Abstracts & Posters

Afternoon Poster Session 1:
Posters 1 - 70

Evening Poster Session 2:
Posters 71 - 142
Cell division is an essential component of B cell differentiation to a plasma cell (PC). Division coupled changes in the expression of transcription factors that coordinate the PC program are known; however, little is known regarding the timing/extent of reprogramming by these factors as the cells are dividing in vivo. Furthermore, how/when these factors coordinate cell division during differentiation has remained unexplored. To address this, we assessed the cell division kinetics of wild-type (WT) B cells responding to LPS in vivo. Interestingly, we found that WT B cells must undergo 8 divisions before differentiating into PCs. Computational modeling of division rates defined a proliferative burst between 48-60 hours after LPS injection that is characterized by a rapid increase in the rate of cell division. In contrast, Interferon Regulatory Factor 4-deficient (IRF4-/-) B cells divided but stalled at divisions 2-6, failing to undergo the proliferative burst. To assess the timing/scope of IRF4-dependent reprogramming, WT and IRF4-/- responding B cells at divisions 0, 1, and 3-6 were sorted for ATAC- and RNA-seq. RNA-seq data revealed hundreds of differentially expressed genes (DEGs) when IRF4 was deleted, a subset of which included Myc target genes. ATAC-seq data also exposed hundreds of differentially accessible regions, the majority of which contained a known IRF4 binding motif and mapped to a corresponding DEG. Interestingly, IRF4-/- B cells progressively became more divergent as they divided when compared to WT B cells in the same division. Together, these data create a road map defining the role of IRF4 throughout B cell differentiation and reveal a critical role for IRF4 in maintaining the proliferative response.
Lynn vaccine strain (GMT 35 vs 217, P<0.0001). The majority of the participants (80%) received their second MMR vaccine ≥10 years prior to study participation. Lower mumps IgG ISR but high avidity, lower GMTs to circulating mumps wild type strain and no detectable mumps-specific MBC in 10% of previous MMR vaccine recipients underscores the need for comprehensive characterization of B and T cell immune responses to mumps vaccine. Strategies are needed to improve the quality and durability of vaccine-induced immunity.

Presenter: Giuseppe A. Sautto
Title: A computationally optimized broadly reactive antigen elicits unique broadly neutralizing antibodies targeting the influenza virus hemagglutinin receptor-binding site
E-mail: gasautto@uga.edu
Co-authors: Greg A. Kirchenbaum, Jeffrey W. Ecker, Rodrigo B. Abreu, Spencer R. Pierce, Ted M. Ross
Affiliation: Center for Vaccines and Immunology, University of Georgia, Athens, GA, USA

The development of broadly protective influenza vaccines will represent the main countermeasure to overcome influenza virus spread and improve the coverage offered by the current standard of care vaccine.

A computationally optimized broadly reactive influenza virus hemagglutinin (HA) antigen (COBRA), named P1, elicits a broadly reactive and neutralizing antibody (Ab) response against multiple seasonal and pandemic H1N1 strains.

In order to understand the mechanism of COBRA P1-conferred breadth of response, we first characterized the breadth of the Ab response at the B-cell level. Specifically, the reactivity of secreted Abs from B cells of BALB/c mice immunized with COBRA P1, seasonal or pandemic vaccine strains was assessed against a panel of H1N1 recombinant HA. Interestingly, while antibody secreting cells (ASC) from COBRA P1-immunized mice exhibited a broader recognition, those from seasonal or pandemic immunized animals showed a predominant homologous response.

In order to dissect the antibody response, a panel of COBRA P1-specific B-cell hybridomas was generated. Subsequently, the corresponding purified monoclonal Abs (mAbs) were assessed for the breadth of HA binding, hemagglutination inhibition (HAI) and neutralizing activity against a panel of H1N1 viruses. Collectively, both mAbs from COBRA P1- and seasonal or pandemic H1N1 HA antigens recognized head or stem HA epitopes. However, only COBRA P1-elicited Abs exhibited a broad spectrum of binding and functional activities, spanning from strain-specific to broadly reactive and neutralizing mAbs, while seasonal- or pandemic strains-elicited mAbs displayed a narrower spectrum of activity.

Importantly, all COBRA P1-elicited mAbs had unique CDRH3 sequences, suggesting different antigen-driven somatic mutations. Finally, epitope mapping studies revealed that COBRA P1-elicited broadly reactive and neutralizing mAbs recognized unique conserved epitopes within the HA receptor binding site.

Collectively, these studies shed light on the mechanism of breadth conferred by COBRA P1 antigen and leverage its optimization for the development of a more effective influenza vaccine.

Presenter: Brianna Swartwout
Title: Lactobacillus reuteri induced IgA production is strain-dependent and bears consequences for neonates
Email: bkswart@vt.edu
Co-authors: Lee G, Mu Q, Hardin K, Reilly CM, Luo XM
Affiliation: 1 Translational Biology, Medicine, Health Graduate Program, Virginia Tech, Roanoke, VA. 2 Virginia Tech-Carilion Medical School, Roanoke, VA. 3 Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College-Veterinary Medicine, Virginia Tech, Blacksburg, VA. 4 Department of Biochemistry, College of Science, Virginia Tech, Blacksburg, VA. 5 Edward Via College- Osteopathic Medicine, Blacksburg, VA.
Current Affiliation: Department of Medicine, Division of Immunology and Rheumatology, Stanford University School of Medicine, CA.
IgA is the antibody responsible for protecting against foreign invaders at mucosal sites. At 50mg/kg produced per day, it is the most abundant antibody in the body. Antibody diversification during early development is strictly regulated, thus the repertoire of endogenous IgA antibodies in neonates are immature, have a lower frequency of somatic mutation, and have a lower affinity for antigen. The mechanism of endogenous neonatal IgA production is still unknown. Mice studies show that early endogenous IgA production can be induced by nursing immunocompetent pups on immunocompromised dams. We hypothesize that the microbiota transferred through milk may play a critical role in instigating this increase in endogenous IgA production. Comparing milk from competent and compromised mice shows an increase in the species Lactobacillus reuteri. Evidence exists that L. reuteri can induce IgA production, but our data suggest that this feature is strain-dependent. Out of 4 strains analyzed (CF48-3A, 6475, 100-23, and 2010), CF48-3A most potently induced IgA production in spleen and intestinal lamina propria cultures. In vitro B-cell cultures showed that inactivated bacteria alone were sufficient to induce increased IgA production, and CF48-3A was consistently more potent than non-stimulatory strains. This phenomenon was found to correlate with increased proliferation of plasmablasts. Therefore, specific strains of L. reuteri, and other stimulatory bacteria, may directly perpetuate the presence of IgA positive B-cells in the neonatal gut by inducing proliferation of antibody secreting cells.

Presenter: Jingjing Ren
Title: Selective histone deacetylase 6 inhibition normalize B cell activation and germinal center formation in a model of systemic lupus erythematosus
Email: renji@vt.edu
Co-Authors: Michelle D Catalina, Kristin Eden, Xiaofeng Liao, Kaitlin Read, Xin Luo, Ryan P McMillian, Hatt Hulver, Matthew Jarpe, Prathyusha Bachall, Amrie C. Grammer, Peter E. Lipsky, Christopher M. Reilly
Affiliation: Virginia Tech, AMPEL Bio Solution, Edward via collage of osteopathic medicine

Autoantibody production by plasma cells (PCs) plays a pivotal role in the pathogenesis of systemic lupus erythematosus (SLE). The molecular pathways by which B cells become pathogenic PC secreting autoantibodies in SLE are incompletely characterized. Histone deacetylase 6 (HDAC6) is a unique cytoplasmic HDAC that modifies the interaction of a number tubulin- associated proteins; inhibition of HDAC has been shown to be beneficial in murine models of SLE, but the downstream pathways accounting for the therapeutic benefit have not been clearly delineated. In the current study, we sought to determine whether selective histone deacetylase (HDAC)6 inhibition would abrogate abnormal B cell activation in SLE. We treated 20-week-old NZB/W lupus mice with the selective HDAC6 inhibitor, ACY-738, for four weeks beginning at 20 weeks-of age. After only 4 weeks of treatment, manifestation of lupus nephritis (LN) were greatly reduced in these animals. We then used RNAseq to determine the genomic signatures of splenocytes from treated and untreated mice and applied computational cellular and pathway analysis to reveal multiple signaling events associated with B cell activation and differentiation in SLE that were modulated by HDAC6 inhibition. PC development was abrogated and germinal center (GC) formation was greatly reduced. When the HDAC6 inhibitor treated lupus mouse gene signatures were compared to human lupus patient gene signatures, the results showed numerous immune and inflammatory pathways increased in active human lupus were significantly decreased in the HDAC6 inhibitor treated animals. Pathway analysis suggested alterations in cellular metabolism might contribute to the normalization of lupus mouse spleen genomic signatures, and this was confirmed by direct measurement of the impact of the HDAC6 inhibitor on metabolic activities of murine spleen cells. Taken together, these studies show HDAC6 inhibition decreases B cell activation signaling pathways and reduces PC differentiation in LN and suggest that a critical event might be modulation of cellular metabolism.

Presenter: Rodrigo B. Abreu
Title: Longitudinal Assessment of Memory B cell and Plasmablast Reactivity
Influenza is a highly contagious viral respiratory disease that affects millions worldwide each year. Annual vaccination is recommended by the World Health Organization with the goal to reduce influenza severity and limit transmission through elicitation of antibodies targeting the hemagglutinin (HA) glycoprotein. The antibody response elicited by current seasonal influenza vaccines is predominantly strain-specific; however, continuous antigenic drift by circulating influenza virus isolates facilitates escape from pre-existing antibodies and requires frequent reformulation of seasonal influenza vaccine. Furthermore, pre-existing influenza immune responses can greatly impact the serological antibody response to vaccination. However, it remains unclear how B cell memory is shaped by annual vaccination over the course of multiple seasons, especially in high risk elderly populations. Here, we systematically profiled the B cell response in young adult (18-34 years old) and elderly (65+ year old) vaccine recipients that received annual split inactivated influenza vaccination for 3 consecutive seasons. Specifically, we quantified changes in frequency of memory B cell and plasma blast in peripheral blood prior, 7 and 21 days after vaccination over 3 consecutive seasons. Additionally, we tracked the frequency of vaccine-elicited H1 and H3-reactive memory B cells \( \mathbb{B}_{\text{mem}} \) by flow cytometry using tetrameric rHA probes ablated for sialic acid binding activity. Moreover, to assess the impact of sequential influenza vaccination on the \( \mathbb{B}_{\text{mem}} \) repertoire, we in vitro-differentiated donor PBMCs collected over 3 consecutive years and evaluated the breadth of \( \mathbb{B}_{\text{mem}} \)-derived antibodies against a panel of rHA by ELISA. In summary, we show that vaccine elicited \( \mathbb{B}_{\text{mem}} \) and plasmablast expansion is impaired in elderly subjects compared to young adults and that the memory B-cell repertoire is transiently reshaped by annual TIV vaccination. Collectively, these studies will shed invaluable insight into how pre-existing immunity shapes the memory B cell response to recurrent influenza vaccination.

Prior to their activation with peptides derived from foreign proteins presented by MHC Class II (pMHC), naïve CD4+ T cells experience weak TCR signals in response to self-pMHC. These weak “basal” TCR signals in response to self-antigens do not result in IL-2 production or cell division, though they may influence responses to foreign antigens. We aimed to determine whether relatively strong basal TCR signaling introduces a bias toward more robust activation to foreign antigen stimulation. Assessing how these basal TCR signals influence T cell function upon foreign antigen stimulation requires a method for visualizing the relative strength of basal TCR signaling in single cells. We found that the combination of a transgenic reporter of antigen receptor signaling (Nur77-GFP) and staining for the surface receptor Ly6C enables visualization of a broad range of basal TCR signaling. Cells experiencing the weakest basal TCR signals are GFP low Ly6C+, whereas cells experiencing the strongest basal TCR signals are GFP high Ly6C–. Though TCR specificity can skew the distribution of Nur77-GFP and Ly6C expression, we find that there is heterogeneity in the naïve CD4+ T cell population, even if they express identical transgenic TCRs, such as the OT2 or AND TCR. Furthermore, adoptive transfer studies indicate that expression of a given amount of Nur77-GFP and Ly6C is relatively stable. Subpopulations of cells experiencing weak, strong, or intermediate basal TCR signals were sorted and stimulated with anti-CD3 antibodies or cognate peptide and antigen presenting cells. Cells experiencing weak basal TCR signaling generate more sustained IL-2 responses and proliferate to a greater extent than cells experiencing moderate basal TCR signal strength. The GFP high Ly6C– cells consistently mounted attenuated IL-2 and proliferative responses, and expressed proteins associated with anergy, such as Grail and Cbl-b. We propose that this population of GFP high Ly6C– naïve CD4+
cells adapts to strong basal TCR signals by initiating negative regulation that attenuates their response to foreign antigen stimulation.

Presenter: Peyton VanWinkle
Title: Characterizing the role of Jagunal homolog 1 protein in neutrophil function
Email: pev18@uab.edu
Co-Authors: Peyton VanWinkle, Tomasz Nawara, Holly Thomas, Eunjoo Lee, Damian Kuna, Piotr Stasiak, Zdenek Hel, John Parant and Elizabeth Sztul
Affiliation: University of Alabama at Birmingham

The highly conserved endoplasmic reticulum protein Jagunal (JAGN1) was first identified as a requirement for Drosophila melanogaster oocyte development. Subsequently, JAGN1 mutations were correlated with Severe Congenital Neutropenia (SCN) in human patients. SCN is characterized by arrested granulocytic maturation in the promyelocyte stage. Neutrophils deficient in JAGN1 have altered granules, diminished fungal-killing capacity, and aberrant glycosylation of proteins. Overall, individuals with mutations in JAGN1 are susceptible to bacterial and fungal infections due to decreased number of circulating neutrophils and impaired release of granule content by the remaining cells. Despite its physiological importance, the cellular role of JAGN1 is poorly understood. Our goal is to use a zebrafish model, along with mammalian cells in culture, to define the molecular functions of JAGN1. The zebrafish (Danio rerio) offers a unique advantage for neutrophil studies as they are translucent, and neutrophil migration, degranulation and pathogen killing can be visualized in intact tissues in real time. Zebrafish have two homologs of human JAGN1 - JAGN1a and JAGN1b, an additional advantage since each may have a distinct function, providing additional information about the molecular mechanism of JAGN1 action. We have generated homozygous JAGN1a-/- and JAGN1b-/- knockout fish, and are now poised to elucidate the role of these proteins by monitoring neutrophil differentiation and by visualizing neutrophil migration and behavior during wound healing in intact animals. A human HL-60 myeloid leukemia cell line capable of differentiation into neutrophil-like cells will be used to analyze granule formation in the absence of JAGN1, define the step of secretion requiring JAGN1 function, and identifying JAGN1 interactors.

Presenter: Zerick Dunbar
Title: DISSECTION OF NATURAL KILLER CELL ANTI-TUMOR RECRUITMENT, MATURATION AND EFFECTOR FUNCTION
Email: zdunbar18@email.mmc.edu
Co-Authors: Anil Shanker
Affiliation:
1Department of Microbiology, Immunology and Physiology, School of Medicine, Meharry Medical College, Nashville, TN
2Department of Biochemistry, Cancer Biology, Neuroscience & Pharmacology, School of Medicine, Meharry Medical College, Nashville, TN
3School of Graduate Studies and Research, Meharry Medical College, Nashville, TN
4Host-Tumor Interactions Research Program, Vanderbilt-Ingram Comprehensive Cancer Center, Vanderbilt University School of Medicine, Nashville, TN
5Vanderbilt Institute for Infection, Immunology and Inflammation, Vanderbilt University School of Medicine, Nashville, TN

An issue in the fight against cancer is the growing discrepancy between resources being applied to treat cancer and stagnant patient survival rates. In part to help decrease this discrepancy by improving patient outcomes, research ongoing into immunotherapy uses one’s own immune cells to combat cancer cells. Cytotoxic innate lymphoid cells, conventionally known as natural killer (NK) cells, play major roles in cancer immunity largely due to their natural cytolytic and immune regulatory functions. However, due to the extensive heterogeneity seen among NK cells, their functions have yet to be fully harnessed. The objective of this research project is to elucidate NK cell maturation, recruitment and effector function in the context of solid tumor microenvironments. Using multi-color flow cytometry and functional assays, we will characterize NK cells over time at different tumor stages, localized versus distant metastatic tumors. We hypothesize that tumor infiltrating NK cells display a unique genetic
and functional profile with a higher expression of development and recruitment markers such as ID2, NCAM1 and Notch receptors, as well as increased effector function compared to non-tumor infiltrating NK cells. Here, we show the heterogeneity that exists among NK cells from different locations, as well as NK cell specific cluster of differentiation markers and gene expression perspectives based on flow cytometry, RNA, and bioinformatics analyses. We will also explore potential mechanisms that can be altered for enhancing NK cell anti-tumor function such as treatment with the FDA approved drug Bortezomib. Understanding and subsequently manipulating NK cells in the tumor microenvironment could prove to be a novel clinical immunotherapy tool for patients and mitigate the resources-results cancer discrepancy.

This work was supported by funds to A Shanker via the following NIH grants: U54 CA163069, U54 MD007593, SC1 CA182843 and R01 CA175370; and supported, in part, by the NIH RISE grant R25 GM059994.

Presenter  Ichiro Misumi
Title  T cell responses to Hepatitis A Virus in a mouse model
Email  imisumi@email.unc.edu
Co-authors  Joseph Mitchell, Makayla Lund, Stan Lemon, and Jason Whitmire
Affiliation  University of North Carolina at Chapel Hill, Department of Genetics

Hepatotropic viruses are major causes of human disease. Hepatitis A virus (HAV) is transmitted from person to person through the oral-fecal route and causes acute hepatitis. Globally, there are ~1.4 million cases of hepatitis A every year. In the US, sporadic hepatitis A outbreaks have been occurring more frequently. More than 15,000 cases and 140 deaths were reported in the last three years (CDC report, 2019). HAV infection can be prevented by a vaccine that induces strong humoral immunity. However, the contribution of T cells to virus control and the underlying mechanisms of pathogenesis have been difficult to study due to the lack of tractable small animal models. Our group recently showed that Ifnar1-/- mice are permissive for HAV infection and develop critical features of type A hepatitis, including acute liver injury and inflammatory immune responses, though declining level of viral RNA could be found for months after infection. Using this model and mouse-passaged HAV, we mapped CD8+ and CD4+ T cell epitopes and tracked T cell responses over time after infection. Murine T cell responses to HAV emerged only after several weeks of infection, similar to responses in infected non-human primates. HAV-specific CD4+ and CD8+ T cells were most abundant in the liver, coincident with declines in fecal virus shedding and liver HAV RNA. Moreover, T cell depletion resulted in an increase in infection and ALT release in both Ifnar1-/- and the chimeric mice. Finally, T cells accumulated PD1 expression over time, and interference with the PD1-PDL1 pathway improved immune control. Collectively, these results demonstrate that T cells protect against HAV infection and do not cause pathogenesis. Further, this study reveals that mice with defects in interferon responses can serve as a model for investigating HAV-specific T cell responses.

Presenter  Bhalchandra Mirlekar
Title  IL-35+ B cells establish immunosuppressive network in pancreatic ductal adenocarcinoma
E-mail  rmirlekar@med.unc.edu
Co-Authors  Yuliya Pylayeva-Gupta
Affiliation  Department of Genetics, University of North Carolina School of Medicine, Chapel Hill, North Carolina, 27599 and The Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, North Carolina.

Pancreatic cancer is the 3rd leading cause of cancer-related death worldwide with a dismal 5-year survival rate of 7 percent. Unfortunately, recent efforts geared towards treatment of pancreatic cancer with immunotherapy have not seen success. A major barrier for immunotherapeutic approaches in pancreatic cancer is marked immunosuppression within the pancreatic tumor milieu. Mechanisms driving immunosuppression in pancreatic cancer are not well known and understanding these mechanisms may help improve efficacy of current
immunotherapy. Our analysis reveals one such mechanism: a specialized subset of B cells, termed regulatory B cells, which promote pancreatic tumorigenesis through production of the immune-suppressive cytokine IL-35. Here, we set out to elucidate the cellular and molecular mechanisms of B cell derived IL-35 in the initiation and progression of pancreatic cancer. Our results demonstrate that IL-35 production by B cells, not by T cells functions in the tumor microenvironment and is essential for suppression of anti-tumor T cell responses. Importantly, while PDAC is typically resistant to anti-PD-1 immunotherapy, we demonstrate robust synergistic reduction in tumor growth when B cell specific IL-35 deficiency is combined with anti-PD-1 treatment. Furthermore, we assessed the effect of anti-IL-35 blockade in combination with immune checkpoint blockade therapy as a pharmacological approach to provide additional invigoration for T cell mediated killing of cancer cells. Collectively, our findings will reveal a potential novel function of B cell derived IL-35 in the pathogenesis of pancreatic cancer and could provide therapeutic insight for patient treatment.

Presenter: Anna Kania
Title: H3K27me3-specific demethylases restrict the magnitude of B cell differentiation
Email: akania@emory.edu
Co-authors: Madeline M. Price, Christopher D. Scharer, Robert R. Haines, Lou-Ella Alexander-George, and Jeremy M. Boss
Affiliation: Department of Microbiology and Immunology, Emory University, Atlanta GA

B cell terminal differentiation into antibody secreting plasma cells (PCs) must be properly regulated to ensure robust humoral immune responses against foreign but not self-antigens. While the role of transcription factors in this process is well-established, there is also a growing appreciation that epigenetic mechanisms also regulate B cell differentiation. The histone modification H3 lysine 27 trimethylation (H3K27me3) is associated with a repressive chromatin state resulting in gene silencing and is highly dynamic during B cell differentiation. EZH2, the enzyme that deposits H3K27me3, is required for proper B cell development, as well as germinal center, and PC formation. However, the role of active demethylation of H3K27me3 by the two demethylases UTX and JMJD3 in B cells is still to be elucidated. To determine the requirements for these enzymes in B lymphocytes, we crossed Utzx/fl/Jmjd3x/fl mice onto the Cd19Cre/+ background (dKO mice). Inoculation of Cd19Cre/+ (CreCtrl) and dKO mice with LPS, a T cell independent antigen, led to a significant increase in CD138+ PCs in UTX- and JMJD3-deficient mice. This increase was attributed to altered differentiation of marginal zone (MZ) but not follicular B cells (FoB) in response to LPS. RNA-seq analysis of PCs generated from MZ and FoB of CreCtrl and dKO mice revealed enhanced expression of genes associated with oxidative phosphorylation metabolism in dKO PCs. To determine the role of UTX and JMJD3 in T cell dependent B cell responses, we infected CreCtrl and dKO mice with PR8 influenza virus. Fourteen days post infection, we observed an increase in GC B cells and skewing in the GC dark/light zone polarity in dKO mice. Taken together, these data place H3K27me3 demethylases as critical enzymes that regulate the epigenetic dynamics of B cell fate and act to restrict the expansion of B cells following T cell dependent and independent stimuli.

Presenter: Robert Haines
Title: LSD1 cooperates with non-canonical NF-κB signaling to regulate marginal zone B cell development
Email: r.r.haines@emory.edu
Co-authors: Christopher D. Scharer, Jeremy M. Boss
Affiliation: Emory University

Marginal zone B cells (MZB) are a mature B cell subset that rapidly respond to blood-borne pathogens and are transcriptionally poised to differentiate. Although the transcriptional changes that occur throughout MZB development are known, the corresponding epigenetic changes and epigenetic modifying proteins that facilitate these changes are poorly understood. The H3K4 mono- and di-methyl demethylase LSD1, an epigenetic and transcriptional
modifier that promotes plasmablast formation, represents a prime candidate for regulating MZB development because of the high degree of overlap between MZB and plasmablast transcriptomes. B cell-conditional deletion of LSD1 resulted in a two-fold decrease in MZB while follicular B cells (FoB) and bone marrow B cell populations were minimally affected. LSD1 repressed genes in MZB that were normally upregulated in FoB and are involved in adhesion, migration, interferon response, transcriptional regulation, and signaling. MZB lacking LSD1 exhibited alterations in chromatin accessibility at transcription factor family motifs, including NF-κB. The importance of NF-κB signaling was examined through an ex vivo MZB development assay, which showed that both LSD1-deficient and NF-κB-inhibited transitional B cells failed to undergo full MZB development. Gene expression and chromatin accessibility analyses of in vivo- and ex vivo-generated LSD1-deficient MZB indicated that LSD1 regulated the downstream target genes of non-canonical NF-κB signaling. Immuno-precipitation experiments demonstrated that LSD1 directly interacted with the non-canonical NF-κB transcription factor p52. Overall, these data reveal that splenic B cell epigenomes are dynamically regulated and that the epigenetic modulation of the NF-κB pathway by LSD1 is an essential process during MZB development.

The systematic profiling of epigenomes in multiple cell types and (differentiation) stages is essential for understanding developmental processes and disease states. For this purpose Chromatin immunoprecipitation (ChIP) has been a commonly used technique that has undergone little change since its inception 30 years ago. This method allows for preservation of the in vivo pattern of protein-DNA complexes through formaldehyde fixation, fragmentation of the genome and immunoprecipitation of targets. Together with the development of next-generation sequencing platforms, this method has led to base-pair resolution mapping of transcription factors. However, the use of formaldehyde is thought to promote epitope masking and false positive binding sites. This finding drove the development of Native-ChIP without crosslinking using ionic conditions to maintain electrostatic contacts, with sensitivity and specificity trade-offs. These uncertainties emphasized the need for methods using different principles, which brought about Cleavage Under Target & Release Using Nuclease (CUT&RUN). This previously described technique tethers the micrococcal nuclease enzyme to protein A through a chimeric fusion, allowing for antibody directed cleavage of the protein-DNA complex of interest into solution for subsequent sequencing. In this report we assessed the sequencing resolution achieved with use of CUT&RUN on mouse lymphocytes. With some modifications to the original protocol we were able to achieve cleavage and release of DNA fragments in murine B- and T-cells for various histone modifications. The released DNA fragments sizes were visualized by bioanalyzer and depicted the presence of a nucleosomal ladder, which is consistent with the presence of histone modifications at nucleosomes. The nucleosomal ladder yield gradually dissipates with lower cell numbers. Targeted cleavage was obtained in as low as 5,000 cells as is evident by our ability to generate a library and sequencing with a high signal-to-noise ratio. Together these findings postulate CUT&RUN as an efficient methodology for epigenetic profiling of lymphocyte populations.

Interleukin-9 (IL-9)-producing CD4+ T helper 9 (Th9) cells are a distinct subset of Th cells induced from naïve CD4+ T cells by Interleukin-4 (IL-4) together with transforming growth fac-
tor-β (TGF-β) cytokine signaling In addition to roles in allergic inflammation and autoimmune diseases, the most intriguing function of Th9 cells is their antitumor activity. We were among the first to report antitumor features of Th9 cells. More recently, we reported that Th9 cells represent a novel third paradigm for T cell therapy – they are less exhausted, fully cytolytic, and hyperproliferative, and only tumor-specific Th9 cells completely eradicated late-stage advanced tumors, a scenario more like that seen clinically. To date, both IL-4 and TGF-β have been considered as the vital cytokines that are indispensable for Th9 cell-priming and differentiation. Here we, for the first time, show that “Th9 cell development” can occur in the absence of TGF-β signaling. We find that when TGF-β has been replaced by IL-1β, namely the combination of IL-1β and IL-4, efficiently promotes IL-9-producing T cells (Th9\textsuperscript{IL-4+IL-1β}). Next, we performed a microarray analysis comparing Th9\textsuperscript{IL-4+IL-1β} cells with other known Th cells. Interestingly, Th9\textsuperscript{IL-4+IL-1β} cells are phenotypically distinct T cells as compared to the classic Th9 cells (Th9\textsuperscript{IL-4+TGF-β}) and other Th cells, including the enrichment in IL-1-signaling and NF-κB-signaling signatures. Inhibition of TGF-β-signaling does not affect IL-9 production from Th9\textsuperscript{IL-4+IL-1β} cells. However, inhibition of NF-κB pathway, mainly RelA, negates the IL-9 production by Th9\textsuperscript{IL-4+IL-1β} cells. Furthermore, Th9\textsuperscript{IL-4+IL-1β} cells are less-exhausted T cells that have been endowed with cytotoxic T effector gene signature and tumor killing function, and exert a superior antitumor response than classic Th9\textsuperscript{IL-4+TGF-β} cells in the in vitro cytolytic killing assay and in mouse melanoma model. Therefore, our study sheds new light on Th9 cell differentiation, and provide an avenue for a potentially more powerful weapon for cancer immunotherapy.

Presenter: Guan Yang¹
Abstract title: Autophagy-related protein Vps34 controls the homeostasis and function of macrophages
Email: guan.yang@vumc.org
Co-authors: Joshua L. Postoak¹, Jian-hua Zhang², Lan Wu¹, and Luc Van Kaer¹
Affiliation: ¹Vanderbilt University School of Medicine; ²University of Alabama at Birmingham

Autophagy plays a central role in regulating immune cell responsiveness to a variety of stimuli, and defects in this process have been linked to a variety of diseases. The phosphatidylinositol-3 kinase vacuolar protein sorting 34 (Vps34) is a key early player in autophagy. Our previous studies found that Vps34 is a critical regulator that controls the development, homeostasis, and function of dendritic cells and T cells. However, the role of Vps34 in regulating macrophage functions is not well understood. We investigated the role of Vps34 in macrophage biology using myeloid cell-specific Vps34-deficient mice. Although these animals develop normally, they exhibit severe ongoing inflammation. Vps34 ablation in macrophages caused a partial impairment in the homeostatic maintenance of Tim-4+ macrophages and defective uptake of apoptotic cells. In addition, Vps34-deficient macrophages had modestly increased surface levels of MHC class II molecules. Interestingly, this altered macrophage phenotype was associated with an expanded population of regulatory T cells in the spleen and peritoneal cavity, but not thymus. Importantly, myeloid cell-specific Vps34-deficient animals showed significantly reduced severity of experimental autoimmune encephalomyelitis (EAE), a primarily CD4 T cell-mediated mouse model of multiple sclerosis. Collectively, our studies establish Vps34 as an important regulator of macrophage functions and macrophage-mediated regulation of EAE. Our findings also have important implications for the development of immunotherapies to treat multiple sclerosis and other inflammatory diseases by targeting Vps34.

Presenter: Esther Zumaquero
Title: IFNγ: an unexpected antibody secreting cell (ASC) fate in Systemic Lupus Erythematosus (SLE)
Email: ezm@uab.edu
Co-Authors: Christopher D. Scharer, Scott A. Jenks, Anoma Nellore, Betty Mouseau, Davide Botta, Alexander Rosenberg, Jeremy M. Boss, Ignacio Sanz, Frances E. Lund
Affiliation: The University of Alabama at Birmingham, Emory University
Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by loss of tolerance, production of autoantibodies (autoAb) and ultimately systemic and organ-specific disease. It is well known that B cells and inflammatory cytokines play an important role in the pathology of SLE. We have identified a population of T-bet\(^{hi}\) (IgD\(^{neg}\)CD27\(^{neg}\)CD11c\(^{+}\)CXCR5\(^{neg}\)) pre-ASCs that are expanded in a subset of SLE with increased systemic inflammatory cytokines, pathogenic autoAb and disease severity. Interestingly, we found that activation of naïve human B cells (B\(_{Na}\)) with allogeneic IFN\(_{g}\)-producing Th1 effectors plus IL-2 and IL-21 or with a cocktail of defined stimuli including anti-Ig, TLR7/8 ligand (R848), IL-2, IL-21 and IFN\(_{g}\), promoted the formation of a T-bet\(^{hi}\) B cell population that was phenotypically, transcriptionally, epigenetically and functionally similar to the SLE-derived T-bet\(^{hi}\) pre-ASCs. Mechanistically, we found that B\(_{Na}\) cells activated in the presence of IFN\(_{g}\) responded to very low levels of R848 and differentiate, expressed higher levels of IL-21R, and were more responsive to IL-21 (as measured by STAT3 phosphorylation). These results were consistent with the identification of a differentially accessible region (DAR) in the IL-21R locus following chromatin accessibility examination. Moreover, we observed that the appearance of the IL-21R DAR was dependent on the presence of IFN\(_{g}\) and IL-2 in the cultures and contained consensus binding motifs for STAT5 and T-bet (IL-2- and IFN\(_{g}\)-induced transcription factors). Interestingly, this IL-21R-associated DAR was also observed in T-bet\(^{hi}\) cells purified from SLE patients. These data suggest that IFN\(_{g}\) synergizes with IL-2 to poise the cells to proliferate, respond to IL-21 and differentiate. Hence, we have identified a novel role for IFN\(_{g}\) in ASC development that is specific for a subset of SLE patients, and which could be therapeutically targeted to prevent the development or differentiation of poised pathogenic pre-ASCs.

Presenter  S. Rameeza Allie
Title  Identification of antigen-specific, lung resident memory B cells after influenza infection
Email  silthyallie@uabmc.edu
Co-Authors  John E. Bradley, Uma Mudunuru, Michael D. Schultz, Frances E. Lund, Troy D. Randall.
Affiliation  Clinical immunology and rheumatology, University of Alabama at Birmingham

B cells display phenotypic and functional heterogeneity in multiple anatomical locations following vaccination or infection. Influenza-specific (Flu+) memory B cells (B\(_{MEM}\)) are found in both lymphoid tissues and lungs. It is unclear whether these cells represent circulating or resident memory B cell (BRM) populations. We hypothesized that a portion of the Flu+B\(_{MEM}\) cell population in the lung would be non-circulating, BRMs. Using a combination of B cell specific antibody infusion (i.v.) and parabiosis of influenza infected, congenically-mismatched mice we identified a protective, Flu+, non-circulating BRM population. The Flu+ BRMs in the lungs consisted of 56% IgM+ and 43.9% isotype-switched BRMs. They were established as early as 15 days after infection and maintained for at least 60 days. The formation of Flu+ BRMs required the germinal center (GC), as blocking CD40L with MR1 antibody, during the primary infection abrogated BRM. However, MR1-treatment of mice with established BRM did not affect BRMs in the lung, even though Flu+ GC B cells could be detected in the LN for up to 90 days. These data suggest that GC-dependent lung-BRMs are established early after infection and maintained independently of GCs. Ongoing analyses of the BRMs shows phenotypic and functional difference during a viral challenge, when compared to their lymphoid counterparts. These findings are important in the development of vaccines that elicit BRMs and they will provide mechanistic information into the function of antigen specific B\(_{MEM}\) cells residing in the mucosa.

Presenter  Jessica E. Thaxton, PhD, MsCR
Title  Metabolic Regulation of Translation Primes T Cell Anti-Tumor Immunity
Email  thaxton@musc.edu
Co-Authors  Katie E. Hurst, Kiley A. Lawrence, Arman B. Aksoy, Molly G. Sekar, Lee R. Leddy, Zihai Li
Affiliation  Medical University of South Carolina
T cells experience loss of function in tumors, but the cell-intrinsic mechanisms that rapidly disable tumor infiltrating lymphocytes (TILs) are unknown. Here we reveal that T cells that commit to high levels of new protein synthesis experience the terminal unfolded protein response (UPR) and lose the ability to control tumor growth. In contrast, we show that limiting T cell protein synthesis relieves the terminal UPR, reduces cell death, and imparts profound capacity of T cells to control tumors. We evidence the mechanisms that determine the extent of protein synthesis in T cells directed by the master energy sensor AMPK. Strikingly, using mouse models and samples from cancer patients, we reveal for the first time that the environment of tumors rapidly disables protein synthesis in conventional T cells. In contrast, we show that AMPK-primed T cells possess a remarkable capacity to overcome AMPK-constrained translation once in the stress of tumors in order to reinvigorate protein synthesis. Our work reveals that the cell response to stress dramatically shapes the ability of T cells to synthesize protein in tumors and we elucidate novel roles for paths to translation to impact CD8 TIL anti-tumor capacity.

Presenter       Rachel V. Jimenez
Title C-reactive protein promotes the expansion of myeloid derived cells with a suppressor phenotype
Email rjmenez@uab.edu
Co-authors Alexander J. Szalai
Affiliation Division of Clinical Immunology & Rheumatology, Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama

In previous studies of acute kidney injury (AKI) in C-reactive protein transgenic mice (CRPtg) our group showed that human CRP exacerbates renal injury. Importantly, the detrimental action of CRP in CRPtg was associated with their heightened renal accumulation of myeloid derived cells that had a suppressive phenotype (MDSC; able to suppress the proliferation of anti-CD3/CD28 stimulated T cells in co-cultures). Herein we provide evidence that CRP modulates both the development and the suppressive phenotype of MDSCs. In vitro CRP i) dose-dependently increased the generation of MDSC from wild type mouse bone marrow progenitors and ii) increased MDSC production of intracellular reactive oxygen species. When added to MDSC:T cell co-cultures, CRP significantly enhanced the ability of MDSCs to suppress anti-CD3/CD28 stimulated T cell proliferation. Experiments using MDSCs generated from FcγRIIB deficient bone marrow showed that while CRP’s ability to expand MDSCs and trigger their increased production of intracellular ROS did not require this inhibitory Fc receptor, CRP’s ability to enhance the T cell suppressive actions of MDSCs was entirely FcγRIIB-dependent. Our preliminary findings indicate that CRP also enables primary human neutrophils to suppress the proliferation of autologous human T cells. In their sum these results suggest that CRP might be an endogenous regulator of MDSC development and suppressive action in vivo, particularly in AKI.

Presenter       Jessica Peel
Title Germinal center organization mediated by T-bet dependent expression of CXCR3 and CCR6
Email jnpeel@uab.edu
Co-authors Sara L. Stone, Christopher D. Scharer, Jeremy M. Boss, Frances E. Lund
Affiliation University of Alabama Birmingham, Emory University

The germinal center (GC) is composed of distinct regions with proliferation and affinity maturation of GC B cells (GCB) occurring in the dark zone (DZ) and antigen-mediated selection and egress of the long-lived plasma cell (LL-PCs) precursors occurring in the light zone (LZ). Despite the key role for GCS in establishing enduring humoral immunity, we know little about the dynamics of the GCB cell response. We showed that the transcription factor T-bet is expressed by GCB cells and that B cell intrinsic T-bet is required for the LL-PC response following influenza (flu) infection. To test whether the loss of LL-PCs in these mice was due to a role for T-bet in regulating the GCB response, we infected 50:50 Tbx21-/-:pepboy chimeric...
mice with flu and monitored GCB cells. We found that Tbx21-/- B cells were overrepresented in the GC relative to the B6 cells and that Tbx21-/- GCB cells were enriched in the LZ. Partitioning of GCB cells in the LZ/DZ is regulated by chemokines and we showed that T-bet controls transcription of two chemokine receptors, CXCR3 and CCR6, which are expressed by flu-specific GCB cells. Using 50:50 Tbx21-/-:pepboy chimeras, we tested whether T-bet regulates GCB cell positioning by inducing CXCR3 and repressing CCR6. We observed that the CCR6+ GCB cell subset, which is known to localize in the LZ, was highly enriched in Tbx21-/- GCB cells. By contrast, the CXCR3+ GCB subset, which was dominated by WT cells, was enriched in the DZ. To determine the role for CXCR3 in GCB cell localization, we infected 50:50 Cxcr3-/-:pepboy chimeras and found that the Cxcr3-/- GCB cells are enriched in the LZ. Therefore, T-bet regulates GCB cell localization and potentially the dynamics of the GC response by controlling CXCR3 and CCR6 expression.

Presenter: Nathan Klopfenstein
Title: Inhibition of SOCS1 improves outcomes during subcutaneous MRSA skin infection
Email: nathan.klopfenstein@vanderbilt.edu
Co-Authors: Stephanie L. Brandt, C.H. Serezani
Affiliation: Vanderbilt University Medical Center

Methicillin-resistant Staphylococcus aureus (MRSA) is the primary cause of skin and soft tissue infections in health care settings and those with diabetes within the United States. MRSA skin infections are characterized by the formation of a neutrophillic abscess that is the hallmark of pyogenic infections to prevent bacterial spread to deeper tissues. However, the events and factors that drive ideal host defense during these infections remains to be fully elucidated. Here we examined the role of the protein SOCS1 and its impact of host defense during MRSA skin infection in both hyperglycemic and euglycemic mice. We have previously shown that hyperglycemic mice are unable to control MRSA skin infection, and demonstrate poor abscess formation. Our data indicate that SOCS1 expression is higher in hyperglycemic mice throughout the course of infection, correlating with their poor infection outcome. Pharmacological or genetic inhibition of SOCS1 in both control and hyperglycemic mice decreased lesion size and improved bacterial clearance in the skin, resulting in better infection outcome. Inhibition of SOCS1 throughout infection correlated with an increase in the production of pro-inflammatory cytokines TNF-α and IL-1β. Furthermore, both phagocytosis and intracellular bacterial killing were increased in macrophages isolated from hyperglycemic and control mice upon inhibition of SOCS1. Overall, these data indicate that the threshold of SOCS1 expression may negatively impact skin host defense in both hyperglycemic and other susceptible patient populations.

Presenter: Meagan Jenkins
Title: Lung-migratory Dendritic Cells traffic into the spleen after influenza virus infection
Email: meaganj@uab.edu
Co-Authors: Holly Bachus1, Beatriz Leon-Ruiz2 and André Ballesteros-Tato1
Affiliation: 1Department of Medicine, Division of Clinical Immunology and Rheumatology, University of Alabama at Birmingham, Birmingham, AL 35294.; 2Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294

Initiation of CD8+ T cell responses to influenza virus requires the trafficking of lung-migratory dendritic cells (mDCs) from the lung into the lung-draining LN (mLN). As such, when mDCs are absent or are unable to migrate into the mLN, T cell responses are severely compromised. Importantly, it is generally considered that mDCs die shortly after reaching the mLN. Thus, the current paradigm suggests that priming of influenza-specific T cell responses by lung-mDC takes place entirely in the mLN. We show here, however, that a fraction of the lung mDCs egress the mLN, enter the blood circulation, and subsequently
traffic to the spleen, where they prime influenza-specific CD8+ T cell responses. Mechanistically, the exit of mDCs from the mLN is controlled by an S1PR dependent mechanism. As a consequence, blockade of S1P/S1PR interactions prevents the egress of mDCs from mLN, thereby precluding the priming of T cell responses in the spleen. Collectively, our results demonstrate a novel DC trafficking pathway and support a new paradigm for how T cell responses to influenza are initiated.

Presenter: Ashlyn Anderson
Title: Understanding the role of STAT4 in CD4 T cell mediated neuroinflammation
Email: aea1294@uab.edu
Co-authors: Ian L. McWilliams, Boyoung Shin, Joy Shepard & Laurie E. Harrington.
Affiliation: University of Alabama at Birmingham

Multiple Sclerosis (MS) is an autoimmune disease that affects over two million people worldwide. MS is characterized by the demyelination of axons in the central nervous system (CNS), leading to vision problems, muscle weakness and poor coordination. Among the various immune cells that contribute to the disease, a subset of CD4 T cells, Th17 cells has been associated with MS pathogenesis. Recently, there has been a distinction between homeostatic Th17 cells, which do not directly contribute to disease and pathogenic Th17 cells that directly influence inflammation. Importantly, IL-23 signaling in Th17 cells is essential for the pathogenicity. The transcription factor STAT4 has been historically correlated with Th1 cells and is downstream of IL-23 signaling. As STAT4 deficiency does not prevent Th17 differentiation, but still limits inflammation, we hypothesize that STAT4 is necessary for the pathogenic properties of Th17 cells in MS. To study the influence of STAT4 in the context of neuroinflammation, our laboratory uses the adoptive transfer model of experimental autoimmune encephalomyelitis EAE. Our laboratory also utilizes in vitro CD4 T cell differentiate to identify how STAT4 influences the expression of genes in CD4 T cells cultured in “homeostatic” Th17 culture conditions compared to “pathogenic” Th17 conditions. We find that while STAT4 expression does not impact the development of Th17 cells but is necessary to induce CNS inflammation. Furthermore, global gene expression analysis indicates that STAT4 regulates the recently described pathogenic and nonpathogenic Th17 gene signatures. In the absence of STAT4, the levels of pathogenic Th17 genes including Tbx21, Il22 and Cxcl3 are significantly reduced, while the expression of nonpathogenic genes including Il10 and Ahr is increased. Furthermore, we find a significant increase of genes that rely on both IL-23 and STAT4 when compared to genes that rely solely on STAT4 in Th17 differentiated CD4 T cells. This leads us to further hypothesize that the IL-23/STAT4 signaling axis is vital to neuroinflammation. Together, these data reveal a novel role of STAT4 in controlling Th17 pathogenicity, which may provide a promising therapeutic target for MS patients.

Presenter: Matthew H. Collins
Title: Epitope targets of the human antibody response to Zika virus infection
Email: matthew.collins@emory.edu
Co-Authors: Matthew H. Collins1,2, Huy A. Tu3,4, Ciara Gimblet-Ochieng5, Guei-Juin Alice Liou5, Ramesh S. Jadi5, Stefan W. Metz5, Ashlie Thomas6, Benjamin D. McElvany4, Edgar Davidson5, Benjamin J. Doranz5, Yaoska Reyes5, Natalie M. Bowman2, Sylvia Becker-Dreps5, Filemón Bucardo7, Helen M. Lazear5, Sean A. Diehl3,4, Aravinda M. de Silva8
Affiliations: 1Department of Medicine, Emory University; 2Department of Medicine, University of North Carolina-Chapel Hill; 3Cellular, Molecular, and Biomedical Sciences Program, University of Vermont; 4Department of Microbiology and Molecular Genetics, University of Vermont; 5Department of Microbiology and Immunology, University of North Carolina; 6Integral Molecular, Inc.; 7Department of Microbiology, National Autonomous University of Nicaragua; 8Departments of Family Medicine and Epidemiology, University of North Carolina-Chapel Hill

Zika virus (ZIKV) transmission became a global public health emergency after the recent
epidemic in Latin America and beyond revealed rare but dire manifestations of infection such as severe birth defects and Guillain-Barré syndrome. ZIKV emerged in areas where other flaviviruses such as dengue are already endemic, and antibody (Ab) cross-reactivity among related flaviviruses creates challenges in accurately diagnosing infections, conducting reliable surveillance, as well as in understanding the distinguishing aspects of the host immune response to ZIKV. Vaccines represent a key strategy for prevention of infectious diseases and typically rely on robust antibody responses. To promote vaccine development and generate fundamental knowledge regarding the protective ZIKV-specific Ab response, we sought to analyze the durable antibody responses in individuals infected by ZIKV as a first flavivirus infection. We observed complex populations of antibodies that bind to epitopes on intact virions, simpler epitopes on envelope protein monomers as well as envelope subdomains. Moreover, strong neutralizing antibody responses that minimally cross-react with dengue viruses were consistently detected. To better understand the molecular determinants of the neutralizing antibody response to ZIKV and to develop tools that could aid vaccine development, we isolated two potently neutralizing monoclonal antibodies (mAbs) from one primary ZIKV case and mapped key amino acid residues involved in mAb binding and neutralization by multiple complimentary methods including generation of neutralization escape mutants and alanine scanning mutagenesis. The mAbs recognize different epitopes centered on domain I and domain II of the viral envelope protein. Functionally, both mAbs were protective in a lethal mouse model of ZIKV infection. Ongoing work is examining the prevalence of these specific Ab responses at the population level. This work provides new knowledge and tools that may be useful as diagnostic reagents or as therapeutics and will advance vaccine development.

Presenter: Amyn Murji
Title: Design and Development of Empirical and Rational Epitope-Focused HIV-1 Vaccine Candidates
Email: amyn.murji@vanderbilt.edu
Co-Authors: Juliana Qin, Ian Setliff, Nagarajan Raju, Allie Greenplate, Clint Holt, Ivelin S. Georgiev
Affiliation: Vanderbilt University

HIV-1 continues to impose a global health burden. Candidate vaccines using HIV-derived antigens have not proven effective to date, and efforts toward protection against new infections remain a high priority in HIV-1 research. In recent years, strategies that target the elicitation of broadly neutralizing antibodies that are capable of neutralizing a large fraction of circulating HIV-1 variants have emerged as a potential avenue to a prophylactic HIV-1 vaccine. The sole target of these neutralizing antibodies is the envelope protein (Env) of HIV-1. However, due to the extensive global diversity of HIV-1, Env-based vaccine candidates so far have only led to the elicitation of antibodies with limited neutralization breadth. To address this challenge, we propose to develop technologies for the presentation of diverse antigens to the immune system. In doing so, we developed candidate multivalent immunogens that recombinantly present on the surface of self-assembling protein nanoparticles: 1) multiple, diverse Envs or 2) conserved domains of the envelope protein. Here, we present progress toward the design and validation of a number of these technologies. We have successfully designed and developed candidate vaccines displaying Envs from two different clades (BG505 and CZA97). Nanoparticle-based immunogens, either as (a) a cocktail of BG505-only nanoparticles and CZA97-only nanoparticles or (b) multivalent nanoparticles simultaneously displaying both BG505 and CZA97 Envs, elicited more robust antibody responses in mice, compared to (c) soluble BG505 and (d) BG505 and CZA97 trimer cocktails. To increase our immunogen production efficiency, we have also developed multicistronic systems that can produce multivalent nanoparticle immunogens from a single transcript. While of particular significance for HIV-vaccinology, the technologies that we have developed will be generalizable to vaccine design for other viruses that exhibit high levels of sequence diversity.

Presenter: Aubrey Schonhoff
Title: Activated border-associated macrophages mediate peripheral cell infiltration in an AAV2 α-syn model of Parkinson Disease
Parkinson disease (PD) is characterized by progressive loss of dopamine-producing neurons in the substantia nigra pars compacta (SNpc) and widespread intracellular inclusions of the protein alpha-synuclein (α-syn). Recent evidence highlights the immune system as a possible mediator of PD progression. In both human patients and rodent models, α-syn pathology is accompanied by microglial activation, T cell infiltration, hyper-reactive monocytes, and increased cytokine and chemokine release. However, the triggers responsible for initiating this immune response and mediating peripheral cell recruitment to the CNS remain poorly understood. Additionally, many previous studies have not distinguished between the resident macrophage populations of the brain: microglia and border-associated macrophages (BAMs, including perivascular, meningeal, and choroid plexus macrophages), complicating the study of innate immune mechanisms. BAMs are uniquely positioned for monitoring the borders between the CNS and periphery, and may act as important antigen presenting cells or cytokine/chemokine producing cells in the disease process. To determine the role of BAMs in models of PD, we utilized an adeno-associated virus (AAV) that overexpresses full-length human α-syn in neurons of the SNpc. We injected this into transgenic reporter mice in which the first exon of CX3CR1 is replaced with GFP. Using flow cytometry and immunohistochemistry, we examined tissue resident CX3CR1+ cells (microglia and BAMs) for activation markers and proliferation. We found that α-syn led to an increase in the number of BAMs and in their expression of MHCII. To determine the functional role of BAMs, we specifically depleted the BAMs without affecting other cell populations, such as microglia or peripheral monocytes, using clodronate liposomes injected i.c.v. Depletion of BAMs had an anti-inflammatory effect, as it significantly decreased infiltration of peripheral immune cells and prevented upregulation of MHCII by microglia. We also investigated the specificity of additional BAM targeting techniques using anti-CSF1R antibodies. Furthermore, we used a CCL2 reporter mouse to provide evidence of CCL2 cytokine production by BAMs in response to α-syn. These results demonstrate the importance of BAMs in the initiation of neuroinflammation and the recruitment of peripheral immune cells subsets in an α-syn PD model.

Memory T cells provide enhanced immunity to previously encountered pathogens. During a primary infection, activation of naïve CD8+ T cells leads to differentiation of memory precursor effector cells, which ultimately develop into long-lived memory cells. However, the transcription factors (TFs) that establish and maintain the memory T cell developmental program are incompletely understood. Motifs encoding binding sites for both ETS- and bZIP-family TFs are highly enriched within cis-regulatory regions whose chromatin accessibility is induced in naïve CD8 T cells during primary TCR stimulation and that are also accessible in mature effector and memory T cell states. The ETS- and bZIP-family TFs are highly encoded by a large number of genes (28 and 60 genes, respectively), and RNA-seq analyses indicate they are dynamically expressed in naïve, early effector, and memory CD8+ T cell subsets during acute viral infection. To clarify their roles during memory CD8 T cells differentiation during acute viral infection, I am applying an in vivo pooled RNA interference (RNAi) screen targeting all genes from both families in naïve CD8+ T cells. Preliminary experiments indicate that insufficiency of one of the mostly highly expressed ETS members, Ets1 impairs effector cell accumulation and increases the relative frequencies of terminal effector cells, which is similar to the phenotypes induced by RNAi of TFs Cbfβ and Runx3. Inhibiting expression of Ets1 also decreases the expression of all the Runx-family members, as well factors that are downstream of Runx3 such as T-bet. Co-transduction of Ets1 shRNAmir and Runx3 cDNA largely rescues the Ets1 RNAi phenotype. These results suggest that Ets1 might function upstream of Runx3, and that both TFs cooperate to establish a memory program in recently
activated CD8+ T cells. Furthermore, the pooled RNAi approach will likely clarify the contribution of additional TFs to memory CTL programming, and lead to information that might ultimately inform strategies to manipulate T cells in therapies to enhance T cell function during infections and cancer.

Enteric viruses encounter epithelial cells amidst diverse microbiota. We thus hypothesized that our unintentional generation of rotavirus (RV)-resistant Rag1-KO mice reflected microbiota influencing RV infection. Accordingly, such RV-resistance was transferred by co-housing and fecal transplant. Interrogation of microbiotas conferring RV-resistance via antimicrobial agents, heat, and filtration, followed by limiting dilution transplant to germfree mice and subsequent fecal DNA sequencing revealed a central role for segmented filamentous bacteria (SFB), which was sufficient to protect mice against RV infection and associated diarrhea. Such protection was independent of lymphocytes (innate and adaptive), interferon, IL-17, and IL-22. Incubation of SFB-containing feces with RV reduced RV infectivity, suggesting direct disabling of this virus. Additionally, colonization of ileum by SFB induce changes in host gene expression and accelerated epithelial cell turnover, which can reduce RV burden. Thus, irrespective of its effects on immune cells, SFB confers protection against certain change enteric viral infections and associated diarrheal disease.

Belatacept, a CTLA4Ig fusion protein, offers promising long term graft survival benefits to transplant patients. However, it is also associated with increased rates of acute rejection which currently limits its clinical use. This increased rejection is potentially due in part to the unintended blocking of CTLA4-mediated coinhibition. Thus, a non-cross-linking Fc-devoid anti-CD28 domain antibody (dAb) was developed to selectively block CD28 costimulation while leaving CTLA-4 coinhibition intact. Anti-CD28 domain antibody was shown to exhibit increased efficacy in mouse and NHP models of transplantation when compared directly with CTLA4Ig. These results have led to the initiation of an NIH-funded clinical trial at Emory University to test the use of selective CD28 blockade in kidney transplant recipients. Patients receiving kidney transplants are routinely treated with lympho-depleting agents prior to transplant. It is unclear what effect selectively blocking CD28 in this setting will have on the immune profile of these patients following homeostatic repopulation of their immune cell compartments. To address this question, we generated a mouse model in which C57BL/6
mice were treated with CD4+ and CD8+ T cell depleting agents at the time of Balb/c skin transplantation in the presence or absence of anti-CD28 dAb every other day for 7 days. Mice receiving only skin transplantation and no depletion or immunosuppressive therapy served as controls. We found that in this system CD4+ and CD8+ T cell compartments began to repopulate at 4 weeks post-depletion. By 6 weeks post-depletion, overall numbers and frequencies of splenic and peripheral tissue CD4+ or CD8+ T cells were not different between mice receiving CD28 blockade and those receiving depletion alone. However, our preliminary results indicate that blockade of CD28 signaling in this setting leads to significant alterations in the expression of T cell activation markers and costimulatory/coinhibitory markers, with the most profound impact observed within T cells found within peripheral tissues. Alterations in the composition and phenotypes of T cell populations in peripheral tissues following homeostatic reconstitution could have implications for protective immunity of these patients following transplantation.

Presenter Paulo José Basso1,2
Title Targeting AMPK to modulate B cell metabolism and treat Inflammatory Bowel Diseases
Email paulo.basso@vumc.org
Co-Authors Thiago M. Steiner1; Fernanda F. Terra1; Shawna K. McLetchie2; Meire I. Hiyane; Vinicius A. Oliveira1; Rafael. R. Almeida1; Mark R. Boothby2; Niels O.S. Câmara1.
Affiliation 1Department of Immunology, Institute of Biomedical Science, University of São Paulo, São Paulo, Brazil.; 2Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN, United States.

zAMPK integrates a group of metabolic sensors that control several intracellular processes such as metabolism, cell growth, autophagy and post-translational modifications. However, its role in B cell metabolism has been little assessed despite the relevance of these cells in both humoral and cellular responses. Thus, our aim was to evaluate the role of AMPK in B cell metabolism and how the B cell-specific metabolic changes could impact immune-meditated inflammatory diseases. Sorted splenic B cells from control (CT) and B cell-specific AMPK knockout (AMPKΔB) mice were cultured with LPS. Both the lactate/glucose ratio and activation markers were mainly increased in AMPKΔB cells after LPS, while few or no differences were observed with other stimuli (CpG, anti-IgM or anti-CD40 + IL-4). Moreover, AMPKΔB cells presented a sharply increase in IL-10 and IL-6 synthesis, CD40/CD86 expression and glucose uptake compared to CT cells but decreased differentiation into plasmablasts. According to these results, CT B cells treated with AMPK agonist decreased IL-10 secretion and CD40/CD86 expression but increased plasmablast differentiation. Similar results were observed in mTORC1-deficient B cells, a downstream target negatively regulated by AMPK. AMPKΔB cells also increased mRNA expression of GLUT1, glycolytic enzymes and PDK1 as well as decreased mitochondrial activity, basal respiration and maximal respiratory capacity. DSS-induced colitis in AMPKΔB mice showed a marked improvement of the clinical signs and increased IL-10 levels in gut compared to CT mice. Finally, B cell-deficient μMT mice that received adoptively transferred AMPKΔB cells had more benign disease scores when compared to those that received CT B cells. These results provide evidence of a regulatory role of AMPK in glycolysis, activation threshold and plasmablast differentiation of B cells. We propose that B cell-specific AMPK inhibition is a potential target to treat inflammatory bowel diseases.

Financial support: FAPESP; CAPES; CNPq

Presenter Ana Beatriz Enriquez
Abstract Title Mycobacterium tuberculosis modulation of Notch ligand expression impedes dendritic cell-T cell crosstalk and limits Th17 polarization
Email ana.enriquez@emory.edu
Co-Authors Jonathan Kevin Sia, Melanie Quezada and Jyothi Rengarajan
Affiliation Emory Vaccine Center, Emory University, Atlanta, GA 30329
Mycobacterium tuberculosis (Mt), the causative agent of tuberculosis (TB), is among the leading causes of death worldwide. While IFN-γ and CD4+ Th1 T cell responses are necessary to mount an immune response to Mt, they are not sufficient to provide protection. Studies from several groups, including our own, have highlighted an important role for IL-17 and Th17 responses in immunity to Mt infection. Previous research from the laboratory has shown that Mt restricts Th17 responses by dampening dendritic cell (DC) responses and has identified CD40-mediated co-stimulation as critical for generating Th17 responses in response to Mt. We showed that exogenous CD40 engagement on Mt-infected DCs enhances Th17 polarization and reduces Mt burden in the lungs in a vaccination model. However, the DC mechanisms that mediate CD40-dependent Th17 polarization and protection have not been defined. Here we show that Notch signaling in DCs modulates DC-T cell crosstalk and influences T cell polarization during infection. Engaging the CD40 pathway on Mt-infected DCs increases the mRNA and protein expression of Notch ligands DLL4 and Jagged1 over the course of infection. Blockade of Jagged1 during a DC-T cell co-culture lowers Th1 responses while blockade of both DLL4 and Jagged1 significantly limits Th17 polarization. These results reveal that during infection, Mt restricts expression of Notch ligands, which impedes Th17 responses during TB.

Presenter: Uma Shanmugasundaram  
Title: M. tuberculosis-specific T cell responses during Latent TB infection of Rhesus Macaques  
Email: ushanmu@emory.edu  
Co-Authors: Uma Shanmugasundaram, Allison Bucsan, Chris Ibegbu, Vijayakumar Velu, Deepak Kaushal, Jyothi Rengarajan  
Affiliation: Yerkes Primate Research Centre, Emory University

Introduction: Mycobacterium tuberculosis (Mt) is transmitted through aerosol route and infection occurs in the lung. Mt infection can either lead to active or latent tuberculosis. While active TB is associated with ineffective control of bacteria, 90% of the individuals develop latent TB infection (LTBI), where bacteria are successfully contained inside lung granulomas. However, the immune responses associated with immune control of LTBI are not known and the CD4 and CD8 T cell responses in blood and lung compartments are poorly understood. We have developed a model of LTBI in rhesus macaques that recapitulates merely all aspects of human infection. Using this model we have characterized the Mt-specific T cell responses involved in immune control during TB latency. Materials and methods: Rhesus macaques (6 animals) were infected through aerosol route with low dose Mt strain CDC 1551. Infection was assessed using Tuberculin Skin test, and Interferon Gamma Release Assays (IGRA) and latency was determined by chest x-ray and lack of clinical signs and symptoms. Animals were longitudinally followed until week 24 we studied the phenotype and function of Mt specific CD4 and CD8 T cells in peripheral blood, BAL and lung by flow cytometry. Results: Mt antigen specific (Mt Cell wall- and ESAT6/CFP10-specific) T cells producing IFNγ and IL-17 were significantly higher in BAL compared to PBMC and CD4+ T cell responses were higher compared to CD8+ T cells. The peak Mt specific responses were seen at week 7 and were maintained at higher levels throughout the latent infection in BAL and in PBMC. Mt antigen specific T cells were mainly of central memory and expressed both CCR6 and CXCR3. Mt specific activated T cells expressing CD38 declined overtime in PBMC whereas in BAL high frequencies were maintained till week 24 of latent infection. Conclusion: Mt latent infection was characterized by high frequency of IFNγ and IL-17 producing T cells and antigen specific central memory T-cells in BAL. Antigen specific activated CD4+ T cells homed to BAL and persisted in BAL throughout the latent infection.
Antiviral proteins (AVPs) such as the oligoadenylate-synthase (OAS), MX, and IFITM families are expressed in epidermal keratinocytes, dendritic cells, and cutaneous T cells in the skin. While inflammatory pathways can induce AVP expression such as IL-27 signaling, the exact temporal pattern of constitutive expression and induction of these proteins in response to viral challenge is unclear. Upon examination of expression of constitutive AVPs in primary human keratinocytes, we found that their expression levels varied over a 24 hr timespan. The circadian genetic clock of BMAL1, CLOCK, and PER/CRY proteins strongly influences cellular immunity, but its role in antiviral protein production is unknown. We hypothesized that the circadian clock may play a pivotal role for innate antiviral immunity. Here, we show by qPCR that in primary human keratinocytes the expression of several antiviral peptides exhibit circadian oscillations over 24 hours. OAS2, MX1, and IFITM1 cycle along with circadian genes BMAL1, CLOCK, and PER2, with AVP peak expression levels correlated to peak expression of the positive circadian regulator BMAL1 ($r=0.736$, $p<0.005$) and AVP expression decreased with low levels of BMAL1 expression. siRNA knockdown of BMAL1 also decreased AVP expression. We next corroborated these findings in vivo. Mouse studies displayed a marked difference in AVP production upon skin wounding depending on time of day. Further exploration of this relationship was undertaken with Bmal1$^{-/-}$ mice. Both in silico and in vivo analysis revealed that skin from Bmal1$^{-/-}$ mice expressed lower levels of AVPs relative to control wild type mice ($p<0.05$, $n=4$) in response to wounding. We conclude from these studies that circadian regulation influences expression of distinct antiviral proteins both in vitro and in vivo. Future directions for this project include determining the mechanism by which the circadian clock influences AVP expression and the functional role of this phenotype for viral infections. Better understanding of this mechanism is relevant in understanding how our skin responds to viral challenges, and may help us understand and manipulate the differential response to cutaneous viral infection in relation to daily timing.

Presenter  Courtney Hegner  35
Title  Determining the role of chromatin regulators in the differentiation of CD8 T cells
Email  hegne22c@mtholyoke.edu
Co-Authors  S. Tsuda, R. Chen, H. Diao, M.A. Frederick, J. Milner, A.W. Goldrath, S. Crotty and M.E. Pipkin
Affiliation  Scripps Research Institute, NSF

Memory cytotoxic T lymphocytes (CTLs) develop from naïve CD8$^+$ T cells, and can provide long-term immunity against intracellular infections and tumors. During a typical acute infection, naïve CD8$^+$ T cells differentiate into a large number of terminal effector cells (TE) that do not become memory CTLs after the infection is cleared. At the same time, a smaller number of memory precursor cells (MP) develop, which give rise to long-lived memory CTLs. This differential process is regulated by transcription, but the chromatin regulators and transcription factors that specify how and when naïve CD8$^+$ T cells differentiate into either CTL population are still unknown. To gain insight into this problem, we developed and applied a pooled in vivo loss-of-function screen using RNAi to individually suppress all putative chromatin regulatory proteins in CD8$^+$ T cells responding to acute lymphocytic choriomeningitis (LCMV) infection. Analysis of the screen indicated that suppression of multiple different histone acetyl transferases (HATs) and bromodomain proteins impaired formation of TE CTLs, whereas a distinct set of HATs and bromodomains impaired development of MP CTLs. Suppression of Brwd3, a bromodomain-containing protein previously implicated in the Jak/Stat signaling pathway in Drosophila appeared to skew differentiation toward TE CTLs and away from MP CTLs. Suppression of Brwd3 with multiple individual shRNAmirs in vivo increased the frequency of TE-like cells at early times after LCMV infection, and increased the number of TE, MP and DP cells at the peak of infection. Moreover, after the peak response, Brwd3-suppression decreased the fractions of central memory-like cells, and enhanced those of peripheral and effector memory-like CD8$^+$ T cells. These results indicate that Brwd3 might normally restrain the differentiation of effector cells, to ensure normal MP development. Given that histone acetylation in chromatin is read by bromodomain-containing proteins, which can recruit factors that regulate the efficiency of RNA Polymerase II elongation from target gene promoters, our results suggest that differential utilization of bromodomain containing
proteins might regulate transcription that governs the differentiation of activated CD8 T cells during infection.

Presenter: Adam Getzler
Title: Chromatin regulator Mll1 initiates memory T cell differentiation and programs stem like properties
Email: agetzler@scripps.edu
Co-authors: Megan Frederick, Huitian Diao, Runqiang Chen, Ananda Goldrath, Shane Crotty, Matthew Pipkin
Affiliation: Scripps Research

During acute viral infections, naïve CD8+ T cells activate and differentiate into both effector and memory lineages. This differentiation is defined by distinct changes in chromatin accessibility and epigenetic marks. Failure to properly establish these changes results in impaired immunological memory and is a hallmark of dysfunctional T cell responses. However, little is known about how chromatin regulators, one of the main class of chromatin modifying proteins, control these processes. To address this question, we performed an in vitro shRNAmir screen against all known murine chromatin regulators for their effect on the expression of co-inhibitory receptors (i.e. PD1, TIM3, and LAG3), whose expression is tightly regulated during typical T cell differentiation. We found that deficiency of histone methyltransferase Mll1 (Kmt2a), a catalytic subunit of the SET/COMPASS family, significantly increased expression of the co-inhibitory genes. RNA-Seq showed Mll1 to be significantly upregulated in memory cells relative to exhausted T cells and most highly expressed during the naïve and memory phases of T cell responses, while ATAC-Seq showed a loss of accessibility for Mll1 in effector and exhausted cell states. This data lead us to hypothesize that Mll1 is playing a role in maintaining naïve and naïve-like memory cell states, consistent with its role in maintaining hematopoietic stem cells, and its down regulation is related to terminal differentiation. To test this hypothesis, we diminished either Mll1 or controls via shRNAmirs in antigen specific CD8 T cells (P14), transferred these cells into naïve mice and infected with lymphocytic choriomeningitis virus (LCMV). Co transfers of cells showed early increases in accumulation and terminal differentiation of Mll1 deficient cells followed by a rapid contraction and loss of memory precursor cells at the peak of T cell response. This contraction was consistent into memory timepoints and upon re-challenge Mll1 deficient cells failed to re-differentiate into effector cells. Together this data points to a role for Mll1 in maintaining T cell stemness and ensuring the proper formation of functional memory CD8 T cells.

Presenter: Mingyong Liu
Title: T cell receptor sequencing reveals recruitment of regulatory T cells from the periphery into omental tumors
Email: miliu@uab.edu
Coauthors: Dmytro Starenki, PhD; Sara J. Cooper, PhD; Troy D. Randall, PhD; Selene Meza-Perez, PhD
Affiliation: 1Department of Medicine – Clinical Immunology and Rheumatology, University of Alabama at Birmingham, 2HudsonAlpha Institute for Biotechnology.

The omentum is an apron-like fatty tissue that connects the spleen, stomach and pancreas, and contains lymphoid tissues termed milky spots. In the advanced stages of ovarian cancers, tumor cells frequently metastasize to the peritoneal cavity and invade the omental adipose tissue. This is accompanied by an increase in CD4+ CD25+ FOXP3+ regulatory T cells (Tregs) in the omentum, which suppress antitumor immunity. Despite the increasing understanding of the roles Tregs play in the context of cancer, how tumor-associated Tregs accumulate in the omentum requires further study. To address this, we intraperitoneally injected EG7 thymomas into syngeneic mice and sorted Tregs for T cell receptor (TCR) sequencing. Although omental Tregs and their splenic counterparts from naïve animals have low degrees of overlap in their TCR repertoires, the similarity between them becomes increased by tumors in the omentum. Due to the phenotypic characteristics shared by Tregs in the omentum and colon, we also examined their repertoire similarity and found it elevated by tumors as
well, albeit more moderately. This indicates Treg recruitment from the periphery during cancer progression. Moreover, since we did not observe dominant Treg clones in the omenta of tumor-bearing mice, the augmentation of Tregs may not be primarily due to local clonal expansion. In fact, we found tumor development increases the repertoire diversity of omental Tregs. Given that Treg clonotypes in the spleen are highly diverse, these data suggest Tregs from secondary lymphoid organs may contribute to the omentum compartment during tumor progression. In line with these findings, the frequency of Ki67+ proliferating Tregs is higher in the tumor-free omentum than in the spleen, but it is significantly reduced in the omentum with tumors. Interestingly, FTY720 treatment does not affect the number of omental Tregs in tumor-bearing mice, indicating Treg trafficking into tumors may be independent of the sphingosine-1 phosphate receptors. Collectively, these results suggest that regulatory T cells from the periphery are recruited to the omentum during tumor development. Specific mechanisms by which Treg traffic to tumors may thus be targeted to enhance antitumor immunity and treat omental tumors.

Presenter: Lacey R. Lopez
Abstract Title: Yersiniabactin drives fibrotic inflammatory bowel disease
Email: laceylop@email.unc.edu
Co-authors: Lopez, Lacey R.; Moore, Lyndsey; Broberg, Christopher A.; Tibbs, Taylor N.; Rothemich, Aaron; Arthur, Janelle C.
Affiliation: 1. Microbiology and Immunology, University of North Carolina - Chapel Hill, Chapel Hill, NC, United States.

Background: Over 30% of inflammatory bowel disease (IBD) patients develop intestinal fibrosis (scarring). Adherent-invasive Escherichia coli (AIEC) are linked to IBD; however, it is unknown how AIEC potentiate IBD-fibrosis. AIEC secrete metal-chelating molecules (siderophores) in metal-limited environments, like the inflamed gut. The Yersiniabactin (Ybt) siderophore is of key interest, as the Ybt biosynthetic gene cluster is prevalent in the microbiota of IBD patients. Our lab developed an IBD-fibrosis model, using gnotobiotic Interleukin-10-deficient mice mono-associated with Ybt-producing AIEC. Ybt-producing AIEC are visualized in the intestinal submucosa, where fibrosis develops. Fibrosis requires Ybt biosynthesis, but not bacterial uptake of Ybt. This suggests Ybt targets the host. We propose that Ybt manipulates host metal status and influences host cell activation/death, which potentiates fibrogenesis.

Methods: In cultured fibrosis-associated cell types (i.e. epithelial cells, macrophages, and fibroblasts), we manipulated metal availability and evaluated expression of metal responsive/extracellular matrix genes and cell death. Metal availability was altered using chelators and purified Ybt in the presence/absence of exogenous metals (e.g. iron and zinc). Ybt-metal binding was validated via mass spectrometry.

Gene expression was evaluated by qPCR and cell death by lactate dehydrogenase release. Results: Ybt induced upregulation of NDRG1, a gene linked to iron starvation. However, more cell cytotoxicity occurred under zinc-depleted versus iron-depleted conditions. Ybt-induced cell death was not rescued by any one metal, suggesting multiple biologically important metals are involved in this process. Lastly, manipulating metal in cultured fibroblasts minimally induced expression of extracellular matrix genes. Conclusions: Our results demonstrate that Ybt is linked with human IBD-fibrosis and drives fibrosis in a relevant mouse model. Although the mechanisms remain unclear, altering metal availability impacts cell death and activation in fibrosis-associated cell types. Furthermore, Ybt induced upregulation of a host iron responsive gene and cell death. Therefore, host metal disruption by Ybt may potentiate IBD-fibrosis.

Presenter: Taylor Tibbs
Abstract title: Escherichia coli siderophore production induces intestinal fibrosis in Il10-/ mice
Email: ttibbs@email.unc.edu
Authors: Taylor N. Tibbs; Aaron Rothemich; R. Ian Cummings; Lacey R. Lopez; Christopher A. Broberg; Yen-Rei A. Yu; Robert M. Tighe; & Janelle C. Arthur
Affiliations: 1Department of Microbiology & Immunology, University of North Caro-
Inflammatory bowel disease (IBD) affects over 3 million Americans. Crohn’s disease (CD), a subtype of IBD, is notorious for developing fibrotic strictures, which are life threatening and require surgical removal. Even with surgery, strictures often reoccur, suggesting that there may be unknown mechanisms in the intestinal microenvironment driving fibrogenic pathology. Adherent invasive Escherichia coli (AIEC) are associated with CD and many harbor a biosynthetic gene cluster for the production of the siderophore yersiniabactin (Ybt); however, the role of Ybt in CD progression and fibrotic development is unknown.

We have developed a gnotobiotic murine model whereby fibrosis develops in germ free 129 Il10−/− mice mono-colonized with Ybt competent AIEC NC101. Colitis and fibrosis was evaluated by H&E and Sirius red stained colon swiss roll sections using established semi-quantitative scoring methods. Colonic tissues were further assessed using immunofluorescent microscopy, flow cytometry, and RNAseq. Human full thickness colon sections from CD, ulcerative colitis, diverticulitis, and unaffected/normal patients were also evaluated by H&E, Sirius red, and immunofluorescent microscopy. Metagenomes and metatranscriptomes of fecal samples from CD and non-IBD donors were analyzed from publicly available consortium data via MetaHIT and the Human Metagenome Project 2 (HMP2).

Evaluation of metagenomes and metatranscriptomes from human fecal samples revealed a higher abundance of Ybt genes and transcripts in CD patients compared to non-IBD patients. In our murine model, fibrosis was dependent on colonization with Ybt+ AIEC. Fibrosis incidence was increased in mice mono-colonized with Ybt+ AIEC deficient in the Ybt uptake system, FyuA. Histological analysis of colon sections from our mouse model compared to human colon resection tissues revealed collagen-rich fibrotic expansions within the submucosa of the colon, mimicking fibrotic lesions in human CD. RNA-Seq data revealed an abundance of extracellular matrix and macrophage genes in colon tissue from fibrotic mice, confirming our histological findings. To further explore the macrophage population involved in fibrosis, we performed immunofluorescent microscopy coupled with flow cytometry and identified a significant expansion of F4/80 hi CD11b hi MHCII lo macrophages within fibrotic colons. We predict that these macrophages are recruited by Ybt-dependent mechanisms and maybe responsible for initiating a cascading inflammatory response that activates fibroblasts to deposit collagen.

Presenter Doan C. Nguyen
Title Human bone marrow plasma cell survival is independent of APRIL
Email doan.c.nguyen@emory.edu
Co-Authors Celia Saney, Iñaki Sanz, F. Eun-Hyung Lee
Affiliation Emory University

A fraction of the circulating antibody (Ab)-secreting cells (ASC) matures into long-lived plasma cells (LLPC) in the bone marrow (BM) microniches. Previous studies showed that ASC survival and longevity require APRIL, which upon binding its receptors, BCMA or TACI, activates PI3K/Akt (and its downstream mTOR) pathways and upregulates the expression of antiapoptotic proteins Mcl-1 and Bcl-2. Later work revealed that APRIL binding to SDC-1 (CD138), a cell-surface heparin sulfate proteoglycan (HSPG), also enables delivery of survival signals. Recently, we showed that APRIL is crucial in the ex vivo survival of early-minted human blood ASC. Here, using an in vitro cell-free BM microniche system, we demonstrate the differential roles of APRIL in the survival of human blood ASC and BM plasma cells (PC) and LLPC. APRIL substantially enhanced the survival of both CD138+ and CD138− ASC populations in the blood, which highly expressed BCMA. In contrast, it had no survival advantage on BM PC or LLPC, despite their high BCMA expression. Additionally, anti-CD138 Ab inhibited the survival of both blood ASC and BM PC and LLPC, including CD138− cell
subsets, suggesting inhibiting the later CD138 upregulation also affects blood ASC survival. These data suggest that APRIL is important for the survival of early-minted blood ASC but not BM PC or LLPC. In conclusion, APRIL is an important survival and imprinting factor of blood ASC but is not required for LLPC maintenance.

**Presenter:** Jaime Sancho  
**Abstract title:** Proteomic analyses of exosome fractions present in peritoneal exudates from pristane-induced lupus mice reveal significant differences in CD47 and TSP-1 abundance between CD38-deficient and WT mice  
**Email:** granada@ipb.csic.es  
**Co-Authors:** José-Ángel Robles-Guirado 1; Elena González-Paredes 1; Alex Mas-Ciurana 1; Miguel-Ángel Palacios-Pedrero 1; Carolina Franco-Herrera 1; Victoria Longobardo 2; Antonio Lario 2; Ana-Belén Jodar 3; Francisco J. Blanco 4; Vivian de los Ríos 5; Ignacio Casal 6; and Mercedes Zubiaur 1.  
**Affiliation:** 1 Department of Cellular Biology and Immunology, Instituto de Parasiología y Biomedicina López-Neyra (IPBLN-CSIC), Parque Tecnológico de Ciencias de la Salud (PTS), Av. Conocimiento 17, 18016 Granada, Spain. 2 Proteomics Unit, IPBLN-CSIC, 18016 Granada, Spain. 3 Facultad de Físicas. Universidad de Granada (UGR). Granada, Spain. 4 Facultad de Medicina. Universidad de Granada (UGR). Granada, Spain. 5 Functional Proteomics Laboratory. CIB-CSIC. Madrid, Spain.

Exosomes are extracellular vesicles thought to be present in all body fluids. Shed by cells, their molecular make-up reflects that of their cell of origin and/or tissue pathological situation. The increased NAD-dependent deacetylase activity of sirtuins in CD38-deficient (CD38ko) mice will cause profound changes in the profile of acetylated lysines (acK) at specific sites (acetylome) in a number of proteins involved in signaling pathways related with inflammation and autoimmunity. Therefore, it will be distinct from that in WT mice, in particular once the lupus disease fully develops. This important post-translational modification can only be identified by high-resolution quantitative proteomics, which involves the enrichment in acK peptides. Our working hypothesis is that analyzing the composition (proteins and microRNAs) and function of exosomes released by specific cell types in the pristane experimental lupus model will allow us to identify predictive or diagnostic biomarkers that might discriminate the autoimmunity process in lupus from inflammatory reactions and/or normal physiological processes. One first observation of our proteomic data in the pristane lupus model is the presence of CD47 and one of its ligands, thrombospondin-1 (TSP-1), in exosomes from peritoneal exudates, particularly in CD38ko mice which develop a milder autoimmune disease. TSP-1 was reported to promote the resolution of inflammatory process and to facilitate phagocytosis of damaged cells. Therefore, TSP-1 may play an anti-inflammatory and immunoregulatory role in SLE autoimmunity. CD47 is also the ligand for signal regulatory protein alpha (SIRPα, also known as CD172a), and the CD47-SIRPα interaction initiates the “do not eat me” signal that inhibits phagocytosis. This may facilitate circulation for a longer time through the bloodstream of CD47-positive exosomes and allow them to reach farther places where they can exert their biological action. This circumstance has been used successfully to direct exosomes to tumor tissues for therapeutic purposes. The functional importance of differential CD47 and TSP-1 expression detected in peritoneal exosomes from CD38ko versus WT pristane-lupus mice requires further studies.

**Presenter:** Kaitlin A. Read  
**Abstract Title:** Ikaros zinc finger transcription factors as novel regulators of T helper cell differentiation and STAT factor activity  
**Email:** kread@vtc.vt.edu  
**Co-authors:** Bharath K. Sreekumar, Michael D. Powell, Jawad Zafar, Devin Jones, Chandra E. Baker, Michael Fox, Irving C. Allen & Kenneth J. Oestreich  
**Affiliation:** Fralin Biomedical Research Institute, Virginia Tech; Biomedical and Veterinary Sciences Graduate Program, Virginia-Maryland College of Veterinary Medicine, Virginia Tech
Cytokine signaling is important for the differentiation and function of a number of cell types, including T lymphocyte populations. One mechanism by which cytokine signals are propagated is via phosphorylation-mediated activation of Signal Transducer and Activator of Transcription (STAT) factors, which directly regulate cell type-specific gene programs. We recently identified a novel regulatory module composed of STAT3 and the Ikaros Zinc Finger (IkZF) transcription factor Aiolos in CD4+ T cells, which positively regulates the T follicular helper (Tfh) gene program. Given conservation between individual members of the IkZF and STAT families, we hypothesized that additional IkZF/STAT complexes may regulate the development of other T cell subsets. Indeed, we find that expression of the IkZF factor Eos is elevated in Tfh1 cells compared to Tfh1 and naive CD4+ T cell subsets. Furthermore, expression of Tfh1 markers, including Blimp-1, IL-2ra, and IL-2rβ, as well as IFN-γ production, were significantly diminished in Eos-deficient Tfh1 cells. Additionally, Eos-deficient CD4+ T cells activated in response to Toxoplasma gondii infection display decreased expression of these markers at the transcript level as compared to their wildtype counterparts. Finally, we find that Eos physically interacts with STAT5 and that these factors are co-enriched at the Prdm1 and Il2ra gene loci in Tfh1 cells. Curiously, we also find that IkZF factor expression correlates with STAT activation, suggesting that, in addition to directly regulating gene expression, IkZF proteins may also influence T helper cell differentiation through the regulation of STAT factor activity. We find that overexpression of Eos, but not Ikaros or Aiolos, results in increased activation of STAT5. Importantly, when we performed these studies using an Eos mutant incapable of interacting with STAT5, the observed increase in STAT5 phosphorylation was lost. We also find that in CD4+ T cells, Eos deficiency correlates with reduced STAT5 activation in Tfh1 cells, while Aiolos-deficient Tfh1 cells display reduced STAT3 activation. Finally, we observe differential IkZF factor expression across CD4+ T cell subsets. When coupled with known differences in STAT factor activation, these findings suggest that IkZF/STAT regulatory modules may broadly regulate T helper cell differentiation.

**Presenter:** Kimberly Cooney  
**Title:** Regulation of Adenosine Receptor 2A and its Role in the Development of Neutrophil Extracellular Trap Formation  
**Email:** kimberly.ann.cooney@emory.edu  
**Co-Authors:** Cooney KA, Xu K, Wang L, Ginn SC, Deppen JN, Levit RD  
**Affiliation:** Division of Cardiology, Department of Medicine, Emory University School of Medicine, 101 Woodruff Circle, Suite 319, Atlanta, Georgia, 30322

**Introduction:** Neutrophils are a sub-type of leukocytes that play a key role in the innate immune system. During times of tissue injury and infection, neutrophils are recruited to the site of inflammation where they are capable of responding to damaged tissue. Neutrophils release their chromatin, known as neutrophil extracellular traps (NETs), into the extracellular environment which can cause inflammation and trigger a host of pro-inflammatory pathways by a process known as NETosis. We discovered that co-culture of mesenchymal stem cells (MSCs) with human neutrophils decreased NETosis and hypothesized as to whether MSC-produced adenosine was involved. Recent literature suggests possible mechanisms that may contribute to NET formation. However, it remains unclear how NETs are regulated and what factors contribute to their production. Methods: Quantification of cAMP production was used to assess adenosine signaling and determine whether cAMP is a regulator of NETosis in both resting and stimulated neutrophils. Other biochemical assays and techniques including liquid chromatography and mass spectrometry will be used to examine potential enzymes implemented in NET formation. Results: We have found that adenosine receptor 2a (A2aR) mediates the anti-NET effects of adenosine. In addition, treatment with an A2a receptor agonist is effective in increasing cAMP signaling in isolated human neutrophils. Conclusion: These preliminary experiments suggest that A2a receptor activation is involved in suppression of NETs and that regulation of downstream signaling factors, such as cAMP, may influence their production. Furthermore, MSCs can reduce NETs in vitro. Reducing NETs in vivo may improve functional recovery after MI/R by reducing inflammation, preventing thrombosis, and other complications. Better understanding of NET properties and examination of downstream mediators may identify therapeutic targets for disease states.
All-trans-retinoic acid (tRA), an active metabolite of vitamin A, can act as an adjuvant to exacerbate autoimmunity, even though it is immunosuppressive under steady state. Previous findings from our group indicated that tRA might expand the pre-existing immunogenic environment in individuals genetically prone to develop lupus. Here, we show that tRA differentially affects the initiation vs. the continuation phase of lupus. To explore the roles of tRA before and after an existing immunogenic status, we utilized the pristane-induced lupus model in Balb/c mice. Oral doses of tRA (1mg/kg) vs. vehicle control were given daily, from 3 weeks of age to the time of lupus induction at 3 months (pre-treatment) or from 3 months to the experimental endpoint at 6 months post lupus induction (post-treatment). Pre-treatment with tRA, but not the post-treatment, aggravated glomerulonephritis as indicated by increased proteinuria and higher anti-double-stranded DNA autoantibodies levels. Mechanistically, tRA induced the activation and the inflammatory phenotype of different subsets of conventional dendritic cells and expanded both the effector memory and gamma delta (γδ) double-negative T cell populations over pristane alone. Additionally, tRA further upregulated the renal mRNA expression of inflammatory cytokines and chemokines including TNF-α, IL-1β, MCP-1, MIP-1-alpha CCL2, and CCL3. Similarly, upregulation of Integrin alpha l-domain of the lymphocyte function-associated antigen-1 (LFA-1) integrin was also observed. Moreover, tRA upregulated the renal transcript level of the matrix protein Laminin β1, which is known to be associated with mesangial proliferative glomerulonephritis. These results indicate enhanced immune cell migration and infiltration to the renal compartment with tRA pre-treatment in the pristane-induced lupus model. Collectively, our findings suggest that tRA promote the initiation of glomerulonephritis by exacerbating the renal immunogenic environment. The transcript profile induced by the pre-treatment will shed light on the molecular mechanism by which it worsened pristane-induced lupus and is currently under investigation.

The devastatingly low survival rate of pancreatic cancer, at only 8%, is further compounded by the limitations of current cancer treatment options. Only 15% of pancreatic cancer patients are candidates for surgical resection due to the location of the primary tumor near or even wrapped around major arteries and bile ducts. The tumor microenvironment is also highly immunosuppressive due to large populations of tumor-associated immune cells such as tumor-associated macrophages and myeloid-derived suppressor cells that have made immunotherapy options ineffective in clinical trials. Furthermore, pancreatic cancer has a high rate of metastasis that can lead to organ failure and death in most patients. Irreversible electroporation (IRE) offers a novel, non-thermal approach to tumor ablation. IRE utilizes short, high frequency electrical pulses to form permanent pores in cancer cell membranes. This leads to a loss of homeostasis and the induction of programmed cell death. In clinical application, IRE has shown significant success and is currently being fast-tracked by the FDA as a treatment option for pancreatic cancer. However, the evaluation of IRE’s biological effects on healthy tissue, the tumor microenvironment, and on tumor progression...
outside of patient survival has been limited. We hypothesize that IRE can alter pancreatic cancer’s immunosuppressive tumor microenvironment to inhibit tumor progression. We utilized healthy porcine tissues, human pancreatic cancer patient-derived xenograft models, and immunocompetent in vivo murine models to evaluate the effects of IRE on the tumor microenvironment, immunosuppressive immune cell populations, and on disease progression. Our studies suggest that IRE can induce pro-inflammatory cell death in pancreatic tumors and alter immunosuppressive cell populations. IRE also leads to increased progression-free survival and lower disease burden with few treatment side effects, potentially due to increased immunosurveillance. These findings could greatly impact clinical application of IRE for pancreatic patients and allow us to identify potential co-therapy options or targets to increase pancreatic cancer patient survival.

Necroptosis, a programmed form of lytic cell death, is initiated by various viral and bacterial pathogens through irreparable ion dysregulation and energy depletion. This cellular damage results in the activation of RIPK1, RIPK3, consecutively, and the activation and membrane targeting of MLKL, the latter responsible for lysis. Recently we have demonstrated that necroptosis of airway epithelial cells is key in the development of the adaptive immune response to asymptomatic colonization by Streptococcus pneumoniae (Spn). Briefly, necroptotic deficient animals or wildtype animals colonized with Spn lacking the necroptosis-triggering pneumolysin toxin, failed to recruit CD11c+ leukocytes to the sites were Spn were present, had dampened serum IgG responses, were delayed in bacterial clearance, and were more susceptible to subsequent lethal re-challenge [Front Immunol. 2019, 10:615]. Herein we examined the role of necroptosis in the generation of an adaptive immune response to influenza A (Flu), a viral pathogen also shown to induce necroptosis of airway epithelial cells. Wildtype, RIPK3 KO, and MLKL KO mice were challenged intranasally with 6,500 PFU of Flu strain A/Puerto Rico/8/1934 (i.e. PR8). Weight loss was monitored over the course of 15 days as a measure of disease severity. At day 15, mice were sacrificed and lungs and mediastinal lymph nodes (mLN) were collected for enumeration of immune cells present. In comparison to wildtype and MLKL KO, RIPK3 KO mice experienced the most severe weight loss, indicating a role for RIPK3 activation independent of MLKL-mediated cell lysis. Enumeration of immune cells by flow cytometry showed no differences in Flu-specific effector (CD62L CD69+CD25+), central memory (CD127+CD62L+), effector memory (CD127+CD62L+), and tissue resident memory (CD69+CD103+) lung and mLN CD8+ T cell responses. Similarly, no differences were observed in Flu specific mLN B cells (CD19+ CD95+ CD38+ IgG1+), or mLN and lung T regulatory cells (CD19+CD25+FOXP3+) and T follicular helper cells (CXCR5+PD1+BCL6+). These results suggest fundamental differences in the role for necroptosis in the development of adaptive immunity against viral versus bacterial pathogens. Ongoing studies are focused on determining the contribution of necroptosis to the innate immune response to Flu in order to explain the observed differences in RIPK3 and MLKL deficient mice.

Functional avidity is a critical determinant of the ability of cytotoxic lymphocytes (CTL) to
function in vivo. High avidity CTL are sensitive to low peptide/MHC, while low avidity CTL require high peptide/MHC. The increased sensitivity of high avidity cells results in improved virus and tumor clearance. Whereas the importance of functional avidity is well established, the mechanisms that determine the sensitivity of a T cell to peptide/MHC are less so. We have identified a new process through which CD8+ T cells can actively modulate their functional avidity. We found CD8+ T cells sense and respond to the level of TCR engagement through the regulated production of IL-4. Stimulation of OT-I CD8+ T cells with high, but not low amounts of peptide induced autocrine IL-4 that resulted in establishment of a lower avidity setpoint. Ca2+ signaling was critical for IL-4 production as demonstrated by promotion of IL-4 production by ionomycin and reduced production following addition of cyclosporine A. High TCR engagement resulted in increased nuclear NFATC1, which has been reported to regulate IL-4 production in CD4+ T cells. Our data support a model wherein the pertinent downstream effect of encounter with strong TCR engagement is an increase in NFATC1 in the nucleus resulting in IL-4 production. These data reveal a novel self-tuning mechanism to control the activation set point of CD8+ T cells through regulated autocrine production and response to IL-4.

Skin conditions that result in intense itching have a significant impact on patient quality of life (QOL). Atopic dermatitis (AD) and bullous pemphigoid (BP) are two mechanistically distinct immune-mediated diseases both characterized clinically by intense chronic itching. Although AD and BP have differences in classic presentation (eczematous eruption versus urticaria and blisters) and population characteristics (young to middle aged persons versus elderly), early stages of BP can appear strikingly similar to AD clinically and histologically. Preliminary data from our group have demonstrated that both AD and BP share similar itch symptoms and QOL impact despite differences in overall clinical presentation. However, the mechanisms for this enhanced pruritus in both diseases are unclear. Previous data have suggested that patients with AD demonstrate an increase in cutaneous nerve endings which may explain the enhanced itch sensation. We explored the role of the sensory neuron and potential influences on immune-mediated pathways that may be perpetuating and/or responding to pruritus in these two seemingly different cohorts of patients. Biopsies were obtained from perilesional skin in patients with AD and BP, examined histologically to detect alterations in intraepidermal nerve fibers, and then compared to skin from healthy controls. Samples were obtained from patients with broad demographic diversity, from various body sites, and with itch duration ranging from 7 months – 15 years. Nerve density was calculated based on the number of PGP9.5 stained fibers in the epidermis. Interestingly, the average epidermal nerve density in the skin of patients was significantly reduced compared to healthy controls (6.89 fibers/mm ± 2.44 vs 11.49 fibers/mm ± 3.69). These data suggest that seemingly different immunologic mediated skin diseases share common clinical endpoints of severe itching, quality of life impact, and reductions in epidermal sensory nerve innervation. It is possible that the decreased epidermal nerve density over time is due to immune-mediated damage; alternatively, active downregulation of sensory nerve function may occur to dampen the itch response. Further research evaluating alterations in corresponding itch specific signaling pathways in these chronic inflammatory diseases may elucidate common pathways and areas for therapeutic intervention.

Presenter: Robin Rolader
Title: Comparing Clinical and Biological Aspects of Pruritus in the Immune-Mediated Diseases Atopic Dermatitis and Bullous Pemphigoid
Email: rrolade@emory.edu
Co-Authors: Taryn DeGrazia, Yuan Liu, Yanyan Xing, Liang Han, Bridget Bradley, Cecilia Thompson, Sandy Francois, Safiyah Rasheed, Sarah Chisolm, Suephy Chen, Ron Feldman
Affiliation: Emory University School of Medicine, Department of Dermatology

Presenter Matthew Cottam
Title Adipose tissue CD4+ and CD8+ memory T cells are retained with weight loss and CD8+ memory T cells expand during weight regain
Email Matthew.A.Cottam@vanderbilt.edu
Bouts of weight loss and weight regain, referred to as “weight cycling”, have been associated with increased risk of cardiovascular disease progression and increased risk of development of type 2 diabetes mellitus in humans. To identify causes and correlation of the progressive metabolic dysregulation observed in humans who weight cycle, we have developed a mouse model of weight cycling in which alternating 60% high and 10% low-fat diets induce cycles of weight gain and weight loss. Glucose tolerance in weight-cycled animals is impaired compared to age, weight, and adiposity-matched high-fat fed control mice. In conjunction with this finding, we have observed an enrichment for CD8+CD62LlowCD44+ memory T cells, but not CD4+CD62LlowCD44+ memory T cells, following weight cycling in mice. Therefore, we hypothesize that the observed impairment in glucose tolerance is due to re-activation of primed memory T cells during weight regain. To test this, we are characterizing T cells and investigating their role in AT insulin resistance during periods of weight gain, weight loss, and weight cycling. In mice that have lost weight, despite a reduction in body mass and adiposity and a concordant resolution of impaired glucose tolerance, CD4+ and CD8+ T cells increased dramatically. Furthermore, CD62LlowCD44+ memory T cells in both CD4+ and CD8+ populations were elevated in weight loss mice compared to obese mice. These findings suggest T cells are retained during weight loss even in the absence of metabolic dysregulation or altered adiposity and may be primed to respond to subsequent weight regain. With these findings in mind, ongoing work to identify AT T cell receptor diversity and common clonotypes is being performed.

Presenter: Taryn McLaughlin
Abstract title: CD4+ T cells in Mycobacterium tuberculosis and Schistosoma mansoni co-infected individuals from Kisumu, Kenya remain functional despite altered lineage phenotypes
Email: taryn.mclaughlin@emory.edu
Co-authors: Jeremiah Khayumbi2, Joshua Ongalo2, Joan Tonui2, Felix Hayara Odhiambo2, Cheryl L. Day1,3
Affiliation: 1Emory Vaccine Center, Emory University, Atlanta, GA; 2Center for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya; 3Department of Microbiology & Immunology, Emory University School of Medicine, Atlanta, GA

It is well established that an effective type 1 T cell immune response is necessary to control infection with Mycobacterium tuberculosis (Mtbb). Determining factors that modulate type 1 immune responses is therefore critical in further defining correlates of protection during Mtbb infection. Helminths stimulate a strong type 2 immune response, which has been shown to antagonize type 1 CD4+ T (TH1) cells. As such, we sought to evaluate whether co-infection with the parasitic helminth Schistosoma mansoni impairs CD4+ T cell responses to Mtbb in a cohort of HIV-uninfected adults in Kisumu, Kenya. Individuals were categorized into six groups by Mtbb and S. mansoni infection status: healthy controls (HC), latent Mtbb infection (LTBI) and active tuberculosis (TB), with or without concomitant S. mansoni infection. We evaluated the frequency and lineage phenotype of CD4+ T cell responses to Mtbb in PBMCs using flow cytometry. Compared to HC, individuals in the LTBI and TB groups had more diverse Mtbb-specific cytokine responses, including production of IL-4 and IL-13. Across all groups Mtbb-specific CD4+ T cells were characterized by expression of both classical TH1 markers, CXCR3 and T-bet, and TH2 markers, CCR4 and GATA3. Additionally, they were enriched for CCR4+CXCR3+ cells as compared to bulk CD4 T cells from the same individual. To further characterize these cells, we stimulated Mtbb-specific CD4+ T cells for five days to evaluate proliferation, cytokine production, and lineage state. CD4+ T cell proliferation was higher in LTBI individuals as compared to HC and TB; however, proliferation capacity did not vary by S. mansoni status. Similar to the short-term stimulation, Mtbb-specific CD4+ T cells expressed both TH1 and TH2 lineage markers. Together these data show that Mtbb-specific CD4+ T cells are not traditional TH1 cells and that this phenotype persists as cells proliferate in vitro. This suggests that Mtbb-specific immune responses may be flexible in response to antigen. In high pathogen settings where co-infection is common and reoccurring, this may
be important in preserving Mtb-specific TH1 responses.

Wild-type measles virus (MeV) infection generates a state of immunosuppression, which does not occur after vaccination. In vivo data in the rhesus macaque model suggest that B lymphocytes are a target of wild-type measles virus. However, the contribution of B cells to the pathogenesis of measles and the role B cells may play in measles virus-induced immunosuppression is not known. Previous data using a recombinant GFP-expressing virus suggests that B cells are infected in vivo and in vitro; however, the replication of measles virus in B cells has not been investigated. This study seeks to examine the replication of wild-type and vaccine strains of measles virus in vitro in isolated primary human B cells. Replication has been investigated by several methods. The expression of viral and recombinant proteins was evaluated by flow cytometry using an anti-MeV hemagglutinin antibody, RT-qPCR using a Taqman assay specific for measles virus N gene and accumulation of reporter protein using a recombinant measles virus encoding GFP. The production of infectious virus by B cells was measured by endpoint dilution (TCID₅₀). Viral replication was not detected in primary B cells that have been infected in vitro by any of the methods used. Preliminary data show accumulation of GFP in B cells when whole peripheral blood mononuclear cells (PBMCs) are infected with recombinant measles virus encoding GFP. However, replication within B cells could not be demonstrated by the accumulation of viral N gene RNA after isolation from infected PBMCs. These findings suggest that B cells may not be productively infected with measles virus, but are acquiring antigen from infected cells.

In contrast with the highly polymorphic MHC-Ia (i.e. MHC-A, -B and -C) locus, non-classical MHC-E contains only two alleles, designated MHC-E*01:01 and MHC-E*01:03, throughout the entire human population. The nonsynonymous mutations existing between these two alleles are outside of the peptide-binding groove, making MHC-E essentially non-polymorphic. In natural infection, MHC-E restricted CD8⁺ T cells are rarely reported. Recent studies have shown that vaccine-induced MHC-E restricted CD8⁺ T cell responses mediated potent protection against challenge with pathogenic SIV. Our goal is to clone MHC-E restricted CD8⁺ T cells induced by HIV infection with the eventual goal of developing MHC-E restricted CD8⁺ T cell immunotherapeutics against HIV.

Here, we present an adapted high throughput protocol to generate both classically and non-classically restricted CD8⁺ T cell clones. CD8⁺ T cells were isolated, plated at 1500 cells/well then expanded 300-500-fold with CD3/CD28 activating beads. T cells from each well were tested in ELISpot for HIV- and HCMV-specific T cell responses. We collected CD8⁺ T cells from reactive wells, stimulated the cells and single cell sorted IFNγ⁺TNFα⁺ cells. We expanded the CD8⁺ T cells 1-8 million-fold by allogenic and cytokine stimulation over the next 8 weeks, successfully yielding clonal HIV- and HCMV-specific classically restricted T cell clones. This method is being adapted to isolate and clone HIV-specific MHC-E restricted CD8⁺ T cells from HIV infected, ART suppressed participants.
Approximately 37 million people are infected with HIV globally, and while effective treatment to suppress viral replication is more readily available, people living with HIV (PLWH) have significant comorbidities driven by inflammation including cardiovascular and neurocognitive diseases. Microbial translocation (MT) is a significant driver of inflammation contributing to HIV pathogenesis even in virally suppressed patients on anti-retroviral therapy (ART). Translocated gram-negative bacteria release lipopolysaccharide (LPS) from their cell wall into the blood, which stimulates and activates monocytes resulting in the release of soluble CD14 (sCD14). Generalized immune activation also results in the release of cytokines such as TNF-α from macrophages. Given that MT is a key driver of chronic immune activation in HIV, we sought to evaluate biomarkers of MT in the plasma of PLWH with (n=17) and without (n=26) viral suppression compared to healthy controls (n=8) by measuring LPS via limulus amoebocyte lysate (LAL) assays and TNF-α and sCD14 levels via ELISAs. In PLWH, LPS (p < 0.01) and TNF-α (p < 0.001) were significantly higher than healthy controls. sCD14 was elevated in PLWH compared to healthy controls who were off ART and had compromised CD4 T cell counts of < 500 cells/mL³ (p < 0.05). However, there was no correlation between the increase in LPS levels and sCD14 or TNF-α in this small study.

Given that biomarkers of MT remain elevated despite viral suppression, these studies suggest the potential for their use in monitoring inflammation contributing to comorbid diseases. Future studies to evaluate this pathway could aid in biomonitoring, identifying patients that would benefit most from targeted therapies to modulate inflammation and improve patient outcomes.

Prolonged exposure to antigen, such as during chronic viral infections and cancer, leads to sustained TCR signaling and ultimately T cell exhaustion, which is in part mediated by up regulation of Programmed cell death 1 (PD-1). Antibody blockade of PD-1 does not work in all instances and can lead to autoimmunity, thus identifying novel tools to manipulate PD-1 are needed. Therefore, we sought to understand the genetic and epigenetic mechanisms that regulate PD-1 during acute and chronic antigen conditions in order to identify additional therapeutic targets to modulate PD-1. We determined the chromatin status at the PD-1 locus and found that activating H3K4me1 and H3K4me2 epigenetic modifications were still present at day 8 during a chronic but not acute LCMV infection. This suggested a failure to remove the active histone marks in chronic infections. Indeed, CD8 T cells lacking lysine-specific histone demethylase 1A (LSD1), a H3K4 demethylase, display significantly higher levels of PD-1 mRNA and protein during acute but not chronic infection. Molecular analysis revealed that Blimp-1 recruits LSD1 to the PD-1 locus to induce silencing during acute viral infection. Despite Blimp-1 binding to the PD-1 locus during both acute and chronic LCMV infection, it fails to recruit LSD1 during the chronic setting. Additionally, failure to remove H3K4 methylation resulted in less DNA methylation at known PD-1 control regions compared to wild-type cells, consistent with the differential expression of PD-1. Taken together, these results demonstrate that LSD1 downregulates PD-1 by interacting with Blimp-1 during acute, but not chronic LCMV infection, facilitating full epigenetic repression of the locus.

Presenter: Allison Dyevoich  Title: TLR and CLR agonists induce antitumor B-1a cell and monocyte responses in peritoneal carcinomatosis
Peritoneal carcinomatosis (PC) is a condition where primary tumors metastasize into the peritoneal cavity and induce malignant ascites. Due to the high mortality rate associated with this disease and the lack of curative treatments available, it is imperative that more effective treatments be developed for PC. The immune system plays an important role in tumor elimination, and one strategy currently being explored to treat malignancies involves leukocyte activation through pattern recognition receptors (PRRs), including Toll-Like Receptors (TLRs) and C-type Lectin Receptors (CLR). Our data shows that mice treated with a combination of a TLR and CLR agonist are afforded significant protection when challenged with an aggressive ascites-forming mammary tumor cell line (TA3-Ha). However, when mice lack B-1a cells, the protection is lost. Consistent with this, the treatment induces a significant increase in IgM and complement (C') bound to tumor cells, both of which are critical components in the antitumor effect. Furthermore, treatment induces a rapid increase in Ly6C+ monocytes into the peritoneal cavity that is proceeded by an increase in Ly6C<sup>low</sup> macrophages. Ly6C+ monocytes were required for the antitumor response, and their recruitment dependent on type I interferon (IFN-I) signaling. Interestingly, IFN-I does not mediate the activation of B cells or secretion of IgM, nor do B-1a cells mediate the recruitment of monocytes into the peritoneal cavity, suggesting an uncoupled mechanism between B-1a cell activation and monocyte function. In summary, our data demonstrates pathogen associated molecular patterns can induce rapid natural IgM production that elicits anti-tumor protection via C' activation. Furthermore, treatment causes essential IFN-I-mediated recruitment of Ly6C+ monocytes into the peritoneal cavity that are recruited independently of B-1a cell activation. Our findings provide a possible mechanism in which tumor metastasis in the peritoneal cavity can be targeted for clearance in an inherently immunosuppressive microenvironment. This work was supported by a Department of Defense Grant, W81XWH-15-1-0585, awarded to K.M.H, and a T32 training grant, 2T32AI007401-26A1 awarded to A.M.D.

**Presenter** Paulo Henrique de Melo

**Title** miR-21 drives proinflammatory and glycolytic program of myeloid cells during sepsis

**Email** paulo.d.melo@vumc.org

**Co-Authors** Annie Roccio Pineiros, Carlos Henrique Serezani

**Affiliation** Vanderbilt University Medical Center

Background: Sepsis is associated with a hyper inflammatory state, switch of metabolic profile of immune cells and impaired innate immune functions of phagocytes, collectively leading to organ damage and lethality. Identification and regulation of molecules with pleiotropic can both prevent exaggerated inflammatory response and decreases morbidities associated with sepsis. MicroRNA-21 (miR-21) is one of the most abundant microRNAs (small noncoding RNA) in phagocytes and plays important roles in both increasing or dampening inflammation. However the roles of miR-21 in the innate immune system during sepsis are inconclusive. Aim: Here, we aim to identify the targets and mechanisms by which miR21 influences glycolytic metabolism and inflammatory profile of macrophage and neutrophils during sepsis. We hypothesized that myeloid miR21 inhibits the production of anti-inflammatory mediators, leading to aberrant glycolysis, inflammatory response and animal lethality. Results and conclusion: WT (C57BL/6), miR21<sup>fl/fl</sup> and miR21<sup>Δmyel</sup> mice were subjected to cecal ligation and puncture (CLP) and peritoneal lavage, serum, bronchi alveolar lavage fluid (BALF) were collected within 18h and the animal survival determined over time. Sepsis enhanced miR21 expression in neutrophils and macrophages from peritoneal cavity and lung after CLP. MiR21 deficient myeloid cells show increased animal survival, which correlated with increased bacterial clearance in both the site of infection (peritoneal cavity) and systemically (blood) and decreased production of inflammatory cytokines, such as IL6 and IL1b, and reduced of heart and liver damage as show by reduced CK-MB and TGO, respectively. Next, we sought to test whether decreased inflammation in miR21 deficient cells is dependent on the glycolytic metabolic program in macrophages/neutrophils during sepsis. Both peritoneal and BALF cells from septic miR-21 deficient myeloid cells showed decreased expression of
glycolytic genes (*Hif1a, Scl2a1, Hk1/2, Pkm, Ldha, Mct4*). Also, decreased HK1 protein was associated with lower lactate release than septic WT mice. Corroborating the *in vivo* data, LPS-challenged BMDM and peritoneal macrophage from miR21Δmyel also exhibited lower expression of glycolytic enzymes and inflammatory cytokines (TNF, IL-1 and IL-6) than miR21fl/fl cells. Both, macrophages (BMDM and Peritoneal) and neutrophils were submitted to glycolytic stress assay (Seahorse) after LPS stimulation in order to confirm the functional impact of miR-21 over glycolysis. Similarity in both cells the lack of miR-21 leads to decrease of ECAR. In conclusion, higher expression of miR-21 after sepsis and LPS stimulation leads to assembly of inflammatory and glycolytic metabolic program in myeloid cells.

**Presenter** Andrea Shiakolas

**Title** High-throughput mapping of B-cell receptor sequences to antigen specificity

**Email** andrea.r.shiakolas@vanderbilt.edu

**Co-authors** Ian Setliff*, Kelsey A. Pilewski, Amyn A. Murji, Nagarajan Raju, Larance Ronsard, Charissa Mynhardt, Rutendo Ziki, Katarzyna Janowska, Masaru Kanekiyo, Juliana Qin, Kevin J. Kramer, Allison R. Greenplate, Barney S. Graham, Priyamvada Acharya, Mark Connors, Lynn Morris, Daniel Lingwood, Ivelin S. Georgiev

**Affiliation** Vanderbilt University

The human B cell repertoire is a rich source of broad and potent neutralizing antibodies against invading pathogens. Recent advances in next-generation sequencing (NGS) allow for high-throughput interrogation of the antibody repertoire, including paired heavy and light chain sequencing. However, little is known about the antigen specificity of the antibodies identified in NGS datasets. To overcome this limitation, we developed a technology to recover antigen specificity from high-throughput sequencing of natively-paired heavy and light chains of human B-cell receptors (BCR). B cells are mixed with DNA-barcoded antigens, such that both the antigen barcode(s) and BCR sequence are recovered during sequencing, enabling direct coupling of antigen specificity and paired heavy/light chain monoclonal antibody sequences. Using a panel of recombinant glycoprotein antigens, including diverse HIV-1 SOSIP trimers and influenza hemagglutinin, we validated this method using human B-cell lines with known BCR sequences and specificities. In this experiment, we demonstrated the ability to simultaneously map thousands of B cells to their cognate antigens with high accuracy, including for B cells cross-reactive to multiple SOSIP variants. Additionally, we have applied this method to study antibody responses in multiple HIV-infected individuals, and were able to identify both known and novel cross-reactive antibody lineages. With minimal modifications, the technology should be easily scalable to enable screening of close to 10^5 single B cells against each of dozens of antigens, overcoming the limits of traditional antigen-specific B-cell sorting. The ability to interrogate antibody-antigen interactions using a sequencing-based readout will be highly beneficial to the fields of immune profiling and antibody discovery.

**Presenter** Aaron Silva-Sanchez

**Title** Neonatal lung CD103+ efferocytic dendritic cells prevent generation of effector CD8 T cells

**Email** asilva@uab.edu

**Co-authors** Selene Meza-Perez¹, Frances Lund², Sara L. Stone², Alex Rosenberg², Troy Randall¹

**Affiliation** The University of Alabama at Birmingham, Department of Medicine¹ and Department of Microbiology².

Vaccination is one of the most important adaptations of human society to reduce susceptibility to infectious diseases. However, vaccination is often much less effective in newborns and infants and the mechanisms that limit the induction of protective immune responses, particularly in the lung, are not completely understood. Here, we established a murine model of pulmonary vaccination to examine the role of lung dendritic cells in the generation of antigen-specific CD8+ T cells in adults and neonates. We found that the lungs and mediastinal lymph...
node (MedLN) of neonatal mice contained a majority of Batf3-dependent XCR1⁺ DCs, while adult mice contained a majority of SIRPa⁺ DCs. However, adult XCR1⁺ DC express high levels of CD103, while the neonate XCR1⁺ DCs contain two distinct subsets with different levels of CD103 expression - the CD103⁺ DCs and the CD103⁻ DCs. The CD103⁺ DCs have a mature phenotype (CCR7⁺, PDL-1⁺, CD40⁺) that occurs independently of microbiota or TLR signaling. Although CD103⁺ DCs spontaneously migrate to the draining lymph node, they fail to capture and cross-present exogenous antigens, as a consequence OVA-specific CD8 T cells primed in neonates fail to differentiate into effector cytotoxic lymphocytes. Transcriptome analysis of CD103⁺ DC showed increased expression of genes associated with the phagocytosis of apoptotic cells. The number and activation status of CD103⁺ DCs was reduced in mice with deficient recognition of apoptotic cells (MerTK⁻). Priming of OVA-specific CD8 T cells in the MedLN of MerTK⁻ neonate mice produced more effector CD8⁺ T cells than in the MedLN of control mice. On the contrary, using apoptotic cells to deliver Ag and prime CD8⁺ T cell in adult mice produced poor effector CD8 T cells. Overall, our results show that the presence of activated efferocytic CD103⁺ DCs in newborn lungs prevents CD8⁺ T cell activation by limiting the cross-presentation of exogenous antigens.

Presenter: Ariel Spurrier
Title: Contribution of B cell subsets to TI-2 Ag-specific antibody production and memory
Email: mspurrie@wakehealth.edu
Co-Authors: Christina A. Daly, Jamie Jennings-Gee, Karen M. Haas
Affiliation: Wake Forest School of Medicine

Antibody (Ab) responses to T cell independent type 2 antigens (TI-2 Ags) depend on innate-like B cell subsets, including B-1b cells and marginal zone (MZ) B cells. However, the roles B cell subsets play in clonal expansion, isotype switching, and memory B cell differentiation in response to these Ags have been understudied. Using B cells from mice expressing the VHB1-8 gene knockin gene, we evaluated the contribution of B-1b, MZ, and follicular (FO) B cell subsets to the TI-2 Ag, NP-Ficoll. All B cell subsets divide in response to NP-Ficoll; nonetheless, Ag-specific B-1b cells more rapidly class switch to IgG and have increased differentiation into Ab secreting cells. All subsets formed memory B cells (CD138⁻CFSE⁻) and expressed memory markers previously identified for TD memory B cells. B-1b cells contributed to the highest number of memory cells in the spleen and peritoneal cavity, which included higher frequencies of IgG and CD80-expressing cells, while MZB and FOB cells were found more frequently in lymph nodes and bone marrow. Despite significant memory formation, secondary immunization of recipient mice 4 weeks after primary immunization did not increase NP-specific IgG levels. However, boosting in B-1b cell-recipient mice occurred when IgG levels declined. Finally, use of a TI-2 Ab-boosting adjuvant enabled significantly higher primary and secondary IgG responses in B-1b cell-recipients. Collectively, these findings demonstrate high affinity B-1b cells generate a functional memory population which can be stimulated to produce significant levels of TI-2 Ag-specific Ab either under conditions of low Ag-specific IgG or in the presence of select adjuvants. Nonetheless, MZB and FOB subsets also significantly contribute to the TI-2 Ag-specific memory B cell pool, which may have implications for regulation of recall responses.

Presenter: Shakyra Richardson
Title: Immunomodulatory capability of VCG in Chlamydia immunity
Email: srichardson@msm.edu
Co-Authors: Roshan Pais, Yusuf Omosun, Stephanie Lundy, Joseph U. Igietseme and Francis O. Eko
Affiliation: Morehouse School of Medicine and Center for Disease Control

Immunization with UV-inactivated Chlamydia trachomatis elementary bodies (UV-EB), the infectious form of Chlamydia, does not protect against infection with live Chlamydia. Vibrio cholerae ghosts (VCG), which are empty V. cholerae cell envelopes derived by genetic inactivation, constitute an effective delivery vehicle for cloned vaccine antigens and promotes the induction of protective immunity in the absence of added adjuvants. In this study, we sought
to investigate the ability of VCG to enhance the innate and adaptive immune responses induced by UV-EB. Thus, bone marrow-derived dendritic cells (BMDC) were pulsed with UV-EB in the presence or absence of VCG and the induction of innate immune responses, T cell proliferation and ability to afford protection against Chlamydia following adoptive transfer into susceptible mice was evaluated. The results show that VCG efficiently modulated the immunostimulatory properties of DCs as indicated by increased secretion of proinflammatory cytokines and expression of toll-like receptors and co-stimulatory molecules associated with DC maturation. Also, UV-EB-pulsed DCs that were exposed to VCG and adoptively transferred into mice resulted in effective chlamydial antigen presentation and protective immunity. The results demonstrate that VCG activate the maturation of DCs leading to enhancement of innate and adaptive immunity to an otherwise non-protective antigen, suggesting their utility as adjuvants for enhancement of protective immunity to inactivated vaccine antigens.

This work was supported by grants from NIH (R01AI126897, 1C06 RR18386) and resource support from CDC.

Presenter: Sarah Mosure
Title: Investigating heme regulation of REV-ERBα activity in Th17 cells
Email: smosure@scripps.edu
Co-Authors: Sean Campbell, Douglas Kojetin, Laura Solt
Affiliation: Scripps Florida

T helper 17 (Th17) cells are important for protective immunity at mucosal barriers, but they are also implicated in autoimmune and chronic inflammatory disease pathogenesis. We recently demonstrated that the nuclear receptor REV-ERBα is a cell-intrinsic negative regulator of Th17 cell development in vitro and Th17 cell pathogenicity in mouse models of autoimmunity in vivo. Mechanistically, REV-ERBα represses core Th17 cell genes by competing for binding at DNA response elements shared with the Th17 cell lineage-defining transcription factor RORγt. As ligand-regulated transcription factors, nuclear receptors like REV-ERBα are susceptible to regulation by natural, or endogenous, ligands, but it remains unclear whether the endogenous REV-ERBα ligand, heme, regulates REV-ERBα activity in Th17 cells. Identifying the roles of endogenous ligands contributes to our understanding of signaling pathways that govern Th17 cell homeostasis and pathogenicity. To investigate a role for heme, we treated primary Th17 cells derived from REV-ERBα/βfl/fl (WT) or REV-ERBα/βfl/fl xCD4-Cre (DKO) mice. While heme treatment repressed the differentiation of both WT and DKO Th17 cells, the repression was not as great in the DKO (or REV-ERBα single KO) cells. These data indicate that heme may have a role in regulating REV-ERBα activity. To test this possibility, we retrovirally transduced a REV-ERBα mutant (H603F) incapable of binding heme in primary Th17 cells. Relative to wild-type (WT) REV-ERBα, the H603F mutant exhibited reduced repression of target genes, despite being present at the protein level in greater amounts compared to WT REV-ERBα. The increase in H603F protein relative to WT indicated that heme binding targets REV-ERBα for proteasomal degradation in Th17 cells, consistent with previous studies using other cell types. Together, these results indicate that heme regulates REV-ERBα activity by multiple mechanisms in Th17 cells, enhancing both REV-ERBα-mediated repression of core genes and REV-ERBα protein turnover. Thus, heme may function as a key regulatory molecule coupling Th17 cell signaling pathways to gene expression through modulation of REV-ERBα activity.

Presenter: Karla Navarrete
Title: Interchromosomal templated mutagenesis accounts for mutation clusters in B cell non-Ig genes
Email: karla.navarrete@emory.edu
Co-authors: Gordon A. Dale, Daniel J. Wilkins, Joshy J. Jacob
Affiliation: Emory University

The immunoglobulin (Ig) genes of germinal center B cells (GCB) undergo somatic hypermutation in order to generate antibody diversity. During this process, non-immunoglobulin (non-Ig) genes can also accumulate mutations. There are two generally accepted methods
Interleukin-23 receptor signaling modulates the stability and function of FOXP3+ regulatory T cells

Methods: To assess whether exogenous IL-23 activates Tregs in vitro, murine Tregs were stimulated with IL-23 or IL-6 and activation of STAT3 was assessed by FACS. The suppressive capacity of Tregs was examined by labeling naïve CD4+ with CellTrace Violet and αCD3αCD28 stimulation in presence or absence of exogenous IL-23, and with or without WT CD25+ Tregs. The in vivo role of IL-23R specifically in Tregs was determined using Il23r-/-Foxp3YFP-Cre mice. To explore whether expression of IL-23R altered Treg stability/survival, YFP+ cells from mice heterozygous or homozygous for the floxed Il23r allele were injected into lymphocyte deficient Rag1-/- mice. Four weeks later, T cells were recovered from peripheral compartments and proliferation of donor Tregs was assessed by EdU incorporation.

Results: Exogenous IL-23 activated STAT3 in Tregs to the same extent as IL-6 in vitro. Moreover, the ability of WT Tregs to suppress proliferation of naïve CD4+ T cells was attenuated in presence of IL-23 in vitro. In vivo, Il23r-/-Foxp3YFP-Cre mice had less YFP+ Tregs compared to Il23r+/Foxp3YFP-Cre mice in the colonic lamina propria. Following adoptive transfer of IL-23R-sufficient or -deficient YFP+ Tregs into Rag1-/- recipient mice, a decrease in YFP+ cells was observed in the mesenteric lymph node and colon in mice receiving IL-23R sufficient Tregs, which was not attributed to increased proliferation of IL-23R-deficient cells.

Conclusions: These data suggest that IL-23R signalling destabilizes FOXP3 directly or by modulating survival or function of FOXP3+ cells in vitro and in vivo. However, the underlying

Background: The cytokine interleukin 23 (IL-23) has been implicated in the multifactorial pathogenesis of inflammatory bowel disease (IBD). IL-23 has previously been associated in the maintenance of Th17 cell responses. However, its pathogenic role in IBD is likely independent of Th17 cell biology as IL-17 blockade with Secukinumab exacerbated disease. The IL-23 receptor (IL-23R) is highly expressed on a subset of colonic highly suppressive regulatory T cells (Tregs) expressing forhead box P3 (FOXP3). We hypothesize that IL-23R signaling in Tregs modifies the transcriptional landscape of the Foxp3 locus leading to reduced function of Tregs, and thereby driving immune dysregulation in the intestine.
Increasingly it is recognized that host genetic variability contributes to infection susceptibility and outcomes. Thus, there has been great efforts made towards leveraging genome and phenome-wide association studies to identify genetic factors that impact infection outcome. Our unbiased phenome-wide association study identified an association between polymorphisms in the gene encoding carnitine-palmitoyl transferase 1a (CPT1a) and an increased risk of infection, including bacterial pneumonia. CPT1a is a mitochondrial outer membrane protein that is required for long-chain fatty acid entry into the mitochondria for (FA) oxidation. To examine whether CPT1a is a host determinant of infection outcome, a murine pneumonia model was used in which animals were treated with the inhibitor of CPT1a, etomoxir, or vehicle control prior to intranasal infection. Results using multiple bacterial pathogens indicate that animals treated with etomoxir exhibited increased mortality and bacterial burdens when compared with vehicle treated animals. Flow cytometric and histological analyses indicate that animals treated with etomoxir had a significant reduction in neutrophils in the blood and recruited to the lungs. To investigate the role of CPT1a in neutrophil recruitment in the setting of bacterial pneumonia, animals treated with etomoxir prior to intranasal infection were assessed for neutrophil production, activation, and egress from the bone-marrow. FACS analysis of the bone-marrow compartment determined that CPT1a function does not play a major role in neutrophil development. However, complete blood count (CBC) revealed that animals treated with etomoxir had fewer circulating neutrophils than PBS treated animals. Serum levels of chemoattractant factors indicate that etomoxir treatment did not affect signals required for neutrophil mobilization during infection, however, FACS analysis shows reduced expression of mobilizing chemokine receptors on neutrophil. These results suggest that CPT1a function may be critical for neutrophil activation and egress from the bone-marrow. Consistent with this, preliminary in-vitro chemotaxis data demonstrate that inhibiting CPT1a function reduced neutrophil migration towards the chemotactic peptide, fMLP, further suggesting that CPT1a function is involved in neutrophil trafficking. Together these data identify CPT1a as a novel host determinant for infection outcome.

An essential question regarding the T cell-inducing influenza vaccine is whether the cellular immunity may drive the virus to rapidly evolve and escape. In our previous modeling study, we have shown an CD8 T cell escape-variant of influenza A virus (IAV) invades extremely slowly given empirical HLA allele frequencies and modest selection pressure. Guided by the model, we aim to quantify the selection from CD8 T cells using a novel digital droplet PCR system under different in vivo settings. Mice were intranasally primed with HKx31 (H3N2) and challenged with a 1:1 mixture of wild-type and mutant PR8, which harbors an N370Q escaping mutation in its NP, immunodominant epitope, on day 30 post-priming. The area under the in vivo viral growth curve (AUC) is used to measure the fitness of wild-type and mutant viruses. Based on the AUCs of C57BL/6 (H-2^{b/b}) and CB6F1 (H-2^{b/a}), the estimated selective advantage of this mutation is 0.28 [0.20, 0.36] and 0.21 [0.10, 0.34], respectively. Interestingly, although the intramuscularly primed C57BL/6 gave a similar estimate for the advantage (0.22 [0.16, 0.30]), the advantage be-
came much more apparent on day 6 and 8 after PR8 challenge. This implies the lung resident memory CD8 T cells may be the source of selection during the initial stage of IAV infection. In combination with the model prediction, an escape-variant of IAV that carries a mutant immunodominant epitope may need 100 to 500 generations (corresponding to 1 to 5 years if the infection period is assumed to be 4 days) to reach 50% of prevalence given the frequency of cognate HLA alleles is 20%. We concluded that the selection from CD8 T cells may not be sufficient to drive the CD8 T cell epitopes of IAV to evolve rapidly like the antibody epitopes.

Presenter: Michael A. Raddatz
Title: Macrophages Promote RUNX2 Activation and Calcification in Aortic Valve Cells
Email: michael.a.raddatz@vanderbilt.edu
Co-Authors: Tessa M. Huffstater, Bradley I. Reinfeld, Jeffrey C. Rathmell, W. David Merryman
Affiliation: Vanderbilt University

Immune cell infiltration characterizes calcific aortic valve disease (CAVD), yet there is little understanding of the role of these cells in pathophysiology. The most common immune cells in both the healthy and the diseased aortic valve (AV) are macrophages, which are known to promote heterotopic calcification in other diseases. To study the role of macrophages in the calcification of aortic valve interstitial cells (AVICs), AVICs were isolated from wild-type (WT) mice, and bone marrow-derived macrophages (BMMs) were generated from WT and Notch1+/− (N1+/−) mice. NOTCH1 haploinsufficiency leads to CAVD in mice and humans. Cells were seeded in monoluculture, in trans-well culture, or in co-culture at physiologic ratio (1 BMM: 7 AVICs) and assayed via immunofluorescence (IF), quantitative polymerase chain reaction (qPCR), Western blot (WB), and calcific nodule (CN) assays. Each cell type was then enriched from co-culture via magnetic-activated cell sorting (MACS) to be assayed via qPCR and/or WB. Separately, to assess the role of hematopoietic cells at large in CAVD, bone marrow was isolated from WT and N1+/− C57BL/6 mice and injected into irradiated WT and N1+/− mice. Mice were evaluated by echocardiography and aged on high-cholesterol diet for 6 months. Mice were then evaluated again by echocardiography and euthanized for isolation of the AV. AVs were stained for calcification via von Kossa, fibrosis via Masson’s Trichrome, immune cell infiltration via IF for CD3 and CD68, and cell-signaling via IF for RUNX2. Co-culture of BMMs and AVICs increases CN formation and expression of Runx2, a key transcription factor in osteoblast differentiation, and osteopontin (Spp1), a marker of osteogenic calcification. IF staining reveals that RUNX2 signaling is increased in the AVICs, not by addition of RUNX2-expressing BMMs. Mice receiving N1+/− bone marrow transplant had an increased rate of disease progression on echocardiography and increased valvular calcification by von Kossa staining. Interestingly, calcification co-localized with CD68-positivity. Translating this impact of N1+/− macrophages to the in vitro model, co-culture of WT AVICs with N1+/− BMMs increases expression of osteopontin. These findings suggest that hematopoietic cells and specifically macrophages play a significant role in promoting calcification in CAVD.

Presenter: Zahraa Mohammed
Title: MiR-155 Positively and Negatively Regulates Mast Cell Mediator Release
Email: zahraa.mohammed@uscmed.sc.edu
Co-Authors: Gregorio Gomez
Affiliation: University of South Carolina

Allergic disease is the 6th leading cause of chronic illness in the U.S. (cdc.gov). Mast cells play a central role in allergic disease through the release of various biological mediators such as pre-stored mediators like histamine, serine protease, lipid-derived mediators like prostaglandins and leukotrienes, and cytokines and chemokines that contribute to allergic inflammation. MicroRNAs (miRNAs) are short non-encoding RNAs that regulate gene expression of various inflammatory mediators by suppressing translation or through mRNA degradation. Several miRNAs have been implicated in the regulation of allergic disease including miR-155, which has been implicated in allergic asthma. However, the role of miR-155 in regulating mast cells function remains unclear. Therefore, the aim of this study was
to characterize the specific role of miR-155 in mast cell activation and release of allergic/inflammatory mediators. Human skin-derived mast cells that were isolated from normal tissue, and bone marrow–derived mast cells (BMMCs) that were generated in vitro from wild type (WT) and miR155 knockout (KO) mice were used as our experimental models. Micro-array analysis and qRT-PCR were used to detect the changes in the miR-155 and gene expression. Degranulation was determined by β-hexosaminidase release assay. PGD2 and leukotriene C4 (LTC4) were measured by enzyme immunoassay. The effect of miR-155 on p38 MAP kinase (MAPK), p42/44 (ERKs), protein kinase B (Akt) and phosphorylated proteins expression was examined by western blot. MiR-155 expression was induced following FcεRI crosslinking with multivalent antigen in human skin mast cells and BMMCs. We found that miR-155 had no effect on FcεRI-induced mast cell degranulation. On the other hand, FcεRI-induced COX-2 was significantly inhibited in the absence of miR-155 suggesting that miR-155 plays a critical role in PGD2 biosynthesis. Importantly, we found that ATF3 mRNA expression increased in BMMCs KO compared with WT, suggesting that ATF3 is a target gene of miR-155 in PGD2-COX-2 pathway. Interestingly, miR-155 had no effect on FcεRI-induced LTC4. Accordingly, arachidonate 5-lipoxygenas (ALOX5) was expressed at the same levels in WT and KO BMMCs. FcεRI-induced cytokine production was diminished in miR-155 KO BMMCs compared to WT. Interestingly, cytokine production from miR-155 KO BMMCs was increased compared to WT following LPS treatment. These findings indicate that miR-155 acts as a positive regulator in the FcεRI pathway, and a negative regulator of the TLR4 pathway in mast cells. The phosphorylation of AKT was significantly decreased in miR-155 KO compared with BMMCs WT, whereas p38, and p42/p44 phosphorylation were the same in both types of mast cells. These data demonstrate that miR155 is both a positive and negative regulator of mast cell mediator release.

Cancer immunotherapy efficacy depends on tumor-specific T cell activity. T cells dramatically increase their metabolic activity upon activation and T cell subsets utilize distinct metabolic programs. Glutaminolysis, the breakdown of glutamine important for anaplerosis and other anabolic processes, is upregulated on T cell activation. Previous studies demonstrate that inhibition of anabolic pathways, such as glycolysis and mTORC1 signaling, decreases effector function in CD8 T cells. In contrast, here we show that blocking glutaminolysis by treatment with Glutaminase inhibitor CB839 during activation of murine CD8 T cells increases effector function. Granzyme B and Perforin levels increase with CB839 treatment, as do effector-associated transcription factors Tbet and Eomes. Intriguingly, Glutaminase inhibition also increases Ki67, BCL2, TCF1, and CD62L levels, suggestive of increased proliferation, survival, and T cell memory. Mitochondria in CB839-treated T cells have reduced inner membrane potential and demonstrate decreased turnover, potentially indicative of improved mitochondrial fitness. Baseline oxygen consumption relative to glycolysis also increases. Correspondingly, murine chimeric antigen receptor (CAR) T cells transiently treated with CB839 sustain higher peripheral blood levels in vivo than non-treated CAR T cells. Overall, our results suggest that Glutaminase inhibition by CB839 increases CD8 T cell effector function as well as in vivo persistence, making T cell Glutaminase an attractive target in anti-tumor immunity. Future directions include comparing transient versus chronic Glutaminase inhibition in T cells and further refining the mitochondrial phenotype of CB839-treated T cells. In vivo, we will assess the role of Glutaminase in T cell memory formation, conduct leukemia survival studies to confirm increased efficacy of CAR T cells treated with CB839, and measure the effect of CB839 treatment on intratumoral metabolite competition between T cells and tumor cells in vivo using 18F-labelled glucose and glutamine.
Previous reports have demonstrated the effects of IL-27 on CD4 T cell activation and differentiation, in addition to a role for IL-27 in cancer therapies mediated by cytolytic CD8 T cells. However, as IL-27 is produced by many cell types during infection, and the IL-27 receptor (IL-27R) is widely expressed on activated CD8 T cells as well as on activated CD4 and NK cells, the direct effects of IL-27 signaling on CD8 T cell function are less well understood. To directly test the role of IL-27R on CD8 T cells following viral respiratory infection in vivo we used a murine model of influenza infection in combination with co-transfer of congenic wild type (WT) and IL-27R−/− OT-I CD8 T cells. We observed a dramatic reduction in the frequency and number of IL-27R−/− OT-I compared to their WT OT-I counterparts within the same host following influenza infection, indicating a CD8 T cell-intrinsic role for IL-27R in the generation of an acute anti-viral response. Further, these IL-27R−/− OT-Is express higher levels of exhaustion markers, fail to proliferate in situ and enter apoptosis at a greater frequency. This effect is not limited to naive IL-27R−/− OT-Is, as memory IL-27R−/− OT-Is also fail to accumulate to the degree of their WT counterparts on heterologous re-challenge. This study reveals a new role for IL-27 in mediating the production of an effective anti-viral CD8 T cell response following respiratory viral infection.

Human cytomegalovirus (HCMV)-specific CD8+ T cells are characterized by a unique, non-exhausting, effector memory phenotype. How HCMV induces this phenotype is poorly characterized. We hypothesized that HCMV-infected fibroblasts may modulate T cell priming producing functionally distinct CD8+ T cells.

To investigate this question, we have generated mesenchymal cells (MSCs) isolated from umbilical cord (UC). MSC and fibroblasts express many of the same phenotypic markers. We standardized culture methods of MSC from UC collagenase-treated Wharton’s Jelly (UC-WJ) resulting in reproducible detection of UC-stromal cells within 10-15 days of initial culture. We corroborated that derived cells (passage #3-6) displayed fibroblastoid morphology and surface markers, such as CD73, CD90, CD105 and CD44, with levels comparable to the fibroblast primary cell line, MRC-5.

Previous studies have reported IFN-γ treatment of fibroblasts induces MHC-II expression, leading to suggestions that fibroblasts can acquire APC-like activity. We used flow cytometry to examine the effects of HCMV infection +/- IFN-γ on MHC-II, CD40, CD80 and CD86 surface expression. Infection with either the HCMV isolate TB40E or the HCMV laboratory-adapted strain AD169 failed to induce MHC-II or costimulatory molecules. HCMV infection +/- IFN-γ induced MHC-II but costimulatory molecules remained undetectable. This suggests HCMV-infected fibroblasts do not acquire APC-like phenotype and that fibroblasts are unlikely to directly prime CD8 T cells.

HCMV-infected UC-fibroblasts may indirectly modulate T cell priming through the release of cytokines or extracellular vesicles. Fibroblasts release extracellular vesicles (EVs), which are important mediators of cell signaling, including antigen cross-presentation. We used electron microscopy to confirm that stromal cells release EVs. Imaging flow cytometry showed that following 5.5-8% of EV collected from the culture supernatant of AD169-GFP infected MRC-5 fibroblasts were GFP-positive. Ongoing studies are investigating whether UC-F modu-
late mDC priming of CD8+ T cells through direct cell-cell contact and/or indirectly, including through the release of cytokines or EVs.

Presenter: Allison E. Norlander, PhD
Title: Prostaglandin I$_2$ signaling is important for Treg function during allergic inflammation
Email: allison.e.norlander.1@vumc.org
Co-Authors: Melissa H. Bloodworth, PhD, R. Stokes Peebles Jr., MD
Affiliation: Vanderbilt University Medical Center

Our laboratory reported that prostaglandin I$_2$ (PGI$_2$) promotes immune tolerance in mice. Thus, we hypothesized that PGI$_2$ promotes the functionality of Tregs in vivo. We utilized mice deficient in the receptor for PGI$_2$, IP (IPKO), as well as two separate adoptive-transfer based models of allergic inflammation. In the first model, splenic ovalbumin (OVA)-specific Tregs were isolated from wild-type (WT) and IPKO mice. OVA-specific CD4+ effector T cells (Teff) were isolated from the spleens of WT mice. RAG1 deficient mice received equal amounts of Teff and either WT or IPKO natural Treg (nTreg). Another group of RAG1 deficient mice were transferred only Teff. RAG1 deficient recipients were challenged intranasally with OVA 1 day prior to and for 3 consecutive days post transfer. On day 4 post transfer the bronchoalveolar lavage fluid (BAL) was harvested and examined for the presence of IL-13 by ELISA, while the lungs were examined by flow cytometry. Compared to mice transferred only Teff, total IL-13 in the BAL was reduced by 46% in the mice transferred WT nTregs; however, there was no decrease in total IL-13 in the mice transferred IPKO nTregs. Furthermore, compared to mice transferred only Teff, total OVA-specific CD4+IL-13+ cells in the lungs were reduced by 32% in the mice transferred WT nTregs, however, there was no decrease in these cells in mice transferred IPKO nTregs. In the second model, WT mice were sensitized with an OVA-alum solution to prime an allergic immune response. Two weeks after sensitization, mice were challenged with nebulized OVA for 3 consecutive days. On day 4 post transfer the BAL was harvested and examined for the presence of IL-13 by ELISA. Compared to mice that received no iTreg, total IL-13 in the BAL was reduced by 60% in the mice transferred WT iTregs; however, there was no decrease in total IL-13 in the mice transferred IPKO iTregs. Our data demonstrate that PGI$_2$ signaling promotes Treg function in vivo and suggests that PGI$_2$ may serve as a novel treatment for allergic diseases.

Presenter: Jing Zhu
Title: Manipulation during B cell maturation ameliorates lupus nephritis in BXSB mice
Email: jing817@vt.edu
Co-authors: Alayna Hay, Ashley Potter, Janie Adkins, Caroline Leeth
Affiliation: Virginia Tech, Dept of Animal and Poultry Sciences, Blacksburg, VA

Systemic lupus erythematosus (SLE) is a complex autoimmune disease associated with the production of autoantibodies. Previous studies demonstrated the majority of pathogenic autoantibodies are mutated IgG, suggesting crucial roles of affinity maturation in SLE pathogenesis. Our hypothesis is that abrogation of this mechanism will retard SLE development by preventing the production of high affinity auto-antibodies. Activation-induced cytidine deaminase (AID, gene name Aicda) is an enzyme required for affinity maturation. In our study, we genetically targeted Aicda in BXSB mice to examine the effects of B cell maturation on SLE. In the absence of AID, mice showed significantly reduced autoantibodies and extended lifespan. While similar levels of immune complex deposition were seen in the glomeruli, AID-deficient mice exhibited impaired complement activation and ameliorated nephritis. Consistent with other models, AID deficiency resulted in enlarged germinal centers. However, the increase in germinal center B cells did not lead to a corresponding increase in follicular helper T cells. In addition, low affinity B cells did not fail to survive or differentiate into plasma cells since no significant difference was found in plasma cell generation either in vivo or in vitro. In conclusion, the success of targeting affinity maturation and class switch to alleviate SLE, especially nephritis, provides potential innovations in SLE treatment.
Iron is essential for many fundamental biological processes including, cellular respiration, DNA synthesis, proliferation, host defense, and cell signaling, and therefore iron homeostasis is an important determinant of immunometabolic health. Tissue iron overload is associated with insulin resistance and glucose dysregulation in rodents and humans; however, the mechanisms or cell types that mediate this phenotype are not completely understood. Given that Mφs are the primary iron-handling cells, it is reasonable to posit that perturbed Mφ iron metabolism is a precipitating factor in metabolic dysfunction. Accordingly, we tested the postulate that disturbed iron trafficking by Mφs drives systemic glucose intolerance in mice. Mice with targeted deletion of ferroportin (Fpn) – the only known mammalian iron exporter – in myeloid cells (FpnΔMφ) were generated using Cre-Lox recombination under control of the LysM promoter and compared to Fpn flox/flox (Fpnfl/fl) littermates as controls. All mice were fed rodent chow and had access to water ad libitum. Body weight, fat mass, and lean mass were not different between Fpnfl/fl and FpnΔMφ mice. Compared to floxed mice, Fpn expression was ≈5-fold lower in bone marrow-derived Mφs in FpnΔMφ animals. To confirm that Fpn depletion causes iron retention, a subset of mice were perfused and fixed with an iron-staining solution (Pearl's Prussian Blue). Gross observation revealed that the spleen, liver, and thymus exhibited positive iron stain that was markedly exacerbated in FpnΔMφ mice, confirming an iron loaded phenotype. Histological evidence in isolated liver sections revealed that iron accumulation in FpnΔMφ mice was localized to extrahepatic cells, presumably resident Kupffer cells. In additional, liver triglycerides were elevated in FpnΔMφ compared to Fpnfl/fl animals. Relative to Fpnfl/fl controls, blood glucose concentrations in response to an intraperitoneal glucose load were increased in FpnΔMφ animals. Collectively, these data suggest that disturbed iron trafficking in Mφs contributes to glucose intolerance in lean mice. Additional research is required to tease-out the mechanism(s) by which blunted iron handling by Mφs disrupts glucose homeostasis.

While checkpoint blockade therapies have demonstrated amazing success treating a variety of cancer patients, significant numbers of patients do not respond to therapy. While it is currently unknown what determines a patient’s response to therapy, increased numbers of T cells within tumors is correlated with longer patient survival and positive response to checkpoint blockade therapy. T cell presence within the tumor can be affected by entry through the endothelium, as well as proliferation or survival within the tumor. What regulates T cell presence within the tumor microenvironment (TME) remains an open area of study. Innate immune cells have been studied for their role in determining T cell activation or suppression within the TME, but their role in driving T cell presence within the tumor has not been thoroughly examined. Among the innate immune cells, NK cells are of particular interest as they can produce inflammatory cytokines (IFNγ and TNFα) that alter homing receptor ligand expression on the vasculature. Increased expression of homing receptor ligands may increase T cell entry into tumors. Using an implantable subcutaneous murine melanoma model, NK cells only produced IFNγ at early time points during tumor development. To determine what effect NK cells have on immune cell presence within the TME, we depleted NK
cells prior to the tumor being established. Homing receptor ligand expression was lowered on the vasculature following NK depletion. In addition, depletion of NK cells resulted in lower number of T cells within the tumor only at late time points. Surprisingly NK cell depletion, while still lowering homing receptor ligand expression, resulted in increased numbers of T cells at early stages of tumor growth. However, T cells were less functional when NK cells were depleted, particularly within early stage tumors. Overall these results suggest that NK cells drive increased levels of T cell numbers and function within late stage tumors, possibly by deleting dysfunctional T cells at early stages of tumor growth. Developing therapies that increase NK cell function within the TME may result in increased T cell function and improve the effectiveness of checkpoint blockade therapies.

Presenter: Marit Melssen
Title: Formation and function of retention integrin expressing CD8 T cells in a murine breast cancer model
Email: mm2xz@virginia.edu
Co-authors: Robin Lindsay, Amanda Briegel, Anthony Rodriguez, Salvador Cyranowski, Cornelis Melief, Sjoerd van der Burg, Craig Slingluff Jr, Victor Engelhard
Affiliation: University of Virginia

Integrins CD49a and CD49b can mediate retention of lymphocytes in peripheral tissues by binding to collagens type IV and type I, respectively. Their expression is upregulated on CD8+ tumor infiltrating lymphocytes (TIL) compared to circulating lymphocytes and presence of CD49a+ TIL improves patient outcome. Little is known about how expression of these integrins is regulated and the functional capacity of the cells expressing them. We hypothesized that CD49a expression identifies more functional T cells in the tumor microenvironment (TME) and expression is induced on effectors and/or early memory cells. To address these hypotheses, CD8 TIL from an implantable breast carcinoma model were evaluated at day (d)14 and d23 for expression of CD49a, CD49b and functional markers, and tumors were assessed for collagen expression. In early stage tumors (d14), T cells were predominantly CD49b single positive (SP) or CD49aCD49b double positive (DP). Later (d23), CD49b SP cells largely disappeared and CD49a SP cells appeared, while DP cells remained unchanged. After treatment with FTY720, to block further T cell infiltration, the switch from CD49b SP to CD49a SP cells was even more pronounced, suggesting the change is not due to newly infiltrating CD49a SP cells. Interestingly, regardless of timepoint, CD49b SP cells were antigen-responsive, whereas DP and CD49a SP cells had features of exhaustion. These data suggest that, contrary to our hypothesis, during tumor progression, CD8 TIL gain CD49a and lose CD49b as they become dysfunctional. To test whether this switch in integrin expression depends on antigen engagement or environmental factors, we transferred activated, non-specific CD49b SP CD8 T cells into tumor-bearing mice. In these tumors, CD49b SP cells upregulated CD49a, followed by downregulation of CD49b. This suggests that the switch in integrin expression is dependent on the microenvironment, not antigen-specific differentiation. Importantly, preliminary data showed that expression of collagen I decreased over time, suggesting changes in ligand availability may play a role in the decrease of antigen-responsive CD49b+ TIL. Future experiments will assess which environmental factors directly play a role in CD49a upregulation, and how processes of retention integrin expression relate to the antigen-dependent generation of exhaustion.

Presenter: Amanda M. Lulu
Title: Pre-existing immune memory to cancer-associated phosphopeptides in healthy donors suggests ongoing immune surveillance
Email: Aml2cu@virginia.edu
Co-Authors: Kara L. Cummings and Victor H. Engelhard
Affiliation: University of Virginia, Department of Microbiology, Immunology and Cancer Biology, Charlottesville, VA

Identifying appropriate antigens is essential to developing effective cancer vaccines. Protein phosphorylation plays a major role in signal transduction pathways that are dysregulated...
and contribute to the malignant phenotype of cancer cells. Our lab has shown that phosphopeptides derived from these proteins can be presented by Major Histocompatibility Complex Class-I molecules and represent a new class of cancer-associated neoantigens. We previously reported that healthy donors without evident prior cancer had unusually robust CD8 T cell responses to a cohort of HLA-B7+ leukemia-associated phosphopeptides, suggesting that they might be due to pre-existing phosphopeptide specific memory T cells. To test this, we evaluated CD8+CD45RO+ memory T cells purified from the blood of healthy donors. These cells showed robust responses to 2-25% of HLA-A2+ and 0-23% of HLA-B7+ cancer-associated phosphopeptides. This demonstrates that healthy individuals with no evident cancer history have pre-existing immune memory to some cancer-associated phosphopeptides. We identified 3 “immunodominant” HLA-A2 associated phosphopeptides, in that memory responses were evident to them in the majority of analyzed donors. Such immunodominance suggests that these phosphopeptides result from signaling processes that occur commonly in transformation- or viral infection-related processes. In contrast, immunodominant phosphopeptides were not evident in the context of HLA-B7. While 2 donors showed pre-existing immune memory to a large number of shared targets, other donors showed memory to only small numbers of non-overlapping phosphopeptides. This suggests that the mechanisms driving expression of these phosphopeptide antigens among healthy donors are much less common. We also found that responses to some phosphopeptides could be detected directly ex vivo. These results suggest that some phosphopeptides in some donors may be the targets of recent or ongoing immune responses composed of effector or effector memory cells. These results provide evidence of regular, ongoing immune surveillance in healthy individuals to cancer-associated phosphopeptides.

Presenter: Anthony B. Rodriguez

Title: Adaptive and innate immunity orchestrate tertiary lymphoid structure formation in tumors

Email: abrodrigu@virginia.edu


Affiliations: 1Beirne B. Carter Center for Immunology Research, University of Virginia School of Medicine, Charlottesville, VA 22908, USA; 2Department of Microbiology, Immunology and Cancer Biology, University of Virginia School of Medicine, Charlottesville, VA 22908, USA; 3Department of Surgery, University of Virginia School of Medicine, Charlottesville, VA 22908, USA; 4Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX 75235, USA; 5Current Address: Department of Pathology, John Hopkins University, Baltimore, MD 21218, USA

Tumor-associated tertiary lymphoid structures (TA-TLS) are ectopic lymphoid aggregates resembling conventional secondary lymphoid organs (SLO). In patients, these structures have been associated with high densities of tumor-infiltrating lymphocytes (TIL) and enhanced survival. We previously found that naïve T-cells enter tumors via high endothelial venules (HEV), which are crucial components of TA-TLS; thus, TA-TLS may be sites for generation of intratumoral immune responses. However, the mechanisms that underlie the formation of TLS in tumors are largely unknown. Here we show that formation of TA-TLS in murine B16 melanomas is orchestrated by podoplanin+ (PDNP+) fibroblasts that resemble lymphoid tissue organizer cells (LTo) and appear distinct from conventional cancer-associated fibroblasts (CAF). These PDNP+ cells establish a reticular network within TA-TLS and express elevated levels of the recruitment chemokines CCL21 and CXCL13 and B-cell survival factors BAFF and APRIL. While expression of these lymphoid tissue organizer molecules depends on tumor necrosis factor receptor (TNFR) signaling, fibroblast proliferation and reticular network formation depends on intratumoral T- and B-cells and lymphotixin-β receptor (LTβR) signaling. Identification of these pathways and their impact on intratumoral fibroblasts provide a platform for manipulating the formation of these structures as a new strategy for cancer immunotherapy.
While the contribution of humoral immunity to systemic lupus erythematosus (SLE) is well established, the role it plays in lupus confined to the skin, chronic cutaneous lupus erythematosus (CCLE), is less clear. One characteristic of SLE is a breakdown of tolerance in autoreactive VH4.34 antibodies that are recognized by the rat anti-human idiotypic antibody 9G4 (9G4+). These antibodies have a germline encoded autoreactivity to glycolipids found on red blood cells and naïve B and are tightly regulated in healthy individuals. In SLE patients this tolerance is broken resulting in high levels of serum 9G4+ IgG which is associated with high disease activity and correlates with anti-dsDNA. This study compared 9G4+ IgG and associated SLE auto-antibodies between CCLE patients and SLE patients. We found 57% of SLE patients were positive for 9G4+ IgG and SLE patients had a significantly higher serum concentration than HC. Surprisingly, CCLE patients also had high levels of 9G4+ IgG, 48% were positive and serum concentrations did not statistically differ from SLE patients. In contrast, while many SLE patients had anti-dsDNA and anti-chromatin, few CCLE patients were positive for these specificities. Consequently, 9G4+ IgG concentration was highly correlated with both anti-dsDNA and anti-chromatin concentration in SLE patients but not in CCLE patients. CCLE patients, however, did have auto-reactive 9G4+, as B cell and apoptotic cell binding 9G4+ antibodies were similar between SLE and CCLE patients. This suggests a two-step model of 9G4 tolerance in SLE, the breakdown of general tolerance of germline encoded autoreactive VH4.34 antibodies and a subsequent development of 9G4+ IgG specific for ds-DNA. CCLE patients clearly have a defect in the first step and the mechanism of this defect is potentially shared between CCLE and SLE. However, the second step is not shared, as CCLE patients with 9G4+ IgG do not have high levels of anti-dsDNA. Regulation of this second step may help determine if otherwise immunologically similar patients may develop SLE or CCLE. Because the specificity of 9G4+ in CCLE is unknown, it remains to be determined whether these antibodies contribute directly to CCLE disease.

Inflammation is a part of the immune system that is activated in response to injury and infection, leading to disease clearance and wound healing. However, chronic inflammation can have a negative affect and lead to metabolic disorders, arthritis, and even some cancers. Neurokinin receptors have been linked to many disease processes such as pain, fibrosis, addictive disorders, as well as acute and chronic inflammation. Published data demonstrate that inflammatory cytokines and bacterial products can elevate neurokinin receptor 1 (NK1R) expression in murine macrophages, though less is known about neurokinin receptor 2 and 3 (NK2R and NK3R, respectively). Previous results have shown that activation of NK3R can lead to inflammation in the brain, but it is unclear how NK3R activation impacts peripheral immune cell function. We treated male sheep with NK3R agonist senktide for 2 hours and examined immune cell populations in the blood by flow cytometry. Sheep treated with senktide had a reduced number of CD44+ activated immune cells in the blood compared to sheep treated with vehicle. Based on the reduction in cell number, we examined if senktide regulates immune cell polarization. By microarray analysis, we found that macrophages express the gene tachykinin-3 receptor (Tacr3), which encodes the protein NK3R, at low levels. We treated J774 mouse macrophages with senktide for 16 hours and examined changes in proinflammatory gene response. Inflammatory cytokine IL1-β was increased 14-fold when cells were treated with senktide compared to untreated cells. This data shows that in vivo,
reductions in immune cell populations by senktide may be due to polarization of activated immune cells to an inflammatory phenotype. These data suggest that NK3R activation induces an inflammatory phenotype in macrophages and is a possible target for treatment of chronic inflammation.

Presenter: Elene Clemens
Title: Evaluation of epitope specificity in the immune response to PR8 H1N1 influenza virus infection in neonatal and adult African Green monkeys
Email: eclemens@wakehealth.edu
Coauthors: Davide Angeletti, Beth C. Holbrook, S. Tyler Aycock, Jonathan Yewdell, and Martha A. Alexander-Miller
Affiliations: 1Wake Forest School of Medicine; 2University of Gothenburg, Sweden; 3University of Tennessee Health Science Center; 4NIAID, NIH

Generation of effective antibody responses following virus infection is challenging in neonates as a result of impairments in the innate and adaptive immune systems that characterize early life. This leaves them particularly susceptible to severe disease following virus infection, e.g. influenza A virus. A potential contributor to the efficacy of the immune response generated following infection is the epitope specificity of the elicited antibodies. Previous studies in adult mice have reported a defined and reproducible pattern of immunodominance among antibodies directed to the neutralizing epitopes on the head of the influenza hemagglutinin (HA) molecule. The impact of age on the immunodominance pattern of HA-specific antibodies has not been explored. To address this, epitope recognition was quantified in plasma collected from newborn and adult African Green monkeys on d14 following PR8 influenza virus infection. For these analyses we used HA molecules engineered to singly express each of the neutralizing epitopes identified on the HA head. In addition, we used a construct that restricts recognition to the stem region of PR8 HA. Our analyses revealed a similar pattern of recognition by HA-specific IgG between infants and adults in the 14 day period following infection. In contrast, the HA-specific IgM pools exhibited distinct binding patterns in these two groups. The most striking of these differences was in the production of antibodies capable of recognizing the stem portion of HA. These data suggest antibody immunodominance patterns may be modulated with age and sheds new light on the regulation of potentially broadly protective stem responses to influenza virus.

Presenter: Marissa A Gonzales
Title: Modulating T cell function through elongation of the mitochondrial network to improve cytotoxic CD8+ tumor infiltrating lymphocyte function
E-mail: mag4bw@virginia.edu
Co-authors: Lelisa F Gemta, Frank M Mason, Timothy NJ Bullock
Affiliation: 1Department of Pathology, University of Virginia, Charlottesville, VA 22908; 2Division of Hematology and Oncology, Vanderbilt University, Nashville, TN 37232

For a number of tumor types, including melanoma, positive clinical outcomes correlate with infiltration of T-cells into the tumor. However, failure to completely control disease progression often occurs, even with immune responses enhanced by checkpoint blockade or other immunotherapy. When T-cells fail to control tumor growth, the tumor infiltrating lymphocytes (TIL) exhibit characteristics of exhaustion such as poor proliferation, poor effector function, and an increase in inhibitory receptor expression. We now know that metabolic activity of T-cells contributes to differentiation and effector function, leading us to investigate the metabolic state of TIL. We previously reported that melanoma TIL have limited OXPHOS capability and low ATP levels. To understand the basis of this using flow cytometry, we find that TIL have lower mitochondrial mass, as well as lower mitochondrial membrane potential when compared to functional effector T-cells. Therefore, we hypothesize that TIL are unable to effectively regulate their mitochondrial network, leading to the degradation of mitochondrial function, and it is likely that one or more fusion or fission machinery proteins has altered expression or can be manipulated to improve TIL function. We find that TIL have punctate mi-
CD8 tumor infiltrating lymphocytes (TIL) are critical for tumor control, and their presence is a positive prognostic factor in many cancer types including melanoma. However, many patient tumors have poor existing T cell infiltration. Additionally, even in tumors that are well infiltrated, the TIL are typically exhausted, characterized by limited cytokine release, cytotoxicity, and proliferation. In order to improve both T cell infiltration and function, we have used a vaccination strategy combining a CD40 agonistic antibody, the TLR3 agonist polyI:C, and the antigen ovalbumin in our B16OVA melanoma model. Vaccination significantly slowed tumor growth when administered both early and once tumors are palpable as a single treatment and corresponded with more than tenfold increases in both CD8 and CD4 T cells in the tumors. By blocking trafficking to the tumor using the S1P receptor agonist FTY720, we have shown that the newly activated T cells generated in the periphery by vaccination are not required to slow tumor growth. Instead, CD8 T cells that have pre-infiltrated tumor by the time of vaccination are sufficient to slow tumor growth. Preliminary studies indicate that this vaccination regimen works with melanocyte self-antigens and neoantigens expressed by this melanoma model, indicating its potential utility for vaccination regimens targeting mutations in human tumors. Current studies are underway to understand the mechanism by which the vaccination is working within the tumor, with initial data suggesting increased cytotoxicity and proliferation by the CD8 T cells and more mature dendritic cells that improve or maintain the CD8 TIL.

Melanoma commonly metastasizes to the brain, resulting in poor prognosis for patients. In contrast to subcutaneous B16OVA tumors, intracranial B16OVA tumors are poorly infiltrated by T cells. We modified the vaccination to target CD8 T cells more directly by replacing the CD40 antibody with a CD27 agonistic antibody, which may be more tolerable in patients. Strikingly, CD27 vaccination was able to both reduce tumor size and vastly increase CD8 infiltration into intracranial B16OVA tumors.
Antibodies are implicated in both protection and pathology against human dengue virus. However, the major cellular factories of the antibody production, plasmablasts, remain poorly characterized in dengue. Here, we provide a first detailed characterization of the magnitude, specificity, and rapidity of the plasmablasts response in acute febrile dengue patients in India. Consistent with previous reports, we found that plasmablasts expand massively. The expansion was highly heterogeneous among individual dengue patients with frequencies ranging from as low as 1% to as high as 85% of the B cells. Stratification of the patients based on the day of illness suggested that the plasmablast responses peak around 4-6 days post onset of symptoms. These plasmablasts were highly proliferating as assessed by Ki67 expression. A vast majority of them were producing dengue specific IgG. At the peak of the response, the frequency of the plasmablasts was slightly higher in secondary dengue patients compared to the primary dengue patients. By identifying patients who have not yet evolved dengue specific humoral response at the time of the clinical presentation, and by performing a longitudinal analysis, we found kinetic differences in the evolution of plasmablasts in primary versus secondary dengue patients. We found that dengue specific IgG antibody secreting cells (ASC) can expand as much as 1000-fold in a short window of just 24-48hrs. This rapid response was preferentially seen only in patients experiencing secondary dengue infections. A rapid IgG secreting plasmablast response early after onset of dengue fever is accompanied by a massive increase of serum neutralizing antibodies against both infecting and heterologous serotypes. By contrast, patients with primary dengue infections showed very little ASC response at the early times after onset of febrile illness and rarely produced dengue neutralizing antibodies. These results suggest that B cell responses might be largely protective during secondary dengue infections.

Systemic lupus erythematosus (SLE) is a multi-system autoimmune disease with no known cure. The crosstalk between the gut microbiota and the immune system plays an important role in the tolerance induction to self-antigens both in the intestinal mucosa and at the systemic level. We previously demonstrated differences between the gut microbiota in lupus mice compared to healthy controls, with a decreased Lactobacillaceae concentration. A mixture of L. reuteri, L. oris, L. johnsonii, L. gasseri, and L. rhamnosus significantly attenuated lupus-like disease in lupus-prone MRL/Mp-Fas"lpr (lpr) mice, by restoring the imbalance between regulatory T cells and T helper 17 cells. To further understand the role of Lactobacillus spp., we treated MRL/lpr mice with the supernatant of the same 5 cultured strains containing the secreted metabolites, given that bacterial metabolites may induce an immunosuppressive response. The results showed a significant attenuation in the inflammation of the spleen and renal lymph nodes similar to the bacterial mixture, but not that of the mesenteric lymph nodes. The results also demonstrated that there was a tendency to decrease double-stranded DNA autoantibodies. Additionally in our previous studies, L. reuteri and an uncultured Lactobacillus species were predominantly identified using 16S rRNA sequencing in MRL/lpr mice. To evaluate the role of L. reuteri in attenuating lupus disease, we treated mice with the single strain. We found L. reuteri did not exert a beneficial effect in MRL/lpr mice phenotypically. This suggests the other remaining strains in the Lactobacillus mixture could have a leading role in ameliorating the disease. Based on these results, we have a better understanding of the pathogenesis of SLE and the role of probiotics Lactobacillus spp. as a potential new therapeutic agent against the disease.
Foodborne infection with *Listeria monocytogenes* (Lm) carries a mortality rate of 20-30% in high-risk individuals and leads to serious complications such as meningitis and miscarriage. Therefore, studies evaluating gastrointestinal responses to *Lm* are needed to better enhance therapeutic interventions. Our data show that adaptive immune responses are dispensable in the first five days of infection since bacterial burden is similar between Rag1-deficient and wildtype mice. Innate Lymphoid Cells (ILCs) are tissue-resident lymphocytes that are enriched at barrier surfaces, and we hypothesize that intestinal ILCs are central mediators of the early response to Lm infection. Using cytokine reporter mice, we demonstrate a robust and early IFNγ response to *Lm* by Group 1 ILCs (ILC1s) in the large intestine lamina propria. In addition, systemic IFNγ response as early as day two post-infection is significantly reduced in the absence of ILCs. Consistent with this, mice devoid of ILCs suffer higher mortality and increased bacterial dissemination compared with ILC-sufficient littermates. Mechanistically, ILC1s lacking the transcription factor STAT4 are unable to produce IFNγ, and STAT4-deficient mice readily succumb to bacterial dissemination and mortality following Lm infection. Interestingly, STAT4-deficient animals with intact ILC populations were no longer protected against infection compared with ILC-depleted littermates, indicating that STAT4 signaling is critical for ILC-mediated protection. Unlike known roles for STAT4 in T cells, regulation of IFNγ by STAT4 in ILCs occurs independently of IL-18Rα and Tbet expression. Further, ATAC-sequencing demonstrated similar accessibility of the *Ifng* gene locus in wildtype and STAT4-deficient ILC1s. However, inhibition of STAT4 activity immediately prior to cytokine stimulation demonstrates that STAT4 directly promotes IFNγ production in ILC1s in response to cytokine activation. Together, these data illustrate a critical role for ILCs in the early responses to gastrointestinal infection with *Lm* and identify STAT4 as a central modulator of ILC-mediated protection.

Ebola viruses are filoviruses which have caused multiple epidemics of viral hemorrhagic fever in Africa. There are no highly effective treatments and control of these outbreaks relies on quarantine, supportive care, and vaccination of individuals at increased exposure risk. Currently-available ebola vaccines are expensive and require cold-chain storage and shipment, limiting the ability to vaccinate the at-risk population. All of these vaccines use the ebola fusion glycoprotein GP as the immunogen, and they are effective. *In vivo*, however, ebola transcribes the majority of the GP gene as an alternate transcript that yields a secreted, truncated, non-fusogenic protein sGP. sGP is efficiently secreted after synthesis and therefore much easier to produce in large quantities than GP. Moreover, sGP forms homodimers via intermolecular disulfide bonds and is stable at elevated temperatures, and could represent a more cost-effective immunogen. Mice immunized with sGP are able to tolerate lethal challenges from ebolavirus, and we suspect this is due to shared epitopes present on both sGP and GP that are known to elicit neutralizing antibodies.

We are exploring modifications to sGP to enhance its immunogenicity. Specifically, we are attempting to delete portions of its C-terminal domain, whose residues differ from GP and are likely to induce antibodies incapable of binding ebola virions. This domain has a cysteine residue required for sGP to assume its native conformation: as a disulfide-linked dimer. Deleting the domain is likely to disrupt sGP folding and assembly.

Through analyzing the electron microscopy structure of sGP, we identified putative residues that, if changed to cysteine, could possibly create a replacement intermolecular disulfide bond. To test this idea, we generated an obligate monomer by mutating native cysteine residues to serine, and then introduced two possible pairs of cysteines. One was able to effectively generate an intermolecular disulfide bond. We thus created a rationally-designed
vaccine antigen which will tolerate deletions of epitopes in sGP that do not induce neutralizing antibodies against GP. It is a precursor for a future cost-effective, heat-stable ebola vaccine that may one day be used to control and prevent future outbreaks in resource-limited settings.

Chronic inflammation in the setting of autoinflammatory diseases and immunosuppression in the setting of tumor microenvironments are both characterized by an imbalance between pro-inflammatory effector T (Teff) cells and anti-inflammatory regulatory T (Treg) cells that leads to dysregulated immune responses. While many currently available therapies broadly target the immune compartment, selectively targeting the specific T cell subsets that contribute to disease may provide a new avenue for the development of improved immunotherapies. In the Rathmell lab, we have shown that Teff and Treg cells can be distinguished by their reliance on distinct metabolic programs, and that this exposes a way to preferentially target specific T cell subsets. Through untargeted metabolomic and proteomic approaches, we have also recently identified the one-carbon metabolism pathways to be differentially critical to Teff and Treg cells. To further dissect these pathways, I have developed in vitro and in vivo approaches to CRISPR-Cas9 based screening in primary T cells. In this method, I design small-scale custom targeted guide RNA libraries, which are transduced into primary T cells. For the in vitro assays, these cells can be cultured under selective pressures, as well as sorted for populations of interest. For the in vivo assays, the transduced cells can be adoptively transferred into host mice of different inflammatory and cancer models, such as airway inflammation and melanoma. At the end of the assay, the transferred T cells are recovered from organs of interest and/or tumors, and processed to yield genomic DNA. The guide RNA sequences are then amplified out and sequenced to identify those that have either been enriched or depleted in the assay, corresponding to favorable and deleterious gene knockouts. This approach provides a powerful tool for identifying critical nodes within metabolic pathways of interest in primary T cells that can potentially serve as therapeutic targets.

A subset of neutrophils identified co-localizing with mononuclear cells, known as low-density neutrophils (LDNs), have increased frequencies in patients with inflammatory conditions including cancer and chronic infections. In cancer, this population is commonly referred to as granulocytic myeloid-derived suppressor cells (g-MDSCs). While the origin and function of LDNs are unclear, immunosuppressive functions have been ascribed to this population. T-cell mediated responses can be suppressed by g-MDSCs by the production of reactive oxygen species, the release of arginase-1 resulting in a downregulation of the TCR ζ chain, and induction of regulatory T cells in the tumor microenvironment. The tumor microenvironment contains g-MDSCs and activated platelets which have been implicated in promoting metastasis and tumor progression. Platelet-neutrophil aggregates are hallmarks of different pathological conditions including inflammatory and myeloproliferative disorders. Platelets produce content which biochemically interacts with neutrophils and the releasate include unprocessed arachidonic acid which can be transformed into leukotrienes promoting neutrophil activation. The primary interaction between platelets and neutrophils is P-selectin- dependent that when amplified can shift neutrophils towards an activated phenotype. Currently, the mechanism of induction of the low-density neutrophil phenotype is unclear. We have
observed a time-dependent increase in the frequency of LDNs following Thrombin Receptor Activator Peptide 6 (TRAP-6) stimulation. LDN induction was abrogated when platelet-neutrophil interactions were blocked via the P-selectin/PSGL-1 axis. A subset of neutrophils from TRAP-6-stimulated blood demonstrated the ability to engulf platelet particles. Pre-treatment with a phagocytosis inhibitor Cytochalasin D reduced the increase in the frequency of LDNs by TRAP-6 while exhibiting no effect on platelet-neutrophil associations. TRAP-6-stimulated platelets activated isolated neutrophils evidenced by an increase in NETosis. TRAP-6-activated platelets shift neutrophils toward an activated low-density phenotype which is partially dependent on a phagocytic mechanism.

Presenter: Holly A. Morrison
Title: Elucidating the Role of the Noncanonical NF-κB Signaling Pathway in Inflammation-Induced Carcinogenesis: Pathway Controls Stem Cell Niche in Colonic Mucosa
Email: hamorrison18@vt.edu
Co-authors: Kristin Eden, Daniel Rothschild, Morgan Stephens, Stephan Brown, Eda Holl, Irving C. Allen
Affiliation: Virginia-Maryland College of Veterinary Medicine

Colorectal cancer is mediated by inflammation-induced tumorigenesis, in which unregulated inflammation causes cells in the colon to accumulate mutations and epigenetic changes that promote carcinogenesis. Thusly, the dysregulation of inflammatory pathways in the colon is attributed to this disease pathogenesis and such signaling may be attributed to NF-κB signaling. This signaling is divided into two distinct pathways – the canonical and noncanonical pathways. The noncanonical pathway is understudied in comparison to the well-defined canonical pathway. Currently, in this field of research, there has been a resurgence in studying the noncanonical NF-κB pathway, particularly the NF-κB inducing kinase (NIK). NIK is a pertinent kinase in the signaling cascade. When activated, the noncanonical NF-κB signaling pathway is attributed to chronic inflammation, production of cytokines associated with inflammation and cancer, development of secondary lymphoid structures in the gut, recruitment of immune cells and cell proliferation. In our research, we observe that complete knockout of the noncanonical pathway via whole-body knockout of Nik in mice results in significant changes in large intestine phenotypes, including reduced stem cell marker expression, diminished regeneration/differentiation capacity upon these inflammatory conditions, altered microbiome composition, and increased predisposition towards inflammation-induced carcinogenesis. Following this preliminary research, novel tissue-specific knockout mice were generated to elucidate the mechanisms relating to the observed phenotypes. These mice either had deletions of RelA/p65 (relating to the canonical pathway) or Nik (relating to the noncanonical pathway) in tissue-specific regions. Here, we observed that the two pathways have distinct roles in regulating inflammation and tumor progression. Specifically, RelA/p65 attenuates disease progression in myeloid cells of the gastrointestinal (GI) tract, while Nik predominately regulates disease progression in GI epithelial cells. Translating this work towards human medicine, colonic biopsy samples from human colorectal patients show significant downregulation of the noncanonical NF-κB pathway. Together, this data suggests that the noncanonical NF-κB pathway has a protective role against colorectal cancer by regulating immune system homeostasis in the GI tract.

Presenter Mildred Perez
Title CD151 as a T cell activation marker
Email mperez14@uab.edu
Co-authors Kelsey Lowman, Lillian Seu, Christopher Tidwell, David Moylan, Olaf Kutsch and Steffanie Sabbaj
Affiliation University of Alabama at Birmingham

We recently described a hyper-responsive T cell sub-population that exhibited an increased, antigen-independent propensity to proliferate in the presence of IL-2 and was identified by expression of the tetraspanin CD151. The frequency of CD151 expression was a function of the memory differentiation state \( T_{\text{naive}} < T_{\text{CM}} < T_{\text{EM}} < T_{\text{EMRA}} \). CD151 was consistently
expressed on senescent CD28-CD57+ T cells, but also identified in T cell populations that were CD28+. We further demonstrated that CD151 in T cells is not a passive marker, but once expressed provided an active outside-in signal that changed cell cycle control and apoptotic process motifs of T cells (Seu et al., J. Immunol., 2017). We now demonstrate that, CD151 expression on unstimulated T cells is not correlated with other T cell activation markers. CD151 expression is upregulated following TCR/CD3 activation and CD151 expression correlates with proliferation capacity. This finding would be consistent with a previous report that CD151 congregates at the T-cell side of the immunological synapse and enhances signaling. The finding further establishes CD151 as a potential marker of currently and previously activated T cells. To test this hypothesis, we compared T cell frequencies in healthy control individuals with the those of HIV/ART patients, who are known to exhibit signs of persistent immune hyperactivation. We found the CD4+CD151+ T cell population in HIV/ART patients is expanded, suggesting an association of CD151+ T cell frequencies with the immune activation status of the individual. Furthermore, we provide evidence that CD151+ T cells can have an inflammatory phenotype, as we found baseline granzyme B expression in CD4+ and CD8+ T cells to be associated with CD151 expression. In regards to HIV/ART patients, our findings suggest that CD151+ T cells are a candidate population that has the potential to serve as a T cell correlate of immune hyperactivation. Overall, our data provide additional evidence that CD151, in a context dependent manner, acts as a T cell activation marker.

Systemic lupus erythematosus (SLE) is a severe systemic autoimmune disease characterized by multiple B cell abnormalities and production of a variety of autoantibodies against nuclear, cytoplasmic and cell surface auto-antigens by antibody secreting cells (ASCs). In the normal non-disease state, the frequency of circulating ASCs in total B cells is extremely low; while following immunization, ASCs increases in a tightly regulated manner. However, during active SLE, ASCs are dysregulated and exhibits a dramatic increase in the circulation. Indeed, the frequency of ASCs in the peripheral blood is correlated with disease activity, as measured by the SLEDAI scoring system. Despite their clinical significance, the heterogeneity, morphology, and molecular mechanisms of circulating ASCs in SLE remain largely unknown. In this study, peripheral ASCs were divided into 4 subsets based on the surface expression of the CD19 and CD138: CD19+CD138- (pop2), CD19+CD138+ (pop3), CD19-CD138- (pop4), and CD19-CD138+ (pop5). CD19+ subsets, and to a larger extent, CD19-subsets, were markedly increased in active SLE patients compared to healthy controls post flu-vaccination. Next, electron microscopy showed ultrastructural changes in the circulating ASCs subsets from active SLE patients in comparison to vaccinated healthy controls, including tighter nucleus, enhanced endoplasmic reticulum volume, increased numbers of mitochondria, and presence of autophagosomes, features which are reminiscent of bone marrow plasma cells. Interestingly, phenotypic characterization of circulating ASCs suggested that those from active SLE patients had significantly elevated expression of CXCR4, a receptor for the homing and survival of plasma cells in the bone marrow, when compared to healthy controls post-vaccination. Furthermore, next generation sequencing was used to analyze the clonality and connectivity between circulating ASC subsets from active SLE patients. Highly polyclonal repertoire and clonal relatedness in all ASC subsets were observed in most flare SLE patients. Together, these data show the increase of ASCs accompanied by the changes of ultrastructural morphology and upregulation of CXCR4 during the flare SLE, indicating their potential of homing to bone marrow and becoming long-lived plasma cells.
Macrophages are abundant within breast tumors and correlate to tumor progression. They may adopt pro- or anti-tumor (inflammatory) phenotypes due to tumor microenvironment interactions. Tumor-associated macrophage (TAM) characteristics are modulated by specific activation of the nuclear factor-kappaB (NF-kB) pathway. Activating canonical versus alternative arms of the NF-kB pathway is one controller of immunostimulatory function in macrophages. We have reported transcript abundance of olfactory receptor (OR) genes among invasive breast carcinoma patients. ORs are members of the G Protein Coupled Protein Receptor (GPCR) family that facilitate the sense of smell. The mechanism behind the ectopic expression of OR genes in breast tumors or their potential role in breast cancer remain unknown. The aim of this study is to validate and characterize OR expression in TAMs correlated with NF-kB pathway activation. Macrophages with tumorigenic (M2) or inflammatory (M1) characteristics were generated by ex-vivo stimulation of bone marrow-derived macrophages (BMDMs). Macrophages were also isolated from a double-transgenic mouse model with expression of CSF1 promoter and P52 by doxycycline induction, corresponding to activation of the alternative NF-kB pathway. In addition, TAMs were harvested from primary mammary tumors of 12-week old PyMT-MMTV mice. OR gene expression was measured by DNA microarray and qPCR. Macrophage inflammatory characteristics were evaluated by qPCR and flow cytometry. Among the top 78 significantly upregulated genes in the double-transgenic macrophages, 21 were ORs (fold change≥1.2). Olfr60, Olfr1487 and Olfr598 showed the greatest levels of upregulation. Comparison among the OR expression in different types of macrophages showed that TAMs demonstrated 47-fold increase in Olfr60 compared to regular macrophages and 21-fold compared to M2 BMDMs. TAMs showed increase in CD206 and Arg-1 compared to regular macrophages, exhibiting more M2-like tumorigenic behavior. However, their levels of CD206 were comparable to M2s. Double-transgenic macrophages showed 1.4-fold increase in Olfr60 expression compared to regular macrophages but not significantly different than M2s and significantly lower than TAMs. They also had comparable levels of CD206 and Arg-1 genes to M2 macrophages. Our findings suggest that OR expression is altered in tumorigenic macrophages, and the tumor microenvironment is critically essential for expression of ORs in breast tumors.

Presenter: Jessica Shartouny
Title: Optimizing frog-derived antimicrobial peptides for use as antivirals
Email: j.r.shartouny@emory.edu
Co-Authors: Joshy Jacob
Affiliation: Emory University

Influenza infections are responsible for much of the annual disease and economic burden worldwide, occurring both as seasonal epidemics and sporadic pandemics. Each year, illnesses worldwide number in the millions with 250-500,000 deaths, so reliable anti-influenza methods are important. The first defense against influenza is vaccination, however in pandemics or when the predicted vaccine strains are mismatched, antiviral drugs become the next option. As resistance to conventional antiviral agents can occur, the development of novel anti-viral compounds is necessary to continue to treat influenza infections. Antimicrobial peptides (AMPs) are one large potential pool of therapeutics, as they are produced innately by most forms of life and often have broad activities. We have synthesized a frog-AMP-derived peptide, Yodha, which is an effective inhibitor of various H1N1 and H3N2 influenza viruses in in vitro focus-forming assays. The peptide appears to form large conglomerates of fibrils in solution that can be imaged with electron microscopy, however, which is a hindrance to in vivo delivery. We have designed and screened several panels of modified peptides based on Yodha's primary structure to examine the structure-function relationship of the antiviral activity. From these screens, we have determined that polymerization is not required for anti-viral activity and several modified peptides have been found to out-perform wildtype Yodha and are primary candidates for the development of a deliverable drug for seasonal and pandemic subtypes of influenza.
T-bet, a cytokine-inducible master transcriptional regulator, is expressed by both T and B lymphocytes. While T-bet is known to regulate Th1 lineage commitment and effector T cell terminal differentiation, the significance of T-bet expression by memory B cells (Bmem) remains unclear. We hypothesized that T-bet expressing Bmem are endowed with "effector-like" properties that distinguishes them from other Bmem subsets. To test this hypothesis, we immunized immunologically healthy adults with the inactivated influenza vaccine (IIV). Within one month of vaccination, we identified two distinct populations of circulating influenza hemagglutinin-specific (HA+) Bmem that differentially expressed T-bet. Interestingly, the presence of the T-bethi HA+ Bmem correlated with the magnitude of the long-lived vaccine serologic response. We also re-vaccinated IIV subjects the following year with the newly formulated IIV that contained the same HA (H1) antigen from the prior season. We observed that the Bmem response to the H1 vaccine antigen shifted from a predominant T-betlo Bmem response in year 1 to T-bethi Bmem response in year 2. Using FcrL5 as a surrogate marker to divide the HA+ Bmem cells into T-bethi and T-betlo subsets, we performed RNA-seq and B cell repertoire analyses. We found that the FcrL5pos (T-bethi) HA+ Bmem transcriptome was enriched in effector memory gene sets relative to the FcrL5neg (T-betlo) HA+ Bmem transcriptome. Moreover, we observed that FcrL5pos HA+ Bmem were clonotypically distinct from FcrL5neg HA+ Bmem, indicating that these cells were likely derived from different precursors. Finally, in a patient revaccinated with IIV, we found that clonotypes from the year 1 FcrL5neg HA+ Bmem subset were preferentially recalled, relative to the year 1 FcrL5hi HA+ Bmem clonotypes, into the year 2 early plasmablast response. Collectively, the data suggest that FcrL5hi (T-bethi) HA+ Bmem may represent a poised effector memory B cell subset that can rapidly differentiate into ASCs after antigen re-challenge.

1Division of Infectious Diseases, University of Alabama at Birmingham, 2Department of Microbiology, Emory University, 3Division of Rheumatology, Lowance Center for Human Immunology, Emory University, 4Department of Microbiology, University of Alabama at Birmingham, 5Informatics Institute, University of Alabama at Birmingham, 6Division of Rheumatology/Immunology, University of Alabama at Birmingham

---

Presenter: Sarah L Hayward
Title: Unrelated respiratory infections compromise established cellular immunity by promoting apoptosis of pre-existing lung-resident memory CD8 T cells
Co-authors: ZR Tiger Li, Jenna L Lobby, Joel O.P. Eggert, and Jacob E Kohlmeier
Affiliation: Emory University School of Medicine

Lung-resident memory T cells (lung T_{RM}) are critical for protective heterosubtypic immunity against influenza viruses. However, the efficacy of cellular immunity against respiratory pathogens such as influenza wanes over time due to the gradual loss of flu-specific lung T_{RM}. One possible mechanism for this decline is frequent exposure to environmental and biological insults resulting in localized inflammation that promotes the death of established lung T_{RM}. We investigated whether unrelated infections could exacerbate the loss of pre-existing, flu-specific lung T_{RM} and reduce the efficacy of cellular immunity to subsequent influenza challenge. Infection of influenza-immune mice with Sendai virus, an unrelated murine parainfluenza virus, resulted in significantly higher viral titers and greater morbidity following influenza challenge compared to PBS-treated controls. This loss of protective cellular im-
munity corresponded to a significant decrease in the number of pre-existing flu-specific lung CD8 T RM compared to PBS controls due to increased apoptosis. This loss of pre-existing lung T RM following Sendai infection was not due to competition for limited resources or tissue niches between flu- and Sendai- specific lung T RM. Sendai infection had no impact on the number of systemic flu-specific memory CD8 T cells in the spleen. The loss of lung T RM required an active infection, as both Sendai and LCMV infection but not intranasal delivery of TLR agonists did significantly reduce the number of pre-existing flu-specific lung CD8 T RM. Together, these data suggest that tissue damage induced by unrelated respiratory infections can promote the loss of pre-existing lung T RM and compromise cellular immunity against respiratory pathogens.

**Presenter** Levelle D. Harris

**Title** Phenotypic and functional profiles of NK cells in individuals with latent *Mycobacterium tuberculosis* infection

**Email** ldharr3@emory.edu

**Co-authors** Jeremiah Khayumbi2, Joshua Ongalo2, Joan Tonui2, Loren E. Sasser1, Felix Hayara Odhiambo2, Cheryl L. Day1, 3

**Affiliations** 1Emory Vaccine Center, Emory University, Atlanta, GA, USA; 2Center for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya; 3Department of Microbiology & Immunology, Emory University School of Medicine, Atlanta, GA, USA

*Mycobacterium tuberculosis* (MtB) is the causative agent of tuberculosis (TB). In 2017 approximately 9 million individuals developed active TB disease and it is estimated that one quarter of the world’s population is latently infected. Latent MtB infection (LTBI) is defined as individuals who have been infected with MtB but do not have clinical signs or symptoms of active TB disease. Although the correlates of immune protection to MtB are not well defined, it is known that numerous innate and adaptive immune subsets are important in mediating control of MtB infection. NK cells are innate immune cells that can kill MtB-infected macrophages *in vitro* by releasing granulysin, activate macrophages through production of IFNy, and regulate CD4 T cells responses through direct and indirect mechanisms. A greater understanding of NK cell biomarkers in LTBI will provide better insights into future therapeutic interventions. In this study, we evaluated NK cell phenotypic and functional profiles associated with LTBI in Kenyan adults. PBMCs were collected from cohorts of MtB-uninfected healthy controls (HC, N=30) and individuals with LTBI (N=31) in Kisumu, Kenya. Individuals classified as LTBI had a positive QuantiFERON-TB (QFT) test, whereas HC were QFT-negative. Flow cytometry was performed on PBMCs to phenotype NK cell subsets and characterize NK cell responses to generic stimuli (MHC class I-devoid cells and antibody-coated target cells) and MtB antigens (MtB whole cell lysate, MtB cell membrane, and MtB cell wall). We found that LTBI is associated with decreased NK cell expression of the natural cytotoxicity receptor NKP46 and decreased expression of the inhibitory receptor TIGIT, compared with HC. While NK cell functional capacity to generic stimuli is similar between LTBI and HC, NK cells from individuals with LTBI exhibit diminished degranulation (CD107a) and activation (CD69) in response to MtB antigen stimulation, compared with HC. Taken together, our results indicate that NK cells are phenotypically altered in LTBI and that these cells have decreased response to MtB antigens when compared with MtB-uninfected individuals.

**Presenter** Casey Butrico

**Title** Neutrophil dysfunction in comorbid *Staphylococcus aureus* osteomyelitis alters bacterial metabolism and abscess architecture

**Email** casey.e.butrico@vanderbilt.edu

**Co-Author** Aimee Potter, Jacob Curry, and Jim Cassat

**Affiliation** Vanderbilt University

*Staphylococcus aureus* is a leading cause of antibiotic-resistant bacterial infections and can infect nearly every organ of the human body. One common manifestation of *S. aureus* disease is invasive bone infection, known as osteomyelitis. Osteomyelitis is considered one
of the most difficult to treat infections and necessitates long-term antibiotic treatment and surgical intervention. Unique to skeletal infections, *S. aureus* clusters around necrotic bone fragments, known as sequestra. These distinct communities are encompassed by innate immune cells and strongly associated with progression to chronic disease. To better understand how *S. aureus* survives during osteomyelitis, we conducted transposon sequencing (TnSeq) to identify genes essential for staphylococcal growth *in vivo*. TnSeq identified a number of central metabolic pathways as crucial for bacterial survival. Mono-infections with *S. aureus* mutants inactivated for various central metabolic pathways revealed that *S. aureus* relies heavily on glycolysis during osteomyelitis. Interestingly, the tricarboxylic acid (TCA) cycle and gluconeogenesis were dispensable for bacterial growth. In line with this finding, anaerobic (replenishing) reactions were also crucial for bacterial survival in bone. To further delineate the role of central metabolism pathways during different stages of infection, we created a pool of select *S. aureus* strains with differential metabolic capabilities. Using this pool, we discovered that mutants which were nonviable during monoinfection were able to persist during a competitive infection. This suggests the ability of *S. aureus* to resource share or reside within different abscess niches *in vivo*. In addition to intra-bacterial influences during osteomyelitis progression, infiltrating neutrophils regulate *S. aureus* growth and physiology in a spatially and temporally resolved manner. Previous work suggests that neutrophil depletion modifies abscess structure during *S. aureus* infection. To elucidate the role of neutrophils in *S. aureus* metabolic regulation, we modeled neutrophil depletion during osteomyelitis. Abscess architecture, infection kinetics, and dissemination to distant organs were altered in the absence of functional neutrophils. Moving forward, novel imaging approaches and fluorescent transcriptional reporters for central metabolism genes will be used to spatially resolve bacteria physiology *in vivo*. Together, our data suggest that abscess architecture and neutrophil function influence *S. aureus* central metabolism.

**Presenter:** Brenna D. Appleton
**Title:** The role of microRNA-22-3p in the dysregulation of lupus T<sub>regs</sub> and the pathogenesis of systemic lupus erythematosus.
**Email:** brenna.d.appleton@vanderbilt.edu
**Co-Authors:** Ashley W. Faust, Danielle L. Michell, Michelle J. Ormseth, Kasey C. Vickers & Amy S. Major
**Affiliation:** Vanderbilt University

Systemic lupus erythematosus (SLE) is an autoimmune disease affecting over 1.5 million Americans and at least 5 million individuals worldwide. Evidence demonstrates autoantibody producing B cells and dysfunctional CD4<sup>+</sup> T cells contribute to SLE pathology, however lack of understanding surrounding mechanisms of disease pathogenesis have prevented therapeutic advancement. Studies indicate one mechanism for dysregulated immune homeostasis in autoimmunity is through microRNAs (miRNAs). MiRNAs are short, endogenous post-transcriptional regulators of gene expression which act by degradation or translation repression of target mRNAs. Our group previously identified miR-22-3p as being increased three-fold in SLE patient plasma compared to age- and gender-matched healthy controls. MiR-22-3p levels were increased in whole CD4<sup>+</sup> T cells (four-fold) and T<sub>regs</sub> (three-fold) from B6.SLE1.2.3 mice compared to B6 controls, and inhibition of miR-22-3p, using locked-nucleic acid (LNA)-22 ameliorated key disease pathologies including glomerulonephritis. There was also a 10% reduction in IFN-γ CD4<sup>+</sup> T cells in mice treated with LNA-22. Based on these data, we hypothesized increased miR-22-3p levels in SLE T cells propagated inflammation directly by skewing naive CD4<sup>+</sup> T cells towards the Th1 phenotype during polarization, or indirectly by reducing the regulatory capacity of T<sub>regs</sub>. Results show neither miR-22-3p deficiency nor overexpression impact the polarization of Th1 or iT<sub>regs</sub> cells. However, inhibition of miR-22-3p in B6.SLE1.2.3 mice increases T<sub>reg</sub> IL-10 production, and overexpression of miR-22-3p *in vitro* decreases IL-10 transcript in iT<sub>reg</sub> two-fold, suggesting heightened levels of miR-22-3p may alter their suppressive function. We conclude that miR-22-3p overexpression in T<sub>reg</sub> alters their suppressive capacity which indirectly contributes to autoimmune T cell dysregulation in SLE.

**Presenter:** Heather Koehler

---

Presenter:  Brenna D. Appleton     98
Title:    The role of microRNA-22-3p in the dysregulation of lupus T<sub>regs</sub> and the pathogenesis of systemic lupus erythematosus.
Email:   brenna.d.appleton@vanderbilt.edu
Co-Authors:  Ashley W. Faust, Danielle L. Michell, Michelle J. Ormseth, Kasey C. Vickers & Amy S. Major
Affiliation:  Vanderbilt University

Systemic lupus erythematosus (SLE) is an autoimmune disease affecting over 1.5 million Americans and at least 5 million individuals worldwide. Evidence demonstrates autoantibody producing B cells and dysfunctional CD4<sup>+</sup> T cells contribute to SLE pathology, however lack of understanding surrounding mechanisms of disease pathogenesis have prevented therapeutic advancement. Studies indicate one mechanism for dysregulated immune homeostasis in autoimmunity is through microRNAs (miRNAs). MiRNAs are short, endogenous post-transcriptional regulators of gene expression which act by degradation or translation repression of target mRNAs. Our group previously identified miR-22-3p as being increased three-fold in SLE patient plasma compared to age- and gender-matched healthy controls. MiR-22-3p levels were increased in whole CD4<sup>+</sup> T cells (four-fold) and T<sub>regs</sub> (three-fold) from B6.SLE1.2.3 mice compared to B6 controls, and inhibition of miR-22-3p, using locked-nucleic acid (LNA)-22 ameliorated key disease pathologies including glomerulonephritis. There was also a 10% reduction in IFN-γ CD4<sup>+</sup> T cells in mice treated with LNA-22. Based on these data, we hypothesized increased miR-22-3p levels in SLE T cells propagated inflammation directly by skewing naive CD4<sup>+</sup> T cells towards the Th1 phenotype during polarization, or indirectly by reducing the regulatory capacity of T<sub>regs</sub>. Results show neither miR-22-3p deficiency nor overexpression impact the polarization of Th1 or iT<sub>regs</sub> cells. However, inhibition of miR-22-3p in B6.SLE1.2.3 mice increases T<sub>reg</sub> IL-10 production, and overexpression of miR-22-3p *in vitro* decreases IL-10 transcript in iT<sub>reg</sub> two-fold, suggesting heightened levels of miR-22-3p may alter their suppressive function. We conclude that miR-22-3p overexpression in T<sub>reg</sub> alters their suppressive capacity which indirectly contributes to autoimmune T cell dysregulation in SLE.

**Presenter:** Heather Koehler 99
The primary function of the immune system is to protect the host from invading pathogens. In response, microbial pathogens have developed various strategies to evade detection and elimination by the immune system.

Z-Nucleic Acid Binding Protein 1 (Zbp1 aka DAI and DLM-1) is an important innate immune protein that was first reported as a cytosolic DNA sensor that leads to increases in type 1 IFN in a redundant fashion to other cytosolic nucleic acid sensors. Later Zbp1 was identified as an adaptor protein for RIPK3 implicated in sensing viral dsRNA leading to virus-induced necroptosis (MCMV, VACV, IAV, HSV). The association of ZBP1 with RIPK3 initiates necroptosis which is a cell death pathway that not only restricts viral replication, but also promotes anti-viral inflammation through the release of damage-associated molecular patterns (DAMPs). This plays a significant role in limiting the pathogenesis of viral infections.

In response, many viruses have developed countermeasures for the pathway in the form of encoded suppressors of cell death. These evolved countermeasures suggest that ZBP1’s role in anti-viral inflammation is of extreme importance and warrants further investigation.

We have recently identified a novel role for Zbp1 in regulating the amplitude of inflammation resulting from viral infections or cytokine stimulation. Zbp1 acts to restrict the magnitude and duration of NFκB and MAPK signaling. Furthermore, Zbp1 dampen the release of inflammatory cytokines such as IL1-β and TNFα following stimulation. Taken together, Zbp1 is an important determinant of favorable outcome of viral infections and innate immune responses.

The commensal gut microbiota critically regulates postnatal skeletal development. We have shown the commensal gut microbiota enhances osteoclastogenesis, which appears to be mediated by T_{17}/IL17 response effects in bone marrow and liver. Of interest, segmented filamentous bacteria (SFB) is a distinct commensal gut bacterium that potently directs T_{17}/IL17-mediated immunity. Study purpose was to delineate the influence of SFB on commensal gut microbiota immunomodulatory actions regulating post-pubertal skeletal development.

Female C57BL/6T germline-associated (GF) littermates were maintained as GF or monoassociated with SFB at 5-weeks-old; euthanized at 9-weeks-old. SFB colonization was confirmed in SFB mice by 16S rDNA analysis. IL17 was substantially increased in the ileum and serum while %T_{17} cells were elevated in spleens and liver-lymph-nodes (LLNs) of SFB mice, validating SFB induction of T_{17}/IL17 immunity. Micro-CT analysis revealed SFB mice had decreased trabecular bone volume fraction in the proximal tibia, which was attributed to reduced trabecular number. TRAP+ osteoclast size and number of nuclei were enhanced in SFB vs. GF mice. To determine the influence of SFB in a complex gut microbiota, female 9-week-old C57BL/6T *excluded-flora (EF) mice (*specific-pathogen-free mice devoid of SFB) and *murine-pathogen-free (MPF) mice (*specific-pathogen-free mice colonized by SFB) were investigated. Paralleling the findings from SFB-monoassociated mice, 16S rDNA analysis validated SFB colonization in MPF mice. MPF vs. EF mice also had increased serum IL17 and ileum Il17a. Trabecular bone volume fraction was blunted in MPF mice. MPF mice had enhanced TRAP+ osteoclast numbers and size in vivo and in vitro. Flow cytometry demonstrated %MDSCs and %T_{17} cells were elevated in marrow of MPF vs. EF mice. MPF
mice had increased %T\textsubscript{H17} cells and decreased %T\textsubscript{REGS} and %naïve CD4\textsuperscript{+} T-cells in the spleen. Notably, %M1-macrophages and %MDSCs were enhanced in mesenteric-lymph-nodes (MLNs), while %T\textsubscript{H17} and %T\textsubscript{H1} cells were increased in LLNs of MPF vs. EF mice. MPF mice had upregulated Lcn2 in liver and marrow and elevated LCN2 in serum, which provides novel mechanistic insight about commensal gut microbiota osteoimmune response effects. SFB colonization enhances innate and adaptive immunity, upregulates osteoclastogenesis, and drives bone loss. This research notably reveals that specific microbes critically impact commensal gut microbiota immunomodulatory actions regulating post-pubertal skeletal development.

**Presenter:** Beth Holbrook  
**Title:** A Novel R848-Conjugated Inactivated Influenza Virus Vaccine Is Effective and Safe in a Neonate Nonhuman Primate Model  
**Email:** bcholbro@wakehealth.edu  
**Co-Authors:** Jong R. Kim, Lance K. Blevins, Matthew J. Jorgensen, Nancy D. Kock, Ralph B. D'Agostino, Jr., S. Tyler Aycock, Mallinath B. Hadimani, S. Bruce King, Griffith D. Parks, Martha A. Alexander-Miller  
**Affiliation:** Wake Forest School of Medicine, Wake Forest University

Influenza virus can cause life-threatening infections in neonates and young infants. Although vaccination is a major countermeasure against influenza, current vaccines are not approved for use in infants less than 6 months of age, in part due to the weak immune response following vaccination. Thus, there is a strong need to develop new vaccines with improved efficacy for this vulnerable population. We developed an innovative nonhuman primate neonate model to test the efficacy of a novel TLR 7/8 agonist R848-conjugated influenza virus vaccine. Newborn animals were housed in a nursery to allow challenge studies to be performed. The results from our study showed that this vaccine induces a robust antibody response after primary vaccination, boost, and challenge, as well as a potent IFNγ–producing T cell recall response. These responses were associated with lessened pulmonary pathology and improved clearance after virus challenge compared with a non-adjuvanted virus vaccine. We also determined the effectiveness of this vaccine in mother-reared infants as well as its ability to promote improved responses at 6 months compared to vaccination in the absence of R848. In agreement with our nursery study, R848 conjugated to influenza virus induced a higher antibody response that was associated with increased antibody at 6 months following vaccination. These data strongly support the utility of R848-conjugated inactivated influenza virus as an effective vaccine in this vulnerable population.

**Presenter:** Jenna Petronglo  
**Abstract title:** The influence of innate immune responses on osteoclast differentiation and function during *Staphylococcus aureus* osteomyelitis  
**Email:** jenna.r.petronglo@vanderbilt.edu  
**Co-authors:** Nicole Putnam, Jacob Curry, Laura Fulbright, Jim Cassat  
**Affiliations:** Vanderbilt University, Molecular Pathology and Immunology Graduate Program

Inflammation in bone, also known as osteomyelitis (OM), is most frequently caused by the bacterial pathogen *Staphylococcus aureus*. While the induction of a strong antibacterial response by host cells is essential to the resolution of bacterial OM, the resulting inflammation drives pathologic skeletal remodeling, which can lead to weak and fracture-prone bone. Bone resorbing osteoclasts (OCs) are essential for maintenance of healthy bone, but are also implicated as drivers of inflammatory bone loss, partially through enhanced differentiation of macrophage precursors into OCs (osteoclastogenesis). In this study, we sought to understand how inflammatory stimuli generated during innate immune responses influence OC differentiation. We focused on the interleukin receptor 1 pathway because of its established roles in antibacterial defense and homeostatic osteoclastogenesis. We hypothesized that this signaling pathway is a major driver of infection-induced osteoclastogenesis. To test this, we stimulated WT bone marrow derived macrophages with the OC differentiation factor receptor activator of NFκB ligand (RANKL) and *S. aureus* supernatants, with or without IL-
1R1 blockade. By quantifying the number of OCs using histochemical staining, we show that IL-1R1 blockade significantly diminishes bacterially-induced osteoclastogenesis. We next assessed the role of IL-1R1 signaling in driving OC-mediated bone loss in an *in vivo* model. Cortical bone of WT and IL-1 receptor type 1 knockout (*Il1r1*−/−) femurs were inoculated with *S. aureus* and harvested at day 14 post-infection. Changes to the trabecular bone were assessed using histomorphometry to quantify osteoclastogenesis and μCT to measure architectural changes in bone. We discovered that loss of IL-1R1 abrogates infection-induced OC differentiation and inflammatory bone loss. Since IL-1R1 drives bone loss by altering OC differentiation, we next stimulated OC lineage cells with bacterial supernatants for 6 hours and measured transcriptional changes in immunologically relevant genes using the Nanostring nCounter platform. We found that cellular responses to bacterial stimulation are strongly dependent on the OC developmental stage. Overall, these data demonstrate the importance of IL-1R1 signaling in driving bone loss in *S. aureus* OM and support further studies into the precise mechanisms by which inflammation impacts cells along the OC differentiation pathway to elicit inflammatory bone loss.

**Presenter:** Elizabeth L. Frost

**Abstract title:** Regulation of myeloid cells by spleen tyrosine kinase contributes to the pathogenesis of experimental autoimmune encephalomyelitis

**Email:** ef2r@virginia.edu

**Co-authors:** Catherine R. Lammert, Ashely C. Bolte, Makenzie A. Scanlon, Calli E. Bellinger, Mariah E. Shaw, and John R. Lukens

**Affiliation:** University of Virginia

Multiple sclerosis (MS) is a debilitating autoimmune disease that is characterized by immune-mediated damage to myelin in the central nervous system (CNS). Although autoreactive CD4+ T cells are required for initiation of MS, myeloid cells—dendritic cells, macrophages, neutrophils, CNS-resident microglia—also participate in pathogenesis at multiple stages of disease. Spleen tyrosine kinase (SYK) is an intracellular signaling protein that has recently been identified to play critical roles in the regulation of inflammatory cytokine production and the clearance of cellular debris. Although SYK has been described to be centrally involved in the pathogenesis of numerous autoimmune and neuroinflammatory disorders, its involvement in MS currently remains unknown. Interestingly, various molecular factors that have been linked to MS disease in genome-wide association studies, including CD37, TREM2, and multiple C-type lectin receptors, are known to primarily signal through SYK. To investigate the contributions of SYK signaling in MS, we have generated multiple novel genetic mouse strains in which SYK has been deleted in defined immune cell populations that are known to play key roles in MS progression. Here, we find that deletion of SYK in myeloid cells under the control of the LysM promoter reduces EAE severity and infiltration of inflammatory immune cells from the periphery. We observe modest defects in priming of the autoreactive T cell response along with decreased chemokine levels in the CNS at early stages of EAE. Current work further investigates the mechanisms that contribute to this protective phenotype in mice with SYK-deficient myeloid cells.

**Presenter:** Ali Nazmi

**Title:** Innate CD8αα+ cells and osteopontin promote ILC1-like intraepithelial lymphocyte homeostasis and intestinal inflammation

**E-mail:** anazmi.abdelnabi@vumc.org

**Co-authors:** Kristen L. Hoek*, Michael J. Greer*, M. Blanca Piazuelo*, Nagahiro Mi-nato* and Danyvid Olivares-Villagómez*1,5,6

**Affiliation:** 1Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN, USA; 2Department of Biomedical Informatics, Vanderbilt University, Nashville, TN, USA; 3Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA; 4Medical Innovation Center, Graduate School of Medicine, Kyoto University, Kyoto 606-8507, Japan; 5Vanderbilt Institute for Infection, Immunology and Inflammation, Vanderbilt University Medical Center, Nashville, TN, USA; 6ORCID, 0000-0002-1158-8978.
Innate CD8α+ cells, also referred to as iCD8α cells, are TCR-negative intraepithelial lymphocytes (IEL) possessing cytokine and chemokine profiles and functions related to innate immune cells. iCD8α cells constitute an important source of osteopontin in the intestinal epithelium. Osteopontin is a pleiotropic cytokine with diverse roles in bone and tissue remodeling, but also has relevant functions in the homeostasis of immune cells. In this report, we present an evidence for the role of iCD8α cells and osteopontin in the homeostasis of TCR-negative NKp46+NK1.1+ IEL (ILC1-like). We show that in the absence of iCD8α cells, the number of ILC1-like IEL is significantly reduced. These ILC1-like cells are involved in intestinal pathogenesis in the anti-CD40 mouse model of intestinal inflammation. The reduction of iCD8α cell numbers and/or osteopontin expression results in a milder form of intestinal inflammation in this disease model. Collectively, our results suggest that iCD8α cells and osteopontin promote survival of ILC1-like IEL, which significantly impacts the development of intestinal inflammation.

Presenter Mei Lan Chen
Title Constitutive androstane receptor directs T cell adaptation to bile acids in the small intestine
Email meichen@scripps.edu
Co-Authors Hongtao Wang, Amber Eliason, Xiangsheng Huang, Guohui Wang, Rui Xiao, Matthew E. Pipkin, David D. Moore, and Mark S. Sundrud
Affiliation 1Department of Immunology and Microbiology, The Scripps Research Institute, Jupiter, FL 33458, USA; 2Department of Pediatrics, Section of Gastroenterology, Baylor College of Medicine and Texas Children’s Hospital, Houston, TX, 77030, USA; 3Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas 77030; Baylor Genetics, Houston, Texas 77021; 4Department of Molecular and Cellular Biology, Baylor College of Medicine, 1 Baylor Plaza, Houston, Texas 77030, USA

Immune competency demands that circulating CD4+ T helper (Tₜ) cells coordinate protective immune responses in diverse tissue microenvironments (i.e., skin, lung, gut). Yet still, the mechanisms enabling Tₜ cell homeostasis and function in discrete non-lymphoid tissues remain ill defined. We have recently discovered that pro-inflammatory subsets of Tₜ cells—including IFNγ-secreting Th1 cells and IL-17A-expressing Th17 cells—upregulate expression of the xenobiotic transporter Mdr1 upon migration into the small intestine lamina propria to maintain homeostasis and suppress Crohn’s disease-like small bowel inflammation in the presence of detergent-like intestinal metabolites, called bile acids. Further, a subset of small bowel Crohn’s disease patients is characterized by overt Mdr1-deficiency. Thus, immune homeostasis in the small intestine involves local interaction between mucosal Tₜ cells and mucosa-associated bile acids, which may be exploited for the development of safer and more selective Crohn’s disease therapies. In exploring these Tₜ cell—bile acid interactions further, we have performed an in vivo RNAi screen and identified the constitutive androstane receptor (CAR; gene symbol Nr1i3) as a nuclear receptor which Tₜ cells use to sense and respond to mucosa-associated bile acids in the small intestine lamina propria. Tₜ cells lacking CAR transfer severe small bowel inflammatory disease in Rag1-/- recipients, and that CAR activates the expression of dozens of cytoprotective genes in Tₜ cells—including Mdr1—that are recognized for detoxifying and removing bile acids in hepatocytes. As a whole, these findings suggest that Tₜ cells infiltrating the small intestine acquire a hepatocyte-like gene expression program to adapt to locally recycled bile acids. In addition, our results shed light on an important new pathway that may be relevant to the understanding and treatment of human Crohn’s disease.

Presenter Gordon Dale
Title Distant genomic sites contribute to somatic diversification of sequences at the IgH locus in mice and humans via templated mutagenesis
Email Gordon.Dale@emory.edu
Co-Authors Daniel J. Wilkins, Michael J. Rowley, Christopher Scharer, Jeremy M.
During the maturation of a humoral immune response, B cells engage in a program of orchestrated mutagenesis known as somatic hypermutation. B cells that undergo this program are localized to a lymphoid structure known as the germinal center, in which a Darwinian evolutionary event occurs. Mutating germinal center B cells, specific for a given antigen, compete for higher affinity binding over multiple alternating rounds of mutation and selection. Those that display reduced affinity compared to their peers are deleted. It has been shown that mutagenesis during the germinal center reaction can occur in two separate ways. In chickens and rabbits, diversity occurs through gene conversion (templated mutagenesis), in which a donor sequence is copied into a homologous, but different, recipient sequence. Conversely, in mice and humans, mutagenesis occurs through the accumulation of pseudorandom point mutations driven primarily by the combination of AICDA and the error-prone polymerase pol η. In animals that undergo gene conversion, it is known that pseudorandom point mutations can occur. However, in mice and humans, the process of gene conversion is largely thought to not occur. Here we show that such a process is active in murine and human B cells by Monte Carlo analysis of mutation clusters within human IgHV gene sequences, as well as unselected transgenes in mice placed at the IgH locus. We performed deep sequencing on the IgHV repertoire of circulating memory B cells from four human donors and analyzed the murine transgene data generated from the Fred Alt laboratory. We find that templated events account for approximately 5-10% of the mutation load of circulating human memory B cells, that donor sites are significantly closer to the IgH locus via Hi-C analysis in germinal center B cells vs. naïve B cells, and that donor sequences are predicted to be primarily within open chromatin regions detected via ATAC-seq. Most significantly, we also find that all human donors acquire mutations interchromosomally from discrete sites in the human genome. These results suggest that templated mutagenesis occurs in human and murine B cells and contributes a non-negligible number of mutations during the maturation of humoral immune response.

Presenter Jakob Habib
Title Investigating the role of type I interferon signaling during costimulation blockade resistant rejection
Email jghabib@emory.edu
Co-Authors Dave Mathews, Ying Dong, Allison Stephenson, Abheek Ghosh, Cindy Breeden, Abe Matar, Brendan Lovasik, Andrew Adams
Affiliation Emory University

Purpose: Costimulation blockade (CoB) is a promising new transplant immunosuppression strategy offering improved long-term patient and allograft survival without the nephrotoxicity of calcineurin inhibitors. However, increased risks of acute rejection have impeded widespread adoption of CoB. Type I interferons (IFN), produced mainly by plasmacytoid dendritic cells (pDCs), induce systemic inflammation that may prime the adaptive immune system for acute rejection. In this study, we examine the contribution of signaling through the type I IFN receptor (IFNAR) to CoB-resistant allograft rejection.

Methods: We performed fully MHC-mismatched skin grafts from BALB/cJ donors to C57BL/6 recipients. Mice were treated with CoB (CTLA4-Ig + anti-CD40L) alone or in conjunction with anti-IFNAR and graft survival was assessed over time. In a subsequent experiment, mice were euthanized 1, 2 and 3 weeks post-transplant and the lymphocytes were analyzed by flow cytometry.

Results: Transplanted untreated mice rejected rapidly (mean survival time=11 days), CoB treatment improved survival but resulted in CoB-resistant rejection (MST=23d), and CoB+antiIFNAR significantly improved survival (MST>60d). IFNAR was highly expressed on murine pDCs (PDCA1+SiglecH+B220+) relative to conventional DCs and T cells. Combination treatment with CoB+anti-IFNAR significantly reduced MHC I expression on pDCs compared to CoB alone, and reduced CD80 expression on pDCs at the peak of rejection, suggesting a role of type I interferon signaling for the activation of pDCs. CoB+anti-IFNAR treatment also led to a reduction in the frequency of activated (CD44+) and effector
(IFNγ+TNFα+) CD8+ T cells compared to CoB alone. Conclusions: These data suggest that signaling through IFNAR augments pDC activation and is associated with CD8+ T cell activation and effector functions in order to promote CoB-resistant rejection.

Presenter: Michael Brandon Ware
Title: Interleukin-6 in biliary tract cancers promotes expansion of immune suppressive myeloid cells and is associated with increased myeloid cells in patient tumors
Email: mbware@emory.edu
Co-Author: Jennifer Yang, Mohammad Y. Zaidi, Alyssa Krasinskas, Thomas A. Mace, Matthew R. Farren, Yiman Li, Wanqi Chen, Zhengjia Chen, Gregory S. Young, Omar Elnaggar, Zheng Che, Shishir K. Maithel, Tanios Bekaii-Saab, Bassel El-Rayes, Gregory B. Lesinski
Affiliation: Emory University

Biliary tract cancers (BTC) are an aggressive malignancy frequently associated with elevated immunosuppressive cell types in patients. These cancers can be grouped into distinct subtypes with unique molecular and genetic signatures. We hypothesized that BTC-derived cytokines act through distinct pathways to promote immune suppressive features of the disease.

Cytokine and chemokine secretion, and activation of associated signaling pathways, were studied in a panel of BTC cell lines. We used flow cytometry and immunoblot to study the effects of BTC supernatants on human peripheral blood mononuclear cells (PBMCs). Based on results, a human BTC tissue microarray (TMA, n=33) was stained for IL-6, GM-CSF and CD33+S100a9+ myeloid cells.

BTC cell lines demonstrated active STAT3 and STAT5 signaling, and secreted several immunomodulatory factors including IL-6, GM-CSF, and MCP-1. Antibody neutralization of IL-6 and GM-CSF in these supernatants limited STAT3 and STAT5 phosphorylation in BTC cell lines and in PBMCs exposed to these supernatants. The expansion of myeloid derived suppressor cells (MDSC) from PBMCs cultured with BTC supernatants was inhibited with antibody neutralization of IL-6 and GM-CSF. TMA analysis revealed significant association between IL-6 expression and CD33+S100a9+ myeloid cell infiltration of tumor samples (p<0.001). Interestingly, analysis of TMA data stratified by disease subtype revealed a significant correlation between higher levels of IL-6 and GM-CSF and earlier recurrence-free survival in extrahepatic cholangiocarcinoma, but not in intrahepatic cholangiocarcinoma.

This study highlights IL-6 and GM-CSF as potential targets in BTC for future clinical trials, as these cytokines promote the expansion of immunosuppressive cell types. Specifically, our data identifies a subtype specific cytokine profile in extrahepatic cholangiocarcinoma. This study suggests subtype specific immune signatures in BTC that may be leveraged in the treatment of these deadly diseases.

Presenter: Anna B. Morris
Title: T cell-expressed FcgRIIB functionally regulates CD8+ T cell immunity via ligation of Fgl2
Email: anna.burcham@emory.edu
Co-authors: David F. Pinelli, Danya Liu, Jeremy M. Boss, Christopher D. Scharer, Mandy L. Ford
Affiliation: Emory University

FcyRIIB is the sole inhibitory Fcy receptor and is known for its inhibitory function on B cells, DCs, and macrophages. Interestingly, we discovered that FcyRIIB is upregulated on a subset of CD44hiCD62Llo effector CD8+ T cells following transplantation and aimed to elucidate its function.

To determine if FcyRIIB plays a cell-intrinsic role in inhibiting CD8+ T cells, we generated
a CD8\(^+\) T cell conditional KO system and observed enhanced accumulation of FcyRIIB\(^{-/-}\) CD8\(^+\) T cells at 14 (p<0.05) and 21 (p<0.01) d post-transplantation relative to WT controls. RNAseq analysis of FACS-sorted, donor-reactive CD8\(^+\) T cells revealed an enrichment of apoptosis-related genes in FcyRIIB\(^{-/-}\) vs. FcyRIIB\(^{-/-}\) cells. Further, FcyRIIB\(^{-/-}\) CD8\(^+\) cells exhibited lower expression of active caspase 3/7 on day 16 following transplant compared to WT cells (p=0.0315). To determine if FcyRIIB on donor-reactive CD8\(^+\) T cells impacted graft rejection, WT recipients of WT or FcyRIIB\(^{-/-}\) CD8\(^+\) T cells were treated with anti-CD28 domain antibody. Animals that received FcyRIIB\(^{-/-}\) CD8\(^+\) cells exhibited accelerated graft rejection relative to those receiving WT CD8\(^+\) T cells. These data demonstrate that FcyRIIB functions in a cell intrinsic manner to functionally regulate CD8\(^+\) T cell immunity.

To determine whether antibodies are the functional ligand of FcyRIIB on T cells, we employed AID\(^+\) animals that are unable to generate class-switched antibodies. WT vs. FcyRIIB\(^{-/-}\) CD8\(^+\) T cells were adoptively transferred into WT vs. AID\(^+\) recipients of skin grafts, and donor-reactive CD8\(^+\) T cell responses were measured at day 14. Results indicated that AID\(^+\) recipients possessed similarly increased frequencies of FcyRIIB\(^{-/-}\) CD8\(^+\) T cells relative to WT CD8\(^+\) T cells, demonstrating that antibodies are not the sole ligand via which T cell-expressed FcyRIIB regulates CD8\(^+\) T cell immunity. Instead, we found that an alternative ligand for FcyRIIB, Fgl2, induced caspase 3/7 activity in FcyRIIB\(^{-/-}\) but not in FcyRIIB\(^{-/-}\) T cells, suggesting that Fgl2 is a functional ligand for FcyRIIB on CD8\(^+\) T cells. Based on these experiments, we conclude that FcyRIIB is a novel, cell intrinsic CD8\(^+\) T cell regulatory pathway that regulates CD8\(^+\) T cell apoptosis and functions through ligation of the immunoregulatory cytokine Fgl2.
The persistence of HIV infection under ART is due to a reservoir of latently infected cells that remain indefinitely despite suppression of virus replication. Defining the mechanisms responsible for the establishment and maintenance of the HIV reservoir under ART has been the focus of efforts aimed at HIV eradication. Several studies have demonstrated that CD8+ T cells inhibit virus replication during untreated HIV/SIV infection; however, the mechanisms responsible for this antiviral effect remain poorly understood.

We used our primary cell based \textit{in vitro} model of HIV latency to study the CD8+ T cell mediated suppression of HIV expression. To examine the impact of CD8+ T cells on the establishment of HIV latency, memory CD4+ T cells from HIV naive donors were infected \textit{in vitro} and then co-cultured with activated CD8+ T lymphocytes (1:1 or 1:5 target:effector ratios). By assessing intracellular Gag expression on CD4+ T cells by flow cytometry, and by quantifying the frequency of integrated HIV DNA by qPCR, we found that HIV expression in CD4+ T cells was reduced when co-cultured with CD8+ T cells an average of 9-fold (p<0.0001) and 18-fold (p<0.0001) at 1:1 or 1:5 ratios respectively, without significantly reducing the frequency of HIV-infected cells (n=21). To assess the role of CD8+ T cells in latency reversal, cells generated in our \textit{in vitro} latency model were stimulated in the presence or absence of activated CD8+ T lymphocytes. We observed a significant suppression of HIV latency reversal, a 6-fold decrease at 1:1 target: effector ratio (p= 0.0156) and 14-fold decrease at 1:5 ratio (p= 0.0156).

Our studies have demonstrated a CD8+ lymphocyte mediated suppression of HIV expression in CD4+ T cells that functions to induce the establishment as well as maintain latency in the presence of activation signaling. Understanding the mechanisms by which CD8+ lymphocytes suppress virus transcription and ultimately promote HIV latency and persistence in ART-treated HIV-infected individuals may provide critical insight to support the design of new approaches for HIV eradication.

Costimulation blockade as a strategy for transplant immunosuppression has given rise to improved long-term outcomes for the first time in thirty-years. Patients treated with belatacept, a high-affinity variant of the CTLA4-Ig fusion protein enjoy superior renal function, and decreased risk of death or graft loss at 7 year follow up. We have previously identified a subset of memory T cells which express high levels of CD127, which are associated with costimulation blockade resistant rejection. We therefore investigated whether signaling downstream of this receptor was necessary for costimulation independent rejection. We examined the efficacy of combined costimulation blockade and anti-CD127 directed therapy in a stringent murine skin allotransplantation model. While costimulation blockade improved survival modestly, all animals eventually rejected (MST=21 days). The addition of anti-CD127 prevented the development of acute allograft rejection and led to indefinite survival (p<.0001, MST>80 days). Signaling downstream of CD127 is known to be critical for proliferation and homeostasis. We therefore investigated the impact of CD127 blockade on T cell proliferation. In a model of graft-versus host disease, CFSE labeled C57BL/6 splenocytes were transferred to irradiated Balb/C recipients, who were then treated with costimulation blockade, or combined
costimulation blockade + anti-CD127, or received no therapy. The addition of anti-CD127 to costimulation blockade significantly abrogated allostimulated proliferative response, nearly reducing proliferation by half (p=.0002). To further define the impact of combined CTLA4-Ig + anti-CD127 on graft specific T cells, we utilized an antigen-specific model (Ova-expressing skin graft, with OT-I and OT-II adoptive transfer). We found mice treated with CTLA4-Ig + anti-CD127 demonstrated reduced expansion and effector function of graft-specific CD8 T cells. When characterized, these cells express high levels of PD-1 and TIGIT, suggesting an exhausted phenotype. By pharmacologically blocking PD-1 in our murine allotransplantation model, the protective benefit of CTLA4-Ig + anti-CD127 therapy was lost (p<.0001), suggesting that the addition of anti-CD127 to costimulation blockade gives rise to graft tolerance via PD1 mediated T cell exhaustion.

Presenter: Donald McGuire
Title: Defining Human CD8 memory subsets following acute viral infection.
Email: dcmguir@emory.edu
Co-Authors: Rama Akondy, Carey Jansen, Shashikala Nagar, Srilatha Edupuganti, Mark Mulligan, Haydn Kissick, Rafi Ahmed.
Affiliation: Emory University

Induction of an T cell memory response is an important mechanism in a protective immune response. The highly effective yellow fever vaccine generates lasting protection and a long-lived CD8 memory. We have previously found that YFV specific CD8 T cell develop into quiescent memory cells that lack most effector proteins. These same cells also have epigenetic marks that suggest a past effector function. The origins and history of this long-lived memory population remains unclear. To trace the lineage of these memory YFV specific CD8 T cells we utilized single cell RNA-seq. Our data from samples taken 14 days to 14 years after yellow fever vaccination identified two populations of cells. The first population closely correlated with cells found 6-14 years after vaccination. These cells expressed fewer effector molecules and expressed markers suggesting affinity to lymphoid structures. The second population expressed high levels of effector molecules and lacked many of the markers associated with lymphoid homing. These cells were more effector like cells were absent in samples taken more than one year after vaccination. Interestingly, both populations re-express CD45RA after the end of viremia. Consequently, these cells do not meet the formal definition of CM or EM cells. However, these populations do closely resemble those of MP/ MPEC and TE/SLEC as defined in mice.

Presenter: Ana Carolina Guerta Salina
Title: Skin resident macrophage-derived microRNA21 impairs skin host defense.
Email: ana.g.salina@vumc.org
Co-authors: Stephanie Brandt, Alexandra Ivo de Medeiros, Carlos Henrique Ser ezani
Affiliation: Vanderbilt University Medical Center and University of Sao Paulo

Introduction. Staphylococcus aureus skin infection is orchestrated by the actions of resident and recruited immune cells. Skin-resident macrophages produce a variety of chemoattractants to recruit neutrophils and monocytes to the site of infection to form an abscess and contain the bacteria. However, excessive production of inflammatory mediators causes unrestricted phagocyte recruitment and tissue damage. Our laboratory aims to identify the role of microRNAs as a small noncoding RNA that inhibit mRNA expression or translation. We have previously shown that miR21 is a homeostatic regulator of macrophage polarization in vivo. Whether miR21 regulates microbial clearance is not well understood. Aim. Here, we hypothesize that miR-21 depletion enhances the expression of antimicrobial effectors, while decreases inflammatory cytokine production, leading to optimal host defense. Methodology. C57BL/6 or miR21∆myel were infected with ~3x10^6 S. aureus CFU s.c.. The size of the lesion was measured during the time, and skin biopsies were collected and processed for qRT-PCR, chemokine for ELISA, colony forming unit (CFU) and, histology. Results. MiR21 expression is enhanced after infection and depletion of skin macrophages, but not neutro-
phils decreased miR21 abundance during infection. Skin infection in miR21KO mice showed reduced lesion size and bacterial burden when compared to WT animals. We detected increased IL-1β, TNF-α, IL-10 and well as Nitric oxide. Importantly, we also detected increased expression of pro-resolution genes, such as CD36, TIM4 and collagen 3 and 4. Increased abundance of inflammatory mediators along with anti-inflammatory genes lead to increased neutrophil migration, highly organized abscess with ticker capsule when compared to infected WT mice. Conclusion. Our results suggest that miR21 is a negative regulator of macrophage actions during skin infection and that treatment of mice with a miR21 antagonim could provide important translational insights to skin infections. Financial Support. FAPESP 2017/04786-0 and 2018/01622-9, HL-103777 and R01HL124159-01.

**Presenter:** Jessica Shannon

**Title:** Adult Epidermal Lgr5+ Stem Cell Regenerative Potential and Immune Functions during Cutaneous Wound Repair is Controlled by Thymic-Stromal-Lymphopoietin (TSLP)

**Email:** jessica.shannon@duke.edu

**Co-Authors:** P Mariottoni2 DL Corcoran3 AS MacLeod1,2,3,4

**Affiliation**

1Department of Immunology, Duke University, Durham, NC, USA; 2Department of Dermatology, Duke University, Durham, NC, USA; 3Genomic and Computational Biology, Duke University, Durham, NC, USA; 4 Department of Molecular Genetics and Microbiology, Duke University, Durham, NC, USA

After skin injury, re-epithelialization is an essential hallmark of successful repair of cutaneous barrier and required for prevention of infections. Among various approaches, stem-cell based therapy has shown great potential in both animal models and human studies to overcome and treat large wounds, such as those observed in burn wound victims and toxic necrolysis patients. However, the underlying mechanisms how stem cell factors contribute to cutaneous wound healing and whether they also contribute to antimicrobial immunity are not known. Genetic fate-mapping wound healing studies revealed that adult Lgr5+ hair follicle stem cells (but not Lgr6+ stem cells) upregulate the cytokine TSLP during the mid-phase of the wound healing response. In contrast to constitutive high expression of TSLP receptor (TSLPR) on skin-resident immune cells, a hair follicle-associated non-hematopoietic Thy1.2+ population upregulated TSLPR expression upon wounding. Notably, when TSLP was administered into skin wounds, it accelerated wound closure compared to vehicle treated wounds (p<0.01). Furthermore, transcriptional signatures from TSLP-treated mouse skin illuminate a set of differentially expressed genes that overlap with transcriptional changes during wound healing in both human and mouse models. This overlap analysis revealed chemotactic factors known to regulate stem cell migration in a time-dependent manner and the RNA helicase DDX6, a protein with known roles in maintaining epidermal stem cell self-renewal. This observation was in agreement with our finding that stimulation of primary human epidermal keratinocytes or human skin explants with recombinant TSLP significantly increased DDX6 expression. Overall, our findings implicate a novel role TSLP in epidermal regeneration which may be mediated by DDX6.
metabolic programs regulate B cell activation and antibody responses. However, the metabolic programs that support the durability of plasma cells and the memory B cell population are unknown. Adenosine monophosphate-activated protein kinase (AMPK) is an evolutionarily conserved serine/threonine kinase that integrates cellular energy status and nutrient availability to intracellular metabolic pathways. Here we show that loss of AMPKα1 in B cells leads to normal memory B cell and plasma cell differentiation but increased circulating antigen-specific immunoglobulin in response to hapten-carrier immunization when compared to wild-type mice. Consistent with the increase in immunoglobulin production observed in vivo in the absence of AMPKα1, LPS-activated AMPKα1-deficient B cells secrete more IgG1 on a per cell basis than AMPKα1-sufficient control B cells. Furthermore, despite enhanced immunoglobulin production, normal plasma cell and memory B cell formation in AMPKα1-deficient mice, loss of AMPKα1 in B cells impaired the long-term survival of the memory B cell and plasma cell populations in vitro and in vivo. AMPKα1-deficient B cells exhibit increased mTORC1 signaling and aberrant mitochondrial activity both in vitro and in vivo after immunization. Moreover, AMPKα1-deficient B cells sustain increased mitochondrial ROS and defects in mitophagy. Collectively, these findings fit a model where AMPKα1 in B cells supports the longevity of the long-lived plasma cell and memory B cell populations by promoting mitochondrial turnover and dampening mTORC1 activity. Surprisingly, however, the findings also reveal evidence that a metabolic sensor regulates the rate of immunoglobulin production by the plasma cell population.

NIH R01AI113292, HL106812, R25-GM062459, NCI T32CA009592-29

Presenter Christopher A. Risley
Title Interferon gamma signaling regulates the generation of influenza-specific memory B cells
Email crisley@uab.edu
Co-authors Christopher A. Risley*, Sara L. Stone, Christopher Scharer, Jerry M. Boss, Frances E. Lund
Affiliation 1Dept. of Microbiology, Univ. Alabama at Birmingham, Birmingham AL 35243; 2Dept. of Microbiology and Immunology, Emory Univ., Atlanta GA 30322

Despite extensive research to prevent or minimize influenza virus (flu) outbreaks, flu remains a serious human health concern. While immunization remains our most effective means of protection from flu infection, the vaccine must be reformulated annually due to constant mutation of the viral hemagglutinin protein that negates antibody (Ab)-mediated virus neutralization. Although neutralizing flu-specific circulating Ab can prevent infection, memory B cells (Bmem), which are elicited with vaccination, also play a key role in reducing morbidity and mortality by rapidly differentiating into protective Ab-secreting cells (ASCs) following reexposure to the virus. Despite the importance of Bmem in immunity to flu, we know little about the signals that induce their formation and program their effector properties. To date, most mechanistic studies of Bmem have been performed in immunized mice using model hapteneated-proteins adjuvanted in alum, which preferentially promotes type 2 immune responses. This is significantly different from immune responses generated during a flu infection that induces a strong host IFN response. Recently, we demonstrated that B cell intrinsic expression of T-bet, an IFNγ-inducible transcription factor, regulates ASC differentiation following flu infection and, while dispensable for memory B cell development, T-bet is required for Bmem differentiation into secondary ASCs. Here, we show that T-bet is expressed across all isotypes of Bmem, including IgM and IgG1, and that there is a B cell-intrinsic requirement for IFNγR signaling to generate these optimal flu-specific Bmem cells. Consistent with this finding, deletion of the IFNγ-inducible transcription factor STAT1 selectively in B cells also alters the development of the flu-specific Bmem population. Additionally, IFNγR signaling, transduced via STAT1, during Bmem generation is critical for Bmem differentiation into ASCs, as their loss dramatically impairs the recall response to heterosubtypic infection. Taken together these data indicate a B cell-intrinsic requirement for IFNγ signaling, mediated by STAT1 and T-bet, in generating robust, transcriptionally distinct and functional Bmem populations following influenza infection. In the future, we hope to use these data to develop better vaccine formulations that will more effectively elicit these protective Bmem cells.
Presenter: Amber Wolabaugh
Title: Lineage-tracing of B cells in human vaccination identifies transcriptional signatures governing the fate of individual clonotypes in vivo
Email: amber.nicole.wolabaugh@emory.edu
Affiliation: Emory University - 1 Division of Microbiology & Immunology, Yerkes National Primate Research Center; 2 Department of Pediatrics, School of Medicine, Emory University.; 3 Yerkes NHP Genomics Core Laboratory, Yerkes National Primate Research Center.; 4 Divisions of Pulmonary, Allergy, & Critical Care Medicine, Emory University, Atlanta, GA; 5 Department of Pathology & Laboratory Medicine, School of Medicine, Emory University.

Background
Vaccinations offer a unique opportunity to study the human adaptive immune system. The advent of single-cell-RNA-Seq now makes it possible to obtain the transcriptional signatures of individual B cells, and distinguish signatures between clonal families exhibiting desirable functional qualities. Recently, we developed a bioinformatics pipeline (BALDR) that accurately reconstructs paired IgH+IgL sequences from sc-RNA-Seq data and allows linking of transcriptional data with clonotypes. Here, we applied this “Clonal lineage-tracing” in the context of a human vaccination to investigate the signatures in vaccine-induced plasmablasts that predict entry into the memory pool.

Methods
Two individuals were vaccinated with 2016/2017 Fluarix quadrivalent vaccines. IgH repertoire sequencing of the naïve and memory populations was performed at Day 0, and at day 28. sc-RNA-Seq of 271 individual plasmablasts at day 7 post-vaccination was performed using SMART-Seq, the Seurat package was used for single-cell DEG analysis, and BALDR for IgH+IgL reconstruction.

Results
We identified several clonotypes from the pre-vaccine naïve and memory populations, and post-vaccine memory pool that were represented in Day 7 plasmablasts. We compared gene expression between plasmablasts that were expanded at day 7 and with plasmablasts represented as singlets, and identified a unique signature for plasmablast expansion. Finally, we contrasted gene expression of plasmablasts with clonotypes of naïve origin with those of memory origin and identified several significant differentially expressed genes.

Conclusion
In this proof of concept study, we combined repertoire sequencing with sc-RNA-Seq to trace clonotypes in vaccine-induced plasmablasts. Lineage-tracing allowed us to identify transcriptional signatures predicting entry into the memory pool. The ability to track B cell gene expression with evolution of antibodies will substantially inform rational vaccine design and allow linking of signatures with B cell and antibody functional properties, enabling unprecedented insight into the molecular mechanisms governing vaccine efficacy.

Presenter: Bradley I Reinfeld
Title: Glycolytic Tumor Associated Macrophages significantly contribute to FDG-PET avidity of tumors
Co-Authors: Mathew Z Madden, Melissa M Wolfe, Allison Cohen, H. Charles Manning, Katy E Beckermann, Jeff C Rathmell, and W Kimryn Rathmell
Email: bradley.reinfeld@vanderbilt.edu
Affiliation: Vanderbilt University MSTP

F18 fluoro-2-deoxyglucose Positron Emission Tomography (FDG-PET) is a fundamental imaging tool to diagnose and stage cancer. For the past 50 years, this technology has been used to evaluate tumor burden based on the principles of the Warburg effect, that tumor cells consume more glucose than non-tumor cells. Intriguingly, Warburg metabolism is implemented by immune cells in order to perform their requisite functions. Because the
heterogenous tumor microenvironment contains a wide variety of these infiltrating immune cells. I hypothesize that the activation state of tumor resident immunocytes contributes to the FDG-PET avidity of tumors. Using magnetic bead cell separation techniques on single cell suspensions obtained from MC38 flank tumors, I am able to successfully purify 3 cell fractions: (1) T cell enriched, (2) myeloid enriched and (3) tumor cell fractions. By combing this separation technique with FDG-PET imaging and gamma counting, we see that immune cells contribute to approximately 70% of FDG avidity in this tumor model. On a per cell basis, the myeloid fraction is the most avid and is enriched with tumor associated macrophages (TAM, CD45+, CD11b+, F4/80+). In this model, F18-glutamine accumulates in the CD45-tumor cells, not the CD45+ immune cells, suggesting that immune cell PET avidity is glucose specific. Additionally, this technique illustrates known aspects as immune-metabolism, where tumor infiltrating T cells utilize glucose to a greater extent than naïve splenocytes. In support of this data, ex vivo seahorse analysis demonstrates that this CD45+, TAM enriched fraction is the most glycolytic tumor resident population in this model. I also observe that tumor infiltrating T cells have a significantly higher glycolytic and oxidative metabolism then their naïve splenocytes counterparts. Therefore, future work will investigate the role of macrophage glycolysis on FDG-PET avidity across mouse and human tumors and in response to new treatment modalities. We will implement genetic and pharmacological tools to deplete macrophages and evaluate how tumor FDG avidity changes in response. These findings challenge the dogma that FDG-PET avidity is solely driven by tumor cell Warburg effect and highlight the significant role of immune cells glycolysis in clinical settings.

Presenter Madhubanti Basu
Title Induction and relationship of HIV Envelope-specific B cells in the peripheral blood and bone marrow following vaccination in humans
Email mbasu@uabmc.edu
Co-Authors Michael Piepenbrink¹, Czestochowa Francois¹, Fritzlaine Roche¹, Christopher Fucile¹, Alexander Rosenberg³, Jane Liesveld², Michael Keefer², and James Kobie¹
Affiliation ¹University of Alabama at Birmingham, Birmingham, AL, ²University of Rochester, Rochester, NY

Induction of long-lived plasma cells producing protective HIV-1 Envelope (Env)-specific antibody (Ab) is a primary goal of HIV vaccine strategies. However, vaccine-induced Env-specific human plasma cells have not yet been identified. In an effort to identify Env-specific bone marrow plasma cells after vaccination, samples were obtained from HVTN 105, a phase I trial in which participants were immunized with the same bivalent gp120 protein, AIDSVAX B/E, as used in RV144, although combined with a DNA immunogen (instead of ALVAC-HIV) in various prime/boost strategies. Peripheral blood and bone marrow samples were analyzed by ELISpot, flow cytometry, in addition to single cell and MiSeq based repertoire analysis. Participants who received a boosting regimen that included AIDSVAX B/E had robust peripheral blood plasmablast responses as measured by Env-specific ELISpot (n=21) and flow cytometry (n=20) after the final immunization. The Env-specific immunoglobulin repertoire of the plasmablasts, assessed by monoclonal Ab generation (>60 mAbs total from 16 participants), was dominated by VH1 gene usage (~60%, VH1-2>VH1-46>VH1-24), and had modest mutation from germline (~5%). Epitope mapping has indicated V3 is a dominant target; however other regions including V1/V2 and C1/C5 were also targeted. The majority of the mAbs are high avidity, and a subset can mediate Ab-dependent cellular phagocytosis. To determine if these plasmablast-derived Env-specific mAbs persisted in long-lived plasma cells, bone marrow was evaluated ~7 months after immunization. Although ELISpot of CD138+ plasma cells (n=3) could readily detect influenza specific IgG Ab secreting cells (ASCs), Env-specific IgG ASCs were not convincingly detected by ELISpot. However, VH deep sequencing of the bone marrow (n=6) identified multiple members of mAb clonal lineages (n=19), of which ~47% were found in CD138+ plasma cell compartment. Our results indicate that HIV-1 Env-specific B cells are present in long-lived bone marrow plasma cell repertoire after vaccination in human, but may persist at very low frequency.

Presenter Bhrugu Yagnik¹ ²

---

69

Presenter Bhrugu Yagnik¹ ²

---

121
Antiretroviral Therapy (ART) against HIV has been successful in suppressing viral burden and improving the quality of life for individuals with HIV/AIDS. However, the high cost of this therapy poses challenges of its accessibility, especially in the developing countries. Moreover, HAART fails to achieve a cure as virus rebounds immediately upon treatment cessation, demanding a continual dependence on the continued therapy. Persistence of latent viral reservoirs during ART and immune dysfunction mediated by inhibitory immune checkpoint receptors are the two major factors that underlay the failure to clear the virus. Therapeutic interventions aiming to reduce the latent viral reservoirs and enhance antiviral immunity are critical in achieving durable remission of HIV. To achieve this goal, we are combining therapeutic CD40L-adjuvanted DNA/MVA SIV vaccine vaccination, PD-1 blockade with ART in SIVmac239 infected rhesus macaque. Nineteen Indian rhesus macaques were infected with SIVmac239 and treated with ART. These were divided into three groups wherein a group of six animals received CD40L-adjuvanted DNA/MVA SIVmac239 vaccine and TLR7 agonist, another group of seven animals, in addition to vaccine, received primatized anti-PD-1 Ab at the initiation of ART and during DNA vaccinations; and another group of six animals served as a control group. We observed that PD-1 infusion at the initiation of ART resulted in increased frequencies of SIV-specific CD8+ T cells. Therapeutic vaccination induced a robust polyfunctional CD4 and CD8 T cells producing cytokines IFNγ, TNFα and IL-2, and targeting an average of 13 CD4 epitopes and 7 CD8 epitopes. These responses persisted for longer period in the PD-1 group. Moreover, vaccine induced CD8 T cells showed improved cytolytic potential depicted by granzyme B and perforin expression. In addition, we also observed increased frequencies of CXCR5+ SIV-specific as well as total CD8+ T cells suggesting induction of antiviral CD8+ T cells with the potential to home to B-cell follicles and limit HIV replication in vivo. Currently, these animals are undergoing ART interruption to evaluate the influence of vaccination on SIV rebound. We hypothesize that the vaccine induced polyfunctional SIV specific CD8+ T cells will contribute to blunting of reemerging viremia following ART interruption.

The incidence of allergic asthma in the United States and other industrialized countries has been increasing over the past few decades. One proposed explanation for this increase is the hygiene hypothesis, which in the context of asthma, states that a lack of early childhood exposure to high levels of environmental lipopolysaccharide (LPS) endotoxins from gram-negative bacteria, increases the susceptibility to developing allergic diseases. Our lab has recently shown that LPS can indeed reduce Th2-driven, allergen-induced airway inflammation by inducing the up-regulation of the transcription factor T-bet in CD11b+ migratory dendritic cells, which we found was required to upregulate T-bet in responding CD4+ T cells. Furthermore, we and others have found that T-bet expression in T cells is required to prevent the Th2 cell differentiation program. Some evidence in the field suggests that IL-12 and IL-18 signaling synergizes to induce robust T-bet expression through the upregulation of...
IFN-γ in T cells. Due to these findings, our lab is now investigating the role of IL-12 and IL-18 in the LPS-mediated suppression of Th2 cell responses. In addition, studies have shown that interleukin-6 (IL-6) reduces Th2 cell responses in murine asthma models, but no clear mechanism has yet been described. Here, we investigate the interplay of IL-12, IL-18, and IL-6 in a house dust mite-induced asthma model in C57BL/6 mice. Through studies with IL-6 knock out and IL-6 receptor knock out mice, we have shown that IL-6 signaling upregulates the expression of the IL-18 receptor on T cells, thus affecting the synergy of IL-18 and IL-12 to induce T-bet and prevent Th2 responses. We propose a model in which IL-6 affects T cell ability to respond to IL-18 and IL-12 by influencing the thresholds of these cytokines needed for T cell responses.

Presenter: Deepti Maheshwari
Title: Massive depletion of non-classical monocytes in systemic circulation during dengue febrile illness.

Co-authors: Kaustuv Nayak², Mohit Singla², Sivaram Gunisetty¹,⁶, Guruprasad Medigeshi³, Rakesh Lodha², Sushil Kabra², Rafi Ahmed⁴,⁵, Anmol Chandel¹, Murali-Krishna Kaja¹,⁴,⁵,⁶

Affiliation: ¹ICGEB-Emory Vaccine Centre, International Centre for Genetic Engineering and Biotechnology, New Delhi, India. ²All India Institute of Medical Sciences, New Delhi, India, ³Translational Health Science and Technology Institute, Faridabad, India, ⁴Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA, ⁵Emory Vaccine Center, Emory University School of Medicine, Atlanta, GA, ⁶Department of Pediatrics, Emory University School of Medicine, Atlanta, GA.

Monocytes in human blood circulation can be divided into three subpopulations according to the expression of the membrane receptor for lipopolysaccharide (CD14), and the low affinity FcγRIII-A receptor for the Fc region of IgG (CD16): CD14++CD16− (classical), CD14++CD16+ (intermediate), and CD14+CD16++ (non-classical). These monocyte subsets display distinct ligand sensing, tissue patrolling and cytokine secretion patterns under normal homeostatic conditions; and are thought to be potential targets for dengue viral infection. While the nonclassical monocytes, which are also often called as patrolling monocytes, are implicated in endothelial interactions, the intermediate monocyte subset is implicated in shaping the adaptive B cell response. Despite this wealth of information, very few studies analyzed the relative abundance of these different monocyte subsets in human blood during dengue febrile infection in vivo. In this study we analyzed the frequency and number of each of the three monocyte subsets in dengue confirmed febrile patients from India. We found that non-classical monocytes undergo a massive depletion whereas the intermediate monocyte subset increases substantially. Similar trends were observed in patients with primary versus secondary infections and in patients with severe versus non severe disease. Taken together, these results suggest that massive changes occur in the relative abundance of the monocyte subsets during dengue febrile illness; and that these changes are reflective of innate response to febrile infection rather than differences mediated by primary versus secondary dengue infections.

Presenter: Luis Enrique Muñoz
Title: Metformin enhances the efficacy of tumor membrane vesicle-based vaccine immunotherapy in a murine model of triple negative breast cancer.

Co-Authors: Ramireddy Bomireddy, Haley Lei Huang, and Periasamy Selvaraj.

Affiliation: Emory University

Breast cancer remains the most commonly diagnosed cancers among women, with over 250,000 new cases per year in the United States. Immunotherapy has emerged as a promis-
ing treatment of breast cancer, however response rates to therapy remain far from ideal. In this study, we investigated the combinatorial effect of tumor membrane vesicle (TMV)-based vaccine immunotherapy, to induce an anti-tumor immune response, with the type 2 diabetes drug metformin, which has been reported to suppress tumor growth with direct and indirect antitumor effects. Our results show that TMV vaccination inhibits primary tumor growth in preclinical tumor models and reduced spontaneous metastasis to the lungs. The combination treatment with metformin further inhibited primary tumor growth and metastatic spread. To dissect the mechanism behind the synergy between TMV vaccine therapy and metformin, we profiled the expression of surface inhibitory markers on tumors and observed a marked decrease in PD-L1 expression after metformin treatment. To determine its effect on T cells infiltrating the tumor we looked at tumor CD8 T cells and found that the TMV vaccine induced a massive increase in T cell infiltration, while the combination with metformin reduced this infiltration despite having better control of tumor growth. Finally, we looked at the quality of the CD8 T cells within the tumor by surface PD-1 and Tim3 expression and found that combination treatment produced more terminal effector CD8 T cells that produced more pro-inflammatory cytokines compared to TMV vaccine treatment alone. This suggests that one way metformin can suppress tumor growth is by reducing the inhibition exerted on T cells by tumor PD-L1 and thus synergizing with TMV vaccine immunotherapy.

Inflammatory bowel disease (IBD) encompasses several gastrointestinal inflammatory conditions manifesting in susceptible individuals as a result of improper immune regulation of the gut microbiota. An estimated 3 million Americans suffer from IBD, for which there is no cure. The exact conditions leading to the onset of this multifactorial disease remain poorly understood. To date, the most successful therapy is a neutralizing antibody to tumor necrosis factor alpha (TNFα), a proinflammatory cytokine that is upregulated in IBD patients. However, 30% of patients are primary non-responders to anti-TNFα therapy and an additional 30 - 40% of patients lose responsiveness within the first year of treatment. We hypothesized that TNFα signaling is involved in shaping the composition of the microbiota both at homeostasis and during intestinal inflammation thereby impacting susceptibility to and chronicity of, murine colitis. Dysregulated colonic IL-10 signaling is known to drive colitis in a microbiota dependent manner. Therefore, we employed a model based on transient administration of an IL-10 receptor blocking antibody (anti-IL-10R) in otherwise immunocompetent mice. Upon 16S sequence analysis of fecal pellets, we have found that the microbiota of anti-IL-10R treated mice are shifted from their pre-treated, homeostatic fecal microbiota, even 30 days after the last anti-IL-10R injection. Anti-IL-10R induces mild, acute colitis in wildtype (WT) mice and severe, chronic colitis in TNF deficient (Tnf-/-) mice as determined by histological analysis, immune cell infiltration, and fecal levels of the inflammation biomarker lipocalin-2. Upon co-housing Tnf-/- and WT mice, anit-IL-10R treated WT mice exhibit more severe colitis compared to their separately housed anti-IL-10R treated counterparts. Our results suggest that exposure to the TNF-naive microbiota is unfavorable for the WT mice, upon disruption of colonic homeostasis. Further, this suggests that TNFα signaling does have a role in condition the microbiota.

Presenter: Rachel Muir
Title: Enhanced susceptibility to colitis upon exposure to TNF-naive microbiota
Email: rqmuir@uab.edu
Co-Authors: Barbara J. Klocke, Casey Morrow, Trenton Schoeb and Craig L. Maynard
Affiliation: University of Alabama at Birmingham

Presenter: Abraham J. Matar
Title: Interruption of Notch Signaling via Blockade of Delta-like Ligands 1 and 4 Prevents Co-stimulation Blockade Resistant Allograft Rejection
Email: Abraham.Jamil.Matar@emory.edu
Co-authors: Ying Dong, Brendan P. Lovasik, David V. Mathews, Cynthia A Breeden, Abheek Ghosh, Allison Stephenson, William H. Kitchens, Andrew B.
Adams

Affiliation: Emory University

Co-stimulation blockade (CoB) has emerged as a promising immunosuppression strategy with the advent of belatacept, a novel CTLA4-Ig fusion protein that blocks CD28-mediated T cell co-stimulation. Belatacept confers long-term advantages in graft survival and function in renal transplant recipients compared to traditional calcineurin inhibitor-based immunosuppression but is associated with increased rates of early acute rejection. Using a mouse model of MHC-mismatched skin transplantation, we investigated the role of Notch pathway inhibition via blockade of Delta-like ligands 1 and 4 (Delta 1/4) on CoB-resistant allograft rejection. In our model of Balb/C to C57BL/6 skin transplantation, combined CoB (CTLA-4Ig + anti-CD154) and Delta 1/4 blockade significantly prolonged skin graft survival compared to CoB alone (MST 20 days vs. >83 days, p = 0.001**). Delta 1/4 blockade did not inhibit T cell proliferation in vivo, but instead induced T cell apoptosis. Anti-donor IgG antibody was also significantly reduced in the combined treatment group. We next examined donor-specific T cell responses using mOVA skin grafts in recipients which received an adoptive transfer of Thy1.1+ ovalbumin-specific OT-I T cells. Combined CTLA-4Ig and Delta1/4 blockade suppressed donor-specific CD8+ T cell accumulation in draining lymph nodes compared to CTLA-4-Ig alone. Further, the combined blockade regimen suppressed both IFN-gamma and TNF production by donor-specific CD8+ T cells. These data demonstrate that Delta 1/4 blockade suppresses donor-specific T cell responses in the setting of CoB and prevents CoB-resistant rejection. We have identified the Notch signaling pathway as a promising target for future large animal and clinical studies of CoB-resistant rejection.

Presenter: Rasha Raheem Alkarkoushi
Title: Indole-3-carbinol ameliorates colonic inflammation in DSS-treated, Helicobacter muridarum-infected mice.
Email: rashaa@email.sc.edu
Co-authors: Ioulia Chatzistamou, Udai P. Singh, Marpe Bam, Yvonne Hui, Haider Alrafas, Prakash Nagarkatti, Mitzi Nagarkatti and Traci L. Testerman
Affiliation: University of South Carolina/ School of medicine /Pathology, Microbiology, and Immunology

Enterohempathic Helicobacter species are epidemiologically linked to increased inflammatory bowel disease; however, little research has been done to elucidate potential contributions of individual species. We hypothesized that Helicobacter muridarum (Hm) would alter the course of DSS-induced colitis and the response to indole-3-carbinol (I3C), an anti-inflammatory phytochemical. We treated Hm-infected C57BL/6 mice with 1% DSS +/- 40 mg/kg I3C and measured inflammatory biomarkers. We found that H. m exacerbated DSS-induced colitis and increased the percentage of Th17 cells in the mesenteric lymph nodes and spleen and increased IL-17 in colon tissue compared to the DSS group. Also, we found that Hm bacteria itself produced inflammation and pathology. I3C, on the other hand, ameliorated colitis and shifted the Treg/Th17 balance in DSS+H.m-infected mice. We found that I3C treatment of DSS + H.m-infected mice decreased the expression of pro-inflammatory IL17 and RORC as well as increased anti-inflammatory Foxp3 when compared to the untreated group. The decreased expression of RORC correlated with increased miR-let7a-2 and miR-29a-3p expression and increased FoxP3 correlated with decreased miR-874 expression following I3C treatment. Moreover, I3C reduced the abundance of certain taxa, such as Clostridiales, Actinobacteria, and Erysipelotrichales, and increased the abundance of Ruminococcus. In summary, H. muridarum causes baseline inflammation and exacerbates colitis via microRNA-mediated increases in Th17 cells, while I3C ameliorates colitis via increased Treg populations. (Supported in part by NIH grants R01AI123947, P01AT003961, P20GM103641, R01 AT006888)

Presenter: John E. Bradley
Title: Generation and validation of B cell tetramers to identify antigen-specific B cells.
Email: johnbradley@uabmc.edu

73
Individual T and B cells express unique antigen receptors that allow them to bind to specific antigens. To identify T cells that recognize particular antigens, immunologists generated recombinant, biotinylated MHC class I and MHC class II molecules, loaded them with an antigenic peptides and “tetramerized” them by binding to fluorescently labeled streptavidin. These tetramers allowed direct characterization of antigen-specific T cells by flow cytometry. More recently, we have generated “B-cell tetramers”, which consist of recombinant proteins in their native configuration expressed with biotinylation sites thereby allowing them to be “tetramerized” by binding to fluorescently labeled streptavidin. These tetramers label B cells specific for a single antigen and allow the B cells to be characterized by flow cytometry. Generating MHC tetramers of different specificities involves swapping out antigenic peptides. However, generating B cell tetramers of different specificities requires making new and sometimes very different proteins. Many of the antigens of interest are surface proteins on viruses or bacteria. Moreover, these proteins often bind to mammalian surface proteins or glycans as a way to mediate viral or bacterial entry into cells or evade immune function. Thus, one must be able to distinguish the ability of tetramers to bind BCRs from their ability to bind non-BCR proteins or glycans on the surface of cells. Here, we will show examples of B cell tetramers and present strategies to overcome some of the difficulties encountered when designing and validating B-cell tetramers.

Presenters: Ariel Ley¹, Doan C. Nguyen¹, Celia Saney¹, Chet Joyner¹, Iñaki Sanz²,³, F. Eun-Hyung Lee¹,³*

Title
In vitro maturation of early blood antibody secreting cells (ASCs) to bone marrow LLPCs

Email
f.e.lee@emory.edu

Affiliation
¹Division of Pulmonary, Allergy, Critical Care, and Sleep Medicine, ²Division of Rheumatology, ³Lowance Center for Human Immunology, Department of Medicine, Emory University, Atlanta, GA, USA

Long-lived plasma cells (LLPCs) are responsible for the secretion and long-term maintenance of serum antibody levels in the absence of antigen re-stimulation. The generation of LLPCs is primarily thought to occur as a result of antibody secreting cell (ASC) differentiation within a germinal center response or extrafollicular response; however, additional evidence suggests that further LLPC maturation can occur within the specialized niche of the bone marrow microenvironment. Previous studies showed that ASC survival and maintenance in vitro requires three bone marrow factors: hypoxia, secreted factors from mesenchymal stromal cells derived from human bone marrow, and APRIL, a TNF family member that binds to BMCA or TACI possibly through SDC-1 (CD138). Using an in vitro cell-free BM microniche system, we observed the maturation of blood ASCs in culture over four weeks. We simultaneously measured antibody production, transcriptional changes and epigenetic changes. From ex vivo early minted blood ASC and BM LLPC, we demonstrate morphological changes that include: an increase in the number of mitochondria, condensation of nuclear chromatin, an increase in ER volume and an increase in the number of autophagosomes. Concordantly, we observe similar morphological changes from the population of early minted ASC that mature in the in vitro cell-free BM microniche. Evidence from both in vitro and ex vivo experiments indicate that there are a number of transcriptional and epigenetic programs that regulate the maturation of early blood ASCs within the bone marrow microniche and substantiates a role for ongoing maturation within the bone marrow.

Presenter
Anusmita Sahoo

Title
Sequence and structure guided HIV-1 Clade C immunogen design

Email
anusmita.sahoo@emory.edu

Co-Authors
Anusmita Sahoo, Rama Rao Amara

Affiliation
Emory University
Limited number of Clade C HIV-1 envelope proteins have been engineered into stable immunogens due to difficulties in folding the envelope proteins into the native trimeric form. 1086C is one such envelopes which is less studied. Use of recent structure guided stabilizing strategies on this protein yielded either aggregates or a trimeric protein with low affinity for apex (V1V2) directed broadly neutralizing antibodies. Antibodies directed against V1V2 region of the envelope has been seen to be associated with decreased risk of infection (RV144 trial), with major contribution by the V2 hotspot directed antibodies. We analyzed the Clade C sequences and mutated the residues in 1086C which varied from the consensus sequences in the hotspot region. All possible mutants were generated with the residues changed to the dominant and sub-dominant residues in the consensus sequence at the selected positions. Two variants were screened which showed significant improvement in binding to multiple V1V2 directed broadly neutralizing antibodies. Few modifications restored the active site binding to one of the V1V2 specific antibodies, while others synergistically helped in folding the protein into better native like trimers. Sequence guided modifications were tested onto very well characterized BG505 SOSIP and were found to have an influence on the folding of the protein. Single site variants at 173 position was seen to influence the ability of the immunogen to modulate and influence the immune response. Two variants (immunogens) at 173 position yielded similar antibody titres against the soluble envelope protein. However, they differed significantly in their ability to recognize native envelope on the cell surface. This observation holds true when binding was monitored against cell surface expressed envelopes from the HIV-1 global panel. We are currently looking into the differences in effector functions of these antibodies generated by the above discussed two variants at 173 position. Structural studies and further analyses will help understand the mechanism of modulation of 173 position on the immune response. These observations will be important for engineering envelopes as immunogens to drive native envelope directed immune response.

Presenter: Ashley Landuyt
Title: Cooperation between T-dependent antibodies and IL-10 reduces inflammatory bowel disease susceptibility
E-mail: ashland@uab.edu
Co-Authors: Klocke BJ1, Schoeb TR2, Maynard CL1
Affiliation: University of Alabama at Birmingham

Inflammatory bowel diseases (IBD) are lifelong autoinflammatory conditions characterized by loss of immune tolerance to gastrointestinal microbes. Currently, the impact of humoral immunity on IBD is largely undefined. We employed mouse models simulating loss-of-function mutations associated with IBD to study the role of T-dependent antibodies in the disease. Inducible T cell costimulator ligand (ICOSL) is encoded by an IBD susceptibility locus. It is also essential for formation of T-dependent antibodies. We observed fewer IgG-producing colonic plasma cells, reduced colon-draining lymph node germinal centers, and lower T-dependent IgG in Icosl-/- mice. Surprisingly, however, we detected more CD4 T cells producing IL-10 in the Icosl-/- colon. IL-10 is an immunosuppressive cytokine that, like ICOSL, is encoded by an IBD susceptibility locus. We hypothesized that the elevated IL-10 production in the Icosl-/- colon compensates for insufficient T-dependent antibodies in preventing intestinal inflammation. We investigated the relationship between IL-10 and T-dependent antibodies by generating mice deficient in CD4 T cell derived IL-10 (Il10cKO) and ICOSL. Icosl-/-Il10cKO pups demonstrated accelerated colitis prior to 4 weeks of age. This early-onset disease could be delayed by fostering Icosl-/-Il10cKO pups to wild type dams. This strongly implies that T-dependent antibodies, even conferred through milk, cooperate with IL-10 to protect from colitis in early life. We then investigated whether this cooperation was required in adulthood. For this, we temporarily eliminated IL-10 producing cells from adult mice. Unlike wild type mice, Icosl-/- mice depleted of IL-10 producing cells rapidly developed colitis. This suggests that T-dependent antibodies also maintain intestinal homeostasis during adulthood. As depletion of IL-10 producing cells from lga-/- mice caused no disease, we determined that the protective effect was not from T-dependent IgA. However, we did determine that Icosl-/- mice have significantly reduced IgG toward colonic mucosa-associated microbes. Treating Icosl-/- mice with antibiotics against Gram-positive or anaerobic commensals averted the colitis upon depletion of IL-10 producing cells. Collectively, our data indicate T-dependent IgG toward mucosa-associated Gram-positive anaerobes synergizes with IL-10 to limit sus-
Costimulation blockade of the CD28 pathway with CTLA-4Ig has shown the ability to attenuate T cell dependent donor-specific antibody (DSA) responses in transplantation. However, CTLA-4Ig’s mechanism of binding ligands CD80/86 indiscriminately blocks both CD28 costimulatory and CTLA-4 coinhibitory pathways, potentially depriving lymphocytes of critical coinhibition. We have previously demonstrated that selective CD28 blockade provides enhanced inhibition of T follicular helper (T_{FH}), germinal center (GC) B cell and DSA responses compared to CTLA-4Ig following transplantation. T_{FH} cells differentially express substantial amounts of CTLA-4 in response to alloantigen and several studies have implicated CTLA-4 as a key regulator of their differentiation and function. Therefore, we hypothesize that the enhanced DSA inhibition observed with selective CD28 blockade is dependent upon preservation of critical CTLA-4 coinhibitory activity. Thus, we utilized a full MHC mismatched BALB/c to B6 skin allograft model to determine whether improved inhibition of the T_{FH} cell-mediated alloresponse with selective CD28 blockade is CTLA-4 dependent. Recipients received anti-CD28 domain antibody (dAb) +/- anti-CTLA-4 for analysis of T_{FH} cell-mediated responses and DSA formation. Selective CD28 blockade in the presence of CTLA-4 reversed the inhibition of DSA formation exhibited by anti-CD28 blockade alone with the development of anti-donor antibodies post-transplantation. Flow cytometric analysis of graft-draining lymph nodes following transplantation revealed significant development of T_{FH}(3.35% vs.0.46%), GC-T_{FH}(36% vs.25%), GC B(63% vs.9.7%) and plasma cell(5.3% vs.4%) responses in CD28 dAb+anti-CTLA-4 treated mice compared to anti-CD28 monotherapy. To determine whether the dependence of CTLA-4 is specific to cognate T_{FH}:B cell interactions and T_{FH} cell intrinsic, we performed ex vivo T_{FH}:B cell co-cultures in the presence of anti-CD28 dAb with and without anti-CTLA-4. CD28 dAb-mediated inhibition of B cell proliferation and differentiation into IgG^+GL7^+ GC-like B cells, and IgG antibody production were reversed with anti-CTLA-4 treatment. Additionally, anti-CTLA-4-mediated reversal was observed when T cells alone (without B cells) were exposed to anti-CTLA-4. Thus, these data indicate that selective CD28 blockade-mediated inhibition of the alloimmune GC response is CTLA-4 dependent and T_{FH} cell intrinsic, supporting the continued development of CD28 blockade-based immunosuppressive strategies that preserve CTLA-4 coinhibitory activity to improve control of GC responses and DSA formation following transplantation.

The omentum is an adipose tissue that contains milky spots (MS). The MS are similar to secondary lymphoid organs and can generate B and T cell immune responses to peritoneal antigens. Moreover, the omentum also collects metastasizing tumor cells in the peritoneal cavity and tumors growing in the omentum are associated with poor clinical prognosis. Here we show that intestinal microbiota affects Tregs activation profile and impairs tumor growth in omentum. Our transplantable tumor model in wild type (WT) mice shows that peritoneal tumors grow progressively in the omentum and peritoneal cavity in WT mice, whereas in germ free (GF) and antibiotic treated mice tumors do not grow. Omental tumor growth in WT mice is associated with an increase in PD-1^+ Tregs, while tumor-specific CD8^+ T are reduced. However, in GF mice numbers of PD1^+ Tregs remain unaltered but tumor specific CD8^+ T cells are still present. After co-housed GF mice with WT mice, ex-GF mice showed tumor...
growth and restore the increase in PD-1+ Tregs with low number of specific CD8+ T cells. These data suggest that intestinal microbiota has a role in omental Treg activation and in the tolerance to peritoneal tumors.

Presenter Saba Tufail1,2
Title Ovalbumin self-assembles into amyloid nanosheets that elicit immune responses and facilitate sustained drug release
Email sabatufail12@gmail.com
Co-authors Mohd. Asif Sherwani3, Shoaib Shoab1, Sarfuddin Azmi1, Najmul Islam1
Affiliation 1Department of Biochemistry, Faculty of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, U.P.-202002, INDIA; 2Department of Biochemistry, Faculty of Life Sciences, Aligarh Muslim University, Aligarh, U.P.-202002, INDIA; 3Department of Dermatology, University of Alabama at Birmingham, AL-35294, USA.

Amyloid fibrils are associated with many neurodegenerative diseases, motivating investigations into their structure and function. Although not linked to a specific disease, albumins have been reported to form many structural aggregates. We were interested in investigating host immune responses to amyloid fibrils assembled from the model protein ovalbumin. Surprisingly, upon subjecting ovalbumin to standard denaturing conditions, we encountered giant protein nanosheets harbouring amyloid-like features and hypothesized that these nanosheets might have potential in clinical or therapeutic applications. We found that the nanosheets, without the administration of any additional adjuvant, evoked a strong antibody response in mice that was higher than that observed for native ovalbumin. This suggests that amyloid nanosheets have self-adjuvanting property. The nanosheet-induced immune response was helper T cell 2 (Th2) biased and negligibly inflammatory. While testing whether the nanosheets might form depots for the sustained release of precursor proteins, we did observe release of ovalbumin that mimicked the conformation of native protein. Moreover, the nanosheets could load the anticancer drug doxorubicin and release it in a slow and sustained manner. Taken together, our results suggest that amyloid nanosheets should be further investigated as either an antigen delivery vehicle or as a multifunctional antigen and drug co-delivery system, with potential applications in simultaneous immunotherapy and chemotherapy.

Presenter Melissa Wolf
Title Fine Needle Aspiration Based Immune Organoids Recapitulate TME in Thyroid Cancer
Email Melissa.m.wolf.1@vanderbilt.edu
Co-Authors Melissa M. Wolf, Brad I. Reinfield, Matthew Z. Madden, W. Kim Rathmell, Vivian L. Weiss
Affiliation Vanderbilt University

Replication of the tumor microenvironment (TME) is a major limitation in organoid development as a model system for cancer research. Studies aimed at understanding the TME are typically performed in an oversimplified 2D cell culture, or utilizing complex mouse model systems. Not only are in vivo mouse studies time consuming and costly, significant differences in the mouse immune system and TME may hinder the applicability of the results in a translational paradigm. Here, we show that human specimens obtained via fine needle aspiration (FNA) from cancerous thyroid and renal cell tumors can grow and be maintained as immune organoids harboring a diverse lymphoid and myeloid population in a matrigel-based culture model. This system provides several advantages over cocultured cell lines with primary immune cells. In our FNA based method, the immune cell population obtained is obtained directly from the TME and therefore may be more representative of a tumor-associated exhaustive and/or regulatory immune phenotype. In addition, these studies provide a framework for future drug development and allow for the evaluation of a 3D organization of tumor cells with immune cells that could recapitulate physiological cancer processes. Ultimately, there is a translational gap between 2D cell culture and animal model testing; therefore, evaluation of the 3D organization of tumor cells within an ideal TME could recapitulate
Anti-nucleotide autoantibodies (e.g., anti-dsDNA) are a defining serological hallmark for systemic lupus erythematosus (SLE), with ~70% or higher of patients exhibiting this specific autoreactivity. Additionally, loss of self-tolerance is also preeminent towards RNA binding proteins/RNA:protein complexes such as Ro antigens, Smith antigens, or ribonucleoproteins. Recent evidence from our group suggests that anti-RNA nucleotide responses are significantly elevated in SLE patients with lupus nephritis, and equally as interesting, that SLE patients exhibiting an enrichment of effector B cell populations such as the CD19hi CD27− IgD− CD21− CD11c+ DN2 B cell and a closely related antigen experienced naïve population, have higher anti-RNA titers. Little is known about the concurrence of anti-DNA and anti-RNA responses in the context of SLE, leading to the overarching question of whether anti-nucleotide responses are mutually exclusive or dependent on one another in the context of SLE. With our previous finding that DN2 B cells are hyper-responsive to RNA containing TLR agonists such as R848, and lead to a serological enrichment in anti-RNA, anti-Sm, and anti-RNP autoreactivities in SLE patients, it would suggest the potential of exclusive nucleotide activation pathways which could lead to improved targeted therapies in this heterogeneous disorder. Here we show, that there is a significant elevation in anti-ssRNA titers in SLE versus healthy individuals. Additionally, this did correspond to an enrichment in DN2 and the clinical presentation of lupus nephritis. Both anti-dsDNA and anti-ssRNA responses correlated well with the presentation of the other, however, there was a stratification of patient groups which included a patient population with strong anti-ssRNA responses but little-to-no anti-dsDNA titers. Patients with the highest titers of anti-ssRNA antibodies were also the patients with the highest titers towards dsRNA (e.g. poly I:C), suggesting the potential of a virally derived disease etiology. Taken together these data initially suggest that anti-RNA autoreactivity can be mutually exclusive in SLE patients, and this may represent a population of patients whose clinical features are driven by a specific environmental trigger such as a viral infection.

Despite successful viral control, HIV-1-infected individuals maintain an increased risk of life-threatening conditions including cardiovascular, liver, and other end-organ diseases. This excess risk is associated with chronic inflammation and immune dysregulation. However, the specific mediators and mechanisms driving these pathologies are not yet understood. Neutrophils, the most abundant leukocytes, are increasingly recognized as a heterogeneous population with critical roles in both immune regulation and disease pathogenesis. Comprehensive characterization of neutrophils and neutrophil subsets is hampered by an inherent instability of neutrophils ex vivo, their tendency to form multiplets with other cell types, and high non-specific staining due to the release of cationic granular proteins following neutrophil activation. Novel methods of neutrophil characterization developed in our laboratory have facilitated the identification of several novel neutrophil subpopulations including two subsets of low-density neutrophils (LDNs). LDNs are known to be expanded in many inflammatory....
conditions including autoimmune diseases, trauma, cancer, and chronic infections. The LDN subpopulations identified in our laboratory (CD16\textsuperscript{high}CD64\textsuperscript{low} and CD16\textsuperscript{CD64\textsuperscript{high}}) have distinct phenotypic and functional properties that likely contribute to inflammation and disease progression. We present data demonstrating that HIV-1/AIDS is associated with significant alterations in the phenotype, metabolism, and gene expression of total neutrophils as well as an elevated frequency of CD16\textsuperscript{CD64\textsuperscript{high}} LDN subset. Observed changes in neutrophil populations correlate with key clinical parameters including CD4+ T cell count, years of exposure to anti-retroviral therapy, and level of liver fibrosis, a key risk factor for liver disease development. Overall, the presented data indicate a critical role of the newly identified neutrophil subsets in the pathogenesis of HIV-1/AIDS and potentially other inflammatory diseases.

---

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease in which faulty B cell tolerance promotes multiple autoantibodies with high disease specificity and clinical involvement in multiple organ systems, including the kidney (lupus nephritis). Individuals with SLE have elevated levels of Abs encoded by the VH4-34 IgH gene which can be detected by the 9G4 idiotype-specific mAb. In lupus nephritis subjects, generation of pathogenic autoantibodies results in tissue damage either through inflammatory immune complexes or by \textit{in situ} binding to the tissue antigens. In-depth analysis of the molecular and antigenic properties of different B cell subsets, which can contribute to this tissue-based response, is critical for our understanding of SLE and end-organ manifestations. To address this, we used a combination of an ultra high throughput methodology for linking the B cell receptor heavy and light chain variable region (VH and VL) on a single-cell level, coupled with next generation sequencing (NGS) for analyzing the B cell repertoire from SLE patients. Plasma blasts (CD19\textsuperscript{IgD}\textsuperscript{CD27\textsuperscript{high}}CD38\textsuperscript{high}CD138\textsuperscript{neg}), switched memory (CD19\textsuperscript{IgD}\textsuperscript{CD27\textsuperscript{+}}), double negative (CD19\textsuperscript{IgD}\textsuperscript{CD27-CD-CXCR5\textsuperscript{-}}), activated naïve (CD19\textsuperscript{IgD}\textsuperscript{CD27-CD21\textsuperscript{-}}\textsuperscript{CD24\textsuperscript{-}}\textsuperscript{CD23\textsuperscript{-}}) and resting naïve: (CD19\textsuperscript{IgD}\textsuperscript{CD27\textsuperscript{+}}) B cells from SLE patients were flow sorted, with subsequent VH and VL transcript linkage using emulsion RT-PCR. IgH, IgL and linked transcripts from blood, kidney biopsies and urine B cells were sequenced using the Illumina Miseq platform. Analysis of B cells from lupus nephritis patients showed a highly polyclonal repertoire of all subsets analyzed in the blood, kidney and urine compartments. B cells in the kidney displayed higher average mutation rate as compared to B cells in the blood compartment. Some of the expanded B cell lineages persisted over a period of 3 years in the blood and were found to be interconnected between the blood, kidney and urine compartments, along with substantial clonal expansions of the SLE-associated VH4-34 clones. Monoclonal antibodies are currently being made from these expanded lineages to test for their auto-reactivity profiles, and nephritogenic antigen binding capacity.

---

Background: Our group has previously used HLA-II associated HIV polymorphisms to predict CD4 T-cell escape in HIV. In both acute and chronic HIV infection, the adapted epitopes (AE) of these polymorphisms elicited weaker CD4 immune responses than the corresponding non-adapted epitopes (NAE). These results suggest that CD4 T cells exert immune-pres-
sure on HIV during infection. Since CD4 AE are encoded by all current clinical HIV vaccine inserts, evaluation of their immunogenicity is essential in order to optimize future vaccine design.

Methods: 88 human PBMC samples were obtained, including both vaccine recipients and placebo controls, from three different HIV-1 vaccine trials (40 samples from HVTN106, 24 from HVTN502, 24 from HVTN505). Using HLA-II binding prediction programs, epitopes containing the HLA-II associated polymorphic sites were designed that fully matched each respective vaccine immunogen sequence. Epitopes were then divided into site-specific pools containing 2-5 peptides, as well as into larger AE and NAE pools. Immunogenicity was then tested using a CD8-depleted IFN-γ ELISpot assay (n=88), as well as the more sensitive flow-based activation induced marker (AIM) assay (n=33) to identify antigen specific CD4 T cells.

Results: In the 88 vaccine samples, we identified 14 IFN-γ ELISpot responses to the site-specific pools. In addition, we saw 10 positive NAE pool ELISpot responses, while we only identified 1 positive AE pool response (p=0.013). Out of the 33 individuals tested, there was a trend towards more AIM responses to epitopes within the NAE pool, with 12 NAE pool responses and only 5 AE pool responses (p=0.09). The frequency of Ox40 and PDL1 co-expression to NAE pool was increased in comparison to the AE pool (p=0.008).

Conclusions: Our initial data demonstrate that CD4 AE contained within past human HIV-1 vaccine trials are poorly immunogenic, suggesting that vaccine inserts could be modified to enhance CD4 responses. Such optimization strategies could thereby enhance vaccine-induced antibodies which are felt to be essential for vaccines designed to prevent infection.

Presenter: Sung Hoon
Title: Hypoxia-Inducible Factors (HIF) in CD4+ T cells promote metabolism, switch cytokine secretion, and T cell help in humoral immunity
Email: sunghoon.cho@vanderbilt.edu
Co-authors: Ariel L. Raybuck, Edna Kemboi, and Mark R. Boothby
Affiliation: Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center

T cell help in humoral immunity includes interactions of B cells with activated extrafollicular CD4+ and follicular T helper (Tfh) cells. Each can promote antibody responses but Tfh cells play critical roles during germinal center (GC) reactions. After re-stimulation of their antigen receptor (TCR) by B cells, helper T cells act on B cells via CD40 ligand and secreted cytokines that guide immunoglobulin class switching. Hypoxia is a normal feature of GC, raising questions about molecular mechanisms governing the relationship between hypoxia response mechanisms and T cell help to antibody responses. Hypoxia-inducible factors (HIF) are prominent among mechanisms that mediate cellular responses to limited oxygen but also are induced by lymphocyte activation. We now show that loss of HIF in CD4+ T cells compromised essential functions in help during antibody responses. HIF-1α depletion from CD4+ T cells reduced frequencies of antigen-specific GC B cells, Tfh cells, and overall antigen-specific Ab. Further, HIF promoted CD40L expression while restraining the FoxP3-positive CD4+ cells in the CXCR5+ follicular regulatory (Tfr) population. Glycolysis increases T helper cytokine expression, and HIF promoted glycolysis in T helper cells via TCR or cytokine stimulation, as well as their production of cytokines that direct antibody class switching. Indeed, interferon-γ elaboration by HIF-deficient in vivo-generated Tfh cells was impaired. Collectively, the results indicate that HIF transcription factors are vital components of the mechanisms of help during humoral responses.

Presenter: Luke Postoak
Title: Thymic epithelial cells require the class III PI3K Vps34 for homeostasis and CD4 T lymphocyte selection
Email: joshua.l.postoak@vanderbilt.edu
Co-Authors: Shiyun Xiao2, Guan Yang1, Lan Wu1, Jinhua Zhang3, Nancy Manley2, Luc Van Kaer1
Affiliation: 1Vanderbilt University, 2University of Georgia, 3University of Alabama at Birmingham
The generation of a functional, self-tolerant T lymphocyte receptor repertoire critically depends upon interactions between developing thymocytes and antigen-presenting cells (APCs) in the thymus. The selection of T lymphocytes occurs in two steps. In the first, thymocytes that have successfully rearranged their antigen receptor and weakly interact with self-peptide/MHC on cortical thymic epithelial cells (cTECs) receive pro-survival signals. In the second step, positively-selected thymocytes traffic to the medulla where thymocytes that strongly interact with medullary thymic epithelial cells (mTECs) or dendritic cells are clonally deleted or induced into a regulatory program. Interestingly, in addition to ubiquitously expressed antigen, a subset of mTECs express the transcriptional regulator AIRE, which mediates ectopic expression and presentation of tissue-specific antigens. The presentation of self-antigens to developing thymocytes involves a variety of cellular processes, including proteolysis, endocytosis, vesicle trafficking, and autophagy. In our current study, we focus on the class III PI3K, Vps34, which has been implicated in autophagy and related processes. To address the contribution of Vps34 to TEC function, we have generated mice with a TEC-specific Vps34 deficiency. We found defects in the homeostasis of TECs in these animals especially in mTECs. Additionally, when TCR transgenic lines were bred to these animals we found profound defects in the positive selection of the CD4 T lymphocyte lineage but not the CD8 T lymphocyte lineage. In future studies, we will further characterize the cellular processes mediated by Vps34 that contribute the noted defects in TEC survival. Also, we will assess the contribution of Vps34 to negative selection by utilizing the OTII/Rip-mOVA model in mice that have an inducible deletion of Vps34 in AIRE-expressing mTECs. Collectively, these studies may identify a role for the PI3K Vps34 in maintaining TEC homeostasis and contributing to the repertoire of selecting ligands processed and presented by TECs.
<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Poster Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abad</td>
<td>Maria</td>
<td>70</td>
</tr>
<tr>
<td>Abdelhamid</td>
<td>Leila</td>
<td>44</td>
</tr>
<tr>
<td>Abreu</td>
<td>Rodrigo</td>
<td>6</td>
</tr>
<tr>
<td>Allie</td>
<td>Rameezza</td>
<td>18</td>
</tr>
<tr>
<td>Anderson</td>
<td>Ashlyn</td>
<td>24</td>
</tr>
<tr>
<td>Au-Yeung</td>
<td>Byron</td>
<td>7</td>
</tr>
<tr>
<td>Basso</td>
<td>Paulo</td>
<td>31</td>
</tr>
<tr>
<td>Brock</td>
<td>Rebecca</td>
<td>45</td>
</tr>
<tr>
<td>Collins</td>
<td>Matthew</td>
<td>25</td>
</tr>
<tr>
<td>Cooney</td>
<td>Kimberly</td>
<td>43</td>
</tr>
<tr>
<td>Cottam</td>
<td>Matthew</td>
<td>49</td>
</tr>
<tr>
<td>Crepeau</td>
<td>Rebecca</td>
<td>30</td>
</tr>
<tr>
<td>Crofts</td>
<td>Kali</td>
<td>47</td>
</tr>
<tr>
<td>Dunbar</td>
<td>Zerick</td>
<td>9</td>
</tr>
<tr>
<td>Dunbar</td>
<td>Paul</td>
<td>69</td>
</tr>
<tr>
<td>Dyevoich</td>
<td>Allison</td>
<td>55</td>
</tr>
<tr>
<td>Edupuganti</td>
<td>Srilatha</td>
<td>2</td>
</tr>
<tr>
<td>Enriquez</td>
<td>Ana</td>
<td>32</td>
</tr>
<tr>
<td>George-Alexander</td>
<td>Lou-Ella</td>
<td>14</td>
</tr>
<tr>
<td>Getzler</td>
<td>Adam</td>
<td>36</td>
</tr>
<tr>
<td>Haines</td>
<td>Robert</td>
<td>13</td>
</tr>
<tr>
<td>Hegner</td>
<td>Courtney</td>
<td>35</td>
</tr>
<tr>
<td>Jacobse</td>
<td>Justin</td>
<td>63</td>
</tr>
<tr>
<td>Jenkins</td>
<td>Meagan</td>
<td>23</td>
</tr>
<tr>
<td>Jimenez</td>
<td>Rachel</td>
<td>20</td>
</tr>
<tr>
<td>Kania</td>
<td>Anna</td>
<td>12</td>
</tr>
<tr>
<td>Kirchner</td>
<td>Stephen</td>
<td>34</td>
</tr>
<tr>
<td>Klopfenstein</td>
<td>Nathan</td>
<td>22</td>
</tr>
<tr>
<td>Li</td>
<td>Zheng-Rong</td>
<td>65</td>
</tr>
<tr>
<td>Liu</td>
<td>Mingyong</td>
<td>37</td>
</tr>
<tr>
<td>Lopez</td>
<td>Lacey</td>
<td>38</td>
</tr>
<tr>
<td>Madden</td>
<td>Matthew</td>
<td>68</td>
</tr>
<tr>
<td>Mclaughlin</td>
<td>Taryn</td>
<td>50</td>
</tr>
<tr>
<td>Melo</td>
<td>Paulo</td>
<td>56</td>
</tr>
<tr>
<td>Melot</td>
<td>Logan</td>
<td>51</td>
</tr>
<tr>
<td>Mirlekar</td>
<td>Bhalchandra</td>
<td>11</td>
</tr>
<tr>
<td>Misumi</td>
<td>Ichiro</td>
<td>10</td>
</tr>
<tr>
<td>Mohammed</td>
<td>Zahraa</td>
<td>67</td>
</tr>
<tr>
<td>Mosure</td>
<td>Sarah</td>
<td>61</td>
</tr>
<tr>
<td>Murji</td>
<td>Amyn</td>
<td>26</td>
</tr>
<tr>
<td>Navarrete</td>
<td>Karla</td>
<td>62</td>
</tr>
<tr>
<td>Neeld</td>
<td>Dennis</td>
<td>54</td>
</tr>
<tr>
<td>Nguyen</td>
<td>Doan</td>
<td>40</td>
</tr>
<tr>
<td>Opsteen</td>
<td>Skye</td>
<td>53</td>
</tr>
<tr>
<td>Patterson</td>
<td>Dillon</td>
<td>1</td>
</tr>
<tr>
<td>Peel</td>
<td>Jessica</td>
<td>21</td>
</tr>
<tr>
<td>Pham</td>
<td>Ly</td>
<td>64</td>
</tr>
<tr>
<td>Raddatz</td>
<td>Michael</td>
<td>66</td>
</tr>
<tr>
<td>Read</td>
<td>Kaitlin</td>
<td>42</td>
</tr>
<tr>
<td>Ren</td>
<td>Jingjing</td>
<td>5</td>
</tr>
<tr>
<td>Richardson</td>
<td>Shakyra</td>
<td>60</td>
</tr>
<tr>
<td>Riegler</td>
<td>Ashleigh</td>
<td>46</td>
</tr>
<tr>
<td>Rolader</td>
<td>Robin</td>
<td>48</td>
</tr>
<tr>
<td>Sancho</td>
<td>Jaime</td>
<td>41</td>
</tr>
<tr>
<td>Sautto</td>
<td>Giuseppe Andrea</td>
<td>3</td>
</tr>
<tr>
<td>Schonhoff</td>
<td>Aubrey</td>
<td>27</td>
</tr>
</tbody>
</table>
### INDEX OF POSTER NUMBERS - SESSION 1
(continued)

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Poster Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shanmugasundaram</td>
<td>Uma</td>
<td>33</td>
</tr>
<tr>
<td>Shi</td>
<td>Zhenda</td>
<td>29</td>
</tr>
<tr>
<td>Shiakolas</td>
<td>Andrea</td>
<td>57</td>
</tr>
<tr>
<td>Silva Sanchez</td>
<td>Aaron</td>
<td>58</td>
</tr>
<tr>
<td>Sponaugle</td>
<td>Alexis</td>
<td>52</td>
</tr>
<tr>
<td>Spurrier</td>
<td>Ariel</td>
<td>59</td>
</tr>
<tr>
<td>Swartwout</td>
<td>Brianna</td>
<td>4</td>
</tr>
<tr>
<td>Thaxton</td>
<td>Jessica</td>
<td>19</td>
</tr>
<tr>
<td>Tibbs</td>
<td>Taylor</td>
<td>39</td>
</tr>
<tr>
<td>Tsuda</td>
<td>Shanel</td>
<td>28</td>
</tr>
<tr>
<td>Vanwinkle</td>
<td>Peyton</td>
<td>8</td>
</tr>
<tr>
<td>Xue</td>
<td>Gang</td>
<td>15</td>
</tr>
<tr>
<td>Yang</td>
<td>Guan</td>
<td>16</td>
</tr>
<tr>
<td>Zumaquero-Martinez</td>
<td>Esther C.</td>
<td>17</td>
</tr>
</tbody>
</table>

### INDEX OF POSTER NUMBERS - SESSION 2

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Poster Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdelnabi</td>
<td>Ali</td>
<td>104</td>
</tr>
<tr>
<td>Aggarwal</td>
<td>Charu</td>
<td>83</td>
</tr>
<tr>
<td>Alkarkoushi</td>
<td>Rasha</td>
<td>128</td>
</tr>
<tr>
<td>Appleton</td>
<td>Brenna</td>
<td>98</td>
</tr>
<tr>
<td>Basu</td>
<td>Madhubsanti</td>
<td>120</td>
</tr>
<tr>
<td>Bradley</td>
<td>John</td>
<td>129</td>
</tr>
<tr>
<td>Breuer</td>
<td>Denitra</td>
<td>79</td>
</tr>
<tr>
<td>Butrico</td>
<td>Casey</td>
<td>97</td>
</tr>
<tr>
<td>Cabana-Puig</td>
<td>Xavier</td>
<td>84</td>
</tr>
<tr>
<td>Cashman</td>
<td>Kevin</td>
<td>137</td>
</tr>
<tr>
<td>Chen</td>
<td>Weirong</td>
<td>91</td>
</tr>
<tr>
<td>Chen</td>
<td>Mei Lan</td>
<td>105</td>
</tr>
<tr>
<td>Cho</td>
<td>Sung Hoon</td>
<td>141</td>
</tr>
<tr>
<td>Clemens</td>
<td>Elene</td>
<td>80</td>
</tr>
<tr>
<td>Connelly</td>
<td>Ashley</td>
<td>138</td>
</tr>
<tr>
<td>Dale</td>
<td>Gordon</td>
<td>106</td>
</tr>
<tr>
<td>Dulson</td>
<td>Sarah</td>
<td>85</td>
</tr>
<tr>
<td>Files</td>
<td>Jacob</td>
<td>140</td>
</tr>
<tr>
<td>Franchitti</td>
<td>Lavinia</td>
<td>111</td>
</tr>
<tr>
<td>Frost</td>
<td>Elizabeth</td>
<td>103</td>
</tr>
<tr>
<td>Gonzales</td>
<td>Lissia</td>
<td>81</td>
</tr>
<tr>
<td>Habib</td>
<td>Jakob</td>
<td>107</td>
</tr>
<tr>
<td>Harris</td>
<td>Levelle</td>
<td>96</td>
</tr>
<tr>
<td>Hathaway-Schrader</td>
<td>Jessica</td>
<td>100</td>
</tr>
<tr>
<td>Hayward</td>
<td>Sarah</td>
<td>95</td>
</tr>
<tr>
<td>Holbrook</td>
<td>Beth</td>
<td>101</td>
</tr>
<tr>
<td>Jenks</td>
<td>Scott</td>
<td>78</td>
</tr>
<tr>
<td>Koehler</td>
<td>Heather</td>
<td>99</td>
</tr>
<tr>
<td>La Muraglia Li</td>
<td>G. Michael</td>
<td>133</td>
</tr>
<tr>
<td>Landuyt</td>
<td>Ashley</td>
<td>132</td>
</tr>
<tr>
<td>Ley</td>
<td>Ariel</td>
<td>130</td>
</tr>
<tr>
<td>Lindsay</td>
<td>Robin</td>
<td>74</td>
</tr>
<tr>
<td>Lulu</td>
<td>Amanda</td>
<td>76</td>
</tr>
<tr>
<td>Last Name</td>
<td>First Name</td>
<td>Poster Number</td>
</tr>
<tr>
<td>---------------</td>
<td>------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Maheshwari</td>
<td>Deepti</td>
<td>124</td>
</tr>
<tr>
<td>Masjedi</td>
<td>Shirin</td>
<td>92</td>
</tr>
<tr>
<td>Matar</td>
<td>Abraham</td>
<td>127</td>
</tr>
<tr>
<td>Mcguire</td>
<td>Donald</td>
<td>113</td>
</tr>
<tr>
<td>McLaughlin</td>
<td>Erin</td>
<td>122</td>
</tr>
<tr>
<td>Mclechtie</td>
<td>Shawna</td>
<td>116</td>
</tr>
<tr>
<td>Melssen</td>
<td>Marit</td>
<td>75</td>
</tr>
<tr>
<td>Meza Perez</td>
<td>Selene</td>
<td>134</td>
</tr>
<tr>
<td>Morris</td>
<td>Anna</td>
<td>109</td>
</tr>
<tr>
<td>Morrison</td>
<td>Holly</td>
<td>89</td>
</tr>
<tr>
<td>Muir</td>
<td>Rachel</td>
<td>126</td>
</tr>
<tr>
<td>Munoz</td>
<td>Luis</td>
<td>125</td>
</tr>
<tr>
<td>Nellore</td>
<td>Anoma</td>
<td>94</td>
</tr>
<tr>
<td>Norlander</td>
<td>Allison</td>
<td>71</td>
</tr>
<tr>
<td>Ong</td>
<td>Krystle</td>
<td>88</td>
</tr>
<tr>
<td>Peek</td>
<td>Chris</td>
<td>110</td>
</tr>
<tr>
<td>Perez</td>
<td>Mildred</td>
<td>90</td>
</tr>
<tr>
<td>Petronglo</td>
<td>Jenna</td>
<td>102</td>
</tr>
<tr>
<td>Postoak</td>
<td>Joshua</td>
<td>142</td>
</tr>
<tr>
<td>Reinfeld</td>
<td>Brad</td>
<td>119</td>
</tr>
<tr>
<td>Risley</td>
<td>Chris</td>
<td>117</td>
</tr>
<tr>
<td>Rodriguez</td>
<td>Anthony</td>
<td>77</td>
</tr>
<tr>
<td>Sahoo</td>
<td>Anusmita</td>
<td>131</td>
</tr>
<tr>
<td>Salina</td>
<td>Ana</td>
<td>114</td>
</tr>
<tr>
<td>Shannon</td>
<td>Jessica</td>
<td>115</td>
</tr>
<tr>
<td>Shartouny</td>
<td>Jessica</td>
<td>93</td>
</tr>
<tr>
<td>Stampfer</td>
<td>Samuel</td>
<td>86</td>
</tr>
<tr>
<td>Stephenson</td>
<td>Allison</td>
<td>112</td>
</tr>
<tr>
<td>Stevens</td>
<td>Aaron</td>
<td>82</td>
</tr>
<tr>
<td>Sugiura</td>
<td>Ayaka</td>
<td>87</td>
</tr>
<tr>
<td>Tomar</td>
<td>Deepak</td>
<td>139</td>
</tr>
<tr>
<td>Tufail</td>
<td>Saba</td>
<td>135</td>
</tr>
<tr>
<td>Ware</td>
<td>Michael</td>
<td>108</td>
</tr>
<tr>
<td>Winn</td>
<td>Nathan</td>
<td>73</td>
</tr>
<tr>
<td>Wolabaugh</td>
<td>Amber</td>
<td>118</td>
</tr>
<tr>
<td>Wolf</td>
<td>Melissa</td>
<td>136</td>
</tr>
<tr>
<td>Yagnik</td>
<td>Bhrugu</td>
<td>121</td>
</tr>
<tr>
<td>Zhu</td>
<td>Jing</td>
<td>72</td>
</tr>
</tbody>
</table>
Cover Art Description and Credit:

“Whole mount isolation of murine olfactory epithelial tissue reveals hematopoietic cells (red, CD45) that interact with the axon tracks of olfactory sensory neurons (green, OMP-GFP). These immune cells are essential for barrier defense of the brain and help maintain tissue homeostasis in the nose.”

Image courtesy of Sebastian Wellford, Duke University.
