IMMUNOLOGY

University of Virginia

Immunology Research Day

Immunology in focus after a global pandemic

May 27, 2022

8:30 AM – 5:30 PM

PINN Hall Conference Center

Center for BRAIN IMMUNOLOGY AND GLIA

Carter Immunology Center

CIIR

Jointly sponsored by the Carter Immunology Center, Center for Brain Immunology and Glia, and the Center for Immunity, Inflammation, and Regeneration

Immunology Research Day Program Schedule

8:30 – 9:00 Continental Breakfast & Coffee (Panera)

9:00 – 9:05 Introduction: Immunology in focus after a global pandemic

9:05 – 9:45 Inspiring UVA Immunology – Vision Statements from the Center Directors

Tajie Harris BIG Steps Forward

Mark Okusa CIIR: Advancing research in immunity, inflammation, and Regenerative Medicine

Coleen McNamara Envisioning Inclusive Excellence in Immunology at the Beirne Carter Center

Session 1. **Co-Chairs: Maureen Cowan and Aman Mangalmurti**

9:50 – 10:05 Michael A. Kovacs
10:05 – 10:20 Xiao-Yu Zhao
10:20 – 10:35 Hema Kothari
10:35 – 10:50 Philip V. Seegren
10:50 – 11:05 Mirna Perusina Lanfranca
11:05 – 11:20 Shuhei Kuwabara

11:25 – 12:10 **Poster Session A.**
12:10 – 1:00 **Poster Session B.**

1:00 – 1:45 Lunch Break (Ivy Provisions – Box Lunches)

Session 2. **Co-Chairs: Harish Narasimhan and Cassidy M. R. Blackburn**

2:00 – 2:15 Andrea R. Merchak
2:15 – 2:30 Xiaoqin Wei
2:30 – 2:45 Tania E. Velez
2:45 – 3:00 John M. Cronk
3:00 – 3:15 Michal Cholko
3:15 – 3:30 Vikram Sabapathy

3:30 – 3:45 Coffee Break (Medical Center Catering)

Session 3. **Co-Chairs: Nabin Poudel and Isaac Babcock**

New Horizons in Immunology

3:45 – 4:05 **Jaeda Coutinho-Budd (BIG)** – Drosophila as a model for investigating neuron-glial interactions in health and disease

4:05 – 4:25 **Sanja Arandjelovic (CIIR)** – Efferocytosis in inflammatory arthritis – friend or foe?

4:25 – 4:45 **Carrie Cowardin (CIC)** – Uncovering the role of the maternal gut microbiome in early life growth and immunity.

5:00 – 5:30 Awards Reception & Closing – (Catered by Feast)

Oral session I Abstracts

9:50 – 10:05

MENINGEAL LYMPHATIC DRAINAGE PROMOTES T CELL RESPONSES AGAINST *TOXOPLASMA GONDII* BUT IS DISPENSABLE FOR PARASITE CONTROL IN THE BRAIN

Michael A. Kovacs[1], Maureen N. Cowan[1], Isaac Babcock[1], Lydia Sibley[1], Katherine M. Still[1], Samantha J. Batista[1], Ish Sethi[1], and Tajie H. Harris[1]
[1]Brain Immunology Center, University of Virginia, Charlottesville, VA, USA

Immunology Center Affiliation: BIG

Toxoplasma gondii is an intracellular protozoan parasite that causes chronic brain infection in a wide range of mammalian hosts. Animal studies have demonstrated that continuous T cell recruitment to the brain is necessary for parasite control. However, it has remained unclear how T cells outside the central nervous system sense and respond to brain-derived microbial antigen. Here, we test the hypothesis that the newly described meningeal lymphatic system promotes T cell immunity during *T. gondii* brain infection. We find that chronic brain infection is associated with significant expansion of parasite-specific T cells in the cerebrospinal fluid (CSF)-draining deep cervical lymph nodes. T cell activation at this site peaks during the late stages of chronic infection after parasite has invaded the brain. Flow cytometric analysis of CSF reveals a population of activated dendritic cells that is not present in the CSF of naïve mice. Mature dendritic cells are also mobilized in the meninges, specifically in areas where CSF protein can be sampled. Disrupting meningeal lymphatic drainage by ligating the collecting vessels leads to impaired T cell responses in the deep cervical lymph nodes. Surprisingly, in spite of reduced T cell activation and IFN-gamma production at this site, T cell responses in the brain remain intact, likely due to ongoing antigenic stimulation in lymph nodes that drain non-central nervous system tissue. Overall, we provide evidence that meningeal lymphatic drainage supports robust parasite-specific T cell responses in the deep cervical lymph nodes. Nonetheless, we find that drainage of central nervous system material to this site is dispensable for host-protective T cell responses in the brain.

10:05 – 10:20

INDUCIBLE NITRIC OXIDE SYNTHASE AT GBP2-TARGETED *TOXOPLASMA GONDII* VACUOLES MEDIATES PARASITE KILLING IN MURINE MYELOID CELLS

Xiao-Yu Zhao[1,2], Bocheng Yin[1,2], Jan C. Urbán Arroyo[1,2], Samantha Lempke[1,2], Sarah E. Ewald[1,2]
[1]Department of Microbiology, Immunology and Cancer Biology; [2]Carter Immunology Center, University of Virginia

Immunology Center Affiliation: CIC

Toxoplasma gondii is an obligate intracellular pathogen of rodents and humans. The parasites' ability to grow and evade cell autonomous immune sensing depends on the parasite vacuole membrane (PVM). The PVM is novel organelle formed from the host plasma membrane during invasion and maintained by dozens of secreted parasite effectors. Interferon-inducible GTPases (IIGs) are central mediators of PVM attack and *Toxoplasma* clearance, however, the precise mechanism linking IIG attack of the PVM, *Toxoplasma* and inflammatory host cell death is not clear. This is linked to a lack of tools to selectively purify the PVM. To identify host and parasite proteins recruited to the PVM, we used Automated Spatially Targeted Optical Micro Proteomics (AutoSTOMP), a novel discovery proteomics technique that uses a confocal microscope to visualize and photo-label proteins associated with the PVM for identification by LC-MS. Using IFNγ to induce IIG attack of the PVM and TLR2 ligand to augment to killing effect, we examined protein recruitment to the PVM in infected bone marrow derived dendritic cells (BMDCs). Using AutoSTOMP we identified over 300 host proteins differentially enriched near the PVM of unprimed, TLR2, IFNγ and IFNγ+TLR2 stimulated conditions. By modifying the AutoSTOMP program to selectively purify proteins on PVMs co-localized with the guanylate binding protein 2 (GBP2), we determined inducible nitric oxide synthase (iNOS) were enriched on parasites that were destined to die. IFNγ priming was sufficient to induce iNOS expression, however, TLR2 co-stimulation dramatically enhanced iNOS upregulation. Interestingly, IFNγ-mediated *T.gondii* restriction was dependent on ins through nitric oxide (NO) production rather than arginine depletion. Mechanistically, GBP2 recruitment to the PVM was unaffected in iNOS-deficient cells, albeit parasite continued to grow in GBP2-targeted PVM, underscoring a novel mechanism of iNOS-mediated parasite killing and/or IRG/GBP regulation. In summary, we report a new tool to study the PVM proteome and identify host regulators of parasite clearance.

10:20 – 10:35

IL-6-INDUCED SIGNALING IN PD-1$^+$ CD4 EFFECTOR MEMORY T CELLS IS ASSOCIATED WITH HUMAN CORONARY PATHOLOGY

Hema Kothari[1,2], Chantel McSkimming[1], Fabrizio Drago[1], Corey M. Williams[3, 4], Eli R. Zunder[3] and Coleen A McNamara [1,2]
[1] Carter Immunology Center, University of Virginia, Charlottesville, VA
[2] Cardiovascular Division, Department of Medicine, University of Virginia, Charlottesville, VA
[3] Department of Biomedical Engineering, University of Virginia, Charlottesville, VA
[4] Cardiovascular Research Center, University of Virginia, Charlottesville, VA

Immunology Center Affiliation: CIC

Objective: Murine data, prospective epidemiological studies, and genetic association data support a potential causal role of IL-6 signaling in atherosclerosis development. IL-6 inhibitors have emerged as potential therapeutics for reducing events in both stable coronary artery disease (CAD) and acute coronary syndromes. IL-6-induced immune regulation plays an important role in atherosclerosis, however, a comprehensive map of IL-6 signaling in human immune cells is currently lacking. We developed a 32-antibody custom mass cytometry (CyTOF) panel to characterize IL-6 signaling across all major human immune cell subsets and applied it to identify IL-6-induced immune signatures linked with unstable atherosclerotic plaque.

Methods: Peripheral blood mononuclear cells from healthy donors and subjects with CAD (N=18) undergoing virtual histology-intravascular ultrasound imaging (IVUS-VH) were stimulated with vehicle and IL-6, stained, and acquired in CyTOF. Unsupervised analysis algorithms (SPADE, UMAP, and Leiden clustering) were used to identify immune cell subsets and IL-6-induced intracellular phosphorylation status.

Results: IL-6 induced STAT1 and STAT3 activation in CD4 and CD8 naïve T cell subsets and CD4 memory T subsets. We identified that IL-6 also activates STAT5 within the CD4 and CD8 naïve T subsets. Other cell types such as CD14$^+$ monocytes, and CD11c$^+$, and CD123$^+$ dendritic cells also showed IL-6-induced STAT activation. Notably, IL-6-induced phosphorylation of STAT1 and STAT3 in PD-1$^+$CD4$^+$ effector memory (EM) T cell subtype was associated with higher CAD burden and unstable plaque features (Table; Spearman correlation analysis).

Conclusions: We have identified a link between PD-1$^+$ CD4 EM T cells and CAD. Work is in progress to understand how IL-6 modulates function of PD-1$^+$ CD4 EM T cells that lead to worsened CAD. Functional immunophenotyping approaches like the one we used could be utilized in clinical trials of IL-6 inhibition to determine if the amount of IL-6-induced or the degree of inhibitor blocking of p-STATs in specific immune cell subtypes might predict response to therapy allowing for a unique customized biomarker for precision medicine.

Fold Change Median		p-STAT1	p-STAT3	p-STAT5
Max. Stenosis (%)	R-value	0.64	0.78	0.12
	P-value	0.004	0.0001	0.64
Atheroburden (%)	R-value	0.76	0.73	0.16
	P-value	0.0002	0.0006	0.52
Fibrous (%)	R-value	-0.63	-0.7	-0.42
	P-value	0.005	0.001	0.08
Fatty (%)	R-value	0.68	0.49	0.08
	P-value	0.002	0.039	0.76
Necrotic (%)	R-value	0.5	0.63	0.43
	P-value	0.03	0.005	0.08
Calcium (%)	R-value	0.48	0.62	0.32
	P-value	0.04	0.006	0.19

10:35 – 10:50

REDUCED MITOCHONDRIAL CALCIUM UPTAKE IN MACROPHAGES IS A MAJOR DRIVER OF INFLAMMAGING

Philip V. Seegren[1, 2], Logan R. Harper[1], Taylor K. Downs[1, 2], Xiaoyu Zhao[2, 3], Shivapriya Viswanathan[1], Marta E. Stremska[2, 3], Rachel J. Olson[1], Joel Kennedy[1], Sarah E. Ewald[2, 3], Pankaj Kumar[4, 6], Bimal N. Desai[1, 2, 5]

Affiliations:

[1]Pharmacology Department, University of Virginia School of Medicine, Pinn Hall, 1340 Jefferson Park Avenue, Charlottesville, VA 22908
[2]Carter Immunology Center, University of Virginia School of Medicine, 345 Crispell dr. MR-6, Charlottesville, VA 22908
[3]Microbiology, Immunology, and Cancer Biology Department, University of Virginia School of Medicine, Pinn Hall, 1340 Jefferson Park Avenue, Charlottesville, VA 22908
[4]Biochemistry and Molecular Genetics Department, University of Virginia School of Medicine, Pinn Hall, 1340 Jefferson Park Avenue, Charlottesville, VA 22908
[5]University of Virginia Cardiovascular Research Center, 415 Lane Rd, Charlottesville, VA 22908
[6]University of Virginia, Bioinformatics Core, 1312A Pinn Hall, 1340 Jefferson Park Avenue, Charlottesville, VA 22908

Immunology Center Affiliation: CIC

Summary

Chronic low-grade inflammation is a hallmark of aging, but its etiology is not well understood. Mitochondrial dysfunction has been linked to both cellular senescence and age-related inflammation or *inflammaging* but the fundamental mechanisms underlying these links are unclear. We analyzed age-related gene expression in different human tissues and discovered that in blood, the mRNA expression of Mitochondrial Calcium Uniporter (*MCU*) and its regulatory subunit *MICU1* inversely correlated with age. MCU is the pore-forming subunit of a Ca^{2+}-selective ion channel complex residing in the mitochondrial inner membrane and is the main conduit for a rapid Ca^{2+}-influx into mitochondrial matrix. We found a significant reduction in the mitochondrial Ca^{2+}-uptake capacity of macrophages derived from aged (80-90 weeks old) mice. These macrophages display amplified cytosolic Ca^{2+} elevations and increased inflammatory output. The *Mcu-/-* macrophages of young mice (<25 weeks old) express more inflammatory genes at baseline and show a hyper-inflammatory response when stimulated. Reduced mitochondrial Ca^{2+}-influx increases cytosolic Ca^{2+} dynamics and potentiates downstream NF□B activation, which is central to inflammation. We have identified a set of genes that are regulated by mitochondrial Ca^{2+} uptake in macrophages. The siRNA-mediated knockdown of *MCU* in human blood-derived macrophages recapitulates the phenomenon – the mitochondrial Ca^{2+}-uptake is greatly reduced, cytosolic Ca^{2+} elevations are more pronounced, and the macrophages are hyper-inflammatory. Our findings pinpoint the MCU complex as a keystone molecular apparatus that links age-related changes in mitochondrial physiology to macrophage-mediated inflammation. Resident macrophages are intrinsic to every organ system and the steady erosion of their mitochondrial Ca^{2+}-uptake capacity in tissue-resident macrophages may play a germinal or exacerbating role in many age-related neurodegenerative, cardiometabolic, renal, and musculoskeletal diseases that afflict us.

10:50 – 11:05

SUSTAINED HOMING RECEPTOR LIGAND EXPRESSION IN MELANOMA-ASSOCIATED ENDOTHELIAL CELLS REQUIRES SIGNALING FROM BOTH TNFα AND IFNγ

Mirna Perusina Lanfranca, Amelia Nachbar, Robin Lindsay, Katarzyna Stasiak, Andrew Dudley, Victor Engelhard. Carter Immunology Center, University of Virginia, Charlottesville, VA, USA.

Immunology Center Affiliation: CIC

In several tumor types, the presence of CD8 T cells is associated with better clinical outcomes. homing receptor ligands (HRL) on tumor-associated vessels regulate CD8 T cells entry but the factors that control their expression are incompletely characterized. Thus, we evaluated regulation of expression of VCAM-1, ICAM-1, and the chemokines CXCL9 and CXCL10 by inflammatory cytokines and its antagonism by the angiogenic factors VEGF and FGF2 in tumor associated endothelial cells (TEC). We analyzed expression by real time PCR, flow cytometry, and immunofluorescence. We found that in TEC from B16F1OVA tumors implanted in either IFNγ KO and TNFα receptor KO mice, expression of these HRL was substantially decreased, demonstrating co-dependence on both signaling pathways. Next, TEC were isolated from mCherry labelled B16-F1-OVA melanoma tumors, implanted in mice with ZSGreen labelled ECs, and cultured *ex vivo* with either or both cytokines. We found that the sustained expression of these HRL required both cytokines. In contrast, TNFα alone upregulated VCAM-1 and ICAM-I, and IFNγ alone upregulated CXCL9 in 3 long-term murine EC lines isolated from normal tissues. Additionally, VEGF had little effect on either cytokine-driven expression of any of these HRL in either TEC or EC lines, while FGF2 was strongly inhibitory.

These results demonstrate that regulation of HRL expression in TEC is complex, and not adequately recapitulated by commonly used EC lines. They suggest that the co-dependence observed in TEC is a consequence of combined deficiencies in signaling pathways intermediates, transcription factors, and/or epigenetic modifications. However, the exclusive regulation of subsets of HRL by individual cytokines in EC lines suggests the possibility that some signaling pathways may be also actively suppressed. In the long term, a better understanding of this regulation in TME can directly lead to improved effectiveness of existing cancer immunotherapies.

Support: USPHS

11:05 – 11:20

NOTCH SIGNALING CONTRIBUTES TO THE RENOPROTECTIVE EFFECTS OF CHOLINERGIC ANTI-INFLAMMATORY PATHWAY

Shuhei Kuwabara, Shuqiu Zheng, Junlan Yao, Mark D. Okusa

Division of Nephrology and Center for Immunity, Inflammation, and Regenerative Medicine, University of Virginia, Charlottesville, VA

Immunology Center Affiliation: CIIR

Abstract

Acute kidney injury (AKI) is a serious disorder because of high mortality, but no effective treatment exists. A novel strategy for the prevention and treatment of AKI is activating the cholinergic anti-inflammatory pathway (CAP), a neuroimmune circuit. The CAP is initiated by vagus nerve stimulation (VNS) and subsequent activation of splenic and peritoneal macrophages (MΦ) to suppress inflammation. We previously showed that an increase in Hairy and Enhancer of Split 1 (HES1) in MΦ contributes to renoprotective effects of CAP. HES1 expression is regulated by Notch signaling pathway, however, a direct relationship between the CAP and Notch signaling has not been reported. In the current study, we focused on Notch signaling in splenic and peritoneal MΦ to unravel the detailed molecular mechanism of CAP.

To test if VNS activates Notch signaling in MΦ, we performed VNS before ischemia-reperfusion injury (IRI). At 24 hours after injury, the expression of Notch2 receptor was upregulated in splenic MΦ of VNS-treated mice. Then, we used optogenetic methods to identify specific neural circuits associated with Notch activation during VNS because the vagus nerve is heterogeneous and composed of efferent and afferent fibers. As a result, optogenetic stimulation of vagal efferent fibers did not alter the Notch2 expression in splenic MΦ. Furthermore, we examined whether Notch signaling mediates anti-inflammatory responses in MΦ. In splenic MΦ of *Notch2* knockout mice, VNS-mediated anti-inflammatory effects were attenuated compared to littermate controls.

Taken together, our findings suggested that: 1) Notch2 signaling in splenic MΦ contributes to renoprotection by the CAP, 2) optogenetic stimulation of the vagus nerve showed that efferent VNS does not mediate Notch2 activation, indicating that afferent VNS may be responsible for initiating Notch2 signaling in splenic MΦ

Oral session II Abstracts

2:00 – 2:15

T CELL ARYL HYDROCARBON RECEPTOR ACTIVITY TUNES THE GUT MICROENVIRONMENT TO SUSTAIN AUTOIMMUNITY

Andrea R. Merchak, Hannah J. Cahill, Lucille C. Brown, Ryan M. Brown, Courtney Rivet-Noor, Rebecca M. Beiter, Erica R. Slogar, Deniz G. Olgun, and Alban Gaultier

Immunology Center Affiliation: BIG

Multiple sclerosis (MS) is an incurable T cell driven autoimmune disease of the central nervous system. The contribution of the environment and, in particular, the gut flora to MS etiology is well documented but not well understood. The aryl hydrocarbon receptor (AHR) is a potential candidate for better understanding the underpinning of this connection. AHR binds numerous molecules present in the environment and is a therapeutic target for MS; however, its precise function in T lymphocytes, the orchestrators of MS, has not been fully described. Most research has focused on how AHR responds to the microbiome to regulate the immune system. Here, we show that AHR knockout in T cells has the capacity to change the gut microenvironment itself. Furthermore, the gut microenvironment resulting from lack of AHR in the T cell compartment has a protective effect in the mouse model of MS, experimental autoimmune encephalomyelitis (EAE). At a mechanistic level, immune cell exposure to the gut microenvironment leads to early apoptosis after activation. This may be attributed to the observed increase in bile acids and short chain fatty acids. The reduced fitness of the immune cells leads to lower immune cell load in the spinal cord lesions and myelin recovery. Our study demonstrates a newly emerging role for AHR in mediating the interdependence between T lymphocytes and the microbiota while identifying new potential molecular targets for the treatment of MS and other autoimmune diseases.

2:15 – 2:30

SIMULTANEOUSLY MITIGATE HYPERINFLAMMATION AND HYPERGLYCEMIA FOLLOWING RESPIRATORY VIRAL INFECTION BY TARGETING MPC

Xiaoqin Wei[1*], Bibo Zhu[1*] and Jie Sun[1,2#]

[1]Carter Immunology Center, University of Virginia, Charlottesville, Virginia, USA
[2]Division of Infectious Diseases and International Health, Department of Medicine, University of Virginia, Charlottesville, VA, 22903, USA.
*These authors contributed equally to this work

[#]Correspondence author, js6re@virginia.edu

Immunology Center Affiliation: CIC

Individuals with hyperglycemia are at great risk of developing exaggerated pulmonary inflammation following infection with respiratory viruses including SARS-CoV-2. Conversely, SARS-CoV-2 infection has been linked to the development of new onset of hyperglycemia and diabetes. Despite global vaccination efforts, SARS-CoV-2 infection continues to be a great public health challenge, and corticosteroids targeting hyper-inflammation remain the mainstay treatment for severe COVID-19. However, steroidal medicine arises the risk of hyperglycemia, limiting its usage. Here, we showed that a second-generation thiazolidines drug MSDC-0602K (MSDC), a mitochondrial pyruvate carrier inhibitor, simultaneously decreased excessive pulmonary inflammation and hyperglycemia in normal and obese hosts during influenza infection, as such, reducing host morbidity and mortality. scRNA-seq analysis revealed that MSDC treatment reduced lung macrophage inflammation, and myeloid-specific ablation of MPC2 led to dampened lung inflammation. Further, MSDC inhibited human lung macrophage inflammatory responses following SARS-CoV-2 infection, and dampened inflammation in lung autopsies of COVID-19 patients. Mechanistically, MSDC treatment destabilized HIF-1α protein, a transcription factor promoting inflammatory responses of lung macrophages, by repressing cellular Acetyl-CoA levels and HIF-1α acetylation. Our results suggest that MSDC has the great potential to treat severe COVID-19 or other respiratory viral infection, particularly in patients with underlying metabolic conditions, by simultaneously repressing hyper-cytokinemia and hyperglycemia.

2:30 – 2:45

EXPANSION OF T REGULATORY CELL SUBSETS IN PULMONARY FIBROSIS

Tania E. Velez*[1,2], Young me Yoon[1], Maile K. Hollinger[1], Kelly Blaine[1], Cathryn Lee[1], Mary Strek[1] and Anne I. Sperling[1,2]

1. Department of Medicine, CIC, University of Virginia, Charlottesville, VA
2. Department of Medicine, University of Chicago, Chicago, IL

Immunology Center Affiliation: CIC

In idiopathic pulmonary fibrosis (IPF), there is evidence of a persistent immune response in the circulation, lung tissue, and lung-associated lymph nodes (LLN), however the role of the immune system remains unclear. We have shown that LLN enlargement in IPF predicts worse prognosis suggesting that immune responses are increasing at end stages of the disease. Currently, the role of T regulatory cells (Tregs) in pulmonary fibrosis in both humans and mice remains controversial, as Tregs can potentially be both anti-fibrotic and pro-fibrotic. Thus, to investigate the function of Tregs in pulmonary fibrosis, we interrogated their phenotype in the LLN of IPF patients. Using spectral flow cytometry, we found subsets of Tregs that specifically expand in IPF patient's LLNs compared to controls. Interestingly, the majority of Tregs in IPF patients' LLN had lower expression levels of CD25 compared to controls. One of these subsets co-expressed ICOS, PD-1, and BCL6, which are markers of T follicular regulatory cells. In conjunction with this, germinal center B cells and T follicular helper cells were increased in IPF patients compared to controls. These data suggest that there are germinal responses that T follicular regulatory cells are unable to resolve, and importantly, that an antigen-driven response is involved. Available databases on the lung cells of IPF patients demonstrates that Tregs in the lung also have low expression of CD25. To further our studies, we have confirmed that similar Treg subsets expand in a model of pulmonary fibrosis in mice. Thus, we have characterized unique subsets of Tregs that are expanded in the enlarged LLN of IPF patients and may be actively involved in disease progression.

2:45 – 3:00

LICENSED NK CELL-MEDIATED ANTIVIRAL IMMUNITY VIA DISPARATE LY49 RECEPTORS COUNTERS MCMV GP34-MEDIATED IMMUNE EVASION

John M. Cronk,[1,2] Karolina H. Dziewulska,[2,3] Rowena B. Crittenden,[2,4] Marie-Louise Hammarskjöld,[1] and Michael G. Brown[1,2,3,5*]

[1]Microbiology, Immunology and Cancer Biology, University of Virginia, Charlottesville, Virginia, USA. [2]Beirne Carter Center for Immunology Research, University of Virginia, Charlottesville, Virginia, USA. [3]Pathology, University of Virginia, Charlottesville, Virginia, USA. [4]Medicine, Division of Nephrology, University of Virginia, Charlottesville, Virginia, USA. [5]Center for Immunity, Inflammation, and Regenerative Medicine.

Immunology Center Affiliation: CIC

The murine CMV (MCMV) immunoevasin *m04*/gp34 binds and escorts MHC I to the surface of infected cells whereby MHC I-gp34 complexes can engage NK cell inhibitory receptors (IRs) and prevent NK cell attack. Despite this viral evasion mechanism, we have shown that MHC I D^k-dependent MCMV control requires self-D^k licensed NK cells expressing the Ly49G2 IR which selectively expand and kill infected target cells during infection. We further found that mAb-mediated neutralization of the Ly49R activation receptor abrogates Ly49G2-mediated antiviral immunity. Whereas both Ly49R and Ly49G2 can bind D^k, the molecular basis by which these functionally disparate receptors sense MCMV and enhance NK cell antiviral immunity remains poorly understood. Here, we show that Ly49R is downregulated during MCMV infection on proliferating Ly49G2$^+$ NK cells in a D^k-dependent and MCMV gp34-independent manner. D^k-specific Ly49R downregulation corresponded with enhanced maturation of and upregulation of the high affinity IL-2 receptor, CD25, on licensed Ly49G2$^+$ NK cells, suggesting a role for Ly49R in recognition of MCMV-modified D^k molecules. Remarkably, in contrast to WT mice, Ly49G2-deficient mice exhibited susceptibility to WT MCMV relative to Δ*m04* MCMV, indicating that Ly49G2 is required to counter gp34-mediated immune escape. To better understand how Ly49G2 facilitates antiviral immunity, we used CRISPR/Cas9 to ablate Ly49G2 in mature primary NK cells and found that the IR is critical for accrual of CD25$^+$ Ly49R^{Lo} antiviral effectors. Furthermore, Ly49G2-mediated licensing enhanced CD25 expression on Ly49R$^+$ NK cells responding to IL-12/15 cytokines or MCMV-infected targets *in vitro*, which may drive proliferation of Ly49R$^+$ NK cells responding to MCMV *in vivo*. Ly49R$^+$Ly49G2$^+$ NK cells additionally required cell intrinsic expression of MHC I D^k for optimal accumulation during MCMV infection. Active sensing of a shared MHC I ligand by activating and inhibitory Ly49 receptors thus may represent an important mechanism of licensed NK cell-mediated viral target detection and host-defense.

3:00 – 3:15

ENGULFMENT AND CELL MOTILITY PROTEIN 1 (ELMO1) ROLE IN ACUTE KIDNEY INJURY

Michal Cholko and Sanja Arandjelovic

Center for Immunity, Inflammation and Regenerative Medicine (CIIR), Department of Medicine, University of Virginia, Charlottesville, VA, USA

Immunology Center Affiliation: CIIR

Acute kidney injury (AKI) is a sudden episode of kidney failure caused by range of health conditions. High mortality in AKI highlights an unmet clinical need. Polymorphisms in the human *ELMO1* gene associate with diabetic nephropathy and lowering *Elmo1* expression in mice was shown protective against severe disease. ELMO1 is a cytoplasmic protein that functions in apoptotic cell clearance and cell migration.

We have previously shown that loss of ELMO1 in neutrophils reduces neutrophil recruitment in inflammatory arthritis, without affecting response to bacterial challenge. Since immune cell recruitment and renal cell death directly affect AKI outcomes, we examined the role of ELMO1 in AKI by subjecting wild type and *Elmo1*$^{-/-}$ mice to three different models of AKI: 1. Renal ischemia-reperfusion (IRI), 2. Cisplatin-induced nephrotoxicity, and 2. Endotoxin-induced septicemia.

In renal IRI, *Elmo1*$^{-/-}$ mice had reduced loss of kidney function and expression of kidney injury markers, as well as lower levels of inflammatory cytokines. Neutrophil-derived granular proteins were reduced in the serum and kidneys of *Elmo1*$^{-/-}$ mice. On the other hand, loss of kidney function in sepsis was not impacted by *Elmo1* deletion, consistent with an intact response to bacterial insults.

Surprisingly, *Elmo1*$^{-/-}$ mice subjected to cisplatin-induced AKI displayed accelerated loss of kidney function and trends toward increased kidney injury. We also noted elevated levels of apoptotic cells in the kidneys of cisplatin-injected *Elmo1*$^{-/-}$ mice, indicating potential for ELMO1 function in clearance of dying cells in this model.

Collectively, our data suggest distinct roles of ELMO1 in acute kidney injury induced by different damage triggers. Our current focus is on ELMO1 function in neutrophil recruitment in renal IRI and apoptotic cell clearance in cisplatin mediated injury. We propose that ELMO1 could represent a new therapeutic target in the context of ischemic renal injury.

3:15 – 3:30

IL-33 (INTERLEUKIN 33)/ST2 (INTERLEUKIN 1 RECEPTOR-LIKE 1) 'ALARMIN' SIGNALING AXIS REGULATES MACROPHAGE RESPONSE IN KIDNEY INJURY

Vikram Sabapathy[1], Gabrielle Costlow[1], Saleh Mohammad[1], Sanja Arandjelovic[1], Rahul Sharma[1]

[1]Center for Immunity, Inflammation and Regenerative Medicine (CIIR), Division of Nephrology – Department of Medicine, University of Virginia, Charlottesville, VA

Immunology Center Affiliation: CIIR

Macrophages (MΦs) following kidney injury play a vital role in inflammation, repair, and fibrosis. IL-33 is a nuclear-localized alarmin cytokine typically released upon tissue damage. IL-33 signals through a receptor complex of IL-1 receptor-like 1 (IL1RL1), also known as ST2. ST2 is expressed in a variety of immune cells, including myeloid-derived cells such as MΦs. However, the MΦ regulation by IL-33 is not fully understood. In this study, we will try to understand the role of ST2+ MΦs using acute and chronic kidney injury models. For myeloid cell-specific deletion of *ST2*, $ST2^{fl/fl}$ mice were crossed with $LysM^{Cre}$ mice.

Summary of Results: We hypothesized that the ST2 receptor could play a vital role in activating and mobilizing immune cells to the injury site. Preliminary results from metanalysis of single-cell RNA seq data indicated high expression of ST2 receptor on macrophages. We performed acute and chronic ischemia perfusion injury studies to examine the physiological relevance of endogenously expressed ST2+ myeloid cells during renal injury. The *in vivo* data from the acute injury model indicated a loss of ST2 on myeloid cells resulted in attenuation of renal injury. On the contrary, results from the chronic injury model showed absence of IL33/ST2 signaling resulted in exacerbation of injury. To understand the role of ST2 on MΦs, ST2 sufficient and deficient MΦs were isolated from the peritoneum and bone marrow and analyzed *in vitro*. RNA seq analysis showed that loss of ST2 downregulated important functional genes. Subsequently, efferocytosis assay on both peritoneal and bone-marrow-derived MΦs demonstrated that the loss of ST2 on MΦs led to decreased functional phagocytosis activity. Through seahorse assay, it was also observed that ST2 deficient macrophages exhibited attenuated mitochondrial metabolism.

Conclusion: Activation of the IL-33/ST2 signaling axis is essential for regulating the functional activity of MΦs.

New Horizons Session Abstracts

3:45-4:05

Drosophila AS A MODEL FOR INVESTIGATING NEURON-GLIAL INTERACTIONS IN HEALTH AND DISEASE

Jaeda Coutinho-Budd, PhD

Glia are critical components of the nervous system that provide trophic factors for neuronal survival, buffer ions & neurotransmitters to regulate neuronal function, insulate axons, facilitate synapse formation and plasticity, and remove harmful debris in disease and injury. Glial dysfunction can therefore lead to a wide array of nervous system conditions including neurodevelopmental disorders, neurodegenerative diseases, and aggressive cancers like malignant glioblastoma. While most of the progress in understanding neuron–glia signaling has been made at synaptic and axonal contacts, exactly how glia interact with neuronal cell bodies in the central nervous system is almost entirely unexplored. Additionally, how glia balance their supportive functions with their roles in recognizing and clearing neuronal debris, and the ways in which these change in neurodegenerative disease like Alzheimer's warrants further investigation. We take advantage of the unparalleled power of *Drosophila* genetics to answer these questions using *in vivo* tools that allow for genome-wide screens and precise spatiotemporal control of different molecules within multiple cell types at the same time. By utilizing this simplified yet sophisticated system for studying universal glial functions, our findings will lay the foundation for mechanistically interrogating these important neuron-glia interactions in nervous system health and disease.

4:05-4:25

EFFEROCYTOSIS IN INFLAMMATORY ARTHRITIS – FRIEND OR FOE?

Sanja Arandjelovic, PhD

Homeostasis in multicellular organisms is exquisitely regulated by cell death via apoptosis (non-inflammatory cell death). Apoptosis plays a defining role during development and is an integral part of the response to tissue damage and infection, leading to resolution of inflammation and return to homeostasis.

Apoptotic cells are present in inflamed joints of patients with rheumatoid arthritis (RA), a chronic inflammatory disease which affects 1 in 100 people globally and millions in the US. Persistence of dying cells in tissues is usually indicative of pathology, as these cells are promptly removed by live neighbor cells in a process called ‘efferocytosis’. When uncleared, apoptotic cells may undergo secondary necrosis (inflammatory cell death) and release their intracellular contents into the surrounding environment to further fuel inflammation.

However, metabolites released by apoptotic cells, also referred to as ‘good-bye’ signals, elicit cellular responses that actively promote tissue resolution and are essential for the maintenance of immune ‘silence’. If and/or when this ‘switch’ from apoptosis to necrosis occurs in vivo is not well described. There may be unappreciated benefits of reduced efferocytosis that could further our understanding of efferocytosis in homeostasis and inflammation and open new avenues in therapeutic designs for the treatment of rheumatoid arthritis.

4:25-4:45

UNCOVERING THE ROLE OF THE MATERNAL GUT MICROBIOME IN EARLY LIFE GROWTH AND IMMUNITY

Carrie Cowardin, PhD

Globally, undernutrition contributes to 44% of childhood deaths under the age of five. Stunting, or reduced linear growth, is a major complication of undernutrition that is associated with poor health outcomes. Stunted mothers are more likely to experience complications during childbirth, and to give birth to stunted children. In turn, many children that are born stunted do not recover despite current therapeutic interventions.
The maternal immune system plays a major role in shaping offspring physical, cognitive and immune development. The gut microbiota likewise influences both local and systemic immunity, raising the possibility that the maternal microbiome could influence fetal and childhood development. To investigate this possibility, we have developed a model of intergenerational undernutrition using human microbial communities to colonize germ-free mice. Using this model, we have found that offspring derived from breeding pairs colonized with undernourished microbiota have altered growth and immune development when compared to offspring of breeders colonized with microbiota from a healthy donor. We plan to pursue these findings by identifying critical microbes that mediate these effects, clarifying when microbial colonization is most impactful on host growth, and investigating immune pathways that mediate microbial signals, with the goal of identifying specific microbial species and immune pathways for targeted intervention to reduce the global burden of undernutrition.

Poster Abstracts

ABSTRACT #A-1

THE ROLE OF CHEMOTACTIC CUES IN DEVELOPMENT OF IN VITRO MODELS OF LIVE LYMPH NODE TISSUE SLICES

Lavoisier Akoolo, Alexander Ball, Rebecca Pompano
Carter Immunology Center Department of Chemistry, University of Virginia

Immunology Center Affiliation: CIC

Live lymph node slice cultures are a promising in vitro system that mimics the functional niche observed in vivo for short-term cultures. The system is however challenged by reduced viability in long-term culture and egress of immune cells, affecting optimal immune function. Here we evaluated the physiological changes in long term culture of LN slices and tested conditions for cellular retention. We considered that retention/egress of T and B cells in lymph nodes is guided in part by the CCL21/CCL19-CCR7 chemokine axis, expressed by fibroblastic reticular cells (FRCs) and T cells respectively, while LTαβ expressed on T cells binding to LTβR expressed on stromal cells is crucial in triggering the expression of chemokines such as CCL19, CCL21. First, we sought to analyze dynamics of these axes in the long-term LN slice culture. Murine LN slices were cultured for 48 hours and analyzed for various chemotactic factors. We observed high numbers of egressed lymphocytes associated with decline in secreted CCL21 after 48 hours (*$p<0.05$), and expression of CXCR3 and CCR7 by CD4 T cells was reduced in egressed cells versus fresh slices, as was LTβR in FRCs after 48 hours of culture (*$p<0.05$). Examination of bound CCL21 showed no change in mean quantity bound, but a redistribution to the periphery at 48 hours. LTα1β2 supplementation increased secretion of CCL21, but did not prevent net cellular loss. We found that recirculation of egressed cells by overlaying them back onto slices after 48 hours of culture resulted in reentry prior to subsequent repeated egress. Our data indicates that deficient chemotactic cues are correlated with cellular egress, and overlay of egressed cells temporarily restored lost cells but did not prevent net egress. We hypothesize that a continuous cellular re-circulation and cytokine supplementation model may provide long term mitigation of cellular egress and optimization of long lymph node slice culture.

ABSTRACT #B-2

DEVELOPING A NOVEL IMMUNOMETABOLISM ASSAY BY IMAGING OXYGEN CONSUMPTION RATE IN LIVE LYMPH NODE TISSUE

Parastoo Anbaei*, Alexander G. Ball[†‖], Marissa A. Gonzales[§‖], Drake Dixon*, Timothy N.J. Bullock[§], and Rebecca R. Pompano*[‡‖]

*Department of Chemistry, University of Virginia College of Arts and Sciences, Charlottesville, Virginia 22904, United states
[†]Department of Microbiology Cancer Biology and Immunology, University of Virginia, Charlottesville, Virginia 22903, United States
[‡] Department of Biomedical Engineering, University of Virginia School of Engineering and Applied Sciences, Charlottesville, Virginia 22904, United States
[§]Department of Pathology, University of Virginia, Charlottesville, Virginia 22903, United States
[‖]Carter Immunology Center, University of Virginia, Charlottesville, Virginia 22903, United States

Immunology Center Affiliation: Other

Understanding the link between immune responses and changing cellular metabolism provides opportunities to understand inflammatory disease mechanisms. Oxygen consumption rate (OCR) is a useful metric of metabolic activity, as many lymphocyte activities are oxygen dependent. Recently, several optical oxygen sensing assays have been reported, however, none provided spatially resolved optical measurement of local oxygen in live ex vivo tissue samples. In this work, we mapped out the OCR in murine lymphoid tissue slices ex vivo, by collecting time-lapse images of an oxygen responsive glass window. We generated oxygen-sensitive luminescent thin films of Pd (II) tetrakis (pentafluorophenyl) porphyrin (PdTFPP) dye and coupled it with a perfusion chamber to create a controlled environment. Using this method, we were able to visualize a range of physiological OCR and observed an uneven and higher rate of uptake in T cell region of naïve slices in comparison to B cell regions. We validated this method against the Seahorse assay, a gold standard method for measuring OCR in cell suspensions. Similar to reported values, the mean OCR in Seahorse and optical methods were $1.79 \pm 0.17 \times 10^{-4}$ and $1.58 \pm 0.60 \times 10^{-4}$ pmol/min/cell, respectively, for isolated T cells and T cell region from naïve lymph nodes, respectively. Finally, we used this method to look at the rate and distribution of oxygen uptake in lymph node of wildtype mice after vaccination. Mice were vaccinated with ovalbumin (OVA) with adjuvant aluminum hydroxide (alum) or OVA in PBS as a control. 14 days after vaccination, there was no significant change in mean OCR in the T cell or B cell zones between vaccination and control mice. We are currently exploring more local variations in the tissue. Looking ahead, we envision that this method will be useful for monitoring region-specific metabolic responses in lymph nodes and other ex vivo tissues.

ABSTRACT #A-3

CMV5^{s} INDUCES SPLENIC TISSUE DAMAGE AND MYELOID POPULATION CHANGES DURING ACUTE MURINE CYTOMEGALOVIRUS INFECTION.

Annis JL[1,2], Crittenden RB[2,3], Brown MG[1,2,3]

1. Department of Microbiology, Immunology, and Cancer Biology. University of Virginia.
2. Beirne B. Carter Center for Immunology Research. University of Virginia.
3. Department of Nephrology; Center for Immunity, Inflammation and Regenerative Medicine. University of Virginia.

Immunology Center Affiliation: CIC

The MHC-linked, C57L-derived *Cmv5*^{s} quantitative trait locus (QTL) suppresses the ability of class I molecule H-2D^{k} to convey resistance to MCMV infection in MA/My background mice. These *Cmv5*^{s}-bearing, MA/My congenic (M.H2$^{k/b}$) mice present with increased viral load, weight loss, and severe splenic tissue damage by 4 days post MCMV infection (dpi). In order to better understand the timeline of infection in our *Cmv5*-disparate mice, we took a closer look at 2dpi hypothesizing that we would see the viral loads diverge in our mice before any tissue damage or weight loss was apparent. Interestingly, at 2dpi there was no difference in the weight loss or viral loads of our *Cmv5*-disparate mice, however we did see initiation of tissue damage in the M.H2$^{k/b}$ mice that was associated with a significant accumulation of neutrophils at the marginal zone, many of which were dead or dying. To determine if this influx of neutrophils was the cause of the extensive tissue damage in the 4dpi mice, we depleted the neutrophils by two separate approaches and did not see an alleviation of tissue damage in either setting. This evidence suggests that the neutrophils are not the cause of the splenic tissue damage and that some other initiating event causes the neutrophils to accumulate in greater numbers, or an issue with the macrophage compartment leads to a defect in clearing dead neutrophils corpses in our M.H2$^{k/b}$ mice. Further investigation into the splenic damage revealed an exacerbation of red pulp macrophage loss in the M.H2$^{k/b}$ mice and we are currently investigating the role of macrophages in this tissue damage phenotype using imaging, flow cytometry, and *in vitro* assay approaches.

ABSTRACT #B-4

REGULATION OF ALVEOLAR MACROPHAGE FUNCTIONS BY PEROXISOMES FOLLOWING INFLUENZA VIRUS INFECTION

Mohammad Arish, Jie Sun

Immunology Center Affiliation: CIC

Abstract: Peroxisomes have long been considered as an essential organelle that plays a critical role in lipids and reactive oxygen species metabolism. However, peroxisomes are now also being recognized as central to cell-intrinsic viral infections. Nevertheless, the role of peroxisomes is elusive in context to immune cells such as alveolar macrophages following viral infection. Here, we have demonstrated during influenza virus infection there is a downregulation of peroxisomes in mice and human alveolar macrophages (AM). Utilizing AM-specific peroxin (pex) 5 deletion, we found that these AM have inflammatory phenotypes and were more prone to cellular death through caspase-1 and -3 dependent manner. Also, metabolic analysis of pex5 deleted AM showed their preference for glycolysis over oxidative phosphorylation that may further lead to their inflammatory characteristic. In addition, our in-vivo study with the infection of pex5 knockout mice with influenza virus showed impaired recovery and significant lung damage, suggesting its importance for alveolar macrophage function. Altogether this study reflects an unexplored role of peroxisomes in alveolar macrophages, which may provide important clues in the generation of the roadmap for future therapies.

ABSTRACT #A-5

MYELOID CELL EXPRESSION OF CASPASE-1 IS NECESSARY FOR OPTIMAL CONTROL OF *T.GONDII* INFECTION IN THE BRAIN

Isaac Babcock, Maureen Cowan, Mike Kovacs, Lydia Sibley, Tajie Harris

Center for Brain Immunology and Glia

Immunology Center Affiliation: BIG

Great strides have been made in our understanding of neuroinflammation but the innate immune signals leading to inflammatory cell death in CNS resident cells during infection remain poorly defined. Using *Toxoplasma gondii* infection as a neuroinflammation model, we are investigating how inflammatory cell death impacts immunity in the brain. *T. gondii* is an intracellular parasite that chronically infects the brain as semi-dormant cysts and spontaneously reactivates resulting in damage and cell death. Our laboratory has shown that the alarmin IL-1α aids in control of parasite replication in the infected brain by promoting the recruitment of inflammatory monocytes. IL-1α is released from microglia via gasdermin D pore formation which is consistent with inflammasome-driven pyroptotic cell death. The caspase cascade that drives inflammatory cell death in the infected brain is unknown. By using genetic knockout mice of canonical and non-canonical inflammasome components we have identified caspase-1 in tissue resident macrophages being a critical component of controlling *T.gondii* in the brain. Mice lacking caspase-1 in *cx3cr1*-expressing cells have increased parasite burden in the brain. Using *ex vivo* cultures of mononuclear cells isolated from the infected brain, we are using agonists and antagonists of canonical and non-canonical inflammasome components to uncover the elements necessary for pyroptosis in microglia. Thus far, our results implicate caspase-1-mediated pyroptosis in microglia as a mechanism to control brain infection.

ABSTRACT #B-6

IMPACT OF ADJUVANT DRAINAGE ON DC SUBSET ACTIVATION

Alexander G. Ball, Parris Anbaei, Jon Zatorski, Rebecca R. Pompano

Carter Immunology Center

Immunology Center Affiliation: CIC

The development of novel vaccine adjuvants that promote strong cellular immunity is critical. One area of adjuvant research focuses on altering the size of the adjuvant i.e., converting TLR agonists into either nanoparticles (np) that drain to lymph node (LN) or larger particulate adjuvants (microparticles/hydrogels) that form a depot and remain at the site of inoculation. Both nanoparticles and larger particulate adjuvants have been shown to induce robust immune responses, yet it is not yet clear how adjuvant drainage impacts dendritic cell (DC) subset activation, specifically DCs at injection site and DCs that reside in the LN. Here, we explore the relationship between adjuvant drainage and DC subset activation through the use of different sized adjuvants. The two adjuvants chosen were alum, a TH2 polarizing adjuvant, and CpG, a TH1 polarizing adjuvant. As the alum adjuvant (Alhydrogel) is already a depot adjuvant, we synthesized alum np that were approximately 150 nm in size that would complement the depot Alhydrogel. The drainage of the alum nps and the Alhydrogel into murine LNs after subcutaneous vaccination was assessed by using inductively coupled plasma optical emission spectrometry to measure the aluminum content in the tissue. The ability of the alum nps and Alhydrogel to stimulate BMDCs in vitro was measured by inflammasome activation, specifically, IL-1β. Additionally, we are generating fluorescent CpG np and microparticle (mp) adjuvants. We will measure CpG adjuvant drainage into the LN by measuring particle fluorescence in the tissue at times 4 and 48 hours post vaccination with wide-field imaging. Our next steps include phenotyping the responses of specific DC subsets and measuring CD4 T cell activation in response to vaccination with alum and CpG adjuvants in their two drainage states. This work lays the foundation to examine the response of DCs to the routes of adjuvant drainage.

ABSTRACT #A-7

UNCOVERING MECHANISMS BETWEEN OLIGOSACCHARIDE GALACTOSE-ALPHA 1,3 GALACTOSE (ALPHA-GAL) SENSITIZATION AND ATHEROSCLEROSIS

Cassidy M.R. Blackburn[1,2], Tanyaporn Pattarabanjird[1,2,3], Hui Qiao[1,4], Fabrizio Drago[1] Melissa A. Marshall[1], Loren D. Erickson[1,4], Coleen A. McNamara 1,2,5

[1]Carter Immunology Center, University of Virginia [2]Cardiovascular Research Center, University of Virginia [3]Department of biomedical Engineering, University of Virginia [4]Department of Microbiology, Immunology, and Cancer Biology, University of Virginia [5]Division of Cardiovascular Medicine, Department of Medicine, University of Virginia

Immunology Center Affiliation: CIC

Recent work by our group and others has shown that individuals with IgE to the oligosaccharide allergen present in mammalian products (□-gal) have increased and more severe atheroma than those without □ -gal specific IgE. These data suggest □ -gal-specific IgE increases plaque severity and vulnerability, yet mechanisms that promote production of IgE to □ -gal are unknown. Previous work from our group utilized multi-omics single-cell analysis of circulating PBMCs from subjects with coronary angiography at UVA and showed subjects with IgE □ -gal sensitization had a higher frequency of CCR6hi switched memory (SWM) B cells and that the CCR6 ligand, CCL20, increased class switching to IgE. To determine mechanisms whereby CCR6 may mediate IgE class switching, we enriched total B cells from healthy donor peripheral blood mononuclear cells and stimulated with 20ng/ml human IL-4 and 10□g/ml agnostic anti-human CD40 with or without 20ng/ml CCL20 for 3 days. After 3 days of treatment, cells were collected and stained with a 15-color panel and analyzed via flow cytometry. Cells were analyzed for surface expression of CD20 and CD3 to gate total B cells. Gated B cells were further separated into CD27+/IgM-/IgD- to define SWM B cell population. CCR6+ SWM B cells have an average of 20% increase of phosphorylated mTOR in total SWM B cells compared to CCR6- SWM B cells. These results suggest CCR6 signaling induces mTOR phosphorylation and activation. Thus, mTOR activation and downstream mediators may be necessary for □ -gal induced B cell class switching. From these preliminary studies, we conclude that CCR6 signaling increases the percent pmTOR+ SWM B cells. We will continue to investigate downstream mTOR mediators in regard to B cell class switching and how □-gal sensitization augments B cell IgE class switching and increases atherosclerosis with our novel □ -gal-/- Apoe-/- mouse.

ABSTRACT #B-8

INVESTIGATING A ROLE FOR ANTIGEN PRESENTATION BY ENTERIC GLIAL CELLS IN MULTIPLE SCLEROSIS

Ryan M. Brown[1], Alban Gaultier[1]

[1]Brain, Immunology, and Glia Center, University of Virginia

Immunology Center Affiliation: BIG

Multiple Sclerosis (MS) is a chronic neurodegenerative disorder marked by an autoimmune response against myelin in the Central Nervous System (CNS). There is a link between the gut and MS as patients frequently present with functional gastrointestinal disorders, suggesting dysregulation of local motility circuits in the Enteric Nervous System (ENS). Enteric glial cells (EGCs) are observed throughout the gastrointestinal tract where they are closely associated with immune cells. EGCs are poised to contribute to intestinal inflammation, as they express several innate immune receptors as well as antigen presentation machinery and costimulatory molecules. Although the ENS is devoid of conventional myelin sheaths, EGCs express several myelin proteins such as Proteolipoprotein 1 (PLP1) and Myelin Basic Protein (MBP). Further, PLP1 and MBP are major constituents of myelin in the CNS and are targeted by the immune system in MS. As such, autoimmune reactions initiated by EGC antigen presentation on MHC I could potentially act to trigger CNS autoimmunity in MS. I aim to use the mouse model of Multiple Sclerosis – Experimental Autoimmune Encephalomyelitis (EAE) – to investigate a role for EGCs in intestinal and CNS pathology. My central hypothesis is that EGCs can present myelin antigen(s) on MHC I to autoreactive CD8 T cells in EAE, contributing to ENS and CNS pathology. I have observed upregulation of antigen presentation machinery in the small intestine of EAE mice prior to the onset of clinical symptoms, supporting a role for T cell mediated intestinal inflammation in disease progression. To further investigate this hypothesis, I will utilize a genetic mouse model by which the MHC I associated protein Beta-2-microglobulin is deleted in EGCs (Plp-Cre^{ERT} x B2m$^{fl/fl}$), resulting in the inability for MHC I to be expressed at the cell surface. These results could provide insights into how the ENS is implicated in MS pathogenesis.

ABSTRACT #A-9

VERSATILE HIGH-DIMENSIONAL TOOL FOR DEFINING COMPLEX T-CELL SIGNATURES IN ASTHMA

Naomi Bryant, MS, Glenda Canderan, PhD, Lyndsey Muehling, PhD, Judith Woodfolk, MBChB PhD, CIC

Immunology Center Affiliation: CIC

Background: Asthma is a heterogeneous disease, owing to differences in clinical presentation and underlying cellular mechanisms. Due to this heterogenicity, asthma has been categorized into subtypes, namely type 2 and non-type 2. While such categorization is important, particularly within a clinical setting, relatively little is known about how complex T-cell signatures relate to different types of asthma. Here, we have designed a novel 28-color spectral flow cytometry panel that enables in-depth interrogation of T-cells in asthma.

Methods: Markers for the panel were selected by analysis of existing T-cell datasets generated from bronchoalveolar lavage (BAL) and/or PBMC specimens from: (1) Children with severe asthma; and (2) Adults with allergic asthma, pre- and post-experimental rhinovirus infection. Key T-cell signatures identified within these data served as the foundation for the final panel. The 28-color panel was validated by staining surface and intracellular markers using matched BAL and PBMC specimens from children (n=11) with severe asthma. Cells were analyzed on a 5-laser Cytek® Aurora and T-cell signatures were identified using uniform manifold approximation and projection (UMAP) and Phenograph clustering.

Results: The 28-color panel identified complex T-cell signatures in both the blood and lower airways of children with severe asthma. Phenograph cluster analysis of BAL specimens revealed a prominence of CD4+ tissue-resident memory cells (CD69+CD103+/-) expressing the Th1 marker CCR5. This population was absent in the periphery. Additionally, cluster analysis identified a CD4+CCR5+ population, in both the blood and lower airways, expressing the canonical Th17 markers CCR6 and CD161. Notably, this population has not been observed previously in severe pediatric asthma.

Conclusions: Our 28-color spectral flow cytometry panel provides a valuable tool for defining complex T-cell signatures in asthma. Additionally, the panels' ability to identify tissue-resident cells allows for the comparison of T-cell signatures across different tissue types.

ABSTRACT #B-10

SURVIVORS OF SEVERE COVID-19 WITH LONG-HAUL RESPIRATORY SYMPTOMS DISPLAY ENHANCED ACTIVATION OF CIRCULATING T CELLS

Canderan G. [1*], Muehling L.M. [1*], Kadl A. [1], Ramani C. [1], Sturek J. [1], Bonham C.A. [1], Murphy D. [1], Wright P.W. [1], Keshavarz B. [1], Wilson J. [1], McNamara C. [1], Barone S.M. [2], Irish J.M. [2], Woodfolk J.A. [1]

[1]Department of Medicine, University of Virginia, Charlottesville, VA. [2]Vanderbilt University, Nashville, TN.

*These authors contributed equally to this work

Immunology Center Affiliation: CIC

Rationale: Some patients who survive severe COVID-19 develop persistent respiratory symptoms. Although increased activation of T-cells has been reported in severe acute disease, little is known about the long-term evolution of T-cells after SARS-CoV-2 infection in patients with long-haul symptoms.

Methods: Circulating T-cells were tracked in a sample of a COVID-19 cohort (n=88) consisting of patients with persistent respiratory symptoms. Cells were obtained during severe acute COVID-19 illness and at 6 weeks and 6-11 months after hospital discharge. Cells were analyzed by high-dimensional immunophenotyping using spectral flow cytometry. Longitudinal changes in complex cell signatures were identified using the T-REX algorithm. Antibodies to SARS-CoV-2 proteins were assessed by ImmunoCAP assay.

Results: Patients with long-haul symptoms who were sampled at 6 weeks after hospital discharge had higher frequencies of activated (HLA-DR^{+}CD38^{+}) and tissue-homing (CCR5^{+}) CD4^{+} and CD8^{+} T-cells compared to healthy subjects and patients with mild acute COVID-19. Higher numbers of terminally differentiated (CCR7^{-}CD27^{-}) CD8^{+} T-cells were also evident. T-REX identified multiple CD4^{+} and CD8^{+} T cell signatures that expanded or contracted by ≥95% up to 6 months after acute infection, including highly activated subtypes (CD3^{+6}CD4^{+7}CD45RA^{+7}CD45RO^{+1}CD38^{+1}HLA-DR^{+4}CD95^{+1}T-BET^{+2}TCF1^{+1}KI-67^{+2} and CD3^{+6}CD8^{+9}CD45RA^{+6}CD38^{+1}HLA-DR^{+5}CD95^{+2}CCR5^{+2}TCF1^{+1}T-BET^{+6} KI-67^{+2}). Fluxes in T-cell signatures were detectable several months after acute infection, even in the presence of declining antibodies to SARS-CoV-2 proteins.

Conclusions: Patients with long-haul respiratory symptoms after severe COVID-19 illness display activated T-cell signatures and marked immune perturbations, consistent with trafficking of T-cells with pathogenic potential and dysregulated homeostasis. Activated T-cells may contribute to airway inflammation long after acute illness resolves.

ABSTRACT #A-11

EXUBERANT PULMONARY T CELLS COMPROMISE LUNG FUNCTION POST-ACUTE COVID-19

In Su Cheon[1,2], Young Min Son[1,2], Chaofan Li[1,2], Ryan Kern[1], Robert Vassallo[1] and Jie Sun[1,2]

Division of Pulmonary and Critical Care Medicine, Mayo Clinic, Rochester, MN[1]

Carter Center for Immunology, Division of Infectious Disease and International Health, Department of Medicine, University of Virginia, Charlottesville, VA[2]

Immunology Center Affiliation: CIC

Severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) has caused a global pandemic, and coronavirus disease 2019 (COVID-19), has led to catastrophic acute pulmonary and extrapulmonary injury, particularly in aged and immunocompromised patients. The immune characteristics associated with COVID-19 have been well-reported in the acute phase, but the local immune traits in the respiratory tract are largely elusive at the convalescent stage. We profiled the immune signatures in the blood and bronchoalveolar lavage (BAL) in a group of aged patients (>60 years old), who have recovered from acute COVID-19 for at least two months. In addition, we performed spirometry and quantitative computer tomography (CT) to determine lung function and tissue pathology of these aged COVID-19 convalescents. We found that a great percentage of these aged COVID-19 convalescents exhibited consistently impaired lung function and increased tissue inflammation and fibrosis. We showed that functional SARS-CoV-2-specific memory T and B cells were enriched at the site of infection compared to those of blood. Of note, the levels of airway $CD103^-$ tissue resident CD8 T cells (Trm) were correlated with increased lung pathology and impaired lung function in COVID-19 convalescents. Moreover, the levels of respiratory dendritic cells (DCs) correlated with lower lung function in the aged COVID-19 convalescents. Our findings support a view that dysregulated local innate and/or adaptive immunity contribute to the development of chronic lung conditions following acute COVID-19. Our findings may inform the development of therapeutic interventions to prevent or alleviate pulmonary sequalae post-acute COVID-19.

ABSTRACT #B-12

MICROGLIAL STAT1-SUFFICIENCY IS REQUIRED FOR RESISTANCE TO FATAL TOXOPLASMIC ENCEPHALITIS

Cowan, M.N., Kovacs, M.A., Sethi, I., Babcock, I.W., Still, K.M., Batista, S.J., O'Brien, C., Sibley, L.A., Thompson, J.A., Harris, T.H.

Immunology Center Affiliation: BIG

Toxoplasma gondii is a ubiquitous intracellular protozoan parasite specialized for establishing chronic CNS infection in a wide range of vertebrates. Constant immune pressure informed by the production and cellular response to a critical cytokine, IFN-γ, is required to prevent fatal Toxoplasmic encephalitis (TE) in both humans and mice. Many groups have characterized the roles of peripheral immune cells in responding to IFN-γ to control both acute and chronic infections, but the role of microglia, the resident immune cells of the CNS, remains relatively under-explored. We propose that brain-resident microglia serve as critical responders to the cytokine IFN-γ, and that their ability to respond to infection is required for controlling infection prior to recruitment and implementation of a fully orchestrated local immune response. Here, we have used the inducible CX3CR1-CreERT2 system to specifically target microglia and block their ability to respond to IFN-γ by genetically excising its downstream transcription factor, STAT1. While mice chronically infected with *T. gondii* display resistance to infection, we find that mice with STAT1-deficient microglia succumb to infection, display worsened histopathology, and harbor a heightened brain parasite burden, despite an overall robust brain-infiltrating immune response and parasite clearance in other tissues. Our data suggest an overall protective role of CNS-resident microglia against *T. gondii* infection, illuminating (1) general mechanisms of CNS-specific immunity (2) and IFN-γ-mediated effector functions for this resident immune cell type.

ABSTRACT #A-13

LOSS OF TEN-ELEVEN TRANSLOCATION 2 (TET2) REDUCES CCR6 EXPRESSION AND INCREASES B1 B CELL NUMBER IN THE PERITONEAL CAVITY

Emily A Dennis[1,2], Sai Vineela Bontha[2], Melissa A. Marshall[2], Prasad Srikakulapu[2], James Garmey[2], Cassidy M.R. Blackburn[2], and Coleen A. McNamara[2,3]

[1]Department of Microbiology, Immunology, and Cancer Biology, University of Virginia, [2]Carter Immunology Center, University of Virginia, [3]Division of Cardiovascular Medicine, University of Virginia

Immunology Center Affiliation: CIC

TET2 is an evolutionarily conserved dioxygenase that catalyzes the conversion of 5-methyl-cytosine to 5-hydroxymethyl-cytosine, promotes DNA demethylation and regulates transcription. TET2 has been reported to regulate germinal center formation and class switch recombination in B2 B cells, yet its role in B1 B cell subsets remains largely unexplored. To investigate the role of TET2 in B1 subsets, flow cytometry of cells from the peritoneal cavity (PEC), spleen and bone marrow of TET2-/- mice and wildtype littermate controls (n = 14/group) was performed. In addition, sort purified PEC B1a, B1b and B2 cells were analyzed for methylation status (n = 24) and RNA expression (n = 24). Results demonstrated increased B1a ($p = 0.0162$) and B1b ($p = 0.0056$) cells in TET2-/-mice in the PEC, their primary niche, but not in the spleen, while in the bone marrow only TET2-/- B1a cells were increased ($p = 0.0021$). RNAseq analysis of PEC B1a, B1b, and B2 cells from TET2-/-and wildtype mice revealed reduced chemokine receptor CCR6 expression in B1a (-4.88 fold change, 8.03E-08 padj.) and B1b cells (-2.17 fold change, 0.35 padj.) from the TET2-/-mice. Further, methylation analysis showed significant hypermethylation of CCR6 in B1a and B1b TET2-/- cells compared to wildtype B1a and B1b cells. This was corroborated by gene pathway analysis of the RNAseq data which showed significant downregulation of chemotaxis and migration pathways in B1a and B1b TET2-/- cells. We conclude that loss of TET2 increases hypermethylation of CCR6 and suppresses CCR6 RNA expression in B1 cells which may impair trafficking out of the PEC to the spleen, but not necessarily the bone marrow, leading to PEC accumulation of B1 subtypes.

ABSTRACT #B-14

ION CHANNEL TRPM7 REGULATES ENVELOPED VIRAL INFECTION VIA ENDOSOMAL pH MODULATION

Catherine A. Doyle[1], Hansa Doppalapudi[1], Volker Kiessling[2,3], Giuseppe Di Caprio[4], Marta Stremska[5], Anwesha Sanyal[4], Wesley Iobst[1], Greg Busey[1], Eric J. Stipes[1], Lukas K. Tamm[2,3], Alex J.B. Kreutzberger[4], Tom Kirchhausen[4], Bimal N. Desai[1,3,6]

[1]University of Virginia Dept. of Pharmacology, [2]University of Virginia Dept. of Molecular Physiology and Biological Physics, [3]Univeristy of Virginia Center for Cell Membrane and Cell Physiology, [4]Boston Children's Hospital Program in Cellular and Molecular Medicine, [5]University of Virginia Dept. of Microbiology, Immunology, and Cancer, [6]University of Virginia Carter Immunology Center

Immunology Center Affiliation: CIC

Summary

Enveloped viruses are responsible for various global outbreaks and epidemics, including Ebola, Lassa, and most recently SARS-CoV-2. While these viruses result in different symptomatic illnesses, they exploit similar mechanisms of entry through the endocytic pathway where they escape from the endosome into the cytosol, thereby infecting their target cell. The decreasing pH in the lumen of the endosome is a key trigger necessary to induce fusion of the viral and endosomal membranes. Understanding host regulators of viral entry may reveal promising therapeutic targets for viral diseases. Using VSV and chimeras of VSV containing the envelope glycoproteins of various viruses, we show that loss of the "chanzyme" TRPM7 protects cells from an assortment of enveloped viral infections including VSV, Rabies, Lassa, LCMV, Ebola, and SARS-CoV-2 variants of interest. Loss of TRPM7 exhibited a stronger inhibition of infection against viruses that require a lower pKa for viral penetration. Pharmacological inhibitors of TRPM7 ion channel activity as well as overexpression of a TRPM7 ion channel pore mutant demonstrate that inhibition of ion channel activity alone is sufficient to prevent viral infection. Combining infection assays and single virion imaging methods, we determined TRPM7 depleted cells can endocytose viral particles into maturing endosomes, but viruses fail to escape into the cytosol. We demonstrate loss of TRPM7 induces a robust acidification defect of endosomes containing viral particles due to a failure of H^+ pumping across the endosomal membrane. We propose a model in which TRPM7 functions directly as a counterion current for the V-ATPase by removing cations from the endosome.

ABSTRACT #A-15

SYK COORDINATES NEUROPROTECTIVE MICROGLIAL RESPONSES IN NEURODEGENERATIVE DISEASE

Hannah Ennerfelt[1], Elizabeth L. Frost[1], Daniel A. Shapiro[1], Coco Holliday[1], Kristine E. Zengeler[1], Gabrielle Voithofer[1], Ashley C. Bolte[1], Catherine R. Lammert[1], Joshua A. Kulas[1], Tyler K. Ulland[1], and John R. Lukens[1]

[1]Brain Immunology and Glia (BIG) Center

Immunology Center Affiliation: BIG

Many neurodegenerative diseases are thought to be caused by impaired containment and/or disposal of neurotoxic material such as amyloid beta (Aβ) and myelin debris. Indeed, recent human genome-wide association studies (GWAS) and animal model studies have begun to reveal critical roles for the brain's professional phagocytes, microglia, as well as various innate immune receptors expressed by microglia in the control of neurotoxic material and subsequent neurodegenerative disease pathogenesis. Yet, the critical intracellular molecules that orchestrate the neuroprotective functions of microglia in degenerative disorders remain poorly understood. In our studies, we have identified the innate immune signaling molecule spleen tyrosine kinase (SYK) as a key regulator of microglial phagocytosis in neurodegenerative disease. We find that targeted deletion of SYK in microglia leads to exacerbated Aβ deposition, aggravated neuropathology, and cognitive defects in the 5xFAD mouse model of Alzheimer's disease (AD). Furthermore, disruption of SYK signaling in this AD model was also shown to impede the development of disease-associated microglia (DAMs) and to cause severe deficits in the ability of microglia to phagocytose Aβ. Importantly, these critical neuroprotective functions of SYK in microglia were not only restricted to Aβ-driven models of neurodegeneration, as we found that SYK is also a critical regulator of microglial phagocytosis and DAM phenotype acquisition in demyelinating disease. Collectively, these results help to break new ground in our understanding of the key innate immune signaling molecules that instruct beneficial microglial functions in response to neurotoxic material. Moreover, these findings suggest that targeting SYK may offer a therapeutic strategy to treat a spectrum of neurodegenerative disorders.

ABSTRACT #B-16

THE ROLE OF MITOCHONDRIAL DYNAMICS IN KIDNEY PERIVASCULAR CELLS

Eibhlin Goggins, Nabin Poudel, Shuqiu Zheng, Mark D. Okusa

Division of Nephrology, Center for Immunity, Inflammation, and Regenerative Medicine, University of Virginia, Charlottesville, VA

Immunology Center Affiliation: CIIR

Background: Chronic kidney disease (CKD) affects over 850 million people worldwide. Understanding the mechanisms leading to the development of CKD is crucial in delaying its progression to end-stage kidney disease (ESKD). Increasing evidence implicates kidney mitochondrial damage in the pathogenesis of CKD. Mitochondria are highly dynamic organelles constantly undergoing fusion, fission, biogenesis and mitophagy under homeostatic conditions. Our lab has previously demonstrated that proximal tubule deletion of dynamin related protein 1, *Drp1*, after ischemia-reperfusion injury (IRI) attenuates progressive kidney injury and fibrosis. However, mitochondrial dysfunction in kidney perivascular cells (PVCs), a major source of myofibroblasts during CKD, has not been explored. We hypothesize that dysregulated mitochondrial dynamics in kidney PVCs mediates fibrosis after injury.
Methods: C57BL/6 mice were anesthetized with an i.p. injection of ketamine (120 mg/kg) and xylazine (12 mg/kg) and underwent unilateral (left kidney, uIRI) or bilateral (bIRI) kidney ischemia reperfusion injury. After flank incision, the left or both kidney pedicles were cross clamped for 26 min. Sham-operated mice underwent same procedures without clamping of the kidney pedicle. In another model of kidney injury, mice were injected with lipopolysaccharide (LPS, 10 mg/kg i.p.) or saline. Kidney PVCs were isolated 24 h (bIRI, LPS) or 14-21 days (uIRI) after. RNA or protein was immediately extracted and analyzed.
Results: mRNA levels of genes related to fission, fusion, biogenesis and mitophagy in kidney PVCs were dysregulated in response to bIRI. In the uIRI model, *Pgc1a*, the master regulator of mitochondrial biogenesis, was downregulated in PVCs isolated from the fibrotic kidney compared with that from both the contralateral kidney and from sham kidneys. Western blots of PVCs isolated from LPS mice showed a similar trend in dysregulation of proteins involved in mitochondrial dynamics.
Conclusion: During acute and chronic injury, mitochondrial dynamics are dysregulated in kidney PVCs. Studies to understand the mechanism of this response are ongoing.

ABSTRACT #A-17

RECIPROCAL INTERACTIONS BETWEEN THE GUT MICROBIOME AND MAMMARY TISSUE MAST CELLS PROMOTE METASTATIC DISSEMINATION OF HR+ BREAST TUMORS

Tzu-Yu Feng, Mitchell T. McGinty, Audrey M. Putelo, Sree H. Kolli, Melanie R. Rutkowski

Immunology Center Affiliation: CIC

Establishing commensal dysbiosis, defined as an inflammatory gut microbiome with low biodiversity, prior to breast tumor initiation, enhances early dissemination of hormone receptor-positive (HR^+) mammary tumor cells. Here, we sought to define mammary tissue mediators of dysbiosis-induced tumor dissemination. We found that commensal dysbiosis increased both the frequency and profibrogenicity of mast cells in the mammary tissue, a phenotypic change that persisted after tumor implantation. Fibroblast activation and tissue remodeling associate with enhanced breast tumor metastasis. We employed pharmacological and adoptive transfer approaches to demonstrate that mammary tissue mast cells from dysbiotic animals enhances dissemination of HR^+ tumor cells. Collagen levels in mammary tissues from HR^+ breast cancer patients correlated with mast cell abundance, suggesting clinical relevance of mast cell-mediated fibroblast activation. Together, these data demonstrate that a gut-mast cell axis exists that induces fibroblast activation and orchestrates early dissemination of HR^+ breast tumors.

ABSTRACT #B-18

DELETION OF DRP1 IN T CELLS INCREASES OXPHOS AND CD8+ MEMORY T CELL POPULATION

Marissa A. Gonzales[1*] and Timothy N.J. Bullock[1*]
[1]Department of Pathology, University of Virginia, Charlottesville, VA
[*]Carter Immunology Center, University of Virginia, Charlottesville, VA

Immunology Center Affiliation: CIC

Memory CD8 T cells can more rapidly respond to a secondary exposure to viruses or tumor antigens than naïve T cells. These memory cells have been shown in vitro to exhibit more elongated mitochondria, more mitochondrial mass, and high spare respiratory capacity, allowing for a rapid response time. During the activation and differentiation of T cells, the metabolic demands fluctuate between the necessity for glycolysis or oxidative phosphorylation (OXPHOS), which is supported by shifting of the mitochondrial network state. We hypothesized that manipulating T cells to elongate mitochondria would provide a metabolic benefit to effector functions and could ultimately be used to increase the function of tumor infiltrating lymphocytes. Using mice lacking Drp1, a mitochondrial fission protein, in T cells as a model of elongated mitochondria, we see increased spare respiratory capacity and OXPHOS as compared to WT T cells with fission capability. Interestingly, we find that these mice are more likely to generate memory precursor CD8 T cells as represented by $KLRG1^{lo}$ and $CD127^{hi}$ during a primary response to αCD40, PolyI:C, and ovalbumin protein. As indicated by the increase in memory precursors, we find that $Drp1^{-/-}$ T cells form more CD8+ memory than WT animals after a 28 day rest period. At this time, mice were challenged with an adenovirus expressing ovalbumin to elicit a recall response. This recall response of CD8 T cells is greater in animals lacking Drp1 in the T cells. Additionally, T cells treated with pharmacological reagents M1 and Mdivi to inhibit mitochondrial fission and induce fusion show increased spare respiratory capacity. Further studies of differentiation and contraction are still required to determine how the memory CD8 T cell population is increased. We ultimately aim to exploit control of mitochondria to increase memory T cell development and metabolism as well as control of solid tumors.

ABSTRACT #A-19

UNDERSTANDING THE SPATIAL REGULATORS OF INTRACELLULAR PARASITE RECOGNITION

Nadia Holness, Samantha Lempke, Xiaoyu Zhao, Bocheng Yin, Jan Urban Arroyo, Sarah E Ewald

Immunology Center Affiliation: CIC

Toxoplasma gondii is an obligate intracellular parasite of rodents and humans that is estimated to infect one-third of the global human population. T. gondii has the remarkable ability to infect most nucleated cells and during the acute phase of infection, *T. gondii* capitalizes on migratory dendritic cells and macrophages to establish chronic infection in tissues amenable to chronic infection. Interferon-gamma (IFNγ) is an indispensable extracellular cue that upregulates interferon-inducible GTPases (IIGs) that are necessary for parasite clearance within infected cells. However, the mechanism of IIG recruitment to the parasite vacuole (PV) and the downstream signaling that leads to vacuole elimination remains unclear. Within an infected dendritic cell culture, we observed that some PVs are targeted by IIGs while others are able to evade IIG targeting. I hypothesize that the host and/or parasite mediators of IIG recognition are differentially expressed in the population of targeted vacuoles relative to immune evasive vacuoles. To identify these mediators, I am utilizing a new technique developed in my lab called Automated Spatially Targeted Optical Micro Proteomics (autoSTOMP). AutoSTOMP uses confocal microscopy to both visualize and photo-chemically biotinylate proteins within 1μm of IIG targeted or IIG evading PVs. Using this approach, we identified 2,394 host proteins differentially enriched near the parasite vacuole samples versus a control region or whole-cell lysate. Using a set of criteria to identify putative sensors of *T. gondii* infection at the vacuole, I identified 11 candidate sensors. At the top of this list are two Leucine-rich repeat-containing (Lrrc) proteins Lrrc25 and Lrrc59, cytosolic proteins with highly conserved ligand-binding domains that have been implicated in the recognition of foreign and damaged self-signals. Using a combination of parasite and host genetics and biochemical assays I will elucidate the mechanism of Lrrc59 and Lrrc25 in IIG targeting and clearance of *T. gondii* from immune cell types.

ABSTRACT #B-20

OVARIAN CANCER INITIATED EMERGENCY MYELOPOIESIS CONFERS CHANGES IN BONE MARROW TLR5 SIGNALING

Kolli Sree. 1, McGinty Mitch. 1, Putelo Audrey. 1, Feng Tzu-Yu (Alkaid). 1, Rutkowski Melanie. 1

1: University of Virginia School of Medicine
Department: Microbiology, Immunology, Cancer Biology
Carter Immunology Center

Immunology Center Affiliation: CIC

Among women, ovarian cancer is a leading oncological cause of death. During the year 2022, an estimated 19,880 women will receive a new diagnosis of ovarian cancer and 12,810 women will die from ovarian cancer in the United States alone. Recently, the human gut microbiome has been proposed to play an important role in the progression of ovarian cancer. To this extent, toll like receptor 5 (TLR5) signaling, whose only known ligand is bacterial flagellin, has been implicated in creating an immune suppressive tumor microenvironment (TME). Leading to the devolvement of cellular phenotypes such as myeloid derived suppressive cells, TLR5 signaling contribute to disease progression by upregulating anti-inflammatory cytokines such as IL-6. With this in mind, the question arises as to what point during myelopoiesis, and differentiation does TLR5 signaling lead to the development of suppressive phenotypes? Given that TLR5-expressing common monocyte and dendritic progenitor cells populate the tumor microenvironment, we hypothesize that immune suppressive activity, intrinsically modulated by TLR5 signaling via commensal bacteria, is derived from the bone marrow and stem cell level. To test this, we induced ovarian cancer using an orthotopic ovarian cell line into WT and TLR5 KO mice, collected bone marrow and stained for hematopoietic progenitor markers. We found that that WT mice bearing tumors tended to develop more common monocytic progenitors at the expense of common dendritic cell precursors when compared to non-tumor bearing WT mice and TLR5 KO mice with or without tumors. These findings suggest that TLR5 signaling causes a shift towards an immune suppressive phenotype earlier in development.

ABSTRACT #A-21

UNDERSTANDING VACUOLE-AUTONOMOUS IMMUNE CLEARANCE OF TOXOPLASMA GONDII USING AUTOMATED SPATIALLY TARGETED OPTICAL MICRO PROTEOMICS

Samantha Lempke, Xiaoyu Zhao, Jan Carlos Urbán Arroyo, Bocheng Yin, Nadia Holness, Sarah Ewald

Carter Immunology Center

Immunology Center Affiliation: CIC

Toxoplasma gondii's remarkable ability to infect and persist within a wide range of host cells is linked to the formation of the parasitophorous vacuole. This dynamic organelle is formed from the host plasma membrane and is constantly remodeled by secreted parasite effectors. Interferon-inducible GTPases (IIGs) are cell-autonomous immune mediators of parasite clearance. However, the precise mechanisms by which IIGs are recruited to the vacuole, disrupt vacuole integrity, and trigger *Toxoplasma* destruction are unclear. These holes in our understanding are linked to limited tools to purify the vacuole for biochemical analysis and heterogeneity in IIG targeting to the vacuole. Specifically, there are multiple pools of IIG in the cell, and our lab and others have shown that within a population of infected macrophage or dendritic cells, no more than 60% of vacuoles are targeted by IIGs at any given time. To understand the mechanism of *Toxoplasma* clearance by IIGs, our lab developed a protein discovery technique called Automated Spatially Targeted Optical Micro Proteomics (AutoSTOMP). AutoSTOMP uses confocal microscopy to visualize the vacuole and selectively, photochemically biotinylate proteins localized to the vacuole membrane for purification and identification by mass spectrometry. Using AutoSTOMP we have identified 2,398 host and 79 *Toxoplasma* proteins that are differentially enriched at IIG-positive vacuoles or IIG-negative vacuoles relative to nearby regions of infected dendritic cells or the total proteome. Using a combination of host and parasite genetic tools, immunofluorescence imaging, and biochemical techniques, we are identifying novel regulators of parasite survival in IIG-mediated clearance.

ABSTRACT #B-22

DEVELOPING A NEW TARGETED THERAPY FOR RHABDOMYOSARCOMA: αVβ3 AND HER2 AS PROMISING TARGETS FOR BISPECIFIC CAR T-CELL THERAPY

Amanda Lulu[1], Drew Cobb[1], Daniel W. Lee[1]

[1]Division of Pediatric Hematology/Oncology, Dept. of Pediatrics, University of Virginia

Mentor: Daniel W. Lee, Dept. of Pediatrics, Division of Pediatric Hematology/Oncology

Immunology Center Affiliation: CIC

Even though rhabdomyosarcoma (RMS) is the most prevalent sarcoma in children, therapy relies on decades-old, multi-agent chemotherapy and radiotherapy. There is imminent need for more effective targeted treatment. Chimeric antigen receptor T-cell (CAR-T) therapy has been highly successful for relapsed/refractory hematologic malignancies. Tisagenlecleucel, which targets CD19 on leukemia, is the only FDA-approved CAR-T for children. Roughly half of responding patients relapse with lost or down-regulated CD19 antigen thereby escaping treatment. **We hypothesize that targeting two RMS tumor antigens with a bispecific CAR-T product will reduce the likelihood of disease escape.** We previously established the efficacy of αvβ3 and HER2 CAR-Ts against diffuse intrinsic pontine glioma/glioblastoma and medulloblastoma, respectively. We identified αvβ3 expression on RMS, and one child with RMS experienced a temporary remission with HER2 CAR-T. Therefore, simultaneous targeting of αvβ3 and HER2 using a CAR-T product approach for RMS may be an ideal therapeutic for relapsed patients.

Our objective was to validate αvβ3 and HER2 as targets for a bispecific CAR-T product in RMS. αvβ3 and/or HER2 are expressed on 6/6 RMS cell lines. Separately, αvβ3 and HER2 CAR-Ts rapidly and robustly kill antigen^{pos} RMS in an antigen- and dose-dependent manner. αvβ3 and HER2 CAR-Ts are sensitive, achieving 70% cytolysis at 300 and 1100 targets/cell, respectively. An ability to kill low-antigen expressing cells would likely preclude disease escape by antigen down-regulation. Co-culture induced increased expression of activation markers demonstrating a strong effector response. Our data support αvβ3 and HER2 as viable targets for a bispecific CAR-T approach in pediatric RMS. These results support continued investigation into αvβ3 and HER2 CAR-T-mediated *in vivo* control of RMS. αvβ3+HER2 bispecific CAR-Ts are being developed, which we expect to perform as well as or better than each CAR-T independently, in addition to limit the likelihood of disease escape by antigen modulation.

ABSTRACT #A-23

ABSENCE OF TOLL-LIKE RECEPTOR 5 CONFERS SURVIVAL IN MICE BEARING OVARIAN TUMORS TREATED WITH ANTI-PD-L1

Mitchell T. McGinty, Sree Kolli, Tzu-Yu Feng, Audrey Putelo, Melanie R. Rutkowski

Immunology Center Affiliation: CIC

Ovarian cancer accounts for more deaths than any other cancer of the female reproductive system. Patients bearing ovarian tumors infiltrated with high frequencies of T cells associate with a greater survival probability. However, therapeutic strategies targeting T cells in ovarian cancers are largely ineffective. We have observed in our models of late-stage murine ovarian cancer that in the absence of Toll-Like Receptor 5 (TLR5) signaling, anti-PD-L1 therapy promotes significant survival and subsequent protection against tumor rechallenge. Ovarian tumors from rechallenged TLR5KO mice exhibited significantly higher frequency/number of both CD4 and CD8 effector memory T cell subsets and greater numbers of antigen experienced T cells. Thus, in the absence of TLR5 signaling, T cells are better able to mount/maintain a response to ovarian tumors after inhibitory receptor blockade. Furthermore, protection against tumor progression in TLR5KO mice corresponds with a significant increase in tumor infiltrating cross presenting and IL-12 producing Dendritic cells (DCs) which could account for the differences observed in T cell infiltrates. We hypothesize that TLR5 signaling functionally alters DCs to become more suppressive in the tumor microenvironment, impacting T cell differentiation and anti-tumor activity, thereby reducing survival during ovarian tumor progression with aPD-L1 therapy. Clinical implications are already present, as roughly 7.5% of the general population harbor a TLR5 SNP that diminishes TLR5 signaling and is associated with increased long-term survival for ovarian cancer patients. Therefore, patients who express the TLR5 SNP may immediately benefit from anti-PDL1 therapy and those without the SNP, TLR5 antagonism.

ABSTRACT #B-24

EVALUATING THE POTENTIAL OF A CHIMERIC INHIBITORY RECEPTOR TO ADDRESS CYTOKINE RELATED TOXICITIES SEEN IN PATIENTS RECEIVING CAR-T CELL THERAPY

Philip Mollica III, Barbara Dziegielewska Ph.D., Drew Cobb Ph.D., and Daniel Lee M.D.
Mentor: Daniel W. Lee, Dept. of Pediatrics, Division of Pediatric Hematology/Oncology
Affiliation: Carter Immunology Center

Immunology Center Affiliation: CIC

Summary: Many forms of cancer remain poorly prognosed as current first-line treatments struggle to elicit adequate therapeutic control, warranting interest in the use of cell-based immunotherapies. Chimeric Antigen Receptor (CAR) T cell therapy has shown promise in providing a means to induce tumor cytotoxicity in a target specific manner; however, it is not without its own limitations. Cytokine Release Syndrome (CRS), is a pathological state seen in the context of CAR T cell therapy characterized by increased cytokine production downstream of the initial T cell mediated immune response, which in turn promotes additional leukocyte recruitment and activation creating a positive feedback mechanism that drives cytokine concentrations above tolerable levels. To address this, we created a Chimeric Inhibitory Receptor (CIR), comprised of an extracellular IFNg specific sensing domain coupled to an intracellular inhibitory signaling molecule, that would allow CAR T cell functionality to be regulated by systemic cytokine concentrations. We hypothesize that co-expression of the CAR and CIR confers a mechanism of self-regulation to engineered T cells that in turn mitigates the risk of developing CRS.

To assess the effects of CIR expression on CAR T cell functionality, we analyzed the relative levels of antigen induced activity between both CAR and CAR+CIR groups. Tumor cell co-culture induced increased activation marker expression in CAR only group when compared to CAR+CIR control. ELISA and intracellular cytokine staining showed evidence of reduced effector cytokine production by CIR expressing CAR T cells following activation. Co-culture with macrophages and dendritic cells highlighted a decline in monocyte specific cytokine production downstream of initial T cell activation in CAR+CIR groups. Cytotoxicity data showed delayed kinetics in CAR+CIR groups but otherwise demonstrated efficacious killing at high effector to target ratios. Collectively, our data illustrates the potential for the CIR to increase CAR-T cell treatment tolerability without sacrificing effective on-target cytotoxicity.

ABSTRACT #A-25

EX VIVO MODEL OF TUMOR CELL SPREAD IN LYMPH NODE METASTASIS IN BREAST CANCER

1Morgaenko, K., [2]Rutkowski, M.R., [1,3]Pompano, R.R.,

Departments of [1]Biomedical Engineering, [2]Microbiology, Immunology and Cancer Biology, and [3]Chemistry, University of Virginia Carter Immunology Center

Immunology Center Affiliation: CIC

In breast cancer, the tumor-draining lymph node (TDLN) is a common site of metastasis that often precedes subsequent tumor spread to distant organs. While it is known that metastatic tumor cells enter and occlude subcapsular sinus of TDLN, the process of tumor cell trans-sinusoidal migration to the lymph node cortex remains poorly understood. Existing experimental models of TDLN metastasis fail to simultaneously capture tumor cell translocation within the TDLN and to measure the factors controlling tumor cell invasion, particularly while retaining the complexity of tissue architecture. Here, to develop an ex-vivo model of the tumor cell spread in TDLN in the absence of lymphatic barriers, highly metastatic 4T1 and poorly metastatic BRPKp110 breast cancer cell lines were seeded onto live murine lymph tissue slices, and their spread was quantified at multiple timepoints during ex-vivo culture. Tumor cell location was determined by immunostaining relative to LN architectural landmarks followed by quantitative image analysis. We identified survival niches in the ex vivo LN that particularly supported tumor invasion, including subcapsular sinus and deep parenchyma. We observed that while both 4T1 and BRPKp110 breast cancer cell lines tended to spread and acquire elongated morphology along the roof and the floor of the subcapsular sinus and form an interconnected network in T-cell zone, the poorly metastatic BRPKp110 tumor cells were notably excluded from B-cell follicles. We hypothesize that tumor cell localization in the lymph node is at least partly influenced by the chemokine gradients designed to guide circulating lymphocytes to the cortex or deep parenchyma of the TDLN. In the future work we aim to analyze tumor cell migration within TDLN relative to chemokine gradients. We propose that elucidating the mechanisms underlying tumor cell traffic to TDLN and metastasis localization is important for identification of druggable targets and advancing therapy.

ABSTRACT #B-26

THE EFFECT OF FGF ON EXPRESSION OF HOMING RECEPTOR LIGANDS OF TUMOR ENDOTHELIAL CELLS

Amelia Nachbar, Dr. Mirna Perusina Lanfranca, Dr. Victor H Engelhard of the Carter Immunology Center

Immunology Center Affiliation: CIC

FGF, or fibroblast growth factor, is a cytokine involved in tissue regeneration and developmental processes that is also implicated in tumor growth. By studying FGF we hope to identify a mechanism to prevent its oncogenic activity. Vascular endothelial cells express homing receptor ligands (HRLs), proteins that allow entry of T cells into tissues. Pro-inflammatory cytokines promote their expression. FGF downregulates expression of these HRLs and consequently tumor infiltration, blocking the immune system from fighting tumor growth.

We hypothesize that FGF will have a dose-response relationship with HRL expression, such that as FGF concentration increases, HRL expression decreases. Additionally, we hypothesize that for each HRL, there will be a cytokine treatment that will correspond with an elevated expression of that HRL.

Three endothelial cell (EC) lines from dermis, pancreas, and skin (MDEC, MS1, and bEnd3 respectively) were used to study the effects of FGF2 (a member of the FGF family) on HRL expression in this experiment. *In vitro* cells were either pretreated or not with FGF2 for 24 hours (0; 0.1; 1 and 10 ng/ml), followed by either no treatment or treatment with IFNγ, TNFα, or both in combination with the previous FGF2 concentration for an additional 24 hours. Total RNA was then extracted and reverse transcribed into cDNA for real time PCR analysis.

We found that increasing FGF2 concentrations was directly related to inhibition of HRL expression. We also identified which cytokine treatments most effectively upregulated expression for the different HRLs in the presence of FGF2.

Not all HRLs were substantially upregulated by the same single or double cytokine treatment. This suggests that each HRL is expressed through a different cellular pathway and that these pathways interact differently with FGF2. Future studies would investigate the specific cellular processes through which FGF2 downregulates HRLs at the translational level.

ABSTRACT #A-27

RESIDENT CD8⁺ T-CELLS DRIVE DYSPLASTIC REPAIR OF AGED LUNGS FOLLOWING VIRAL PNEUMONIA

Harish Narasimhan, Nicholas P. Goplen, In Su Cheon and Jie Sun.

Carter Immunology Center

Immunology Center Affiliation: CIC

Global studies have established age as the most critical risk factor in determining morbidity and mortality following respiratory viral infections such as influenza and SARS-CoV2. Previous studies from our group identified non-resolving inflammation in aged lungs post influenza infection, primarily driven by the persistence of tissue resident memory (T_{RM}) CD8 T-cells. We also observed sustained impaired lung function in aged COVID19 convalescents, which negatively correlated with levels of pulmonary CD8 T_{RM} cells. While critical for acute antiviral responses, we have demonstrated that persistence of this population is detrimental rather than protective in the chronic phase during aging, evidenced by improved outcomes upon its depletion.

Upon severe lung damage during viral infection, epithelial progenitor cells characterized by cytokeratin 5 (Krt5) expression are mobilized for repair. However, these cells are known to be incapable of differentiating into functional ATI and ATII cells, instead persisting as dysplastic epithelial scars – a process known to be exacerbated with age. Thus, in addition to mortality during acute infection, aged individuals also experience adverse long-term outcomes due to dysfunctional repair of the lung.

Notably, we observe these dysplastic regions in close proximity to CD8⁺ T_{RM} populations in both human and mouse lungs following viral pneumonia, suggesting potential immune-epithelial crosstalk. Furthermore, upon depletion of pulmonary CD8 T_{RM} cells, we observed a reduction in Krt5 cysts, increased ATI and ATII levels, and a concomitant improvement in lung function. Therefore, we hypothesize that the interactions between CD8 T_{RM} cells and Krt5 cysts are responsible for sub-optimal repair of the lung following injury. We aim to elucidate the molecular basis of this crosstalk to deepen our understanding of pulmonary immune-epithelial communication, and ultimately manipulate these interactions to promote successful regeneration of the aged lung following

ABSTRACT #B-28

ANTI-INFLAMMATORY VAGUS NERVE STIMULATION INDUCES SPLENIC CONTRACTION, ALTERED IMMUNE CELL DISTRIBUTION, AND SUPPRESSED INTERFERON-γ PRODUCTION.

Nash WT, Tanaka S, Okusa MD
Center for Immunity, Inflammation, and Regenerative Medicine

Immunology Center Affiliation: CIIR

Vagus nerve stimulation (VNS) provides protection from inflammatory conditions by maintaining tissue morphology, preserving organ function, and reducing likelihood of mortality. While the neural circuits that contribute to this effect have been extensively studied, little is known about the impacts of VNS on the immune system. Our work seeks to better understand immune implications of VNS and gain insight into mechanisms of protection. We explored this by electrically stimulating the vagus nerve in B6 mice and assessing immune cells in the spleen. The vagus nerve was exposed at the neck and stimulated with electrical pulses for 10 minutes. After 48 hours, spleens were processed into single cell suspensions and immune cells were analyzed via flow cytometry, to quantify immune cell populations, and ex vivo stimulation, to determine inflammatory cytokine production. Ex vivo cells were incubated with agonists for the inflammatory toll-like receptors (TLR) TLR3, TLR4, or TLR9. After 20-24 hours, media was collected for cytokine analysis via 32-plex Luminex assay. Spleens from VNS-treated mice weighed less (sham avg=0.064±0.01 g; VNS avg=0.046±0.01 g) and exhibited reduced abundance of most immune cell populations. The most significantly impacted cells were T cells (*p=0.007*), B cells (*p=0.01*), NK cells (*p=0.01*), dendritic cells (*p=0.01*), and basophils (*p=0.02*). Ex vivo cytokine production was also suppressed. The profile of cytokines affected varied depending on TLR agonist, but a subset exhibited regulation across multiple TLR-stimulations (IFNγ, CCL2, CXCL9, VEGF, CCL3, CXCL10, GM-CSF, CCL4, IL-10, and IL-9). Interestingly, IFNγ production was decreased in all conditions tested. We conclude VNS modulates immune cell prevalence and distribution, which may contribute to the anti-inflammatory state. VNS suppressed different cytokine profiles for each TLR agonist, possibly due to different cells responding in each condition based on TLR expression. Additionally, the apparent ubiquitous impact on IFNγ provides a good candidate for further inves

ABSTRACT #A-29

AUGMENTING THE EFFICACY OF $\alpha_v\beta_3$.BBζ CAR T CELLS FOR THE TREATMENT OF GLIOBLASTOMA

Katherine M. Nowak, Dustin A. Cobb, Catherine M. Gorick, E. Andrew Thim, Mark R. Schwartz, Daniel W. Lee, Tajie H. Harris, & Richard Price

Brain Immunology and Glial Center (BIG)

Immunology Center Affiliation: BIG

Due to the aggressive and highly heterogenous nature of glioblastoma multiforme (GBM), only about 5% of patients diagnosed with GBM will survive 5 years post-diagnosis. Current standard of care therapies include surgical resection, radiation, and chemotherapy, which are often accompanied by side effects and toxicities. Furthermore, barriers to effective therapies for GBM include the blood-brain-barrier, low mutational burden and/or the lack of neoantigens, intra-tumoral heterogeneity, and the immunosuppressive microenvironment. Recent advances for the treatment of GBM utilize cancer immunotherapies to promote immune-mediated tumor growth control. In particular, chimeric antigen receptor (CAR) T cells targeting $\alpha_v\beta_3$ integrin have been successfully employed to treat U87-eGFP-luc glioblastoma tumors. Not only is $\alpha_v\beta_3$ integrin overexpressed on glioblastoma cells, but also the tumor vasculature making $\alpha_v\beta_3$ CAR T cells uniquely equip to target both the cancer cells as well as their blood supply. While infusion of 10×10^6 $\alpha_v\beta_3$.BBζ CAR T cells has shown promise in controlling GBM tumor growth and promoting long-term survival in immunocompromised mice, such high concentrations often lead to cytokine release syndrome and/or immune effector cell-associated neurotoxicity syndrome in humans. Therefore, reducing the concentration of CAR T cells required to control tumor growth while limiting toxicities is crucial to the translational success of the therapy. Here we show that 3×10^6 $\alpha_v\beta_3$.BBζ CAR T cells administered intravenously is insufficient to control GBM tumor growth and survival. These results open an avenue for improving low dose $\alpha_v\beta_3$.BBζ CAR T cell therapy using focused ultrasound (FUS)-mediated blood-brain-barrier opening in combination with an immunotherapeutic agent to overcome the GBM immunosuppressive microenvironment and enhance CAR T cell efficacy.

ABSTRACT #B-30

TARGETING IMMUNOSUPPRESSION FOR ENHANCED FOCUSED ULTRASOUND EFFICACY IN TRIPLE NEGATIVE BREAST CANCER.

Lydia E. Petricca[1] (CIC), Alexandra R. Witter[1], Natasha D. Sheybani[2], Eric A. Thim[2], Timothy N.J. Bullock[1] and Richard J. Price[2]
[1] Univ. of Virginia, Charlottesville, Dept. of Pathology
[2] Univ. of Virginia, Charlottesville, Dept. of Biomedical Engineering

Immunology Center Affiliation: CIC

Immunologic rejection of triple-negative breast cancer (TNBC) is rare. Focused Ultrasound (FUS) promotes antitumor immunity by inducing tissue destruction and inflammation. We hypothesized that thermally-ablative FUS (tFUS) would increase the immune response to TNBC. However, TNBC is commonly accompanied by the expansion of immunosuppressive myeloid cells. By implanting a metastasizing TNBC cell line, 4T1, into BALB/c mice, we showed that a combinatorial therapy of tFUS and Gemcitabine (GEM), a myeloablative chemotherapy, was able to control primary tumor growth and reduce mortality. This response is dependent on T cell-mediated immunity. While this treatment remarkably resulted in some cures, the effects were not durable in every case; suggesting immunosuppression may be re-established. We hypothesize GEM is synergizing with FUS to immunologically control tumor growth by depleting myeloid derived suppressor cells (MDSCs). To test this, we will use a Ly6G-specific 1A8 antibody to deplete the MDSC population. Additionally, we have current studies focused on understanding alterations and the role of CD4 and CD8 T cells in tumor control following FUS+GEM therapy. While the CD8 T cell compartment does not appear to change as a result FUS treatment, there is a shift in the CD4 compartment. There is a decreased frequency of regulatory T cells (Tregs) accompanied by an increase in CD4 Helper T cells within both the tumor microenvironments and tumor draining lymph nodes of FUS+GEM treated mice. This suggests that CD4 T cells may be responsible for the primary growth control induced by FUS+GEM therapy. Notably, similar therapeutic approaches are now ongoing as a clinical trial at our institution. We expect our studies to reveal the mechanistic basis in which FUS and GEM are eliciting an immunological response resulting in primary tumor growth control and an overall survival advantage.

ABSTRACT #A-31

PANNEXIN 1 CHANNEL REGULATES CELL DEATH AND INFLAMMATION DURING ACUTE KIDNEY INJURY

Nabin Poudel[1], Marissa Yee[1], Shuqiu Zheng[1], Nataliya Skrypnyk[1], Eibhlin Goggins[1], Christopher B. Medina[2], Diane L. Rosin[1], Kodi S. Ravichandran[2], Mark D. Okusa[1]
[1]Division of Nephrology and Center for Immunity, Inflammation, and Regenerative Medicine, [2]Department of Microbiology, Immunology, and Cancer Biology, University of Virginia, Charlottesville, VA

Immunology Center Affiliation: CIIR

Background: Pannexin 1 (Panx1) is a heptameric membrane associated channel that serves as a conduit for release of small metabolites upon activation during cellular stress and injury. We have previously shown that pharmacological inhibition or genetic deletion of *Panx1* in mice prior to injury is protective against renal ischemia-reperfusion injury (IRI). How Panx1 contributes to acute kidney injury (AKI) pathology is unknown. We hypothesized that Panx1 induces cell death by mediating release of metabolites that influence both intracellular and extracellular events.

Methods: We performed bilateral IRI or cisplatin-induced AKI in a transgenic mouse that overexpress human isoform of PANX1 globally (*PANX1*^{Tg}) or specifically in kidney proximal tubule epithelial cells (*PTEC*^{PTg}) and assessed extent of kidney injury. For mechanistic studies, we challenged *PANX1* overexpressing proximal tubule epithelial cells (OX) and their controls with cisplatin *in vitro* and assessed cell death and mitochondrial changes. We next used flow cytometry to assess immune cell infiltration in the kidneys of PANX1 overexpressing animals after cisplatin AKI.

Results: *PANX1*^{Tg} mice had significant rise in plasma creatinine and expression of kidney injury marker *Ngal* in the kidneys in both models of AKI compared to their littermate controls. *PTEC*^{PTg} mice also had significantly higher injury compared to their littermates in both cisplatin as well as IRI induced AKI. Assessment of mitochondria in kidneys showed a significant reduction in Drp1 levels in *PANX1*^{Tg} kidneys compared to wildtype littermate controls after cisplatin challenge. *In vitro* studies showed that OX cells had significantly higher cell death compared with wildtype controls. Furthermore, in a co-culture model in which both wildtype and Panx1 overexpressing cells were cultured together, OX cells had greater cell death compared to wildtype controls during cisplatin challenge. Conditioned media from cisplatin challenged OX cells also induced greater cell death compared to that from cisplatin challenged control cells. The higher cisplatin induced cell death was associated with reduced mitochondrial biogenesis, mitochondrial function, increased mitochondrial ROS production, and altered mitochondrial quality control. Flow cytometric analysis revealed a higher infiltration of neutrophils, CD8-positive T cells, and CD11b-positive dendritic cells in the kidneys of *PTEC*^{PTg} animals compared to their littermate controls.

Conclusions: Our data demonstrate that PANX1 overexpression results in overt renal injury during AKI that is in part mediated by reduced mitochondrial function and increased inflammation. These results provide strong rationale for the development of selective strategies to inhibit Panx1 in the prevention or treatment of AKI.

ABSTRACT #B-32

DISTAL METABOLIC REGULATION OF PRE-CANCEROUS MAMMARY TISSUE BY THE GUT MICROBIOME DRIVES HR+ BREAST TUMOR METASTASIS VIA IMMUNE-MODULATED CROSSTALK

Putelo A. 1, Feng T. (Alkaid). 1, Kolli S. 1, McGinty Mitch. 1, Rutkowski M. 1

1: University of Virginia School of Medicine
Department: Microbiology, Immunology, Cancer Biology
Carter Immunology Center

Immunology Center Affiliation: CIC

Hormone receptor-positive (HR^+, $HER2^-$) is reported as the most prevalent subtype of breast cancer in the US. Though first-line treatment options have increased long-term survival for HR^+ breast cancer patients, reducing mortality amongst those who develop metastatic disease remains a challenge. Previous work in our lab found commensal dysbiosis, defined as an inflammatory gut microbiome with low biodiversity, enhances the metastatic potential of HR^+ breast tumors. Furthermore, in response to commensal dysbiosis, we observe long-term cellular, molecular and immunological changes in normal (non-tumor bearing) mammary tissue that resemble a favorable environment for tumor dissemination. This notion is congruent with evidence that defines crosstalk between the adjacent tissue and tumor microenvironment— often immune-mediated and preceding tumor diagnosis— as a driver of metastatic disease. Herein lies the question of exactly how dysbiosis-induced perturbations to the gut microbiome distally regulate normal mammary tissue to ultimately promote breast tumor dissemination. Using an *in vivo* model, we established commensal dysbiosis by gavaging mice with broad-spectrum antibiotics to evaluate systemic and mammary tissue-resident changes, prior to tumor initiation, that may enhance HR^+ tumor dissemination. To understand how immune-mediated crosstalk impacts related signaling pathways in normal tissue, we metabolically profiled mammary fat pads from dysbiotic and non-dysbiotic murine cohorts. Metabolomics revealed increased accumulation of acylcarnitine species in dysbiotic mammary tissue. Acylcarnitine metabolites, which are byproducts of incomplete β-oxidation of fatty acids, induce inflammatory cytokines in immune cell subsets and are associated with adipose tissue inflammation and linked to metabolic diseases, possibly by contributing to insulin resistance. In this regard, we tested how dysbiosis impacts insulin-glucose dynamics. Non-fasted glucose levels and fasted glucose levels in response to insulin challenge were significantly elevated in dysbiotic mice, accompanied by reduction in signaling downstream of the insulin receptor in the mammary tissue, muscle and liver. Altogether, we propose that commensal dysbiosis triggers a systemic metabolic shift that enhances HR^+ breast cancer metastasis by shaping the immune landscape of pre-cancerous mammary tissue.

ABSTRACT #A-33

A SECRETORY PHENOTYPE ASSOCIATED WITH ENGULFMENT AND CELL MOTILITY PROTEIN, ELMO1

Aspen Robinson and Sanja Arandjelovic

Center for Immunity, Inflammation and Regenerative Medicine

University of Virginia

Immunology Center Affiliation: CIIR

Inflammatory diseases, such as Rheumatoid Arthritis (RA), affect millions of individuals worldwide. However, mechanisms of inflammatory immune cell activation and potential therapeutic targets, remain an area of ongoing research. Engulfment and cell motility protein 1 (ELMO1) binds to Dock family members to form a guanine exchange factor for Rac GTPase to induce rearrangements of the actin cytoskeleton. ELMO1 has been shown to be a promoter of apoptotic cell clearance and cell migration and contributes to neutrophil trafficking and osteoclast function in mouse models of RA, suggesting that inhibition of ELMO1 may open a new therapeutic avenue in inflammatory disease.

In addition to the inflammatory capabilities of mast cells most often associated with allergy, degranulation of mast cell TNF has recently been shown to influence neutrophil extravasation into injured and inflamed tissues, indicating a role for mast cell degranulation as a modulator of inflammatory signaling. We noted high expression of ELMO1 in mast cells. However, ELMO1 has not previously been linked to a secretory phenotype. Here, we show that bone marrow derived mast cells (BMMC) from *Elmo1*$^{-/-}$ mice secrete fewer inflammatory cytokines after stimulation *in vitro*, compared to wild type BMMC. Surprisingly, this difference was not at the level of cytokine expression, suggesting reduced exocytosis in the absence of ELMO1. In a degranulation assay, we observed reduced release of the mast cell granule proteins from stimulated ELMO1 deficient mast cells. Our current studies are focused on testing the role of ELMO1 in an *in vivo* model of mast cells activation, passive cutaneous anaphylaxis.

Taken together, these findings suggest that ELMO1 may contribute to the secretory function of mast cells and that loss of ELMO1 leads to reduced cytokine secretion and inflammatory capabilities. We propose that ELMO1 may also be an attractive new target for therapy in mast cell mediated allergy.

ABSTRACT #B-34

TRAUMATIC BRAIN INJURY INDUCED LYMPHATIC DYSFUNCTION AGGRAVATES TAU DISEASE AND COGNITIVE DECLINE IN THE P301S MODEL OF TAUOPATHY.

(2 presenters) Royo Marco A., Bruch K., Bolte A., Lukens J. BIG center

Immunology Center Affiliation: BIG

Traumatic Brain Injury (TBI) is a known risk factor for developing a neurodegenerative disease later in life, including Alzheimer's Disease (AD) and other forms of tauopathy such as chronic traumatic encephalopathy (CTE) and frontal-temporal dementia (FTD). Although several studies highlight the relationship between the severity and frequency of a TBI and the progression of these tauopathy diseases, the mechanism behind this association remains unknown–as does a potential rescue therapy. While post-TBI inflammation is beneficial for clearing debris and promoting tissue repair, decreased meningeal lymphatic drainage and prolonged neuroinflammation have shown to be a cause of secondary injury, rendering the brain susceptible to earlier neurodegeneration. In our work, we explore how mild TBI (mTBI) in young, three-month-old mice expressing P301S mutant human tau (PS19) accelerate the disease course and severity, and whether the administration of VEGFc, a promoter of lymphangiogenesis, could rescue these long-term deleterious effects. Here we show with immunofluorescence (IF), that four months after mild TBI, the brains of PS19 exhibit signs of chronic neuroinflammation and increased tau burden in the ipsilateral hemisphere. These findings correlate with our behavior studies showing earlier signs of hyperactive locomotion, cognitive decline, and poor neuronal health in the TBI group. We hypothesize that the administration of VEGFc within 24-hours after mTBI will reduce the accelerated tau burden and clinical symptoms in the TBI group.

ABSTRACT #A-35

FUNCTION OF CASPASE-8-DEPENDENT APOPTOSIS IN CONTROLLING CHRONIC *TOXOPLASMA GONDII* INFECTION

Lydia Sibley, Isaac Babcock, Maureen Cowan, Mike Kovacs, Sydney Labuzan, Tajie Harris
Center for Brain Immunology and Glia
University of Virginia

Immunology Center Affiliation: BIG

Toxoplasma gondii is a protozoan parasite that lives chronically encysted in immune privileged tissue, i.e. the brain. Continual immune activity is necessary to combat and control parasite replication and spread during a chronic brain infection. Recent studies have demonstrated that one method of *T. gondii* survival is by interfering with the immune defense of infected cells, specifically blocking the activation of caspase-dependent apoptosis. Here we investigate the role of caspase-8, a regulator of the extrinsic apoptosis pathway, during *T. gondii* infection. We found that chronically infected RIPK3$^{-/-}$Casp8$^{-/-}$ mice have an 8-fold increase in the number of *T. gondii* cysts in the brain in comparison to controls. However, the RIPK3$^{-/-}$Casp8$^{-/-}$ mice mounted strong immune T cell and myeloid cell response as well as IFN-γ and iNOS production, the main mediators of parasite killing, in the brain. We hypothesize that parasite infected cells typically undergo apoptosis as a mechanism of controlling infection. In RIPK3$^{-/-}$Casp8$^{-/-}$ mice cells that are unable to undergo apoptosis become a niche for parasite survival. This leads to a greater potential for parasite replication, leading to an increase parasite burden that cannot be maintained by the robust immune response.

ABSTRACT #B-36

THE ROLES OF *CLOSTRIDIOIDES DIFFICILE* BINARY TOXIN AND TOLL-LIKE RECEPTOR 2 IN THE PATHOGENESIS OF *C. DIFFICILE* INFECTION

Simpson M, Bilverstone T, Frisbee A, Leslie J, Donlan A, Uddin MJ, Kumar P, Schwan C, Minton N, Petri Jr. WA

Immunology Center Affiliation: CIC

Clostridioides difficile, the causative agent of the most common hospital-acquired gastrointestinal infection, is responsible for approximately 500,000 infections and 29,000 deaths in the US each year despite readily available antibiotic therapy. Increases in the rates of infection and disease severity have been linked to the emergence of hypervirulent strains of *C. difficile* expressing the binary toxin CDT. While CDT expression has been shown to enhance virulence during infection, there is still much that is unknown regarding the mechanisms responsible.

Our lab has previously demonstrated that TLR2 recognizes CDT to induce downstream inflammation. To further explore this interaction, we utilized an NF-κB reporter cell line and determined that CDT was recognized by the TLR2/6 heterodimer to induce an NF-κB response. Furthermore, the binding component (CDTb) alone was sufficient to induce this response and could induce IL-1β transcript expression in bone marrow-derived dendritic cells. To study the contribution of CDTb during *in vivo* infection, we developed novel isolates of *C. difficile* expressing the separate components of the binary toxin and tested them using a hamster model of CDI. Infection of hamsters with an isolate of *C. difficile* expressing CDTb without CDTa resulted in more severe disease as compared to those infected with an isolate deficient in both components, indicating that CDTb enhances virulence during infection. Interestingly, CDTb expression in the absence of CDTa did not affect virulence in the mouse model, highlighting the impact that selection of an animal model can have on *in vivo* infection studies.

Overall, we found that CDTb alone interacts with the TLR2/6 heterodimer to induce downstream inflammation and that CDTb expression enhances virulence in a hamster model of CDI. It is vital to understand how virulence factors like CDT can affect the host response during infection, as this knowledge can help identify novel targets for therapeutic treatment.

ABSTRACT #A-37

INTRACRANIAL TUMOR IMMUNOGENICITY IS LIMITED BY ANTIGEN TRANSPORT FROM TUMOR TO MENINGES

Katarzyna Stasiak[1,2], MA Kovacs[3,4], AC Bolte[3,4], RS Lindsay[1,2] , M Perusina Lanfranca[1,2,], JR Lukens[3,4], TH Harris[3,4], VH Engelhard[1,2]. [1]Carter Immunology Center, [2]Dept of Microbiology, Immunology, Cancer Biology, [3]Center for Brain Immunology and Glia, [4]Dept of Neuroscience, Univ of Virginia, Charlottesville, USA.

Immunology Center Affiliation: CIC

While intratumoral CD8 T cells (TIL) in cutaneous melanoma correlate with longer survival, intracranial (IC) melanomas are poorly infiltrated. IC B16 melanomas had fewer TIL than subcutaneous (SC) tumors, and were larger, suggesting lack of immune control. To test homing to IC tumors we determined that expression of VCAM1, ICAM1, and E-selectin, relevant homing receptor ligands (HRL), on tumor vascular endothelial cells (TEC) was lower on IC TEC relative to SC. Only ICAM1 levels were higher on IC TEC versus normal brain endothelial cells (bEC). Upregulation of VCAM1 and ICAM1 on SC TEC depends on IFNγ^+ CD8 effectors. However, no changes of HRLs were found on IC TEC from IFNγ or TNF receptor knock out mice. Thus, the IC tumor inflammatory tone is distinct. Surprisingly, exogenously activated CD8 T cells infiltrated both tumors equally, suggesting IC tumors do not elicit a robust immune response. Additionally, more TIL infiltrated IC tumors in mice immunized against a tumor Ag than in naïve mice and SC tumors in immunized mice.

Dendritic cells (DC) in tumor draining lymph nodes (tdLN) lacked tumor Ag, suggesting poor immunogenicity resulted from impaired drainage from tumor via meningeal lymphatic vessels (MLV) to tdLN. However, MLV drainage of intra-cisterna magna injected beads or naïve T cells to tdLN was not altered. Numerous tumor Ag carrying DC were present in IC tumors, but a lower fraction were Ag positive and they were less mature than in SC tumors. Tumor Ag carrying DC were sporadically observed in the meninges, but intra cisterna magna transferred T cells were not activated there. Our results suggest that: 1) brain microenvironment limits DC Ag acquisition and maturation; and 2) poor antigen and/or DC transport from tumor to MLV, likely via glymphatics, limits IC tumor immunogenicity

ABSTRACT #B-38

ASSESSING TREATMENT-RESISTANT CANCERS THROUGH CELLULAR INTERACTION NETWORKS

Anne M. Talkington[1,2], Sepideh Dolatshahi[1,2]

[1]Department of Biomedical Engineering, University of Virginia

[2]Carter Immunology Center, University of Virginia

Immunology Center Affiliation: CIC

In recent years, pharmacogenomic approaches have greatly advanced our understanding of hallmarks of cancer progression and differential responses to treatment based on large gene expression datasets. These approaches hold even greater potential with the advent of single-cell RNA sequence analysis (scRNA-seq). Utilizing the resolution of scRNA-seq data, we examined the tumor microenvironment (TME) of biopsied tissue from 19 melanoma patients and 18 colorectal cancer patients from publicly available data sets. More specifically, we implemented network inference methods to infer interactions between T cells, tumor cells, and macrophages in the TME. Here we applied CytoTalk, a *de novo* inference method, to infer intercellular communication and downstream intracellular signaling mechanisms both on average in the population and at the individual patient level. Recurring intercellular interactions included VIM -> CD44, CD58 -> CD2, PVR -> CD96, and other adhesion processes between tumor and (CD8+) T cells. We also observed the ligand-receptor pairs HLA (T cell) -> LILRB (macrophage) and GAL9 (macrophage) -> TIM3 (T cell), indicating that immune cells may be responsible for suppressing one another in addition to responding to signals they receive from the tumor. We further considered these interactions in the context of post-translational modifications, namely, the glycosylation profile of the TME, which has been shown to be associated with the malignancy of solid tumors. Of genes known to associate with later stages of melanoma, we found the highest and most variable expression of ST3GAL1 and ST6GAL1 in the TME across patients, followed by ST3GAL3 and FUT8, indicating the potential for upregulation of sialylation and core fucosylation processes in these systems. Ultimately, these network inference strategies taken in the context of the post translational landscape hold potential for informing *in silico* models for patient prognosis.

ABSTRACT #A-39

MUCOSAL IMMUNITY AGAINST SARS-COV-2 VARIANTS OF CONCERN INCLUDING OMICRON FOLLOWING VACCINATION

Jinyi Tang[1,2,3*], Cong Zeng[4,5*], Thomas M. Cox[3], Chaofan Li[1,2,3], Young Min Son[1,2,3], In Su Cheon[1,2,3], Yue Wu[6], Supriya Behl[7], Justin J. Taylor[8], Rana Chakaraborty[7], Aaron J. Johnson[9], Dante N Shiavo[3], James P. Utz[3], Janani S. Reisenauer[3], David E. Midthun[3], John J. Mullon[3], Eric S. Edell[3], Mohamad G. Alameh[10], Larry Borish[11], Mark H. Kaplan[12], Drew Weissman[10], Ryan Kern[3], Haitao Hu[13], Robert Vassallo[3], Shan-Lu Liu[4,5#] and Jie Sun[1,2,3,6#]

1. Carter Immunology Center, University of Virginia, Charlottesville, VA, USA 22908
2. Division of Infectious Disease and International Health, Department of Medicine, University of Virginia, Charlottesville, VA, USA 22908
3. Division of Pulmonary and Critical Medicine, Department of Medicine, Mayo Clinic, Rochester, MN, USA 55905
4. Center for Retrovirus Research, The Ohio State University, Columbus, OH, USA 43210
5. Department of Veterinary Biosciences, The Ohio State University, Columbus, OH, USA 43210
6. Department of Immunology, Mayo Clinic, Rochester, MN, USA 55905
7. Department of Pediatrics and Adolescent Medicine, Mayo Clinic, Rochester, MN 55905, USA
8. Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA
9. Department of Immunology, Mayo Clinic, Rochester, MN 55905, USA
10. Department of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
11. Division of Asthma, Allergy and Immunology, Department of Medicine, University of Virginia, Charlottesville, VA, USA 22908
12. Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN 46074, USA
13. Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX, USA 77555

*, # These authors contribute equally

Email: liu.6244@osu.edu, js6re@virginia.edu

SARS-CoV-2 mRNA vaccination induces robust humoral and cellular immunity in the circulation; however, it is currently unknown whether it elicits effective immune responses in the respiratory tract, particularly against variants of concern (VOCs), including Omicron. We compared the SARS-CoV-2 S-specific total and neutralizing antibody (Ab) responses, and B and T cell immunity, in the bronchoalveolar lavage fluid (BAL) and blood of COVID-19 vaccinated individuals and hospitalized patients. Vaccinated individuals had significantly lower levels of neutralizing Ab against D614G, Delta and Omicron in the BAL compared to COVID-19 convalescents, despite robust S-specific Ab responses in the blood. Further, mRNA vaccination induced significant circulating S-specific B and T cell immunity, but in contrast to COVID-19 convalescents, these responses were absent in the BAL of vaccinated individuals. Using an animal immunization model, we demonstrate that systemic mRNA vaccination alone induced weak respiratory mucosal neutralizing Ab responses, especially against SARS-CoV-2 Omicron; however, a combination of systemic mRNA vaccination plus mucosal adenovirus-S immunization induced strong neutralizing Ab response, not only against the ancestral virus but also the Omicron variant. Together, our study supports the contention that the current COVID-19 vaccines are highly effective against severe disease development, likely through recruiting circulating B and T cell responses during re-infection, but offer limited protection against breakthrough infection, especially by Omicron. Hence, mucosal booster vaccination is needed to establish robust sterilizing immunity in the respiratory tract against SARS-CoV-2, including infection by Omicron and future variants.

ABSTRACT #B-40

SINGLE CELL RNA SEQUENCING REVEALS MECHANISMS UNDERLYING A SENESCENCE-LIKE PHENOTYPE OF ALVEOLAR MACROPHAGES DURING AGING

Yue Wu[1,2,5*], Bibo Zhu[3,5,6*], Ruixuan Zhang[3,5,6], Nick P. Goplen[4], Xiaochen Gao[2,3], Harish Narasimhan[5,6], Ao Shi[1], Yin Chen[1,2], Ying Li[7], Haidong Dong[2,8], Thomas J. Braciale[5,6] and Jie Sun[1,2,3,4,5,6#]

1. Mayo Clinic Graduate School of Biomedical Sciences, Rochester, MN 55905, USA.
2. Mayo Clinic Department of Immunology, Rochester, MN 55905, USA.
3. Division of Pulmonary and Critical Medicine, Department of Medicine, Mayo Clinic, Rochester, MN 55905, USA.
4. Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, MN 55905, USA.
5. Carter Immunology Center, University of Virginia, Charlottesville, VA 22908, USA.
6. Division of Infectious Disease and International Health, Department of Medicine, University of Virginia, Charlottesville, VA 22908, USA.
7. Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN 55905, USA.
8. Department of Urology, College of Medicine, Mayo Clinic, Rochester, MN 55905, USA

* equal contributor # correspondence author

Immunology Center Affiliation: CIC

Alveolar Macrophages (AMs) are unique innate immune cells that reside in the alveolar space and accommodate the ever-changing need of the lungs against internal and external challenges. During homeostasis, AMs maintain themselves through self-renewal without input from adult hematopoietic stem cells. Currently, little is known about how aging influences AM dynamics, heterogeneity and transcriptional profiles. Here, we identified CBFβ as an indispensable transcription factor that ensures AM self-renewal. Deficiency in CBFβ led to decreased proliferation and self-renewal ability of AMs. Moreover, with single cell RNA sequencing analysis of AMs from young and aged mice, we discovered that despite for the similar transcriptome in the proliferating cells, AMs from the aged mice had reduced embryotic stem cell-like features. Those aged AMs also showed diminished DNA repairing ability, which could contribute to their abrogated capacity to pass through the cell cycle checkpoints and elevation of senescence markers. In accordance with the analysis, we observed a reduced number of AMs in the aged mice, which had defective self-renewal ability and were more sensitive to the reduction of GM-CSF. Interestingly, decreased CBFβ was observed in the cytosol of the AMs from the aged mice. A similar phenotype was also found in human AMs. Taken together, we concluded that AMs in the aged host harbor a senescence-like phenotype, and CBFβ could play a role in such phenotype.

ABSTRACT #A-41

SEX BIAS IN MATERNAL IMMUNE ACTIVATION NEURODEVELOPMENTAL DISORDERS INITIATE AT THE PLACENTA

Kristine E. Zengeler, Catherine R. Lammert, Daniel A. Shapiro, Katherine Bruch, Hannah Ennerfelt, John R. Lukens

Center for Brain Immunology and Glia, Department of Neuroscience, School of Medicine, University of Virginia, Charlottesville, VA

Immunology Center Affiliation: BIG

Inflammation during pregnancy is increasingly recognized as a significant risk factor for the onset of neurologic disorders in offspring including autism spectrum disorder (ASD). The murine maternal immune activation (MIA) model of ASD recapitulates the male sex bias and behavioral abnormalities observed in the human condition. To address whether sex differences in fetal neurodevelopment begin at the placenta, the first site that the fetus encounters maternal inflammation, RNA-sequencing was conducted on placental tissue from MIA and control dams. Transcriptional signatures indicated a robust immune response, largely driven by Interferon signaling, 3-hours post-initiation of maternal inflammation that was resolved by 48 hrs. We identified notable baseline sex differences in the placental transcriptome that were completely dampened by MIA which could explain some of the behavioral sex bias observed in MIA offspring. The resident immune cells of the brain, microglia, are known to be sensitive to environmental stimuli and may contribute to neurodevelopmental alterations. RNA-sequencing conducted on sorted microglia from MIA offspring across development revealed no distinct changes to the microglial transcriptome. These findings suggest that sex-specific changes to neurodevelopmental may begin at the placenta.

ABSTRACT #B-42

POTENTIAL OF A NEW INSULIN SENSITIZER TO MITIGATE ACUTE VIRAL PNEUMONIA

Bibo Zhu[1], Xiaoqin Wei[1], Jie Sun[1*]

[1], Beirne B. Carter Center for Immunology Research (CIC),

University of Virginia School of Medicine

*, Corresponding author

Immunology Center Affiliation: CIC

Abstract

Viral pneumonia including COVID-19 is a serious complication associated with hyper-inflammation of the lungs caused by respiratory viral infection. These diseases are particularly dangerous to people with existing comorbidities, including obesity and/or diabetes. Although vaccines are the best for preventing viral pneumonia, emergence of novel variants and decline of vaccine-induced immunity highlight the need for the development of effective therapeutics targeting hyper-inflammation and hyperglycemia in populations with metabolic or diabetic conditions. In this study, we test the efficacy of a novel insulin sensitizer MSDC-0602k, which targets mitochondrial pyruvate carrier (MPC), in mitigating viral pneumonia. Our data showed that MDSDC-0602k treatment reduced host mortality and improved tissue recovery following influenza virus (IAV) infection in both lean and obese mice. Importantly, we discovered that MSDC-0602k can simultaneously diminish high blood glucose and dampen excessive pulmonary inflammation following IAV infection in a mouse model of type 2 diabetes mellitus. Mechanistically, MSDC-0602k treatment mainly targeted alveolar macrophage (AM) inflammatory responses to diminish host morbidity and mortality. Further, we observed reduced pulmonary inflammation in mice with myeloid-specific ablation of MPC2, that was sufficient for viral pneumonia amelioration. Further, MSDC-0602k treatment reduced human alveolar macrophages inflammation following SARS-CoV-2 infection, and MSDC-0602K suppressed inflammatory gene expression by lung cells isolated from COVID-19 lung autopsy samples. Thus, targeting MPC with MSDC-0602k in acute viral pneumonia is promising to simultaneously repress of hyperglycemia and hyper-cytokinemia following respiratory viral infection including SARS-CoV-2, particularly in those patients with underlying metabolic syndrome.