic cues guiding their function remain incompletely defined. We demonstrate that the IL-33/ST2 axis critically governs Treg metabolic fitness and regenerative capacity. Transcriptomic analysis using bulk RNA sequencing of the splenic Tregs and single-cell RNA sequencing of renal Tregs post-ischemic injury demonstrated that ST2-deficient Tregs had an attenuated expression of genes related to Treg suppressive function, migration, and tissue repair. The ST2-deficient Tregs also had a marked reduction in the expression of genes related to mitochondrial metabolism. Therefore, we performed untargeted metabolomics, which revealed that ST2-deficient (ST2KO) Tregs exhibit significant downregulation of tricarboxylic acid (TCA) cycle intermediates and ATP synthesis pathways, accompanied by suppressed expression of Acetyl-L-carnitine and DL-carnitine, key metabolites involved in mitochondrial fatty acid oxidation. Lipidomic profiling further revealed a marked reduction in triglycerides in ST2KO Tregs, underscoring impaired lipid utilization. Seahorse extracellular flux analysis showed that ST2KO Tregs had diminished oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), indicative of defective oxidative phosphorylation and glycolysis. Notably, IL-33 stimulation restored mitochondrial respiration in murine and human Tregs, increasing OCR levels. Functionally, co-culture of wild-type (WT) Tregs with tubular epithelial cells enhanced epithelial proliferation in scratch assays and maximized OCR under hypoxic conditions. These findings establish ST2 signaling as a critical regulator of Treg metabolic programming—integrating TCA cycle flux, lipid oxidation, and bioenergetic output—to drive epithelial repair during kidney injury.

Abstract #: S23

Cellular Signatures of Hybrid Immunity Underpinning Superior Mucosal Protection

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Abstract:

Understanding mucosal immunity is vital for designing vaccines against respiratory viruses like SARS-CoV-2. Immunity resulting from vaccination followed by infection may offer superior mucosal protection, yet the underlying mechanisms remain unclear. This study reveals the mucosal humoral, cellular, and molecular signatures underpinning this enhanced protection. Mice received two Spike mRNA doses and were challenged with mouse-adapted SARS-CoV-2. In these mice, bronchoalveolar lavage (BAL) single-cell RNA sequencing showed increased adaptive immune cells, enriched T and B cell activation pathways, enhanced T cell differentiation, and upregulated B cell IgA production. Flow cytometry confirmed increased SARS-CoV-2-specific IFN-γ and TNF-α producing $CD4^+$ and $CD8^+$ T cells. The group exhibited elevated mucosal RBD-specific B cell levels and significantly higher IgA responses, leading to the highest respiratory neutralizing antibody levels. In contrast, mucosal IgG levels were similar across all groups. Using K18-hACE2 mice infected with Omicron, we found that despite minimal lung pathology, these mice showed increased respiratory T and B cell responses and elevated mucosal and plasma IgA levels compared to vaccination or infection alone. This study offers insights into the cellular signaling networks providing superior mucosal protection following combined vaccination and infection, informing vaccination strategies against respiratory pathogens.

Abstract #: S24

SYSTEMS MODEL PREDICTS MECHANISMS OF NEUTROPHIL-INDUCED CARDIAC INFLAMMATION

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Heart disease remains the leading cause of death worldwide. In every case of cardiac injury an immune response is initiated, with neutrophils—the most abundant immune cell type in humans—serving as the first responders to tissue damage. While they are crucial for defending against extracellular pathogens and promoting tissue repair, excessive activation can prolong inflammation and exacerbate injury.

To elucidate the molecular mechanisms underlying neutrophil activity in cardiac inflammation, we developed a computational model using logic-based ordinary differential equations constructed with continuous ‘AND’ and ‘OR’ gates to simulate protein-protein interactions. This model integrates experimental findings from 165 publications and is validated against 35 independent publications, accurately recapitulating over 80% of observed in-vitro experimental outcomes. Using this validated model, we found that IL-1β induces oxidative bursts via a Rac feedforward circuit, and we are currently experimentally testing the mechanisms driving this response.

Our work provides a systems-level framework to better understand the complex dynamics of neutrophil signaling in different contexts, potentially leading to novel therapeutic approaches for heart disease.

Abstract #: S25

Microglia-dependent SYK signaling coordinates cerebral T cell responses and neurodegeneration in tauopathy

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Tauopathy is a major driver of neuronal dysfunction and inflammation in multiple neurological conditions including frontotemporal dementia and Alzheimer's disease. Recent studies have uncovered pivotal roles for microglia in tauopathy progression. Yet, the specific molecular pathways that microglia deploy to shape tau pathology currently remain poorly defined. In our unpublished studies, we have identified that spleen tyrosine kinase (SYK) signaling in microglia is a major driver of tauopathy pathogenesis in the PS19 mouse model. Here we found that conditional deletion of SYK in brain-resident macrophages leads to improved spatial learning, decreased tauopathy, and attenuated microglial activation in PS19 mice. At the mechanistic level, we show that SYK-deficient microglia are defective in antigen processing and presentation, and that this limits their ability to support the neurotoxic T cell responses that underlie degeneration and neuroinflammation in this tauopathy model. These findings indicate that SYK-dependent antigen processing by microglia contributes to T cell–mediated neurotoxicity in tauopathy and further suggest that SYK-targeting therapies may offer strategies to treat tau-driven neurodegeneration.

Abstract #: S26

PARP inhibitors boost antitumor effects of ADAR1 inhibition regardless of BRCA1 status

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The RNA editing enzyme adenosine deaminase 1 (ADAR1) is highly expressed in metastatic breast cancer. Targeting ADAR1 is effective in interferon-stimulated gene (ISG)-high but not ISG-low tumors, limiting its use in clinical settings. Here, we aim to enhance the efficacy of ADAR1 inhibition through combination therapy. We focused on triple-negative breast cancer (TNBC), the most aggressive form of breast cancer, which lacks effective therapies.

Using a genome-wide CRISPR screen, we found that silencing ADAR1 sensitized TNBC cells to type I interferon-mediated killing, which can be induced by poly (ADP-ribose) polymerase inhibitors (PARPi). PARPi are approved for BRCA-mutated TNBC. Notably, using both murine and human TNBC cells, we discovered that combining PARPi with ADAR1 siRNA synergistically induced cell death in both BRCA1-deficient and BRCA1-proficient TNBC cells. Additionally, RNAseq analysis suggest that the combination therapy enhanced tumor immunogenicity more effectively than PARPi or ADAR1 siRNA alone. Using both genetic and pharmacological tools, we are currently evaluating the efficacy of combination therapy in GEM models of advanced TNBC.

Taken together, our study investigated the mechanism of action for combining PARPi with an ADAR1-targeting agent, highlighting the therapeutic potential of this combination. This approach could expand the use of single agents and benefit more patients with advanced TNBC.

Abstract #: S27

Gut microbiota shape immune patterns in naïve and glioma-bearing humanized microbiome mice

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Glioblastoma (GBM) is the most common primary malignant brain tumor. While anti-PD-1 immunotherapy has increased survival in GBM mouse models, clinical trials demonstrated no survival benefit. This failure of preclinical models to replicate in the clinic may be due to inadequate consideration of the gut microbiota. Our laboratory was the first to report that human gut microbes, transplanted into the gastrointestinal tracts of mice (humanized microbiome—HuM—mice), modulate immunotherapy response in murine GBM. We identified distinct microbiomes associated with immunotherapy response (responders) and resistance (nonresponders), but the mechanisms by which gut microbes modulate systemic and glioma immunity remain unclear. To address this, we investigated immune differences between HuM mouse lines under tumor-free conditions. 16S rRNA sequencing revealed that responder mice had a higher abundance of gram-positive, lipoteichoic acid (LTA)-producing bacteria compared to nonresponders. Supporting this, single-cell RNA sequencing analysis of colon immune cells revealed an increase in expression of the LTA receptor *Tlr2* in myeloid, B cells, and $CD8^+$ T cells of responder mice. Using flow cytometry, I found that responder mice had higher frequencies of Th17 cells in the gut- and brain-draining lymph nodes. Colonic T cells from responders also had higher expression of migratory receptors (*Ccr5, Cx3cr1* and *S1pr1)*, suggesting a potential increase in circulation of immune cells from the colon. Together, these findings suggest that gut microbes in immunotherapy responder microbiome mice may prime the colonic immune landscape to support systemic immune activation and trafficking, potentially enhancing antitumor immunity in GBM.

Abstract #: S28

IL-33/ST2-independent activation of type 2 responses in allergic asthma

Emily M. Grayson, Maile K. Hollinger, Tania E. Velez, Fernando A. Oliveira, Caroline M. Ferreira, Faith C. Wells, Jacqueline J. Powers, Christopher F. Pastore, Mariam A. Okhowa, Anne I. Sperling

Allergic asthma is a chronic inflammatory lung disease causing 1,000+ deaths per day. The innate immune response is driven by alarmins like IL-33, which signals through its cognate receptor ST2. Given its early role in the allergic cascade, IL-33 is an attractive therapeutic target for downstream asthma control. Nevertheless, α-IL-33 treatments have shown limited clinical efficacy. In prior literature, mice lacking IL-33 (IL-33-/-) or ST2 (ST2-/-) exhibit reduced allergic responses. However, we have reproducibly found that our IL-33-/-, ST2-/-, and double-knockout mice respond to allergen similarly to wild-type controls suggesting the IL-33/ST2 axis is dispensable in our mice. To address whether this phenomenon is environmentally driven, we compared the allergic responses of our ST2-/- mice to those from another facility (Facilities A and B, respectively). Excitingly, ST2-/- mice from Facility B exhibited a reduced allergic response consistent with prior literature. In analyzing the cellular landscape in the lungs of naïve ST2-/- mice from both facilities, we found that the ILC2 compartment was expanded only in ST2-/- mice from Facility A. Thus, environment drives the generation of distinct cellular landscapes in the lung in the absence of IL-33/ST2**.** We are exploring alternative pathways of allergic induction and the role of the microbiome in allergic response severity to explain variability in clinical therapeutic efficacy and uncover novel therapeutic targets.

Abstract #: S29

Investigating the role of effector B cells in the brain during CNS viral infection

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B Cells and their antibody secreting cell (ASC) progeny have a profound ability to protect us from potentially deadly viral infections. Early after infection and detection of their cognate antigen, B cells proliferate and form a wave of early ASCs that produce large quantities of antibodies – most of which funnel into circulation to provide the host protection from the invading pathogen. In addition, studies of lymphocyte migration have uncovered the ability of ASCs to migrate into infected sites for local antibody secretion. This same capability has not often been considered for B cells themselves. However, our lab and others have recently identified B cells, alongside ASCs, directly at infected sites. We employed a viral-specific BCR transgenic mouse to detect antigen-specific B cells and ASCs responding directly to viral encephalitis. While B cells have been identified in different inflammatory contexts in the brain, how they function locally and how they impact clearance of an invading pathogen is still largely unexplored. In vesicular stomatitis virus (VSV) infection, the B cell population in the brain is majorly class-switched (IgM-IgD-) and a fraction express CD95 and uptake EdU in the tissue, suggesting that these B cells are antigen-experienced and may be capable of local activation-induced effector function and division. To further understand the local role of this B cell population in the anti-viral immune response, we have identified phenotypic and functional features of B cells as well as their ability to differentiate into ASCs in the infected brain.

Abstract #: S30

Network Model Predicts Anti-inflammatory Mechanisms of Colchicine

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Pericarditis affects around 35,000 people in the United States every year. The NLRP3 inflammasome and IL1b secretion is of particular interest in pericarditis due to the use of colchicine to suppress the inflammasome. Our goal is to develop the first computational model of NLRP3 inflammasome signaling to test the role of network motifs and synergy governing priming and activation and predict mechanisms of anti-inflammatory drugs for pericarditis. To do this, we built a logic-based differential equation model of NLRP3 inflammasome signaling and colchicine treatment including 28 nodes and 30 reactions using 17 articles from existing literature. The model has been validated against *in vitro* imaging of inflammasome responses to independent priming and activating stimulation. Published *in vivo* data validated the model responses to zymosan along with treatments including NLRP3 inhibitors and colchicine. Using the model, we made predictions of the changes in the network activity in response to stimulation with zymosan and inhibition of inflammasome priming via NFkB inhibitors or activation via NLRP3 inhibitors and colchicine. In future work, we will further analyze network motifs in the inflammasome and their role in governing synergy of priming and activation.

Abstract #: S31

Shifts in small intestine epithelial immune phenotypes are associated with intestinal bacterial overgrowth in a mouse model of undernutrition

Caroline Danielski[1,2], Claire Williams[1,2], Yadeliz A. Serrano Matos[1,2], Lindsey Bihuniak[2], Carrie Cowardin[1,2]

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Early life acute undernutrition and growth stunting remain a major area of public health concern, with stunting associated with lifelong risk for health complications. Stunting is often associated with poor nutrient absorption, dysbiosis, immune dysregulation, and increased epithelial barrier permeability. Intra-epithelial lymphocytes (IELs) are known to function in intestinal immune regulation, nutrient sensing, and barrier maintenance, but their role in undernutrition is poorly understood. To further characterize the response of IELs to the nutritional risk factors for growth stunting, young mice were maintained on a diet deficient in protein and micronutrients modeled after human undernourished diets (referred to as the “Malawi-8” or “M8” diet) for four weeks. Diet treatment significantly shifted the immune cell population of the intestinal epithelium, with M8-fed mice having significantly depleted CD8+ IEL populations, with preferential reduction of CD8αα “natural” IELs. These mice also exhibited increased intestinal epithelial infiltration of eosinophils and neutrophils. Immune shifts were accompanied by bacterial overgrowth in the distal small intestine and intestinal lengthening, suggesting a role for IELs in regulating both the commensal population and host epithelial homeostasis. However, the mechanism behind these changes remains poorly characterized. Data from germ-free mice suggest that the IEL shift is diet-driven, but the specific dietary component responsible is still unknown. Future work might identify the dietary component driving IEL shifts and mechanism of innate cell recruitment, with the goal of targeting nutritional therapies to support intestinal immune function in stunted children.

Abstract #: S32

Authors: Asmaa Mohamed[1], Gabriel F. Alencar[1], Madison G. Burnett[2], Yapeng Su[2], Raphael Gottardo[2], Stefan Bekiranov[1], Philip D. Greenberg[2,3], Kristin G. Anderson[1]

Institutions: University of Virginia[1], Fred Hutchinson Cancer Center[2] and the University of Washington[3]

Title:

Engineered T cell therapy modulates tumor-infiltrating CD4 T cell differentiation in ovarian cancer

Abstract:

Ovarian cancer is the deadliest gynecological cancers in the US. Patients with advanced disease have a 5-year survival rate of 31%, underscoring the need for innovative treatment strategies. Engineering T cells to target tumor-specific proteins could enhance treatment efficacy while minimizing toxicity to healthy tissues. Mesothelin (Msln) is minimally expressed in healthy tissues, overexpressed in ovarian cancer cells and contributes to malignancy, making it an excellent immunotherapy target. In a mouse model, T cells engineered to express a Msln-targeting T cell receptor (TCR_{MSLN}) delayed tumor growth, but inhibitory signaling in the tumor microenvironment diminished T cell function, resulting in disease progression. Combining TCR_{MSLN} T cells with PD-1, Tim-3, and Lag-3 triple checkpoint blockade led to significantly improved T cell function and overall animal survival.

Single-cell RNA sequencing of tumor-infiltrating lymphocytes revealed distinct gene expression changes after treatment. Given the critical role of CD4 T cells in cancer immunity, we examined each treatment's impact on CD4 T cell differentiation. CD4 T cells in untreated tumors expressed stem-like signatures. After treatment with TCR_{MSLN} T cells, tumor-infiltrating CD4 T cells expressed genes associated with T_H2 differentiation. Checkpoint blockade alone led to T cell activation (but no change in tumor control). However, CD4 T cells exposed to the TCR_{MSLN}+triplet antibody treatment had increased cytotoxic gene expression. These results suggest that treatment combinations can dramatically alter endogenous immune responses and could potentially be targeted to rationally improve adoptive T cell therapy efficacy and ovarian cancer patient outcomes.

Abstract #: S33

Tunable Transcription Factor Control for T Cell Programming via Clinically Approved Drug

Authors: Fucong Xie[1], Zaid Syed[1,2], Qidang Ye[1,2], H. Kay Chung[1,3]

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Transcription factors (TFs) play a critical role in regulating T cell differentiation. In the tumor microenvironment (TME), persistent antigen exposure often leads to T cell exhaustion and diminished anti-tumor responses. Precise TF control in T cells represents a promising strategy to manipulate T cell differentiation to overcome T cell dysfunction, restore cytotoxic potential, and improve the efficacy of cancer immunotherapies. A central challenge in leveraging TFs for cell therapy is their dynamic functionality and context-dependency. Limitations of blank TF overexpression include unpredictable transcriptional rewiring, loss of cell identity, or triggering of compensatory pathways that compromise efficacy. Therefore, TFs must be tightly controlled to avoid undesired effects such as unchecked proliferation or impaired effector differentiation. To address this, we built upon the Small-Molecule Assisted Shutoff (SMASh) system and engineered a clinically approved, drug-controllable ON/OFF switch for TF expression in CAR T cells. We validated the controllability of this system both in vitro and in an adaptive cell therapy model. By leveraging clinically approved drugs, we demonstrated the tunable expression of effector or stemness-associated TFs (e.g. KLF2, TCF7, etc). This positions the SMASh ON/OFF system as a novel tool to enhance the precision, safety, and therapeutic efficacy of T cell-based cancer immunotherapies, including CAR-T cell treatments.

Abstract #: S34

Title: Enhancing aspartate production improves the persistence of engineered T cells in pancreatic cancer

Authors: Yaning Li, Breanna M. Bates, Rossymar Rivera Colón, Lucas B. Sullivan & Kristin G. Anderson

Affiliations: University of Virginia and Fred Hutchinson Cancer Research Center

T-cell receptor (TCR)-engineered T cell therapy, which involves reprogramming cytotoxic CD8+ T cells to selectively target tumor-specific antigens and elicit tumor killing, holds great promise for the treatment of pancreatic cancer. Mesothelin (Msln) is a promising tumor antigen in pancreatic cancer and has limited expression in healthy cells. We have previously demonstrated that Msln-targeting TCR (TCR_{Msln})-engineered T cells safely provide therapeutic benefit in a murine pancreatic tumor model. However, our data also revealed some immune-suppressive features of tumor microenvironment that restricted engineered T cells efficacy. The nutrient- and oxygen-deprived tumor microenvironment induces mitochondrial stress of infiltrating T cells, dampening persistence and anti-tumor function. Specifically, reduced mitochondrial respiration impairs *de novo* aspartate synthesis and inhibits cell proliferation in tumors. Unlike poorly membrane-permeable aspartate, its precursor asparagine is available to cells by environmental acquisition. We hypothesized providing T cells with a mitochondria-independent supply of aspartate by asparaginase-mediated conversion would overcome this limitation. We engineered mouse T cells to express a high-activity asparaginase (ASNase1) combined with TCR_{Msln} and confirmed that ASNase1 can restore aspartate production in the TCR_{Msln} T cells. Our results revealed that ASNase1 is functional in TCR_{Msln} T cells and enhances T cell persistence *in vitro* and *in vivo* in mouse pancreatic tumors compared to control TCR_{Msln} T cells. These findings suggest that bolstering aspartate levels with ASNase1 expression could be a novel and feasible strategy to advance T cell therapy against cancers.

Abstract #: S35

Infection-experienced alveolar macrophages sustain airway T cell memory

In Su Cheon[1], Young Min Son[1,2], Jun-Sub Im[1], Yue Wu[1] and Jie Sun[1]

Carter Center for Immunology, Division of Infectious Disease and International Health, Department of Medicine, University of Virginia, Charlottesville, VA[1]

Department of Systems Biotechnology, Chung-Ang University, Anseong, Republic of Korea[2]

Emerging evidence has suggested that primary infection and/or insult can “train” or “prime” long-term alterations in the transcriptional and epigenetic profiles of innate immune cells, particularly macrophages. Currently, it is unknown whether those “infection-experienced” macrophages can facilitate adaptive immune memory responses after infection resolution. Here, we show that influenza virus infection induces lasting transcriptional and epigenetic changes in lung alveolar macrophages (AMs) compartment following the resolution of primary infection. Further analysis showed that the IL-1β was persistently upregulated in the “infection-experienced” AMs at the memory phase compared to those of AMs from the naïve mice, and the “primed” AMs represent the major source of IL-1β in the infection-experienced lungs. Strikingly, blocking IL-1β or ablating it in myeloid cells during the memory phase led to decreased number of antigen-specific CD8 resident memory T cells (Trm) specifically in the airway but not the lung parenchyma. Consistently, IL-1β blockade caused diminished Trm-mediated protective immunity during heterologous influenza virus re-challenge. Our data suggest that infection-primed innate immune compartment facilitates protective local adaptive immune memory in the respiratory mucosa after the resolution of primary infection. Our findings may inform future vaccine design aiming to induce strong mucosal CD8 T cell immunity against respiratory viral infections.

Abstract #: S36

Function of tissue-resident helper T cells in chronic lung sequelae following viral pneumonia

In Su Cheon[1], Young Min Son[2], Harish Narasimhan[1], Jun-Sub Im[1], Robert Vassallo[3] and Jie Sun[1]

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Abstract

Respiratory viral infections such as influenza and SARS-CoV-2 can lead to the development of chronic lung disease and impaired lung function long-term after the recovery from acute morbidity. However, the mechanisms underlying the development of chronic diseases post-acute respiratory viral infection are largely elusive.

Using a model of age-related development of chronic lung disease after acute influenza infection, we found that a population of lung resident $PD1^{Hi}CD4^{+}$ T helper cells (TRH), which exhibit characteristics of both follicular helper T cells and resident memory cells, was greatly increased after primary influenza pneumonia. Furthermore, TRH cells were located near the dysplastic/inflammatory lung parenchyma after the resolution of primary viral pneumonia. Strikingly, genetic ablation of TRH or the blockade of TRH-derived IL-21 signaling diminished chronic lung pathology and improved lung function following viral pneumonia in aged animals. Moreover, TRH cell presence was associated with worse lung function and increased lung pathology in COVID-19 convalescents. Our data suggest that exuberant TRH responses contribute to the development of chronic lung sequelae following viral pneumonia. Additionally, our findings may pave the way to develop novel preventive and/or therapeutic options treating chronic lung diseases following acute viral pneumonia including long COVID.

Abstract #: S37

IL-4 signaling drives Lag-3 expression macrophages.

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Immunotherapies that target immune checkpoints have extensively been shown to reverse immune cell exhaustion, however patient responses to immune checkpoint inhibitors remain heterogeneous. Lymphocyte-activating gene 3 (Lag3) has recently emerged as a promising and potent checkpoint inhibitor expressed on various immune cells. While research studies have focused on the role of Lag3 in T cell exhaustion, questions remain regarding Lag3 biology and expression. Here, we have identified tumor associated macrophages as an additional Lag3 expressing immune cell in a tumor microenvironment. In an effort to further explore this, M1- and M2-like bone marrow derived macrophages were stimulated with IFNγ and IL-4, respectively, and shown to have upregulated Lag3 expression after 24 hours. Specifically, M1-like macrophages showed variable expression of Lag3, with an initial upregulation at 4 hours, but subsequently reduced to a baseline level resembling that of unstimulated macrophages. However, M2-like macrophages maintained high Lag3 expression over a prolonged period of time. Interestingly, M2-like macrophage Lag3 expression seems to be glycolysis dependent because incubation with the inhibitor, 2-DG, reduced expression on M2-like macrophages, but only minimally affected levels on M1 macrophages. Additionally, macrophages isolated from MC38 tumors exhibit Lag-3 expression. The dynamic expression of Lag3 by macrophages indicates that this immune population may serve as a potential therapeutic target in cancer.

Abstract #: S38

Post-translational nitration at Rop5 locus shapes virulence and immune evasion in *Toxoplasma gondii*

Pierce DiMare (presenter), Samantha Lempke, Nadia Holness, Xiao-Yu Zhao, Sarah Ewald

UVA School of Medicine Beirne B. Carter Center for Immunological Research, Ewald Lab

Toxoplasma gondii, an obligate intracellular parasite of the phylum Apicomplexa, is a remarkably successful parasite that has infected over 30% of humans globally. The major parasite virulence locus encodes tandem repeats of the Rop5 secreted effector genes (Rop5A, B and C). The Rop5 complex phosphorylates and inactivates host cell immunity-related GTPase (IRG) proteins, which target the parasite vacuole (PV) for degradation. Our lab has recently shown that inducible nitric oxide synthases (iNOS) expression and the nitration of proteins localized to the PV is required for host cells to efficiently clear parasite infection. We hypothesized that nitration of parasite effectors involved in immune evasion may be may a mechanism of iNOS-mediated parasite clearance. Using cysteine-nitration pull down and mass spectrometry, we found that Rop5 was among the most abundantly nitrated proteins isolated from infected, wild-type cells compared to infected iNOS knockout cells. To understand how nitration post-translational modifications regulate Rop5 function, we generated Rop5-deficient Toxoplasma, then complemented this strain with alleles of Rop5A, B or C where the cysteine nitration sites have been mutated to alanine or serine. To assess which residues are the significant targets of nitration regulating parasite control, the ability of each allele of Rop5 to interact with the rest of the IRG complex and defend against clearance from the host will be evaluated, with a reductionist approach utilized to determine which post-translational modifications and how their prevalence at the Rop5 locus ties into the virulence trends between the main types of T. gondii.

Abstract #: S39

Bin-Bin Schell
SIS 2025 Abstract

Title
Defining REV-ERBα's Ligand-Mediated Transcriptional Mechanisms in T_H17 Cells

Authors
Bin-Bin Schell[1,2], Sarah Mosure[1,2], Tim O'Leary[2], Adrianna Wilson[1,2], Jonathan Chuck[1,2], Sean Campbell[2], Robin Kobylski[1,2], Laura Solt[1,2]

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Abstract
T_H17 cells are a subset of $CD4^+$ T cells that mediate autoimmune and chronic inflammatory pathology. We have demonstrated that REV-ERBα, a member of the nuclear receptor superfamily of ligand-regulated transcription factors, acts as a critical repressor of this cell type. In line with this finding, REV-ERB agonism has been shown to ameliorate disease in several mouse models of chronic inflammation. Much work has been done to identify the target genes of REV-ERBα across multiple tissues. However, little is known about the transcriptional machinery driving its function. Our goal is to identify key proteins that drive REV-ERBα-mediated gene repression in pathogenic T_H17 cells and further understand how ligands modulate this process. Therefore, we have coupled MiniTurboID proximity labeling with label free proteomics to capture the REV-ERBα interactome in T_H17 cells. Comparison of the REV-ERBα interactome induced by the presence and absence of endogenous ligand has provided insight into the transcriptional complexes that govern REV-ERBα activity and the role of ligands in their formation. Specifically, we have identified canonical and non-canonical co-repressors that potentially mediate distinct mechanisms of repression. Ultimately, these efforts will bolster our understanding of REV-ERB, nuclear receptor, and T_H17 biology and inform the development of focused therapeutics for treatment of autoimmune and chronic inflammatory diseases.

Abstract #: S40

Title: Sexual Dimorphism in Macrophage Function Restricts Immune Checkpoint Blockade in Pancreatic Cancer

YuJie Chen[1], Zihan Chen[1,2,3], Tuisha Gupta[1], Tenzin Passang[1], Fanyuan Zeng[1], Jian-Ming Li[1], Cindy R. Giver1, Kiranj Chaugadar[1], Edmund K. Waller[1]

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Pancreatic ductal adenocarcinoma (PDAC) remains one of the deadliest cancers, with notably poorer survival in males. The limited efficacy of immune checkpoint inhibitors (ICI) in PDAC has been linked to an immunosuppressive tumor microenvironment (TME) driven by M2-like tumor-associated macrophages (TAM). Our previous studies showed that dual blockade of PD-1 and the vasoactive intestinal peptide (VIP) receptor VPAC enhances $CD8^+$ T cell activity and eradicates tumors in ~50% of PDAC models. We hypothesized that combinatorial ICI therapy targets TAM-driven immunosuppression and boosts T cell–mediated anti-tumor immunity in PDAC.

We tested this using in vivo tumor growth and survival studies in syngeneic PDAC models, supported by RNAseq of polarized bone marrow–derived macrophages (BMDMs) and scRNAseq analysis of TAMs from PDAC patients using public datasets.

In syngeneic PDAC models, tumors grew slower in VIP-knockout mice, with females exhibiting superior responses. Depletion of phagocytic tumor-associated macrophages (TAM) accelerated tumor growth in females but not in males, highlighting sexually dimorphic macrophage function. Androgen receptor (AR) inhibition reprogrammed macrophages to a less suppressive phenotype, reducing Arg1, CD206, and PD-L1 expression. In vivo, AR inhibition or PD-L1 loss enhanced the efficacy of VPAC blockade in males. Mechanistically, AR inhibition upregulated VPAC1 in macrophages, mirroring the female-like immune profile.

These findings suggest that androgen and VIP pathways mediate sexual dimorphism in TAM phagocytosis, which limits ICI efficacy in PDAC. VPAC1 and PD-L1 are identified as potential sex-specific phagocytosis checkpoints, providing promising therapeutic targets to overcome sex-based differences in PDAC immunotherapy outcomes.

Abstract #: S41

SHP-1 negatively regulates microglial innate immune responses in Aβ amyloidosis

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Microglia, the brain-resident macrophages, have emerged as key players in Alzheimer's disease (AD) where they are critical responders to amyloid beta (Aβ) plaques. Previous investigation identified that microglial inhibitory immune receptors dampen neuroprotective microglial functions, making them of high clinical relevance. While many of these inhibitory receptors contain immunoreceptor tyrosine-based inhibitory motifs (ITIM), the molecular regulators executing this immune inhibition in microglia are not well understood. One ITIM receptor-associated regulator that has known roles in inhibiting peripheral immune functions is the SH2 domain containing phosphatase SHP-1. While *PTPN6* (the gene encoding SHP-1) is almost exclusively expressed by microglia in the brain, little is known regarding its role in Aβ pathology. To study how loss of microglial SHP-1 impacts microglial immune responses to Aβ plaques, we generated 5xFAD *Ptpn6*$^{fl/fl}$ *Cx3cr1*^{Ert2Cre} mice to selectively delete SHP-1 in microglia in a mouse model of Aβ-driven AD. Here, we show that SHP-1 deletion in microglia leads to a reduction in Aβ plaques, enhances microglial recruitment to Aβ plaques, and increases microglial plaque containment and Aβ engulfment when compared to 5xFAD *Ptpn6*$^{fl/fl}$ littermate controls. Additionally, transcriptional analysis of isolated SHP-1-deficient microglia indicates that SHP-1 functions downstream of microglial ITIM-containing receptors, including CD22 and CD72, to regulate microglial migration, lipid metabolism, and phagocytosis. Collectively, these results identify SHP-1 as a regulator of microglial immune functions in Aβ amyloidosis and suggest that targeting SHP-1 may offer a promising strategy to treat AD.

Abstract #: S42

Title: First glimpse of immune surveillance during premalignant progression of triple negative breast cancer

Authors: **Xian Zhou**, Jianhao Zeng, Elaina K. Ball, Alexys Riddick, Patcharin Pramoonjago, Victor H. Engelhard, Hui Zong

Affiliation: University of Virginia

Immune surveillance is believed to eliminate pre-cancerous cells to prevent cancer. However, the mechanisms remain unclear, as pre-cancerous cells are difficult to detect. To gain a glimpse into immune surveillance, we developed a genetically engineered mouse model recapitulating human triple negative breast cancer (TNBC), which generates rare, *p53-Brca1* mutant cells with unequivocal GFP labeling. Guided by the visualization of premalignant cells, we observed tertiary lymphoid structure (TLS)-like immune aggregates near mutant ducts long before tumor formation. GeoMx-based spatial profiling showed not only activation signatures in T and B cells but also elevated interferon response genes in mutant ducts associated with immune aggregates when compared to those free of immune infiltration. FTY720 treatment at early premalignancy led to increased mutant cells expansion and reduced T cell infiltration and aggregates formation, demonstrating the restraint of mutant cell growth by the immune system. To clarify the roles of specific immune cells, we plan to deplete CD8 T, CD4 T, and B cells at premalignancy. We will also conduct longitudinal studies on T and B cells along the tumor evolution process from premalignancy to malignancy to understand how immune surveillance eventually falters. Taken together, our studies are poised to elucidate key mechanisms of immune surveillance in the premalignant stage of TNBC.

Abstract #: S43

Title: CD34 serves as an intrinsic innate immune guardrail protecting stem cells from replicating retroviruses.

Presenter : Amrita Haikerwal

Authors: Sijia He1 ‡†, **Amrita Haikerwal**1 †, Sameer Tiwari1 †, Deemah Dabbagh1,2†, Mohammed Z. Alam1 , Janice L. Yoon1 , Brian Hetrick1 §, Yang Han1 , Liang Shan3,4, Christopher Lockhart 5 , Yuntao Wu1 *

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Abstract

Stem cells are highly resistant to viral infection compared to their differentiated progeny, and this resistance is associated with stem cell-specific restriction factors and intrinsic interferon stimulated genes (ISGs). In HIV infection, proviral DNA has been detected in certain bone marrow hematopoietic stem cells, yet widespread stem cell infection in vivo is restricted. Intriguingly, exposing bone marrow stem cells to HIV in vitro led to viral replication selectively only in the CD34- population, but not in the CD34+ cells. The mechanism dictating this CD34-based HIV restriction remained a mystery, especially since HIV has a capacity to antagonize restriction factors and ISGs. CD34 is a common marker of hematopoietic stem and progenitor cells. Here, we report on the intrinsic antiviral properties of CD34. Expression of CD34 in HIV-1 producer cells results in the loss of progeny virion infectivity. Conversely, removal of CD34 using CRISPR/Cas9 knockout or stem cell differentiation cytokines promotes HIV-1 replication in stem cells. These results suggest that in addition to restriction factors and intrinsic ISGs, CD34 serves as a host innate protection preventing retrovirus replication in stem cells. Mechanistically, CD34 does not block viral entry, integration, and release. Instead, it becomes incorporated onto progeny virions, which inactivates virus infectivity. These findings offer new insights into innate immunity in stem cells and highlight intriguing retrovirus-host interactions in evolution.

Abstract #: S44

Intestinal Dysbiosis and Inflammation in Lupus: A Multi-Omics Approach

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Department of Microbiology, Immunology, and Cancer Biology, University of Virginia

Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by systemic inflammation and the production of autoantibodies. The pathogenesis of SLE has been linked to changes in the gut microbiome, which plays key roles in immune development and tolerance. However, most studies focus on global taxonomic changes. Therefore, a high-resolution exploration of individual species and functional changes in the microbiome is needed to elucidate specific mechanisms of host-microbe interactions and how they are altered, contributing to autoimmunity. We hypothesize that in lupus, along with changes in intestinal microbial abundance, bacterial gene functions and metabolic pathways are altered, disrupting intestinal immune and epithelial processes. In this study, we used an inducible mouse model of SLE and collected intestinal samples to perform whole shotgun metagenomics and bulk RNA-seq. Upon lupus induction, RNA-seq analysis demonstrated a decrease in host antimicrobial peptide expression and tight junction proteins. Pathways associated with epithelial barrier function and mucosal homeostasis were downregulated, while inflammatory pathways were upregulated. This is supported by our observation of increased total fecal IgA in mice with lupus. Additionally, we observed a dysbiotic state in the lupus microbiome, characterized by reduced overall microbiome diversity and altered global microbiome functionality. These findings suggest that in our lupus model, intestinal inflammation occurs and is correlated with the loss of epithelial barrier function, dysbiosis of the gut microbiota, and changes in microbial functional potential.

Abstract #: S45

IL-33 Restores Humoral Immunity to Prevent Recurrent *C. difficile* Infection

***Naz F,* Hagspiel N, Young M K, Uddin J, Tyus D, Boone R, Brown A, Ramakrishnan G, Rigo I, Fleming C, Madden G R, and Petri W.A.**

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Abstract

Clostridioides difficile infection (CDI) recurs in approximately one in five patients. Monoclonal antibodies targeting the virulence factor TcdB have been shown to prevent disease recurrence, suggesting that a suboptimal anti-TcdB immune response contributes to recurrence. In patients with CDI, elevated IL-33 levels at diagnosis were predictive of future recurrence, prompting an investigation into the role of IL-33 signaling in promoting humoral immunity during CDI. Using a mouse model of recurrent infection, we found that IL-33 is essential for producing anti-TcdB antibodies. IL-33 signaling activates $ST2^+$ ILC2 cells, which in turn support the generation of germinal center T follicular helper (GC-Tfh) cells required for antibody production. The role of ILC2s in antibody production was further confirmed by utilizing ILC2 knock-out mice. Notably, protection from reinfection was antibody-dependent, as demonstrated by the lack of protection in MuMT KO mice and those treated with anti-CD20 mAb. These results highlight the pivotal role of IL-33 in facilitating humoral immunity to prevent recurrent CDI.

Keywords: *Clostridioides difficile*, IL-33 signaling, ILC2s, toxin-specific antibody, GC-TFH, recurrent *C. difficile* infection

Abstract #: S46

Title:
Deciphering the interplay between fever and neutrophil immunometabolism during infection

Authors:
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Main Text:
The development of a fever is a fundamental response to infection and inflammatory diseases, yet temperature is often overlooked when studying immune development and function. Recent findings show that subsets of CD4 T cells cultured at febrile temperatures above 37°C have altered metabolism leading to enhanced downstream effector functions, providing insight into how temperature changes affect the immune response. Neutrophils are the backbone of the innate immune response and possess an arsenal of antimicrobial processes that are crucial in the host's response to infection. Herein, we identify that neutrophils cultured at elevated temperatures have decreased mitochondrial respiration while maintaining similar levels of glycolytic function. Since mitochondria play a critical role in regulating neutrophil effector functions, neutrophils were cultured with the bacterial pathogen *Staphylococcus aureus*. When neutrophils are cultured with *S. aureus* at febrile temperatures, neutrophils preferentially elicit phagosomal oxidative stress rather than release neutrophil extracellular traps resulting in augmented antibacterial activity. These results indicate febrile temperatures impact metabolic homeostasis within neutrophils thereby heightening the capacity of neutrophils to persist and produce an oxidative burst implying synergistic interplay between fever and oxidative stress.

Abstract #: S47

Title:

Untangling the immunologic imbalance in SLE NET release

Authors:

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Main Text:

The deposition of neutrophil extracellular traps (NETs) is thought to contribute nuclear antigens that exacerbate inflammation in the autoimmune disease systemic lupus erythematosus (SLE). We showed that mitochondrial lactate dehydrogenase (LDH) is essential to suicidal NET release (NETosis) in response to lactate producing microbes, like *Staphylococcus aureus*. However, SLE neutrophils do not undergo NETosis in response to *S. aureus* but instead undergo chronic vital NET release. We sought to determine the immunologic perturbations dysregulating neutrophil inflammation in SLE. Herein, we demonstrate that activation of TLR7 and TLR9 inhibit and TLR2 and TLR4 promote expression of LDHB, which is necessary to drive mitochondrial oxidative stress and suicidal NETosis in response to *S. aureus*. Furthermore, excessive type 1 interferon exacerbates vital NET release in response to *S. aureus*. As such, treating murine (MRL/*lpr*) and human SLE neutrophils with anifrolumab to inhibit the interferon alpha/beta receptor prevents chronic vital release, while inhibiting TLR7/9 signaling with hydroxychloroquine restores LDHB expression and suicidal NETosis in response to *S. aureus*. These findings decipher two of the immunologic perturbations dysregulating NETosis in SLE and indicate that NET release during SLE-related inflammation and in response to bacterial pathogens are two independent and possibly antagonistic processes.

Abstract #: S48

Chlamydia trachomatis evades a novel intracellular defense pathway

Chlamydia trachomatis (*Ct*) represents the most common sexually transmitted bacterium in the US and can cause severe sequelae in infected women. No vaccine is currently available. In response to *Ct* infection, host lymphocytes produce the cytokine interferon-γ (IFNγ), which acts on infected epithelial cells and induces innate immune functions in these cells through the induction of IFNγ-stimulated genes (ISGs). Most ISGs remain poorly characterized, but are collectively known to restrict or kill many intracellular pathogens. Unlike these pathogens, *Ct* evades this form of defense in the human epithelium. The mechanisms of this evasion are still under investigation.

To identify how *Ct* evades immunity in epithelial cells, we performed a screen of *Ct* mutants with large alterations in the bacterial chromosome. This approach identified a single bacterial gene, the secreted factor IncS, as a novel virulence factor that drives evasion of innate immunity in human epithelial cells. Additionally, IncS mutant *Ct* strains have developed our understanding of human anti-bacterial defense. This work has revealed a novel IFNγ-inducible pathway that eliminates mutant *Ct* in an intracellular, proteasome-dependent manner. Together, this research represents the identification of a novel human anti-bacterial defense pathway and a novel evasion mechanism by *Ct*.

Authors: Jeffrey R. Reitano[1], Stephen C. Walsh[1], Robert J. Suchland[2], Haley Adcox[3], Maria-Eugenia Cortina[3], P. Scott Hefty[4], Isabelle Derré[3], Jörn Coers[1]

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Abstract #: S49

Title: Paradoxical Inverse Duration-dependent Inhibition of IL-1β Release in Response to Short- and Long-Term Prednisolone Treatment: from Anti- to Pro-Inflammatory

Authors: Jazmin Kelly, Jonathan Daly, Antonio Abbate, Stefano Toldo

Affiliation: University of Virginia – Robert M. Berne Cardiovascular Research Center. 415 Lane Rd (MR5), Charlottesville, VA 22903

Abstract:

Prednisolone (PRED), a widely prescribed and potent anti-inflammatory glucocorticoid, has been recently associated with worse outcomes when used long-term in patients with inflammatory diseases. PRED inhibits inflammatory pathways such as the inflammasome, which regulates Interleukin-1β (IL-1β) release. We aimed to assess the effects of different doses and treatment durations of PRED on IL-1β release in vitro. We used J774A.1 mouse macrophages, exposed to PRED (10, 1, 0.1 μM) for short-term (24 or 48 h) or long-term (2 or 4 weeks) on the induction of the inflammasome by priming with Lipopolysaccharide (LPS,1 μg/ml, 6 h) and triggering Adenosine triphosphate (ATP - 5 mM, 30 min), assessing treatment with PRED before or after priming. IL-1β concentration was measured by Enzyme-Linked Immunosorbent Assay. PRED administered before priming showed a dose-dependent reduction in IL-1β release after 24 and 48 hours. Treatment with PRED after priming showed the same dose-dependent reduction in IL-1β at 24 hours, suggesting that PRED interfered with triggering more than priming. Paradoxically, long-term treatment for 2 or 4 weeks significantly increased IL-1β levels at all tested doses. In conclusion, short-term PRED exhibits a dose-dependent inhibitory effect on IL-1β release in macrophages, interfering with inflammasome triggering, whereas long-term exposure to PRED exacerbates IL-1β release.

Groups with LPS+ATP	IL-1β [pg/ml]
Control – 24 h	983±2
PRED 10 μM – 24 h	31±1 ****
PRED 1 μM – 24 h	57±3 ****
PRED 0.1 μM – 24 h	402±4 ****
Control – 4 w	632±78
PRED 10 μM – 4 w	734±17 #
PRED 1 μM – 4 w	923±25 ####
PRED 0.1 μM – 4 w	763±74 ##

****p<0.0001 vs Control – 24 h

#p<0.05 vs Control – 4 w

##p<0.01 vs Control – 4 w

####p<0.0001vs Control – 4 w

Abstract #: S50

Title: Dimethyl sulfoxide reduces Lipopolysaccharide/Nuclear factor Kappa B (NF-kB)-dependent inflammation

Authors: Gabrielle McDowell[(1)], Stefano Toldo[(2)], Jazmin Kelly[(2)]

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Abstract:

Dimethyl sulfoxide (DMSO) is a solvent that interferes with cell signaling and viability. It has been proposed to be an NLRP3 inhibitor at a 2% concentration in human macrophages. Characterizing a novel NLRP3 inhibitor (NLRP3-i), stimulating mouse macrophages (J774.1) with LPS (Lipopolysaccharide,1 μg/ml, 6h, to activate NF-kB-dependent gene expression) and ATP (5 mM, 30 min, to activate NLRP3), we found that the NLRP3-i (20 to 0.02 μM) in 1% DMSO before LPS, strongly inhibited the Interleukin (IL)-1β release (NLRP3 dependent) but also the Tumor Necrosis Factor (TNF)-α secretion (NLRP3-independent) (>70% reduction of release for all tested doses; $p<0.001$). We, therefore, hypothesized that DMSO could inhibit NLRP3-dependent and -independent cytokine release. To test this, we exposed J774.1 cells to DMSO (0.2, 0.5, 1%) at the time of LPS or after 6 hours (together with ATP, to inhibit NLRP3 activation). IL-1β and TNF-α levels were determined using an ELISA. 1% DMSO significantly reduced IL-1β after LPS+ATP stimulation (205±12 vs 709±7 pg/ml; $p<0.0001$); 0.5% and 0.2% DMSO had no effect. 1% DMSO administered together with ATP did not inhibit NLRP3-dependent release of IL-1β (708±16 vs 709±16; p=NS). 1% and 0.5% DMSO reduced TNF-α levels (1348±37 and 2672±25 vs 3125±27 pg/ml; $p<0.0001$); while 0.2% DMSO had no response. However, this effect was lost when 1% DMSO was given together with ATP (3047±38 vs Control: 3125±27 pg/ml; p=NS). In conclusion, DMSO has a dose-dependent inhibition of IL-1β and TNF-α, preventing LPS / NF-kB-dependent gene expression, which is an NLRP3-independent mechanism.

Abstract #: S51

Title: Discovery of Cross-reactive RSV/hMPV antibodies using LIBRA-seq

Authors: Parastoo Amlashi[1,2], Alexandra Abu-Shmais[1,2], Perry Wasdin[1,2], Ivelin Georgiev[1,2]

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Respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) are two common respiratory viruses associated with high morbidity and mortality in certain populations, such as infants, elderly, and immunocompromised individuals. Most known neutralizing antibodies target the fusion (F) glycoprotein—which is critical for virus and host membrane fusion and is structurally conserved between RSV and hMPV. Three distinct antigenic regions on the F glycoprotein—sites III, IV, and V—have been shown to elicit cross-neutralizing responses. Using LIBRA-seq, a high-throughput sequencing method developed in our lab, we discovered a subset of five novel, cross-reactive antibodies against the fusion (F) glycoprotein. These antibodies were used to further define the antigenic landscape of RSV and hMPV F proteins and were validated against a variety of clinically relevant variants. Overall, by using LIBRA-seq against a panel of diverse, viral pathogens this work has led to the analysis of human antibody repertoire, discovery of novel antibodies, and furthering our knowledge of antibody-antigen interactions which ultimately aid in vaccine design and therapeutic development.

Abstract #: S52

Targeting Bcl-xL with A-1331852 inhibits breast cancer relapse following chemotherapy-induced tumor dormancy

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Breast cancer (BCa) mortality is primarily driven by distant recurrence, often due to the establishment of tumor dormancy following initially successful treatment. Current clinical management offers no intervention to prevent relapse in cancer survivors, except for hormone therapy in ER-positive cases, which still leaves a substantial risk of recurrence. To identify a potential therapeutic target in dormant tumor cells, we investigated the expression of survival pathways in chemotherapy-induced dormant mammary carcinoma cells (MMC) derived from FVBN202 mice. We found that Bcl-xL, an anti-apoptotic protein, is upregulated in dormant tumor cells. We hypothesized that targeting Bcl-xL during dormancy could prevent tumor relapse. Using a low-dose immunogenic chemotherapy regimen (FAC: 5-FU, Adriamycin, Cyclophosphamide), we established tumor dormancy in MMC cells and treated them with or without the selective Bcl-xL inhibitor A-1331852. In vitro, the combination therapy inhibited tumor regrowth following chemotherapy. In vivo, neoadjuvant low-dose FAC combined with A-1331852 significantly reduced tumor relapse, although off-target toxicity limited the dosing of A-1331852. These findings support the development of tumor-targeted delivery systems for A-1331852 and its potential use following neoadjuvant chemotherapy to prevent recurrence in patients with residual dormant disease, including those who fail to respond to initial neoadjuvant chemotherapy.

Funding: This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs through the Breast Cancer Research Program under Award No. W81XWH2210793, and the VCU Cancer Mouse Models Core Shared Resource (CMMC) supported, in part, by funding from the NIH-NCI Cancer Center Support Grant P30 CA 016059.

Abstract #: S53

Phase 2 Study of Perioperative Lenvatinib with Pembrolizumab in Patients with Locally Advanced Nonmetastatic Clear Cell Renal Cell Carcinoma

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Combination therapy with checkpoint inhibitors and targeted therapy represents a new horizon of treatment for renal cell carcinoma (RCC). Lenvatinib is an oral multi-tyrosine kinase inhibitor of VEGFR1-3, FGFR1-4, PDGFRα, RET, and c-Kit. Pembrolizumab is a humanized immunoglobulin G4 monoclonal antibody binding to the programmed cell death 1 (PD-1) receptor. We conducted a phase 2, single-arm trial of lenvatinib with pembrolizumab for 12 weeks in 17 patients with locally advanced biopsy-proven non-metastatic clear cell RCC before surgical resection and 13 cycles of adjuvant pembrolizumab monotherapy. Three patients (18%) experienced a partial response, and 14 patients (82%) had stable disease. There was no disease progression while on neoadjuvant therapy. No treatment grade 4 or 5 adverse events related to lenvatinib or surgery occurred. For correlative studies, we first used flow cytometry to measure phenotypic changes in T-cell surface markers for each patient. The peripheral blood was isolated from patients at different time points: baseline/Cycle 1 Day 1 (C1D1), C1D8, C2D1, C3D1, C4D1, post-treatment, pre-surgery, post-surgery, and C5D1. We measured Ki67+ proliferation marker and HLA-DR+ and CD38+ activation markers in T cells, which indicate recent activation and have been associated with response to immunotherapy. We found that after the two cycles (C1D8 and C2D1) of lenvatinib and pembrolizumab treatment, there was a significant expansion of both CD4+ and CD8+ T cells expressing Ki67 and HLA-DR+CD38+ in the blood compared to the baseline time point. This data suggests that lenvatinib with pembrolizumab treatment was clinically active and safe in the neoadjuvant setting and T cells are activated in the peripheral blood.

Abstract #: S54

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LCK knockout enables allogeneic CAR-T activity

CAR-T technology has achieved significant success in treatment of liquid cancers, but many challenges have hindered development, including poor quality and quantity of the patient's own T cells, and the time taken to produce sufficient CAR-T cells for therapy. "Off-the-shelf" allo-CAR-T could solve these problems, but can potentially cause Graft versus Host Disease (GVHD). Additionally, autologous CAR-T cells have not so far been very effective against solid tumors. We recently showed that CD28-CAR signaling was triggered in the absence of the SRC family kinase LCK, which is essential for TCR signaling. We found that LCK-disrupted CAR-T cells (*disLCK*-CAR-T) signal strongly through CAR and have significantly better *in vivo* efficacy in both liquid and solid tumor models. This is because of their enhanced persistence, induction of memory, and reduced exhaustion phenotype. The *disLCK*-CAR-T blocks activation through endogenous TCR as well as TCR KO (*TRAC*-targeted) CAR-T, both methods blocking the development of GVHD. *disLCK*-CAR-T also shows superior *in vivo* persistence compared to TCR KO T cells, probably because of retained tonic signaling. This non-canonical signaling in CAR-T cells provides new insight into the initiation of TCR and CAR signaling and has important clinical implications for improvement of both autologous and allogeneic CAR-T cells. *disLCK*-CAR-T is being further developed as an allogeneic CAR-T.

Abstract #: S55

Title: Exploring Racial Impact on Endometrial Cancer Immune Landscape

Authors: Natalia Jaeger[1], Aleksandr Taraskin[1], Martina Zocchedu3, Ahmad Alimadadi[4], Yali Zhu[1,3], Michael Nakama[1], Akash Shah[1,4], Sharad Purohit[1,5], Diane Hopkins[4], Richard McIndoe[4], Sharad Ghamande[4], David Mysona[4], Catherine C. Hedrick[1]

Affliations: [1]Immunology Center of Georgia, [2]Georgia Cancer Center Integrated Genomics, [3]La Jolla Institute of Immunology, La Jolla, CA, [4]Medical College of Georgia at Augusta University, [5]Center for Biotechnology and Genomic Medicine

Abstract: Black patients experience higher mortality rates across most cancers, including endometrial cancer (EC), where incidence and death rates are rising over twice as fast as in White patients. Despite controlling for socioeconomic and pathologic factors, Black patients with EC have worse outcomes, yet the biological mechanisms underlying these disparities remain unclear. This study investigates how race influences immune responses in early-stage EC. Using a well-matched cohort of Black and White patients from MCG, we conducted a pilot CITE-seq study in 15 patients with serous and endometrioid EC. Unsupervised clustering of surface protein and gene expression identified 15 immune cell clusters, including CD4 and CD8 T cells, γδ T cells, B cells, NK cells and, monocytes. Preliminary data indicate that γδ T cells are significantly reduced in the circulation of Black EC patients and exhibit an exhausted phenotype, suggesting racial differences in immune regulation. Independent from the CITE-seq cohort, we performed CosMx SMI RNA and protein analysis on 10 and 7 patient tumors, respectively. This technology enables precise, single-cell analysis of immune cell frequencies, localization, interactions, and gene and protein expression within the tumor. Clustering identified four main populations: fibroblasts/smooth muscle cells, cancer cells, endothelial cells, and immune cells. Black patient tumors exhibited fewer total immune cells, particularly myeloid cells. In conclusion, this research highlights significant differences in immune cell composition and localization by race in EC. By defining the impact of race on immune system changes, we aim to inform future strategies and treatments to address racial disparities in EC outcomes.

Abstract #: S56

MECHANISM OF ACTION OF THE IBD SUSCEPTIBILITY FACTOR CREM IN THE DSS-COLITIS MODEL

Shelby L. Schenck, Audrey C. Brown, Christopher F. Pastore, Md Jashim Uddin, and William A. Petri Jr.

Over 2.1 million Americans are afflicted with inflammatory bowel disease (IBD), a number that is increasing (+169%; 2001-2006 vs. 2016-2021) despite advances in therapeutics. Genome-wide association studies (GWAS) conducted by the Petri laboratory have uncovered risk loci *(rs11010067, $p = 1 X 10^{-25}$; rs34779708, $p = 2 x 10^{-25}$)* that confer IBD susceptibility for the gene cAMP-response element modulator (CREM). Furthermore, *Crem* mRNA levels are significantly increased in the gut of patients with ulcerative colitis, suggesting CREM may be involved in IBD pathogenesis and not simply act as a biomarker. CREM possesses multiple isoforms, including ICER, which is theorized to function in autoinflammation and autoimmunity by altering T cell function in patients with systemic lupus erythematosus. I find that under the oft-utilized murine model of IBD, DSS-colitis, inducible full-body deletion of *Crem* confers protection from acute DSS treatment (*%Original Weight, $p < 2.2 x 10^{-16}$; disease activity index, $p = 1.78 x 10^{-8}$, Type III ANOV*A). Preliminary experimentation suggest that this phenomenon is at least partly mediated by the immune system, characterized by significant increase in T helper 17 (Th17) and RORγT^{+} regulatory T cells (Tregs) during acute disease within the colon ($p = 0.023$, $p = 0.007$, respectively). Work is underway to determine the mechanism that inducible CREM deletion employs to ameliorate colitis.

Abstract #: S57

MICE HARBORING A HUMAN AFRICAN G6PD GENE VARIANT CAUSING G6PD DEFICIENCY HAVE DECREASED ATHEROPROTECTIVE IgM^{OSE} AND INCREASED ATHEROSCLEROSIS.

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Epidemiological studies suggest a role of glucose 6-phosphate dehydrogenase deficiency ($G6PD_{def}$) in exacerbated atherosclerosis (AS). To date, no studies have established a causal relationship between the two. To address this, we engineered novel humanized mice harboring the African $G6PD_{def}$ variant ($hG6PD_{A-}$) or the nondeficient G6PD allele ($hG6PD_{ND}$) and tested them for diet-induced AS induced by PCSK9 AAV and Western diet (WD). Sudan-IV en face staining revealed increased AS in the $hG6PD_{A-}$ compared to $hG6PD_{ND}$ mice ($hG6PD_{ND}$ n=8, $hG6PD_{A-}$ n=9, P=0.0227). As $G6PD_{def}$ promotes oxidative stress and IgM to oxidation-specific epitopes (IgM^{OSE}) protects from AS, we assayed IgM^{OSE} levels in our $hG6PD_{A-}$ compared to $hG6PD_{ND}$ mice. Hyperlipidemic $hG6PD_{A-}$ mice had reduced IgM^{OSE}, specifically to phosphorylcholine and a trending decrease in IgM to MDA-LDL (IgM$^{MDA-LDL}$) ($hG6PD_{ND}$ n=8, $hG6PD_{A-}$ n=9, P=0.0114). To determine if differences in IgM^{OSE} in $G6PD_{def}$ mice is due to $G6PD_{def}$ in B cells, we utilized a G6PD specific inhibitor (G6PDi-1) *in vitro* on cultured peritoneal B cells. In parallel with the trending reduction in plasma IgM$^{MDA-LDL}$ in $G6PD_{def}$ mice, G6PD inhibition significantly reduced LPS-induced production of IgM$^{MDA-LDL}$ (Vehicle n=5 G6PDi-1 n=5, P<0.0001). In parallel, human B cells treated with G6PDi-1 resulted in a significant reduction in CpG-induced production of IgM$^{MDA-LDL}$ (Vehicle; G6PDi-1 n=10, P<0.0001). Together, these data are the first to demonstrate that $hG6PD_{A-}$ promotes AS and provide evidence that this effect may be mediated by reduced B cell IgM^{OSE} production. Studies to investigate the cellular and metabolic pathways by which $G6PD_{def}$ reduces the production of IgM^{OSE} in mice and human are ongoing.

Abstract #: S58

TOWARDS MODELING VACCINE DRAINAGE USING EX VIVO LYMPH NODE SLICES IN AN ORGAN-ON-CHIP DEVICE

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The development of particle-based vaccines targeting the lymph node (LN) is becoming increasingly important for disease prevention and treatment. However, predicting their effectiveness remains difficult without long-term trials in animals and humans. After injection, vaccine nanoparticles either drain passively or are transported by antigen-presenting cells to the LN, where they initiate an adaptive immune response. By recreating these processes ex vivo, we believe it will be possible to predict vaccine efficacy using fewer animals and providing richer spatiotemporal insights compared to traditional in vivo methods. Here, we present the development of a microfluidic organ-on-chip model that utilizes ex vivo lymph node slices to mimic the drainage pathways of nanoparticles from the injection site to the LN, designed to later evaluate the immune response to vaccination. These tissue slices preserve native architecture, while the microfluidic platform provides controlled, physiologically relevant fluid flow. We previously showed that this system replicates early events in the lymph node after injection of a molecular vaccine. To simulate upstream events in a particulate vaccine, we are incorporating a cell culture insert containing antigen presenting cells embedded in a gel matrix to model nanoparticle vaccine uptake and transport to the downstream LN slice on-chip. Once fully integrated, this system will allow us to evaluate immune responses to particle-based vaccine delivery and compare them directly to in vivo outcomes. Ultimately, this platform could serve as a predictive tool to guide the design of more effective vaccines with fewer preclinical animal studies.

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Abstract #: S59

CD38 Regulates Age-dependent Protective and Pathological Functions of $CD8^+$ T_{RM} Cells

Gislane de Almeida Santos, Arka Sen Chaudhuri, Harish Narasimhan, Jinyi Tang, Yue Wu, In Su Cheon, and Jie Sun

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Aging increases susceptibility to persistent pathology and sustained inflammation following respiratory viral infections. Our previous work demonstrated that this pathology is associated with excessive $CD8^+$ tissue-resident memory T cell (T_{RM}) responses. However, the mechanisms driving abnormal T_{RM} accumulation and pathological activity remain unclear. In this study, we found that $CD8^+$ T_{RM} cells from influenza-infected young and aged mice express elevated CD38 levels, with aging further enhancing this expression. We also observed an increased frequency of $CD38^+$ $CD8^+$ T_{RMs} in the BAL of elderly human patients following SARS-CoV-2 infection, where this increase inversely correlated with lung function. Furthermore, anti-CD38 treatment in influenza-infected aged mice reduced lung pathology, improved lung function, and decreased $CD8^+$ T_{RM} numbers, suggesting CD38 expression on $CD8^+$ T_{RM} drives pathology during aging. Experiments with influenza virus-infected young CD38KO mice, mixed bone marrow chimeras (WT x CD38KO), and $CD38^{fl/fl}CD8^{cre}$ mice revealed that CD38 intrinsically defines $CD8^+$ T_{RM} maintenance and responses after viral clearance. Additionally, CD38KO mice rechallenged with X31 influenza were more susceptible to infection than WT mice, suggesting reduced T_{RM} numbers impair protective immunity in young mice. These findings reveal that CD38 expression on $CD8^+$ T_{RM} has age-related protective or pathological impacts on lung responses during influenza infection.

Abstract #: S60

Novel Identification of HIV-1 truncated TW10 epitope SW9 dually restricted by HLA-E.

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Numerous factors have made HIV vaccine development extremely difficult. Chief among them is the vast diversity of circulating viral strains which is caused in large part by escape from CD8 T cell responses. Another challenge is identifying epitopes capable of inducing CD8 T cell responses irrespective of variations in the human genetic makeup. The non-canonical HLA-E is of particular importance in that there are mainly 2 polymorphisms in the global population. Furthermore, it was previously demonstrated that CD8 T cell SIV epitopes restricted by major histocompatibility complex E (MHC-E), a homolog of HLA-E, were essential for protection against the establishment of HIV infection. We therefore wanted to identify and characterize HIV epitopes restricted by HLA-E.Thus, we studied the well described HLA-B*57 epitope TW10 and its variant/truncated forms for HLA-E dual restriction. Here we show a truncated epitope of TW10, SW9 elicits polyfunctional CD8 T cell responses (TNF, IFNγ, etc) when restricted by HLA-E and HLA-B*57. Through our HLA-E expressing cell lines we demonstrate SW9 stabilizes HLA-E on the cell surface in contrast to TW10 stabilizing only HLA-B*57 cell surface expression. Through advanced technology we generated HLA-E and HLA-B*57 monomers of which bound SW9 at around 5-8%, and 90-98% respectively. Lastly, Using the golden standard of dextramer generation we detected an ex-vivo HLA-E restricted response using PBMC's of 1 person living with HIV(PLWH). Our results demonstrate that HLA-B*57 described epitopes and their derivatives can be dually restricted by HLA-E, and induce polyfunctional CD8 T cell responses in PLWH paving the way for a promising strategy for HIV vaccine design.

Abstract #: S61

Role of Serum IgA Glycosylation in Modulating HIV Vaccine Efficacy

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HIV remains a global health challenge, with no efficacious vaccine despite 10 efficacy trials over four decades. Despite their limited success, these trials have revealed immune correlates of HIV risk, notably a circulating IgA response with defined specificities that was negatively associated with protection in three distinct trials. This IgA response correlated with reduced vaccine efficacy and negatively modified the protective effects of antiviral Fc effector functions. The mechanisms underlying this apparent immune inhibition of IgA are unclear. Given the immunomodulatory role of IgA glycosylation, we hypothesized that anti-inflammatory glycan profiles contribute to this phenomenon. We analyzed the glycosylation of serum-derived HIV-specific IgA using capillary gel electrophoresis. Preliminary findings reveal distinct glycan profiles for IgA specificities linked to increased HIV risk, suggesting glycosylation differences may distinguish inhibitory from non-inhibitory IgA. Ongoing studies aim to identify glycans responsible and how they interact with innate effector cells. Elucidating IgA's role in attenuating HIV vaccine efficacy could guide improved vaccine and immunotherapy design.

Abstract #: S62

Reprogramming CD8 T cell differentiation through gain-of-function genetic manipulation

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In the context of chronic viral infections and cancer, CD8 T cells differentiate into a dysfunctional lineage state referred to as exhaustion. To define signals that may allow for favorable reprogramming of exhausted T cells, we developed a genetic screening approach to uncover transcription factors that modulate T cell exhaustion when overexpressed *in vivo*. We uncovered gain-of-function alterations that modulate T cell differentiation trajectories and stemness during chronic infection and cancer. We identified undescribed roles of canonical regulators of T cell differentiation such as Blimp1 and KLF2, and we also uncovered unexpected roles for transcription factors that are conventionally silenced in T cells. We found that sustained expression of T-bet led to enhanced formation of TEX-KLR cells that retain cytotoxic activity during chronic viral infection. Last, we found that sustained expression of the transcription factor Nanog increased features of CD8 T cell stemness during infection. Our study identifies new gain-of-function alterations that may inform CD8 T cell reprogramming strategies in infection and cancer.

Abstract #: S63

Title: Investigating the role of α_{2A} adrenergic receptor in acute *C. difficile* infection pathology

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Abstract: *Clostridioides difficile* infection (CDI) is the most common healthcare-related infection in the U.S., with half a million cases annually. CDI mortality and histopathology were previously shown to be significantly reduced upon pharmacological blockade of α2 adrenergic receptors (α_2ARs). Genetic knockout of α2A adrenergic receptor (α_{2A}AR) also improved survival, but where and how it acts in acute CDI remains unknown. *C. difficile* toxins are major cause of pathology and can directly act on colonic epithelium, causing cell damage and cytokine release. Given that the epithelium is a major site of α_{2A}AR expression in the colon, I hypothesize that α_{2A}AR stimulation enhances epithelial response to toxins and the release of pro-inflammatory cytokines. Bulk RNA-seq analysis of CDI mice colon showed profound changes in cell cycle and upregulation of *Mki67* upon treatment with α_2ARs blocker, suggesting barrier repair as a potential mechanism. Further studies will confirm this effect on colonic epithelium. Additionally, neither norepinephrine (α_{2A}AR ligand) nor α_2ARs blocker altered sensitivity to toxin B measured by cell rounding. Future experiments will test if α_{2A}AR activation enhances toxin-induced epithelial cytokine production and release. In summary, α_{2A}AR potentially regulates colon epithelium proliferation without affecting sensitivity to toxin B during acute CDI. Future studies will confirm the anti-proliferative effect and test if α_{2A}AR alters epithelial cytokine release.

Abstract #: S64

Mitochondrial DNA replication stress triggers a pro-inflammatory endosomal pathway of nucleoid disposal

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Mitochondrial DNA (mtDNA) engages innate immune sensors when released from mitochondria, contributing not only to antiviral resistance, but also pathogenic inflammation within many disease contexts. Cells experiencing mtDNA stress due to depletion of the mtDNA-packaging protein, TFAM, or HSV-1 infection exhibit elongated mitochondria, enlarged nucleoids, and mtDNA-cGAS-STING signaling. However, the relationship between enlarged nucleoids, altered mitochondrial dynamics and mtDNA release remains unclear. Here we show that, under a variety of mtDNA replication stress conditions and during herpes simplex virus-1 infection, enlarged nucleoids that remain bound to TFAM exit mitochondria. Enlarged nucleoids arise from mtDNA experiencing replication stress, which causes nucleoid clustering via a block in mitochondrial fission at a stage when endoplasmic reticulum actin polymerization would normally commence, defining a fission checkpoint that ensures mtDNA has completed replication and is competent for segregation into daughter mitochondria. Chronic engagement of this checkpoint results in enlarged nucleoids trafficking into early and then late endosomes for disposal. Endosomal rupture during transit through this endosomal pathway ultimately causes mtDNA-mediated cGAS–STING activation. Thus, we propose that replication-incompetent nucleoids are selectively eliminated by an adaptive mitochondria–endosomal quality control pathway that is prone to innate immune system activation, which might represent a therapeutic target to prevent mtDNA-mediated inflammation during viral infection and other pathogenic states.

Abstract #: S65

EVALUATING EFFICACY AND SIGNALING OF A NOVEL CHIMERIC INHIBITORY RECEPTOR (CIR) FOR REGULATING CD19 CAR-T CELL RESPONSES

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Chimeric Antigen Receptor (CAR)-T cell therapy has emerged as a promising cancer immunotherapy option. While particularly effective against hematologic malignancies, CAR-T overactivation can sometimes trigger Cytokine Release Syndrome (CRS) and produce neurotoxicity, which can be life-threatening. To mitigate this, our lab has developed novel Chimeric Inhibitory Receptors (CIRs), which are co-expressed in T-cells and recognize CRS-associated cytokines. This triggers an inhibitory signal to be sent within the T-cell to regulate potential CAR overactivation. We first developed a CIR prototype with an scFv to recognize CRS-associated cytokines (IFN-γ) and TIGIT as an inhibitory molecule to relay the inhibitory signal to the T cell. This study aims to characterize the differentiation state of CAR-CIR T cells following ex vivo manufacture and expansion, determine anti-tumor efficacy, and evaluate downstream signaling events affected by CIR co-expression. To compare the differentiation profiles of CAR-T and CAR-CIR T cells, we conducted memory subset profiling using flow cytometry analysis. Our results portrayed comparable differentiation patterns amongst CAR-T and CAR-CIR CD4+ cells, but a moderate increase in the proportion of effector memory T cells within CD8+ cells. Following comparison of differentiation profiles, CAR-T and CAR-CIR T cells were introduced to leukemia cell lines as part of a cytotoxicity assay, resulting in similar tumor cell killing. Lastly, in order to assess the effects of CIR regulation on T cell signaling pathways, we evaluated the PI3K/AkT pathway as it has been seen to be regulated by TIGIT signaling. By evaluating AkT phosphorylation (pAkT) within stimulated CAR-T cells, we observed that CIR co-expression limits pAkT signaling, suggesting that CIRs can be mitigating CAR-T overactivation. These findings enhance our understanding of CIR mechanisms and contribute to their potential in improving the safety and efficacy of CAR-T cell therapy.

Abstract #: S66

Fine-tuning chimeric antigen receptor signaling to minimize pro-inflammatory programs in human regulatory T cells

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Abstract
Regulatory T cells (Tregs) hold tremendous therapeutic promise in autoimmunity and transplant rejection. Chimeric antigen receptor (CAR) engineering enables new antigen specificities and disease indications. Yet, CAR Tregs have shown limited efficacy in solid organ transplant rejection and autoimmune disease models, even in conjunction with non-specific immunosuppression. In contrast, antigen-specific T cell receptor (TCR) transgenic Tregs alone can reverse autoimmune disease. We hypothesized that CAR signaling leads to diminished Treg activity compared to endogenous TCR. Indeed, we found that, while CAR-activated Tregs remained stable ($FOXP3^+HELIOS^+$), they exhibited reduced suppression of antigen-presenting cells and effector T cells. Surprisingly, CAR Tregs secreted high levels of pro-inflammatory mediators, such as IFNγ, and acquired CD40L expression. Sorting CD40L-expressing CAR Tregs enriched for inflammatory cytokine-producing cells. Single-cell RNA-seq identified a pro-inflammatory $FOXP3^+HELIOS^+$ Treg subset uniquely induced by CAR activation. Of note, reducing CAR affinity mitigated pro-inflammatory activity by CAR Tregs, underscoring receptor affinity as a critical modulator of CAR Treg biology. Ongoing studies are elucidating the signaling cascades triggered by high-affinity CAR binding in Tregs and dissecting their impact on CAR Treg function.

Abstract #: S67

PATIENTS WITH IGE SENSITIZATION TO GALACTOSE-α-1,3-GALACTOSE HAVE INCREASED CORONARY ARTERY DISEASE ASSOCIATED WITH CXCR5 EXPRESSION ON CIRCULATING MEMORY B CELL POPULATIONS

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IgE-sensitization to galactose-α-1,3-galactose (α-Gal) – the allergen involved in red meat allergy – was shown in a small study to associate with increased coronary artery disease (CAD), as measured by intravascular ultrasound with virtual histology (IVUS-VH). We sought to test this in a larger cohort and using a second coronary artery imaging modality, coronary computed tomography angiography (CCTA). We also performed spectral flow cytometry on PBMCs from 207 new subjects undergoing IVUS-VH (n=61 α-Gal IgE-sensitized; α-Gal+) to identify features of circulating immune cell populations that are associated with increased CAD specific to the α-Gal+ subjects.

In subjects ≤65 years of age undergoing CCTA, the α-Gal+ subjects (n=18) had increased coronary artery calcium (CAC) scores (p=0.0649) and increased CCTA risk scores (p=0.0445) compared to non-α-Gal-sensitized (α-Gal-) subjects (n=35). Using IVUS-VH, α-Gal+ subjects ≤65 years of age (n=35) had increased atheroma burden (p=0.0033) and necrotic core (p=0.0079) compared to α-Gal- subjects (n=85). Moreover, in α-Gal+ subjects, increasing atheroma burden correlated with increased expression of CXCR5 on switched memory B cells (r=0.3241, p=0.0108), and increased frequency of DN1 B cells (r=0.2757, p=0.0315) – neither of which correlate with CAD severity in α-Gal- subjects. CXCR5, which is highly expressed on DN1 B cells, has previously been shown to promote B cell recruitment to antibody-producing niches. Therefore, we hypothesize that trafficking via CXCR5 plays a causal role in atherogenic B cell responses in these patients. Further mechanistic studies are required to better understand B cell involvement in atherogenesis in the context of α-Gal sensitivity.

Abstract #: S68

Investigating the combinatorial advantages of myeloid manipulation and focused ultrasound

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Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer (BrCa) owing to its lack of targetable receptors and resistance to chemical and molecularly targeted therapeutic approaches. While chemotherapy and surgical resection remain the standard of care, these agents have significant side effects and varying patient outcomes. Focused ultrasound (FUS) - a non-invasive and non-ionizing therapy that utilizes targeted acoustic energy to debulk tumors – has displayed immunomodulatory effects in BrCa. However, FUS as a monotherapy has had limited clinical efficacy. I hypothesize that FUS efficacy is, in part, limited by the recruitment of immunosuppressive myeloid populations, particularly in TNBC tumors which have a greater level of myeloid-derived suppressor cell (MDSC) infiltration than other BrCa malignancies. One proposed way to counter the immunosuppressive barrier posed by these cells are through myeloablative chemotherapies; however, our data suggest that while these chemotherapies (gemcitabine and doxorubicin) are systemically myeloablative, they have no myeloablative effects in the TME which may explain its limited efficacy when combined with FUS. I have overcome this by using targeted antibodies (αGr1 or α1A8) which transiently ablate intratumoral myeloid cells. Another proposed way to counter immunosuppressive myeloid cells is through repolarization by TLR agonism. Using two different TLR agonists, I see no evidence of repolarization, but both TLRs promote DC mobilization and activation. Therefore, I hypothesize that (1) overcoming myeloid driven immunosuppression through αGr1 mediated depletion and (2) boosting the immune response through TLR agonism will enhance FUS efficacy.

Abstract #: S69

Abstract Title: $\alpha_v\beta_3$-CAR-T Cell Efficacy is Maintained Following Subtotal Focused Ultrasound Mechanical Ablation in an Orthotopic Breast Tumor Model

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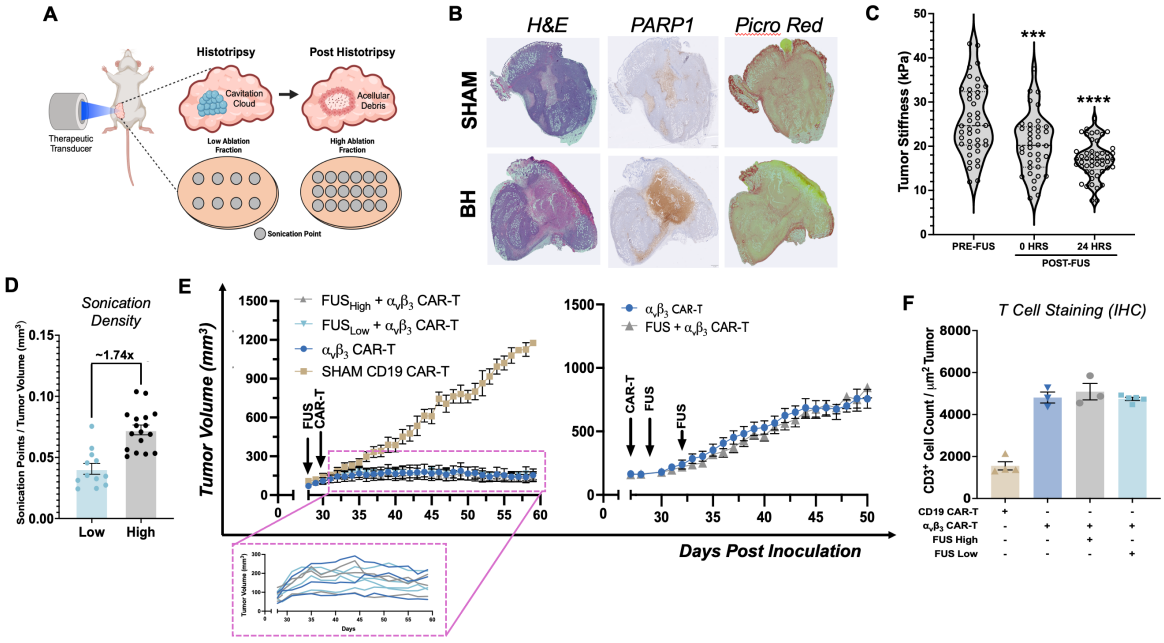
Objectives: To investigate the impact of FUS mechanical ablation (boiling histotripsy; BH) on the efficacy of $\alpha_v\beta_3$-targeted CAR-T cell therapy in breast cancer.
Methods: Orthotopic triple-negative breast tumors (MDA-MB-231) were treated with partial mechanical FUS ablation (BH; Fig.1A) using a custom multi-element ultrasound-guided FUS system (3.78 MHz), followed by i.v. CD19- or $\alpha_v\beta_3$-targeted CAR-T cell administration. Strategies using varied CAR-T doses, distinct sonication densities, and fractionated ablations were evaluated. Tumor outgrowth was monitored via digital calipers. Ablation was characterized by IHC and shear-wave elastography (SWE). Spatial distribution of T-cells was assessed in tumors at select endpoint.
Results: Post-FUS IHC showed clear evidence of BH-induced cell death and liquefaction, demonstrated by H&E, PARP-1 (apoptosis enrichment), and picrosirius red staining (Fig.1B). SWE confirmed a significant decrease in mean tumor stiffness, from ~25.89 kPa to ~16.98 kPa by 24 hours post-FUS (Fig.1C). Strikingly, at two distinct sonication densities (Fig.1D), ablation did not impair $\alpha_v\beta_3$-targeted CAR-T efficacy; this was conserved in a fractionated ablation regimen combined with titrated CAR-T dose (Fig.1E). IHC for CD3+ T cells revealed no significant differences in local distribution or staining density of CAR-T cells (Fig.1F).
Conclusions: While histology and ultrasound imaging showed clear evidence of ablation, subtotal BH did not impact the ability of $\alpha_v\beta_3$-targeted CAR-T cells to effectively control triple-negative breast tumor burden. Future studies will expand ablation regimens and investigate other solid tumor and CAR-T settings.



Abstract #: S70

Suppression of SARS-CoV-2 Pathogenesis through NLRX1

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As of January of 2025, SARS-CoV-2 has been responsible for 7.08 million deaths worldwide, with 3.04 million in the United States alone. COVID-19 induced ARDS is hypothesized to be the result of an overzealous and dysregulated inflammatory response. NLRX1, a pattern recognition receptor, is known to negatively regulate inflammatory signaling during viral infections. However, its actions can depend on cellular context and pathogen. Its role in SARS-CoV-2 infection, however, remains poorly defined. We demonstrate a protective role for NLRX1 in the context of SARS-CoV-2 infection. Single-nucleus RNA sequencing (snRNA-seq) from COVID-19 lung autopsy samples reveal downregulation of *NLRX1* in airway epithelial cells, with differential enrichment of pathways modulated by NLRX1. In murine models, *Nlrx1*$^{-/-}$ mice infected with mouse-adapted SARS-CoV-2 exhibited elevated viral titers, increased airway inflammation and greater epithelial denudation at 2 days post-infection compared to wild-type controls. Furthermore, human airway epithelial cells overexpressing NLRX1, not only had decreased viral replication and increased cell death. SnRNA-seq also revealed dysregulated metabolic pathways in the airways of patients with lethal COVID-19. Notably, infection of NLRX1 overexpressing Calu3 cells altered lactate levels compared to wildtype control, suggesting a role for NLRX1 in modulating host energy metabolism during infection. These findings indicate NLRX1 antagonizes SARS-CoV-2 through multiple mechanisms, including inflammation and metabolic regulation. Elucidating NLRX1's function may be key to understanding the molecular drivers of protective verses pathological immune responses to viral infections.

Abstract #: S71

Glycosylation-Mediated Drivers of Immune Checkpoint Inhibitor Response in Melanoma

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Though immune checkpoint inhibitors (ICIs) have been successful in targeting immunosuppressive tumor-immune interactions in multiple cancer types, resistance is a major clinical challenge. Glycosylation is a post-translational modification that is perturbed in multiple cancers. Elevated sialylation and fucosylation associate with poor prognosis in melanoma. As the tumor microenvironment contains various cell types that communicate through a variety of secreted and surface proteins, many of which depend on appropriate glycosylation for function, we have taken a systems-level approach to uncover glycosylation-mediated cell populations and interactions indicative of response.

We performed interaction inference on publicly available single cell RNA-sequencing (scRNA-seq) data from melanoma patients using LIgand-receptor ANalysis frAmework (LIANA). The resulting networks were used in a supervised statistical learning framework to identify interactions that were predictive of response pre-treatment and that shifted pre- to post-treatment. Immune-immune interactions involved in T cell activation, chemotaxis, and adhesion were upregulated in responders pre-treatment and predicted survival in bulk RNA-seq. An independent scRNA-seq dataset recapitulated expected trends in differences between responders and non-responders. Additionally, tumor cell expression of sialylation pathway genes was correlated with increased CD8+ T cells, decreased CD4+ T cells, and interactions involved in antigen presentation via MHC-I. We find that inclusion of glycosylation in multidimensional systems analysis has potential to improve success of existing immunotherapies and identify targets for combination therapies.

Abstract #: S72

Mechanisms of superior respiratory IgA responses after mucosal vaccination

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Abstract #: S72

Summary

Mucosal immunization and respiratory IgA offer significant promise in protecting against airborne pathogens, including SARS-CoV-2. However, the conditions and mechanisms that lead to the robust induction of respiratory IgA responses following mucosal vaccination remain poorly understood. It is also currently debatable whether mucosal vaccination is still warranted given that most individuals in developed countries have established a hybrid immunity from vaccination and infection. Here we characterized respiratory mucosal immune responses after SARS-CoV-2 infection, vaccination or both in humans. We found that hybrid immunity resulted in moderately increased respiratory IgA and neutralizing antibody responses compared to infection or vaccination alone. However, a direct comparison of hybrid immunity and a mucosal adenovirus-based booster vaccination in animal models revealed that respiratory booster immunization elicited markedly stronger and more durable respiratory IgA, T cell response, and protective immunity against SARS-CoV-2, supporting the promise of respiratory mucosal vaccination. Mechanistically, we found that mucosal booster immunization induced local IgA-secreting cells in the respiratory mucosa, aided by pulmonary $CD4^+$ T cells *in situ*. Strikingly, local IL-21-producing Blimp-1^+ Th1 effector cells were critical in mediating the $CD4^+$ T cell help for IgA production. Furthermore, lung macrophages were important for this mucosal IgA response via the production of TGF-β. Consequently, Spike mRNA coated with pulmonary surfactant (PS) containing liposomes, rationally designed to target lung macrophages, elicits a stronger mucosal IgA response. Collectively, our results uncover a local cellular network supporting enhanced respiratory IgA responses, with implications for the development of optimal mucosal immunization strategies against SARS-CoV-2 and other respiratory pathogens.

Abstract #: S73

RORα regulates the differential development of CD8$^+$ memory subsets.

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The establishment of immunological memory is crucial for antiviral immunity, forming the foundation of vaccine design and enhancing memory responses to combat infections. The retinoic acid receptor-related orphan receptor alpha (RORα), a member of the nuclear receptor family of ligand-regulated transcription factors, has emerged as a potential transcriptional regulator of CD8$^+$ T cell immunity, though its role in these processes remains poorly understood. To explore this, we utilized a mouse model with T cell-specific RORα deletion which resulted in reduced cytotoxic T cell numbers, accompanied by higher $LCMV_{ARM}$ titers in the spleen on day 5 post-infection, compared to wild-type controls. RORα deficiency also promoted T cell circulation leading to redistribution of cells into non-lymphoid tissues, specifically within the liver and adipose tissue during acute LCMV infections. Overall, RORα deficiency promotes the development of classical memory precursors (**MP**), peripheral memory (**TPM**) and tissue-resident memory (**TRM**) cells. Taken together, these results suggest that the RORα promotes terminal effector (**TE**) cell formation and regulates effector function. Our study offers significant insights into the transcriptional regulation of CD8$^+$ T cell differentiation. As a ligand-regulated transcription factor, these findings underscore the potential of RORα as a target for small molecule drugs that may enhance CD8$^+$ T cell responses in the context of vaccines and adoptive cell therapies.

Abstract #: S74

Tissue-specific vulnerabilities of Foxp3-driven transferrin receptor deletion

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During activation, T cells increase transferrin-bound iron uptake via the transferrin receptor, also called CD71. We previously demonstrated that targeting CD71 with an antibody which lowers cellular iron can modify T cell function, with different effects on Th1, Th17, and regulatory T cells (Treg). Tregs had no loss of viability or differentiation, and Foxp3 expression was increased. However, a deletion of *Tfrc* (the gene for CD71) driven by Foxp3-Cre causes a lethal autoimmune phenotype similar to scurfy mice. Despite the severe phenotype of CD71 knockout (KO) Tregs, the mechanisms responsible for maintaining immune homeostasis have been difficult to study. Here, we examined both the Foxp3-^{YFP}-Cre KO mouse model as well as a tamoxifen-inducible KO model in adults to determine the role of CD71 expression in Tregs. We hypothesized that due to a lack of iron that supports mitochondrial metabolism, KO Tregs become unstable due to metabolic adaptations that rely heavily on glycolysis, promoting pro-inflammatory ex-Tregs. KO Tregs were able to suppress disease pathology of the colon in a colitis model, although slightly less effectively than WT Tregs. Necropsy analyses revealed tissue-specific inflammation. The colons of mice with KO Tregs appeared healthy, whereas skin and lung tissue were severely inflamed. Tregs in affected tissues were unstable, adopting T-bet and RORγt expression in addition to Foxp3. Metabolically, KO Tregs had increased Glut-1 expression and a significant decrease in their glycolytic capacity. Thus, Tregs rely on CD71 for their stability and function in tissue microenvironments that promote increased oxidative stress or have low transferrin-bound iron availability such as the lungs and the skin but not the colon.

Abstract #: S75

Characterization of a Murine Model of Enterovirus D68 Associated Acute Flaccid Myelitis and Antibody-Based Therapeutics

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Acute Flaccid Myelitis (AFM) is a poliomyelitis-like syndrome caused by Enterovirus D68 (EV-D68), that results in severe motor deficits and long-term disability in children. To study AFM neuropathogenesis and identify therapies, we developed a neonatal EV-D68 model using IFNAR-/- mice that replicates key clinical features. Mice infected intramuscularly on postnatal day 8 with strain US/IL/14-18952 developed progressive motor deficits starting in the inoculated limb and spreading to contralateral and upper limbs. Viral dissemination kinetics were measured in peripheral organs, brain, and spinal cord. Neuroinvasion was visualized by Basescope, showing viral RNA localized to motor neuron–rich spinal cord regions. Histopathology revealed inflammation and immune cell infiltration correlating with motor neuron injury. Flow cytometry identified CD8+ T cells and macrophages in the spinal cord, with upregulation of interferon-stimulated genes at peak disease.

To evaluate antibody-based therapies, we tested neutralizing, poorly neutralizing, and non-neutralizing anti–EV-D68 monoclonal antibodies (mAbs). The neutralizing mAb EV68-228 as expected eliminated paralysis and improved survival. Notably, the poorly neutralizing mAb EV68-41 also conferred partial protection, increasing survival from 0% to 55% and reducing neurologic symptoms, suggesting Fc effector mechanisms may contribute to efficacy.

This model provides insight into AFM pathogenesis and a platform to evaluate combinatorial therapies. We are now testing EV68-228 with a small-molecule antiviral and immune modulator to enhance viral clearance, suppress neuroinflammation, and improve functional recovery in EV-D68-infected animals.

Abstract #: S76

Epstein-Barr Virus Drives Atypical B Cell Differentiation via an IFNγ-Independent, EBNA2-T-bet Axis

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Abstract

Epstein-Barr virus (EBV) infection of naïve B cells drives their activation and proliferation through viral transcription factors and signaling proteins. This dynamic, heterogeneous process forms lymphoblastoid cell lines (LCLs) *in vitro*. *In vivo*, EBV persists mainly in resting memory B cells, including autoreactive clones, suggesting a link to multiple sclerosis (MS). Another link between EBV and MS is the correlation of EBV viremia with CNS-resident T-bet+/CXCR3+ atypical B cells (ABCs). The mechanism of EBV's role in ABC expansion remains unclear.

Our study found *in vitro* EBV infection of naïve B cells generates a distinct ABC population peaking at day 5 post-infection, persisting at low levels in LCLs. ABC induction increased with age, highest in older females. Mechanistically, EBV induced ABCs independent of IFNγ, instead through EBV nuclear antigen 2 (EBNA2) induction of T-bet. EBNA2 binding sites near the *TBX21* locus were critical for T-bet and CXCR3 expression in infected B cells, unlike ABCs induced by IFNγ, IL-21, and TLR7 agonist. Importantly, we found EBV preferentially persists in ABCs within PBMCs of both early MS and EBV viremic lung transplant patients.

Our findings reveal a novel EBV-driven IFNγ-independent, ABC formation mechanism by EBNA2-mediated T-bet regulation. EBV persistence in ABCs suggests a reservoir with implications for autoimmunity and cancer, providing insight into EBV's contribution to pathogenesis by modulating B cells linked to inflammation.

Abstract #: S77

Exploring the Role of the Genetic Risk Factor RIN3 in Microglia Biology and Alzheimer's Disease

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Alzheimer's Disease (AD) is a neurodegenerative disease characterized by the buildup of protein aggregates resulting in irreversible neuronal loss and subsequent memory and cognitive decline. Recent studies have highlighted the role of microglia, brain-resident macrophages, as critical players in AD pathogenesis. Microglia have crucial roles in engaging amyloid-beta (Aβ) plaques to preserve neuronal health, but genome wide association studies have identified AD-risk genes emphasizing the involvement of the microglial endolysosomal system. One identified risk gene is Ras and Rab Interactor 3 (*RIN3).* Functioning as a guanine exchange factor, RIN3 is a key regulator of RAB5 activity, a small GTPase that regulates early endosome dynamics, organization and function. While it is not known what role RIN3 plays in microglial biology or AD pathology, we hypothesize that RIN3 is vital for proper endocytic organization and adequate microglial responses to Aβ. To test this hypothesis, we generated 5xFAD *Rin3*$^{fl/fl}$ *Cx3cr1*^{Ert2Cre} mice to selectively delete microglial *Rin3* in a mouse model of neurological amyloidosis. Preliminary work has showed that mice deficient in microglial *Rin3* have higher cortical plaque counts, reduced plaque compaction, impaired microglial recruitment to plaque surfaces, and an exacerbation of dystrophic neurite pathology. Looking at microglia responses, *Rin3*-deficient microglia exhibit reduced levels of the TAM receptor Axl and show an increase in lipid droplet burden. Together, this data suggests that *Rin3* deletion results in maladaptive microglial responses to Aβ and a worsened environment for neuronal health. Thus, a deeper investigation into RIN3 functions in microglia and AD pathology is prompted.

Abstract #: S78

Impact of Focused Ultrasound Ablation on Innate Immune-Targeted Antibody Access in Solid Tumors: A Theranostic PET Imaging Study

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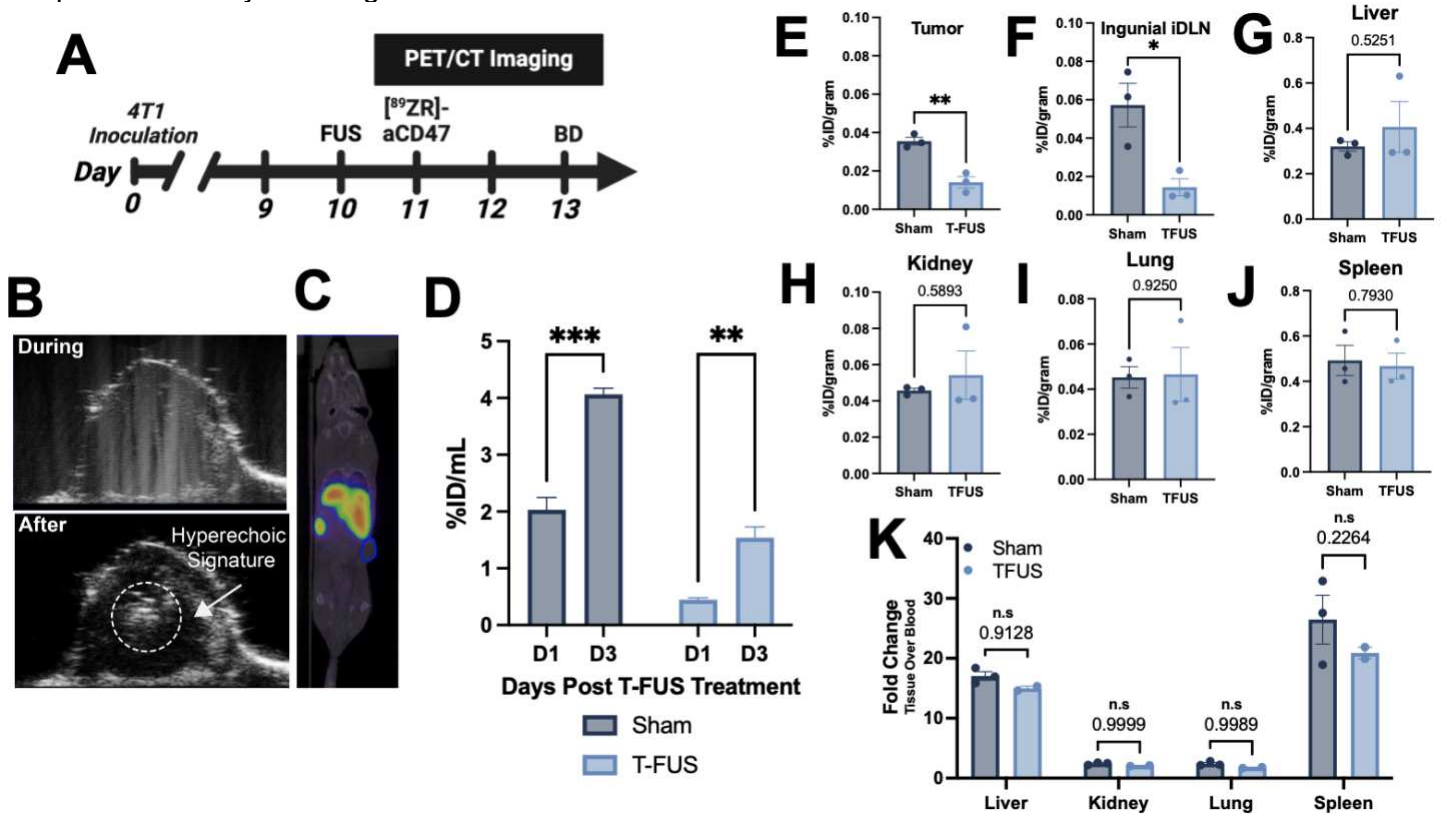
Objective: We aim to use theranostic PET to map the spatiotemporal kinetics of CD47 blockade therapy following subtotal thermal ablation of breast tumors with focused ultrasound (FUS)

Methods: Breast tumor (4T1)-bearing mice underwent FUS thermal ablation T-FUS) with a custom ultrasound-guided FUS system (3.78MHz, 18W, 15s). Sham control and T-FUS mice received a single intravenous injection of [^{89}Zr]-αCD47. Serial static PET/CT imaging was performed serially for up to three consecutive days (Fig 1A). At a terminal time point, ex vivo biodistribution analysis was performed and quantified via automated gamma counter.

Results: Ultrasound images capture before and during T-FUS revealed a hyperechoic signature, which was used as an indicator of ablation (Fig 1A-B). Furthermore, co-registered PET/CT scans revealed the spatiotemporal distribution of [^{89}Zr]-αCD47 uptake. (Fig 1C). Measurement [^{89}Zr]-αCD47 from tumor volumes of interest on standardized uptake value (SUV)-corrected PET/CT images revealed significantly damped antibody penetrance following T-FUS, with a temporal increase across both groups from D1 to D3 (Fig 1D). Ex vivo biodistribution analysis corroborated significantly decreased [^{89}Zr]-αCD47 in ablated tumors (Fig 1E). This trend was mirrored in tumor-draining inguinal lymph nodes (Fig 1F). No appreciable changes in [^{89}Zr]-αCD47 uptake were noted between sham and T-FUS groups across other peripheral organs (Fig 1G-K).

Conclusion: A theranostic PET approach revealed that T-FUS can impact tumor-targeted antibody penetrance, herein demonstrated for [^{89}Zr]-αCD47. Interestingly, an elevation in intratumoral [^{89}Zr]-αCD47 accumulation was noted over days subsequent to T-FUS exposure. Future studies will expand the temporal window of tumor-drug exposure assessment and contextualize findings with analysis of tumor tissue architecture and cellular composition acutely following FUS.



Abstract #: S79

Desmosterol in regulation of B cell subset functions in atherosclerosis.

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Inflammation requires proper metabolic reprogramming, including cholesterol metabolism, to meet the increased energy demands of the cells. Maintaining cholesterol balance is essential in regulating toxic levels of cholesterol accumulation. This process is likely dysregulated in a hyperlipidemic, atherosclerotic environment. While it is known that different B cell subsets utilize divergent metabolic pathways, little is known about B cell subset-specific cholesterol metabolism.

Desmosterol, the last intermediate in the biosynthesis of cholesterol precursor, serves as a negative regulator of macrophage functions. Atherosclerosis induces the accumulation of desmosterol in B cells. To test desmosterol functions in B cells, we generated a mouse model with overexpression of 24-dehydrocholesterol reductase (DHCR24), an enzyme that converts desmosterol specifically in B cells (*Dhcr24$^{fl/fl}$Cd19$^{cre/+}$Ldlr$^{-/-}$ mice*). Reduced desmosterol levels in B cells increase B cell activation in response to BCR-induced activation with increased calcium flux, pSYK, and pBTK. Significantly, reduced desmosterol levels in B cells accelerated atherosclerosis in *Dhcr24$^{fl/fl}$Cd19$^{cre/+}$Ldlr$^{-/-}$* vs control *Dhcr24$^{+/+}$Cd19$^{cre/+}$Ldlr$^{-/-}$* mice. Depletion of desmosterol led to increased mLDL uptake, elevated mitochondrial ROS, and defective complex-I activity in the B cells, particularly in B1a cells. Thus, our data uncover a potential role of cholesterol metabolism intermediates in the regulation of B cell subset functions in health and disease. These findings also highlight the critical function of desmosterol in atherogenesis by reducing inflammation via integration of B cell metabolism in the modulation of the humoral immune response.

Abstract #: S80

Abstract Title: **The Balancing Act: STAT4 As A Regulator In Insufficient Sleep Associated Atherosclerosis And Survival**

Investigator(s): Alina Moriarty, W. Coles Keeter, Shelby Ma, Alex Coleman, Basudha Habisyasi, Larry Sandford, and Elena Galkina

Department(s): *Biomedical and Translational Sciences*, *Division of Cardiopulmonary and Metabolic Diseases*, *Macon & Joan Brock Virginia Health Sciences at Old Dominion University*

Signal transducer and activator of transcription 4 (STAT4) is a transcription factor known for its role in driving T_h1 and T_h17 differentiation. We have shown STAT4 is expressed in neutrophils and functions as a critical regulator of neutrophil activation in health and infection. STAT4-deficient neutrophils exhibit reduced ROS production, faulty neutrophil extracellular trap (NET) formation, and decreased migratory ability resulting in poor survival of animals during bacterial challenge. Interestingly, we have shown the deletion myeloid specific deletion of STAT4 reduced atherosclerosis and improved plaque stability in *Ldlr*$^{-/-}$ mice, highlighting the context-dependent role of STAT4 in neutrophils in various diseases. Sleep is an essential component of life. Atherosclerosis has been extensively linked to insufficient sleep. We showed that poor sleep accelerates atherosclerosis and destabilizes atherosclerotic plaques in a neutrophil dependent manner. Specifically, neutrophils from sleep fragmented mice have enhanced ROS production, neutrophil extracellular trap (NET) formation and chemotaxis. Therefore, we hypothesized that STAT4 is involved in the neutrophil-dependent destabilization of plaques in sleep fragmented, atherosclerotic mice. Survival studies revealed increased intestinal and peritoneal inflammation and decreased survival in atherosclerotic mice which lacked STAT4 in myeloid cells. Interestingly, not all mice died and those which survived the study had reduced atherogenesis. These data suggest that STAT4 is involved in sleep fragmentation-induced myeloid activation and deleting STAT4 in myeloid cells reduces atherosclerosis but it makes the animal vulnerable to any additional immune challenges.

Abstract #: S81

Abstract

Differential adaptative responses of T cells to mild heat stress

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Systemic fever and increased local temperature during inflammation are low-grade heat stresses that shape the immune response. Although generally associated with inflammation, how $CD4^+$ helper T cell subsets differentially regulate their stress response pathways upon heat stimulus is less understood. Understanding the mechanistic underpinnings of heat-related metabolic stress may uncover pathogenicity and potential targets in inflammatory diseases. We found that T_H1 and T_H17 cells cultured at fever-range temperature (FRT, 39°C) have increased reactive oxygen species (ROS) and cytokine production but vary in their response and adaptation to heat stress. T_H1 cells exhibit selective sensitivity to heat resulting in DNA damage and increased death of some cells and a survival adaptation of others through increased mitochondrial mass. In contrast, T_H17 cells readily adapt and avoid cell death through reliance on glutaminolysis. Differential gene expression and pathway analysis on bulk RNA sequencing data of T_H1 and T_H17 cells cultured at 37 or 39°C at different timepoints showed that T_H1 cells were enriched in transposable element (TE) as early as 30 min and 1h of 39°C exposure whereas T_H17 cells did not. This includes the ERV and LTR subfamilies ERVL, ERVK, ERV1, ERV-MaLR and non-LTR LINE subfamily member L1. T_H1 cells' unique TE enrichment in response to heat may prime them for increased genomic instability, resulting in cell death. Future studies will investigate the role of transposable elements in driving this unique stress responses in these subsets as well as the components of the integrated stress response and unfolded protein response involved in the process. Understanding the involvement of these pathways in the overall enhanced T cell responses at FRT may reveal actionable targets for treating autoimmune conditions and other inflammatory diseases.

Abstract #: S82

Title: The helix-loop-helix transcription factor Id3 regulates Marginal Zone B cell development and homeostasis.

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Abstract:

Localized to the splenic marginal zone, Marginal Zone B cells (MZBs) have well-established functions such as orchestrating innate antibody responses to infections. However, mounting evidence supports an expanded role for MZBs beyond infection, especially in the context of autoimmunity. Given their importance in antigen shuttling and immune co-regulation, MZBs are attractive targets for cell-based immunotherapies. As such, a more complete understanding of the molecular mechanisms dictating MZB maintenance and function is needed. One regulator of B cells is the helix-loop-helix protein Id3, although the mechanism through which it modulates MZB cells is unclear. Using flow cytometry, we analyzed B cell-specific Id3-deficient mice and controls and confirmed that Id3 deficiency results in decreased MZBs, despite increased transitional 1 (T1) and follicular-II (FO-II) B cells, two known MZB progenitor populations. To investigate how loss of Id3 leads to these changes, we performed bulk-RNAseq on FACS-purified T1, FO-II, and MZBs as well as scRNAseq with paired BCRseq. We found that loss of Id3 has contrasting effects on T1/FO-II and MZB transcriptomes and GSEA revealed that Id3-deficient MZBs have decreased Notch pathway gene set enrichment, suggesting that Id3 may regulate Notch signaling in MZBs. These findings suggest that Id3 regulates both MZB differentiation and effector functions and motivate further studies of how these effects may impact autoimmunity.

Abstract #: S83

THE RELATIONSHIP BETWEEN ACUTE RESPIRATORY VIRAL INFECTION, CHRONIC INFLAMMATION, AND ACCELERATED AGING

Elizabeth Fink[1,2], Harish Narasimhan[1,2,3], Arka Sen Chaudhuri[1,2], Gislane de Almeida Santos[1,2], Megan Yi[1,2], & Jie Sun[1,2,3]

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In the past three centuries, influenza A viruses have caused 12 pandemics. As seen by the millions of deaths from the COVID-19 pandemic, respiratory viral infections will continue to pose a major public health threat. Despite the decline in new daily cases, many people who were infected by SARS-CoV-2 are now exhibiting long-term symptoms, known as post-acute sequelae of SARS-CoV-2 (PASC). Beyond lung fibrosis and pulmonary symptoms, other organs such as the heart, brain, and kidneys are also affected. Recent studies have shown that not only are aged individuals more prone to severe COVID-19 infection, but that COVID-19 infection is correlated with higher rates of long-term mortality, especially in elderly individuals. As our lab established that SARS-CoV-2 infection can lead to chronic inflammation, we are interested in investigating the relationship between acute respiratory viral infection and consequent chronic inflammation as a potential driver of accelerated aging and long-term mortality. Preliminary data reflected this phenomenon observed in humans, as we showed that compared to age-matched uninfected controls, severely infected middle-aged mice had a decreased median lifespan. Additionally, expression of several inflammatory genes was persistently upregulated in infected middle-aged mice compared to age-matched controls. While the total number of immune cells in the lungs did not show a significant difference, certain subsets of immune cells, including neutrophils, were found at higher levels. As we further investigate features to differentiate age-matched controls, the goal is to develop therapeutic strategies to mitigate chronic inflammation following viral infection to reverse the accelerated aging process.

Abstract #: S84

Accelerated Aging as a Result of Viral Infection

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University of Virginia

Aging is an expanding area of public health interest as an increasing proportion of the world's population reaches old age. At the biological level, aging involves various cellular and molecular processes known as the hallmarks of aging.[1] One of these hallmarks, cellular senescence, occurs when a cell loses its ability to proliferate, and viral infection—a major cellular disruption—can trigger senescence pathways.[2,3] Acute respiratory viral infections, including flu and COVID-19, have been associated with shortened median lifespan in mice and humans, even after recovery.[4] Post-acute sequelae of viral infection further increase the risk of mortality, organ dysfunction, and health burden.[5] However, the specific mechanisms and molecular actors involved in biological aging post-infection are not fully understood. Thus, we established a mouse model of post-flu lifespan to investigate these processes in vitro. Using β-galactosidase assays, we found that tissue from mice with a history of infection had significantly higher levels of senescent cells compared to naive controls. Further, qPCR data showed that infected organs upregulate senescence-associated genes such as *Cdkn1a*, *Cdkn2a*, and *Pai-1*. These findings suggest that respiratory viral infections may contribute to biological aging by promoting cellular senescence pathways. Our findings point to interventions that target these pathways post-infection, improving healthspan and resilience against biological aging.

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Abstract #: S85

Targeting Ifnγ-dependent Pros1 signaling to improve the macrophage anti-tumor response

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Although adaptive checkpoint inhibitors have dramatically changed the therapeutic landscape of melanoma, many patients are unresponsive to immunotherapy or will later relapse. Targeting the innate immune response to reduce tumor growth and improve therapeutic effectiveness is a promising, yet understudied area. Recently, we identified that cancer-secreted Pros1 activates an innate immune checkpoint. Indicating the importance of Pros1 in tumor progression, mice bearing Pros1 deficient tumors show a 63±10% (mean ± SEM) decrease in tumor growth, as well as increased pro-inflammatory immune infiltration. In the context of macrophages, Pros1 lessened the expression of key mediators of the complement, interferon and NOD-like receptor cascades indicating a broad role in suppressing innate immune activation. Unfortunately, the mechanisms governing cancer cell expression of Pros1 are still unknown. Using bulk RNA-seq, we determined that Ifnγ induces the expression of Pros1, as well as other well studied anti-inflammatory mediators, including Pd-l1, Ido1 and Tgf-β. Pros1 expression is increased via the Ifnγ:Ifngr1/Ifngr2 axis and appears to be conserved among several types of tumor cells, including melanoma, breast, lung and pancreatic cancer. In conclusion, we have identified a mechanism through which cancer cells secrete Pros1 in response to Ifnγ signaling. A better understanding of the mechanisms that modulate Pros1 expression may allow us to improve innate immune activation within the tumor and increase the efficacy of chemotherapy in melanoma patients.

Abstract Submissions that are not Presenting Posters			
Abstract #	**Name**	**Institution**	**Title**
1	Michel Enamorado	Icahn School of Medicine at Mount Sinai	Microbiota-Induced Sensory Neuron Hyperinnervation Exacerbates Itch
2	Mahfuzul Islam	Virginia Tech	The Role of EGR2 in B cell development in Systemic lupus erythematosus
3	H. Kay Chung	University of North Carolina Chapel Hill	Multi-Omics Atlas-Assisted Discovery of Transcription Factors for Selective T Cell State Programming
4	Uday Tak	University of Virginia	Bacterial cGAS-like pathways in antiviral immunity
5	Xiaoqin Wei	University of Virginia	Macrophage peroxisomes guide alveolar regeneration and limit SARS-CoV-2 tissue sequelae
6	Jackie Bader	Vanderbilt University Medical Center	Obesity reprograms bone marrow derived macrophages to promote anti-tumor immunity
7	Nandeeni Suryawanshi	Augusta University	Decoding the immune landscape of sarcoidosis through integrative systems immunology
8	Adil Ijaz	Augusta University	Olfr2 Regulates oxLDL Signaling and Processing, Promoting a Proinflammatory Foamy Macrophage Phenotype
9	Brandon Pratt	The University of North Carolina at Chapel Hill	Single-cell gain-of-function genetic screens reveal synthetic regulators of T cell stemness in cancer.
10	Khalia Cummings	Augusta University	Olfactory Receptor 2 Signaling Enhances Monocyte Chemotactic Migration in the Atherosclerotic Aorta
11	Joseph Choi	Vanderbilt University	Defects in B cell differentiation and antibody production due to biallelic TANK mutation

12	Recep uyar	Augusta University	MXene Nanomaterials Exhibit Anti-Inflammatory Properties by Modulating Macrophage Polarization and Gene Expression Profiles
13	Joshua Lopez	UNC Chapel Hill	Regulatory T Cells exhibit lasting transcriptional changes in response to mutein IL-2 treatment in a non-human primate model
14	SohamSoma Sonawane	Emory University	Sex-based Differences in Macaque Models of HIV Pathogenesis and Persistence

Abstract #: 1

Microbiota-Induced Sensory Neuron Hyperinnervation Exacerbates Itch

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The interaction between the immune system and the somatosensory nervous system is essential for modulating itch sensation and maintaining survival. Sensory C-fiber hyperinnervation of the skin is a common feature of chronic itch. However, the mechanisms driving nerve overgrowth and its contribution to itch pathogenesis remain poorly understood. Here, we demonstrate that microbiota-elicited T cells promote skin sensory neuron hyperinnervation, exacerbating itch sensation during inflammation. Using a psoriatic itch model induced by Imiquimod, we observe that mice previously colonized with the bacteria *Staphylococcus aureus* (*S. aureus*) exhibit enhanced CGRPα^{+} sensory neuron hyperinnervation and heightened itch responses. Single-nuclei RNA-sequencing of dorsal root ganglion neurons reveals a transcriptional signature associated with axonal growth in itch-exacerbated mice compared to controls. Mechanistically, CGRPα^{+} sensory hyperinnervation is mediated by the interleukin-17A/interleukin-17A receptor (IL-17A/IL-17RA) axis. As such, disruption of this signaling pathway attenuates sensory hyperinnervation, and reduces itch severity. These findings establish a direct link between microbiota-induced immune activation, sensory neuron plasticity, and skin innervation, identifying sensory hyperinnervation as a pivotal mechanism in psoriatic itch pathogenesis.

Abstract #: 2

Abstract

The Role of EGR2 in B cell development in Systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with an unclear pathogenesis and no effective cure. It is characterized by B cell hyperactivity, which results in the production of pathogenic autoantibodies that contribute to inflammation and tissue damage in multiple organs. Therapeutic depletion of B cells in human lupus patients had mixed success due to the complex heterogeneity of B cell subsets, differences in autoantibody production, and the persistence of antibody-secreting plasma cells (PCs). Our laboratory reported that the transcription factor Early Growth Response 2 (EGR2), a key regulator of immune cell function, was markedly upregulated in the lymphocytes of murine and human lupus. Further, we reported that conditional deleting Egr2 in lymphocytes in lupus-prone B6/lpr mice suppressed pathogenic anti-dsDNA autoantibodies. Notably, Egr2-/-B6/lpr mice exhibit an increased germinal center B (GCB) cell population but fail to differentiate progress into fully differentiated PCs. These data suggest a novel and unexpected regulatory role of EGR2 in the transition from GCB cells to antibody-secreting PCs. These findings provide compelling evidence that EGR2 functions aberrantly in lupus, contributing to disease pathology by facilitating the generation of autoreactive PCs. To further investigate the novel role of EGR2 on B cells, we are conducting extensive experiments using newly developed CD2Cre-Egr2$^{-/-}$ MRL/lpr mice, which have Egr2 deletion in both T and B lymphocytes and CD19Cre-Egr2-/-MRL/lpr mice, where Egr2 is deleted specifically in B lymphocytes to fully understand the role of EGR2 on B cell development. Understanding these pathways will provide critical insights into the signaling mechanisms that promote autoreactive PC development in lupus.

Abstract #: 3

Multi-Omics Atlas-Assisted Discovery of Transcription Factors for Selective T Cell State Programming

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Transcription factors (TFs) orchestrate the differentiation of $CD8^+$ T cells into diverse functional states. To enable precise programming of therapeutically beneficial T cell states in cancer and viral infections, we generated a comprehensive transcriptional and epigenetic atlas of nine distinct $CD8^+$ T cell states. Our analysis catalogued TF activity fingerprints of each state, uncovering new regulatory mechanisms that govern selective cell state differentiation. Leveraging this platform, we focused on two critical T cell states in tumor and virus control with opposing functions: terminally exhausted T cells (TEX_{term}), which are dysfunctional, and tissue-resident memory T cells (T_{RM}), which are protective. Despite their contrasting roles, differences, these states share significant transcriptional and anatomical similarities, making it both challenging and essential to engineer T cells that avoid TEX_{term} differentiation while preserving beneficial T_{RM} characteristics. Through *in vivo* CRISPR screening combined with single-cell RNA sequencing (Perturb-seq), we validated the specific TFs driving the TEX_{term} state and confirmed the accuracy of TF specificity predictions. Importantly, we discovered novel TEX_{term}-specific TFs such as ZSCAN20 and ZNF324, whose deletion enhanced tumor control and synergized with immune checkpoint blockade. Notably ZSCAN20 perturbation also improved the therapeutic efficacy of human CAR-T cells. In parallel, we identified multi-state TFs such as HIC1 and GFI1, which are vital for both TEX_{term} and T_{RM} states. Furthermore, our global TF community analysis and Perturb-seq experiments further revealed how distinct TFs regulate divergent pathways—such as protein catabolism—underlying TEX_{term} development. Together, this work establishes a scalable platform for decoding TF networks across $CD8^+$ T cell states, enabling rational design of T cell-based therapies through targeted manipulation of transcriptional programs.

Abstract #: 4

Bacterial cGAS-like pathways in antiviral immunity

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Bacteria encode ancestral versions of the mammalian cGAS-STING pathway which mediate antiviral immunity against bacteriophages. During phage infection, bacterial cGAS-like enzymes produce nucleotide second messengers including cyclic GMP–AMP (cGAMP), which activates a cognate receptor to execute programmed cell death. This restricts viral replication and protects the bacterial population by preventing phage spread. Several of these receptors are predicted to associate with membranes but are of unknown function. Here, we determine that a widespread class of transmembrane receptors are novel cGAMP-activated channels that restrict phage replication via initiating premature host cell lysis. Remarkably, we identified that the activating nucleotide is 2',3'-cGAMP, which is the first observation of this signaling molecule outside of metazoans. Structural and biophysical analysis reveals that these receptor-channels display selectivity, and a unique assembly mechanism that is distinct from canonical pore-forming proteins such as gasdermins or perforins, but instead resembles known structural features of the mammalian cGAMP receptor: STING which was recently proposed to act as a proton channel. We further demonstrated that a transmembrane STING homologue in bacteria also protects against phage by inducing cell lysis, suggesting pore-formation as a conserved feature of STING receptors. Collectively this work reveals that cyclic dinucleotide receptors in bacterial and metazoan immune pathways may broadly function as membrane-perforating channels.

Abstract #: 5

Macrophage peroxisomes guide alveolar regeneration and limit SARS-CoV-2 tissue sequelae

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Abstract #: 5

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Abstract: Peroxisomes are vital but often overlooked metabolic organelles. Here we found that excessive interferon signaling remodeled macrophage peroxisomes. This loss of peroxisomes impaired inflammation resolution and lung repair during severe respiratory viral infections. Peroxisomes were found to modulate lipid metabolism and mitochondrial health in a macrophage type-specific manner and enhanced alveolar macrophage-mediated tissue repair and alveolar regeneration after viral infection. Peroxisomes also prevented excessive macrophage inflammasome activation and IL-1β release. They protected against stress-induced activation of multiple cell death pathways (apoptosis, pyroptosis, and necroptosis) that fuel an inflammatory storm and exacerbate lung injury. Finally, this protective effect limited the accumulation of $KRT8^{high}$ dysplastic epithelial progenitors following viral injury. Pharmacologically enhancing peroxisome biogenesis mitigated both acute symptoms and post-acute sequelae of COVID-19 (PASC) in animal models. Thus, macrophage peroxisome dysfunction contributes to chronic lung pathology and fibrosis after SARS-CoV-2 infection.

Abstract #: 6

Obesity reprograms bone marrow derived macrophages to promote anti-tumor immunity

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Obesity has been identified as the number two modifiable risk factor for cancer, behind only smoking. This prolonged exposure to excessive nutrients and altered systemic metabolism can promote a distinct immunosuppressive state for tumor immunosurveillance. Obesity has also shown to have a detrimental effect on bone homeostasis by altering bone marrow and hematopoietic stem cell differentiation by shifting stem cells in favor of myeloid progenitors. Further these obesity-induced changes in the bone marrow can lead to dramatic bone marrow remodeling, compromising immune cell functions, which can impact host response to inflammatory diseases and regulation of systemic metabolism. Interestingly, we found that bone marrow derived macrophages (BMDMs) taken from obese (HFD) mice maintain an altered metabolic phenotype, exhibiting increased basal OCR and ECAR compared to BMDMs from lean mice. Additionally, HFD BMDMs exhibited increased mitochondrial ROS, increased lipid uptake and increased pro-inflammatory markers such as MHCII and CD86. Transplanted bone marrow from obese mice into irradiated lean mice resulted in significantly reduced tumor size when subcutaneously injected with MC38 tumor cells. Further, mice that received HFD bone marrow exhibited increased macrophages and CD8 T cell infiltration into tumors. Together these data suggest that an “innate memory” of obesity is preserved in the bone marrow which can impact subsequent anti-tumor immunity and tumor growth.

Abstract #: 7

Decoding the immune landscape of sarcoidosis through integrative systems immunology

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Abstract

Sarcoidosis is a complex systemic inflammatory disease characterized by granulomatous inflammation affecting multiple organs. Epidemiological evidence suggests that sarcoidosis initiation likely involves systemic infection, followed by an antigen-driven immune response leading to T cell activation. However, the underlying immunological mechanisms remain largely unclear. To elucidate the landscape of sarcoidosis, we established a comprehensive systems immunology framework integrating single-cell RNA sequencing (scRNA-Seq), T cell receptor (TCR) profiling, proteomics, and lipidomics. Our results reveal significant insights: (1) we successfully identified expanded T cell clones in sarcoidosis patients, comprising 8.2% (1,099) of all detected clonotypes; (2) we detected interactions between CD4 T cells and CD14 monocytes; (3) proteomic and lipidomic analyses further revealed dysregulation of inflammatory mediators and lipid metabolism in sarcoidosis; and (4) the activation of immune signaling pathways and alterations in lipid biosynthesis are closely associated with immune cell functions and granuloma formation. These insights enhance our understanding of sarcoidosis immunopathogenesis and lay a strong foundation for biomarker discovery and potential vaccine development. Our integrative systems immunology approach offers a powerful strategy for elucidating complex autoimmune diseases and advancing translational research.

Abstract #: 8

Olfr2 Regulates oxLDL Signaling and Processing, Promoting a Proinflammatory Foamy Macrophage Phenotype

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Atherosclerosis is a lipid-driven inflammatory disorder characterized by plaque formation in the arterial intima, primarily due to the accumulation of modified low-density lipoproteins (LDL) such as oxidized LDL. After oxLDL exposure, macrophages acquire a lipid-loaded “foamy” phenotype. When overwhelmed, foamy macrophages can switch to a proinflammatory state, leading to disease progression. This mechanism is still unknown. In this study, we described that Olfr2, whose depletion has been shown to be protective in atherosclerosis, is involved in the proinflammatory switch of foam cells that leads to atherosclerosis progression. We found that the lack of Olfr2 in BMDMs mediates less oxLDL accumulation, significantly reducing the lipid marker (BODIPY) signal compared to WT. Gene expression analysis showed no significant changes in scavenger receptors such as *CD36* and *Lox1* expression. Whereas the genes regulating LXR pathway and oxLDL efflux genes like *Abcg1*, *Abca1* were significantly upregulated in Olfr2 KO BMDMs compared to WT. Moreover, anti-inflammatory genes such as *Il10, Il1r2, and Trem2* were also upregulated in Olfr2 depleted macrophages compared to WT, while Il1β was downregulated in Olfr2 KO macrophages. We observed a marked increase in surface Trem2 expression in Olfr2 KO BMDMs compared to WT. We hypothesize that activation of LXR pathway in olfr2 depleted macrophages have dampening effect on nitric oxide (NO) production which is demonstrated by lower NOS2 gene expression and nitric oxide production in olfr2 knockout macrophages compared to WT. Delineating Olfr2 downstream signaling effects and its interplay with Trem2 receptor might help to identify a potential target to lower the disease burden of atherosclerosis in humans.

Keywords: Foamy macrophages, Olfr2, oxLDL, atherosclerosis

Abstract #: 9

Single-cell gain-of-function genetic screens reveal synthetic regulators of T cell stemness in cancer.

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Adoptive cell therapies (ACTs) represent a promising cancer immunotherapy approach with curative potential. Although ACT has revolutionized the treatment of certain hematologic malignancies, the immunosuppressive tumor microenvironment of solid malignancies limits the efficacy of ACT. An intriguing advantage of ACT is that T cells can be genetically engineered for augmented anti-tumor function. However, our understanding of the genes controlling T cell responses in cancer are primarily limited to loss-of-function experiments and screens (using tools such as ‘knockout’ mice or CRISPR/Cas9 strategies). T cells can also be genetically engineered to overexpress or artificially activate genes, empowering gain-of-function (GOF) manipulations. Assessing how GOF alterations in T cells impact ACT efficacy in vivo represents a relatively unexplored area in tumor immunology. We have recently established an in vivo single-cell GOF screening approach that allows us to profile concurrent GOF manipulations in mouse T cells in vivo at single-cell resolution. Using this approach termed “MORF-seq,” we uncovered undescribed roles for numerous transcription factors influencing T cell stemness, cytotoxicity, and exhaustion. Notably, we established crucial roles for KLF4, OCT4, and SOX2 in programming CD8 T cell stemness during infection and cancer. Last, we demonstrate that rational GOF manipulation of T cells can enhance ACT efficacy in mouse models of cancer. These studies highlight the therapeutic potential of synthetic reprogramming of CD8 T cells in cancer.

Abstract #: 10

Olfactory Receptor 2 signaling Enhances Monocyte Chemotactic Migration in the Atherosclerotic Aorta

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Atherosclerosis is an inflammatory disease of the arterial wall initiated by monocyte recruitment and macrophage (Mφ) function. Human and mouse vascular macrophages, mouse bone marrow-derived macrophages (BMDMs), and monocytes express several olfactory receptors (ORs), including Olfr2 and its human orthologue OR6A2. Ligation of Olfr2 and OR6A2 with their ligand octanal activates the NLRP3 inflammasome pathway while knocking out (KO) Olfr2 in mice reduces atherosclerosis progression in vivo. Octanal, a product of lipid peroxidation, is proposed to derive from endothelial dysfunction (ED), which leads to atherosclerosis initiation. Recently, we identified Olfr2/OR6A2 protein expression in about 20% of monocytes, with expression increasing in disease states in humans and mice. However, the role of Olfr2 in monocytes is unknown. Interestingly, competitive adoptive transfer experiments with labeled WT and Olfr2 KO bone marrow monocytes injected into CD45.1 Apoe KO mice, revealed that WT monocytes were significantly enriched in the aorta compared to Olfr2 KO monocytes and could differentiate into Olfr2+ macrophages. In vitro, BMDMs significantly migrate in response to octanal while Olfr2 KO do not. Overall, these findings reveal Olfr2 as a previously unrecognized chemotactic receptor in monocytes and macrophages, highlighting its potential role in directing immune cell migration within the atherosclerotic aorta and as a potential therapeutic target.

Abstract #: 11

Defects in B cell differentiation and antibody production due to biallelic TANK mutation

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Exposure to bacterial respiratory pathogens is commonplace, but severe recurrent disease requiring hospitalization may suggest an underlying inborn error of immunity (IEI). We studied a pair of siblings with decade-long histories of severe recurrent lower respiratory tract infections and positive cultures for *Streptococcus pneumoniae*. These patients had normal total B cell frequencies, but reduced generation of memory B cells and low antibody levels. CyTOF analysis showed all other immune cell subsets were present at normal frequencies. No mutations in known IEI-causal genes explained these patients' phenotypes. By whole exome sequencing, we identified a novel mutation in TANK, which segregated in an autosomal recessive manner. This mutation caused a frameshift and early truncation of the TANK protein, and complete TANK deficiency in the patients' cells. TANK is as adaptor protein with poorly characterized roles in both canonical and non-canonical NF-kB signaling. Using an *in vitro* B cell differentiation assay, we know that TANK-deficient patients' B cells seem 'blocked' at the $IgD^{-}CD27^{-}$ double-negative stage and proliferate poorly. Using scRNA-seq of patient and healthy control samples, we show an accumulation of intermediate B cells with unique gene expressions, such high *SOX5* and *ZEB2*. Mechanistically, TANK suppresses the canonical NF-kB pathway and serves as a critical determinant of B cell proliferation and differentiation, antibody secretion, and protection from respiratory pathogens.

Abstract #: 12

MXene Nanomaterials Exhibit Anti-Inflammatory Properties by Modulating Macrophage Polarization and Gene Expression Profiles

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Macrophages are innate immune cells with diverse phenotypes and functions shaped by their microenvironment. The M1/M2 classification, despite its limitations, has been pivotal in understanding their role in inflammation. Recent single-cell technologies have uncovered a broader spectrum of macrophage activation states, each contributing to immune balance and disease progression. Identifying and modulating these subsets remains a key challenge. MXenes, a novel class of two-dimensional transition metal carbides and nitrides, are emerging as promising biomedical tools due to their biocompatibility and unique surface chemistry. These properties enable their detection at single-cell and tissue levels using mass cytometry (CyTOF), making them highly attractive for immune profiling. We observed that MXenes are readily internalized by myeloid precursors, monocytes, and macrophages without compromising cell survival or differentiation. Early uptake is detectable within 30 minutes, partially via clathrin-mediated endocytosis. RNA sequencing and CyTOF analyses demonstrated that MXenes modulate monocyte and macrophage phenotypes, promoting an anti-inflammatory, M2-like response by reducing pro-inflammatory markers and upregulating anti-inflammatory markers such as CD28. In vivo studies further confirmed similar phenotype shifts in peritoneal macrophages. These results position MXenes as powerful tools for tracking and regulating macrophage function, with significant potential for future biomedical applications.

Key words: MXene, transcriptome, macrophages, macrophage molarization

Abstract #: 13

Regulatory T Cells exhibit lasting transcriptional changes in response to mutein IL-2 treatment in a non-human primate model

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Graft-versus-host disease (GVHD) remains a major complication of organ transplantation, often leading to graft failure. Mutein IL-2 (mIL-2), an engineered cytokine, has shown promise in reducing GVHD severity by selectively promoting the expansion of regulatory T cells (Tregs), which suppress cytotoxic and inflammatory immune responses, particularly from $CD8^+$ T cells. However, the long-term transcriptional and functional effects of mIL-2 treatment on Tregs remain unclear. To address this, we administered mIL-2 to non-human primates and performed single-cell RNA sequencing (scRNA-seq) on peripheral blood samples collected at multiple time points post-treatment. Transcriptomic analysis in R, including gene set enrichment approaches, revealed that Tregs appear to adopt a less inflammatory and less stress-responsive state following mIL-2 exposure. Specifically, we observed negative enrichment of gene sets associated with oxidative stress, hypoxia, apoptosis, and UV response. These findings provide new insights into the immunomodulatory effects of mIL-2 and will assist its further development as a therapeutic strategy to prevent GVHD.

Abstract #: 14

Title: Sex-based Differences in Macaque Models of HIV Pathogenesis and Persistence

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Background: Women make up the majority of people living with HIV globally but remain underrepresented in cure research. Biological sex influences HIV pathogenesis, as women often show stronger immune responses and lower viral set points during acute infection. However, studies on viral persistence during antiretroviral therapy (ART) have produced inconsistent results, highlighting the need for more research into how sex impacts HIV outcomes and the development of effective cures.

Methods: We conducted a meta-analysis of 18 studies to explore the impact of biological sex on viral persistence in the rhesus macaque (RM) SIV/SHIV infection model. Inclusion criteria included infant or adult studies of SIV or SHIV infection and daily ART treatment with data on viral dynamics and reservoir size. Data were extracted from 296 RMs, 70% (207) male, and 86% (142) adults. Variables included viral strain, plasma viral load (PVL), ART duration, and reservoir measures in peripheral blood (PB), lymph node (LN), and gastrointestinal (GI) tract. We compared peak PVL, cell-associated viral DNA, and intact proviral DNA levels by sex.

Results: Female adult macaques had significantly higher peak PVL compared to males (median 1.8×10^7 versus 1.3×10^7 copies/ml plasma, p=0.0062). Among SIV-infected RMs treated with suppressive ART, females had significantly higher SIV DNA levels in CD4+ T-cells from PB (2.1×10^3 versus 3.4×10^2 copies/million cells, p<0.001), LN (median 2.1×10^3 versus 4.4×10^2 copies/million cells p=0.0164), and GI tract (median 3.3×10^1 versus 7.7 copies/million cells, p=0.0004). Levels of CD4+ T-cells with intact provirus were similar in females and males (PB: 1.2×10^3 versus 8.8×10^2 copies/million cells, p=0.1604; LN: 1.2×10^3 versus 9.2×10^2 copies/million cells, p=0.7074). No significant differences were observed in SHIV-infected RMs.

Conclusion: These analyses revealed that, compared to males, females have higher viral loads prior to ART, a greater number of infected cells in multiple tissues during ART, but similar intact viral reservoir size. Underlying drivers may include enhanced viral spread pre-ART due to increased immune activation combined with clearance of intact reservoirs during ART due to better antiviral immunity in females. Our data support increased inclusion of women in HIV cure research for more accurate assessment of the impact of cure-directed interventions on virus persistence.