VANDERBILT INSTITUTE FOR INFECTION, IMMUNOLOGY AND INFLAMMATION (VI4) ANNUAL SYMPOSIUM

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THURSDAY, APRIL 5, 2018 8:00am - 6:00pm Student Life Center Ballrooms A, B, C, & Board of Trust Room



Vanderbilt Campus 310 25th Ave. South Nashville, TN 37240

VANDERBILT VUNIVERSITY MEDICAL CENTER

2018 VI4 Symposium

8:00 - 8:30 a.m. Coffee and Registration

8:30 a.m. Welcome

- 8:35 9:10 a.m. Jonathan Powell, M.D., Ph.D., Johns Hopkins Medicine *"Targeting metabolism to enhance Cancer Immunotherapy"*
- 9:10 9:45 a.m. Katrine L. Whiteson, Ph.D, University of California, Irvine "Metabolites, germs and people: Eavesdropping on human-associated microbial communities"
- 9:45 10:00 a.m. Break
- 10:00 10:35 a.m. Sergio D. Rosenzweig, M.D., Ph.D., National Institutes of Health *"IKAROS deficiency, from haploinsufficiency and CVID to dominant negative and PCP"*
- 10:35 11:10 a.m. Adam Ratner, M.D., M.P.H., New York University School of Medicine "Group B Streptococcus: from colonization to disease"

11:10 a.m. - 12:00 p.m. Lunch

- 12:00 1:45 p.m. Poster Session
- 1:45 2:15 p.m. Trainee Presentations, Ian Setliff and Jessica Sheldon, Ph.D.
- 2:15 2:50 p.m. Ward Wakeland, Ph.D., UT Southwestern Medical Center "Genetic Analysis of SLE Pathogens"
- 2:50 3:25 p.m. Jun Liu, Ph.D., Yale School of Medicine *"High-throughput cryo-electron tomography: Visualizing host-pathogen interactions at high resolution"*
- 3:25 3:40 p.m. Break
- 3:40 4:15 p.m. Gabriela Andrejeva, Ph.D., Vanderbilt University School of Medicine *"MTHFD2 as an immune modulatory target in inflammation and Multiple Sclerosis"*
- 4:15 4:50 p.m. Matt Waldor, M.D., Ph.D., Harvard T.H. Chan School of Public Health; Brigham and Women's Hospital *"Re-engineering the cholera pathogen as a probiotic for rapid protection against cholera"*
 - 4:50 p.m. Wine and Cheese Reception Begins

Notes

Vanderbilt Institute for Infection, Immunology and Inflammation (VI4)

The future of biomedical science requires a renaissance in microbiology and immunology to develop a more detailed understanding of the microbial world and our immune and inflammatory responses to it. The Vanderbilt Institute for Infection, Immunology, and Inflammation (VI4) positions Vanderbilt at the leading edge of this renaissance. With its membership of more than 100 accomplished faculty, VI4 capitalizes on the strengths of Vanderbilt University and Vanderbilt University Medical Center in areas such as personalized medicine, structural biology, vaccinology, immunometabolism, and nutrition, while simultaneously creating infrastructure required to support research into the microbiome. Through VI4, Vanderbilt fulfills its mission to be an internationally recognized leader in research that bridges the discipline of immunology and infection biology to improve human health.

The Division of Molecular Pathogenesis, Department of Pathology, Microbiology and Immunology

Center for Immunobiology

The immune system and inflammation are found more and more to influence a wide range of pathologies, including infections, diabetes, cancer, and others. The Vanderbilt Center for Immunobiology (VCI) and Vanderbilt Center for Translational Immunology and Infectious Disease (VCTIID), therefore, were designed to cross Vanderbilt University and Vanderbilt University Medical Center and promote immunology-related basic and translational science and graduate education. With approximately 70 associated faculty, the VCI and VCTIID encompass all fields of immunology and interact closely with a wide range of other Vanderbilt departments, divisions, centers, and institutes to foster and grow a community for immunologists.

Adult Infectious Diseases, Department of Medicine

The Vanderbilt Department of Medicine Division of Infectious Diseases is dedicated to enhancing prevention and treatment of infectious diseases through discovery and application of new knowledge that is seamlessly integrated with mentoring trainees to become the next generation of national leaders in the field. This mission is enhanced by our strong emphasis on interdisciplinary collaboration, social and intellectual diversity, commitment to life-long self-learning, and professionalism dedicated to team work and collegiality.

Pediatric Infectious Diseases, Department of Pediatrics

The Division of Pediatric Infectious Diseases at Vanderbilt is committed to the generation of new knowledge through research, teaching and training of physicians and scientists, and delivery of superb clinical care with the overarching goal of improving childhood health. The Division enjoys a national and international reputation of excellence in pediatric infectious diseases. Research interests of Division investigators span microbial pathogenesis, vaccine development, human immunology, clinical evaluation of vaccine safety and efficacy, infectious disease epidemiology, antimicrobial stewardship, and infection control. The Division is strengthened by several groups at Vanderbilt, including the Institute for Global Health, Lamb Center for Pediatric Research, Vanderbilt Vaccine Center, and Vanderbilt Vaccine Research Program.

The Division of Rheumatology & Immunology, Department of Medicine

Systemic autoimmune inflammatory diseases and musculoskeletal disorders are among the most common causes of morbidity and disability. Clinicians and scientists in the Division of Rheumatology & Immunology are focused on basic discovery and translational studies that bring the latest scientific advances to patients with rheumatic diseases. The Division has important efforts focused towards the understanding of diseases such as rheumatoid arthritis and systemic lupus erythematosus. Within the unique environment at Vanderbilt, we are using advanced technologies spanning basic discovery to human physiology to informatics approaches to epidemiology as we seek to improve the outcomes of our patients. Our educational programs assure that future generations of clinicians and scientists will be well prepared to apply new knowledge towards prevention, treatment and cure of rheumatic diseases.

VI4 Symposium Hosts

The Vanderbilt Pre³ Initiative - Preventing Adverse Pregnancy Outcomes and Prematurity

Adverse pregnancy outcomes are a major cause of death and disability for mothers and babies. Globally, nearly 50 Million pregnancies suffer from at least one major complication each year. Preterm birth (occurring prior to the 37th week gestation) is a major contributor to this burden and increases the risk for other complications such as neonatal death, infection of the newborn, and serious end-organ disease in surviving children. The Vanderbilt Pre³ Initiative (Preventing adverse Pregnancy outcomes & Prematurity) represents a trans-disciplinary community of faculty and learners with a shared interest in improving pregnancy outcomes through discovery, education, innovation, and implementation. The Vanderbilt Pre³ Initiative is supported by a Vanderbilt Transinstitutional Program award.

Center for Structural Biology

The Vanderbilt Center for Structural Biology (CSB) is part of a major transinstitutional initiative started in the year 2000. The initiative was designed to significantly upgrade the capabilities in Structural Biology at Vanderbilt by bringing additional faculty and state-of-the-art instrumentation to campus. The CSB was developed to promote the broad use of structural biology approaches in all life science research and to provide a focal point that bridges medicine and biology to math, chemistry, and physics.

The philosophy of the CSB at Vanderbilt is to integrate the application of all techniques that can provide the atomic resolution structure of biomacromolecules. The merging of applications of the traditional high resolution structural biology disciplines, X-ray Crystallography, NMR Spectroscopy, and Computational Biology, along with electron microscopy and fluorescence and EPR spectroscopies, is especially unique. This strategy is being increasingly recognized as the necessary approach to solve fundamental structural problems in medicine and biology. Establishing an environment that provides access to all available tools provides Vanderbilt investigators with key competitive advantages. This philosophy is being successfully applied in exciting ways in collaborations with investigators from a range of Departments in both the College of Arts and Science and The School of Medicine.

The Vanderbilt Microbiome Initiative

The Vanderbilt Microbiome Initiative (VMI) will provide Vanderbilt the opportunity to become the first university that unifies a major precision medicine initiative with personalized microbiome studies. The VMI will blend Vanderbilt's clinical, basic, translational and educational endeavors into a community of 100+ microbiome scholars who will catalyze development of new microbiome research and education projects. As 21st century health care moves its focus toward precision medicine and self-tracking, microbiome samples that affect health and disease will need to be tracked, databased and integrated with human genome data to develop diagnostic, preventative and therapeutic approaches for improving health.

This project brings together multidisciplinary, but complementary expertise in Biology, Microbiology, Virology, Genetics, Nutrition, Metabolism, Pathology, Computer Engineering and Education to create an infrastructure that unifies the microbiome community at Vanderbilt. The initiative will also catalyze campus-wide research and teaching activities, and deploy state-of-the-art technologies and methods to advance the microbiome sciences.

Symposium Speakers



Gabriela Andrejeva, Ph.D.

Vanderbilt University School of Medicine

Gabriela Andrejeva has research interests lying in the understanding of how cell metabolism influences the function, stability and longevity of T cells and how different transcriptional and signaling programs are interdependent with T cell metabolic phenotypes. She is a Postdoctoral Research Fellow, in the Rathmell Lab, at Vanderbilt University School of Medicine.

Jun Liu, Ph.D. Yale School of Medicine



Jun Liu has been working in the field of electron microscopy for 20 years. In particular, he gained expertise in cryo-electron tomography (cryo-ET) after working with Prof. Ken Taylor at Florida State University and Dr. Sriram Subramaniam at NIH. Since he started his own laboratory in 2007, he has been dedicated to developing high-throughput cryo-ET pipeline in which both data collection and image analysis are streamlined and automated. The high-throughput cryo-ET pipeline is becoming increasingly powerful, enabling his laboratory to visualize over 100,000 cells from 100 different bacterial species. More importantly, the massive data from cryo-ET has been systematically utilized to gain structural insights into fundamental biological processes related to signaling transduction, flagellar assembly, protein secretion, phage adsorption DNA translocation, and host-pathogen interactions. Dr. Liu has published more than 60 papers in journals that include Nature, Science, PNAS, and Cell.



Jonathan Powell, M.D., Ph.D.

Johns Hopkins Medicine

Jonathan Powell is a Professor of Oncology and Pharmacology at Johns Hopkins University School of Medicine. He received his AB from Dartmouth College and his M.D. Ph.D. from Emory University School of Medicine. His Post-graduate clinical training included the Osler Internal Medicine Residency Program at Johns Hopkins and Fellowship training in Hematology-Oncology at The Brigham and Women's Hospital in Boston and NHLBI at the NIH. While at the NIH Dr. Powell was a post-doctoral fellow in the Laboratory of Dr. Ronald Schwartz. He joined the faculty of Johns Hopkins in 2001 and heads up a basic research lab that focuses on T cell biology and immunometabolism. Clinically Dr. Powell's focused has been Bone Marrow Transplantation and Immunotherapy. Dr. Powell is currently an Associate Director of the Bloomberg~Kimmel Institute for Cancer Immunotherapy.

Symposium Speakers



Adam Ratner, M.D., M.P.H. New York University School of Medicine

Adam Ratner is the Division Director of Pediatric Infectious Diseases at New York University (NYU)-Langone Medical Center and an Associate Professor of Pediatrics and Microbiology at NYU. Adam received his B.A. from Yale University and his M.D. and M.P.H. degrees from Columbia University. He completed his pediatric residency at Columbia University / Babies' Hospital and fellowship training in Pediatric Infectious Diseases at the Children's Hospital of Philadelphia. Dr. Ratner heads an active, NIH-funded translational research laboratory focused on understanding and preventing infections in pregnant women and newborn infants, including Group B Streptococcus. His areas of particular interest include new vaccine development and combining molecular, small animal, and human studies to understand the biology of infectious diseases.

Sergio D. Rosenzweig, M.D., Ph.D.



National Institutes of Health

Sergio D. Rosenzweig is a pediatrician and clinical immunologist with more than 20 years of experience in the field of primary immunodeficiency. Sergio is the Chief of the Immunology Service, Department of Laboratory Medicine, NIH Clinical Center, NIH, as well as the Co-Director of the Primary Immunodeficiency Clinic, National Institutes of Allergy and Infectious Diseases, NIH.

Dr. Rosenzweig earned his medical degree and his PhD from the University of Buenos Aires, Argentina in 1989 and 2006, respectively. He completed his pediatric residency in 1993 and his clinical immunology fellowship in 1996, both at the National Pediatric Hospital, in Buenos Aires, Argentina. Between 2000 and 2003 he joined the Immunopathogenesis Unit, Laboratory of Host Defenses, NIAID, NIH under Dr. Steven M. Holland where he studied the interferon gamma signaling pathway and the molecular basis for genetic susceptibility for mycobacterial diseases. After leaving NIH, he continued with his interferon gamma pathway research at the National Pediatric Hospital, in Buenos Aires. In 2009 he returned to NIH as Chief of the Infectious Diseases Susceptibility Unit, Laboratory of Host Defenses, and Co-Director of the Primary Immunodeficiency Clinic, NIAID, NIH. In 2013 he joined the Department of Laboratory Medicine, NIH Clinical Center, under Dr. Thomas A. Fleisher, where he serves as the Immunology Service Chief.

Dr. Rosenzweig has extensible published in the field of primary immunodeficiency and genetic susceptibility to particular infectious diseases; he is an associated editor for different journals in the field of clinical immunology, and he has been elected president for the Clinical Immunology Society.

Symposium Speakers



Ward Wakeland, Ph.D. UT Southwestern Medical Center

Edward K. Wakeland an internationally recognized immunologist, holds the Edwin L. Cox Distinguished Chair in Immunology and Genetics and is a Professor in the Department of Immunology. He joined the faculty of the University of Texas Southwestern Medical Center in 1998 and is the founding Director of the Genomics and Microarray Core facility. He is also the Scientific Director of the Next Generation Sequencing Clinical Laboratory and its Research Genomics Core.

An expert on the genetic basis for susceptibility to autoimmune disease, Dr. Wakeland's labs have made significant advances in the study of lupus. He is the author of more than 220 scientific publications. He also pioneered the application of NGS to the analysis of genetic risk loci for systemic autoimmunity in human populations. The primary focus of his current research has been the development of genomic technologies for the advancement of personalized medicine.

Dr. Wakeland was a member of the National Science Foundation Advisory Panel for Eukaryotic Genetics and the National Institutes of Health Genetics of Health and Disease, Immunological Sciences and Biological Sciences Study sections. He was also the Chair of the NIH Special Emphasis study section, the Chair of the Genetics Initiative Planning Committee for the Alliance for Lupus Research, and a member of the American Cancer Society National Scientific Advisory Committee for Immunology.

He has served on the editorial boards or review boards of Current Opinion in Immunology, Arthritis and Rheumatology, and Immunogenetics. Dr. Wakeland has mentored nearly 50 doctoral students and postdoctoral fellows from the United States, Europe and Asia. He has been a visiting Professor at the University of Cambridge, Tokyo University and the Chinese National Academy of Science.

Matt Waldor, M.D., Ph.D. Harvard T.H. Chan School of Public Health Brigham and Women's Hospital



Matthew Waldor is Professor of Medicine and a physician in the Division of Infectious Diseases at Brigham and Women's Hospital, and an investigator of the Howard Hughes Medical Institute. His lab investigates pathogen-host interactions, particularly in the context of animal models that closely mimic human infections. Work is primarily focused on enteric pathogens including Vibrio cholerae, enterohemorrhagic Escherichia coli (EHEC) and Shigella. Recently his group developed a combined probiotic and vaccine for cholera.



Katrine L. Whiteson, Ph.D.

University of California, Irvine

Katrine Whiteson is an Assistant Professor at University of California Irvine and the Associate Director of the recently launched UCI Microbiome Initiative. She studied Biochemistry at UC Berkeley (BA, 2000) and University of Chicago (PhD, 2007). She first had the opportunity to study human-associated microbes at the University of Geneva Hospitals in 2008. Dr. Whiteson focused on the oral microbial communities of healthy Europeans, and malnourished kids in Niger who develop a devastating facial gangrene known as noma. In 2011 she moved to Prof. Forest Rohwer's lab at San Diego State, where she undertook breath and sputum metabolite analysis to better understand the activity of Cystic Fibrosis patient microbial communities. Combining sequencing and metabolomics data is a powerful approach that Dr. Whiteson has continued to employ since starting a lab at UCI in 2014.

1 Inhibition of Coronaviruses by β-D- N^4 -hydroxycytidine (NHC)

Maria L. Agostini, Erica L. Andres, James D. Chappell, Amy C. Sims, Rachel L. Graham, Timothy P. Sheahan, Michael G. Natchus, George R. Painter, Ralph S. Baric, Mark R. Denison

The recent emergence of very virulent epidemic coronaviruses (CoVs), such as severe acute respiratory syndrome (SARS)-CoV and Middle East respiratory syndrome (MERS)-CoV, emphasizes the need for broadly active antivirals that inhibit a range of CoVs. There are currently no approved antivirals or vaccines for the treatment and prevention of CoV infections. Development of nucleoside-based antivirals for CoV infections has been hampered by the presence of a viral proofreading 3'-5' exoribonuclease (ExoN). β -D- N^4 -hydroxycytidine (NHC), also known as EIDD-1931 (Emory Institute for Drug Development), has been reported to inhibit multiple viruses, including several alphaviruses, Ebola virus, and hepatitis C virus. Here, we report that NHC inhibits CoVs, including murine hepatitis virus (MHV) with a 50% effective concentration (EC₅₀) value of 0.17 μ M and no cytotoxicity across a wide range of concentrations. Addition of exogenous cytidine and uridine restored viral replication in the presence of NHC, suggesting that NHC competes with natural pyrimidine nucleotides for metabolism and incorporation into CoV RNAs. To specifically address the role of ExoN in CoV inhibition by NHC, we tested a proofreading-deficient mutant [ExoN(-)] of MHV. ExoN (-) virus exhibited increased sensitivity to NHC compared to wild-type virus, manifested by lower infectious yields and lower EC₅₀ values. Together, these results support further development of NHC for treatment of CoV infections as well as investigations into the mechanism by which NHC inhibits CoV replication.

2 Modified Dendrimer Targeting the translation initiation complex of ZIKV Inhibits Virus Replication: Implications for Therapy

Waldemar Popik, Atanu Khatua, James E.K. Hildreth, Benjamin Lee and Donald J. Alcendor

Background: Zika virus (ZIKV) infection has been associated with a sporadic increase in the incidence of Guillain-Barré syndrome and microcephaly in infants. Currently there is no treatment or vaccine for ZIKV infection. Here we explore the use of dendrimer based technology targeting the nucleotide translation initiation complex site of ZIKV for antiviral development.

Methods: The modified dendrimer DWK1 targeting the nucleotide translation initiation complex site of ZIKV was used in an infectivity assay at 10 uM for inhibition of ZIKV replication. Inhibition of ZIKV infectivity in human glomerular podocytes treated with DWK1 was analyzed by quantitative real time polymerase chain reaction (qRT-PCR), western blot analysis, immunofluorescence, and fluorescent focus assay (FFA). We also examined proinflammatory cytokines induced in ZIKV infected podocytes pretreated with DWK1 treatment.

Results: We show a 1438-fold or 99.9% reduction in ZIKV transcription by qRT-PCR in glomerular podocytes after a 24 h pretreatment with 10uM of DWK1 followed by 72h exposure to ZIKV in the absence of DWK1. In addition, we demonstrate by immunoblot analysis a complete suppression of ZIKV protein expression in infected podocytes treated with DWK1. We also show highly reduced levels of ZIKV proteins expressed in infected podocytes treated with DWK-M1 by immunofluorescence and fluorescent focus assays. We observed a suppression of ZIKV induced proinflammatory cytokines namely RANTES (regulated on activation, normal T cell expressed and secreted), TNF- α (tumor necrosis factor- α), MIP-1 α (macrophage inflammatory protein 1 alpha), and INF β (interferon beta) in podocytes pre-treated with DWK1, compared to controls.

Conclusions: This study shows that the ZIKV specific modified dendrimer DWK1 is a novel antiviral that can effectively inhibit ZIKV replication and suppress ZIKV induced inflammation in vitro. DWK1 is a non-toxic compound, stable at room temperature that can penetrate target cells. DWK1 has unique features of a potential therapeutic antiviral for ZIKV.

3 Identification of an Inflammatory Monocyte Transcriptional Profile and Potential Novel Role for Lactotransferrin in Human Hypertension

Matthew R Alexander, Allison E Norlander, Fernando Elijovich, Ravi V. Atreya, Juan S. Gnecco, Cheryl L. Laffer, Cristi L. Galindo, & Meena S. Madhur

Hypertension affects approximately half of all individuals in the United States and is the leading risk factor for global morbidity and mortality. Emerging evidence suggests an important role for monocytes in the pathogenesis of hypertension, though the mechanisms by which monocytes promote hypertension are unclear. We set out to determine, in an unbiased fashion, whether patients with hypertension exhibit altered gene expression in peripheral monocytes, and whether differentially expressed genes may play a pathogenic role in human hypertension. RNA sequencing of peripheral monocytes identified 60 differentially expressed transcripts in patients with hypertension compared to controls, with significant over-representation of transcripts related to inflammation by gene ontology analysis. In addition, of the differentially expressed genes, expression of four genes significantly correlated with mean arterial pressure in hypertensive and/or control patients by univariate and multivariate stepwise regression. Three of these genes, lactotransferrin (LTF), peptidoglycan recognition protein 1 (PGLYRP1), and interleukin-18 receptor accessory protein (IL18RAP), remained significantly elevated in patients with hypertension in a separate validation cohort. An initial phenome-wide association study of a missense single nucleotide polymorphism in LTF (rs1126478) that decreases antimicrobial activity and increases protein levels revealed a nonsignificant trend for over-representation of hypertension ICD9 codes in patients with the minor allele. A subsequent retrospective case-control study found increased frequency of rs1126478 minor allele homozygosity in patients with hypertension relative to controls (odds ratio 1.16; p=0.005). In addition, minor allele homozygosity significantly increased odds of hypertension in a logistic regression analysis controlling for age, gender, body mass index, coronary artery disease, and diabetes mellitus (p=0.017). Taken together, these data demonstrate that monocytes exhibit enhanced pro-inflammatory gene expression in hypertensive patients and identify LTF, an iron-binding glycoprotein with antimicrobial and pro-inflammatory function, as a potential novel mediator of human hypertension.

4 IL-21 modulates dendritic cell function in response to Helicobacter pylori

Sharia Yasmin, Beverly R.E.A. Dixon, and Holly M. Scott Algood

Helicobacter pylori is a dominant member of the gastric microbiota in a majority of the world's population. *H. pylori* colonization can lead to gastritis, peptic ulcers and gastric cancer. We identified interleukin-21 (IL-21), a cytokine produced by many subsets of activated CD4+ T cells and NK cells, as a critical driver of gastritis during *H. pylori* infection. IL-21 is a pleiotropic cytokine and its receptor is present on a number of cell types including lymphocytes, dendritic cells (DCs) and epithelial cells. Our published data indicate that concomitant with protection from chronic inflammation, *H. pylori*-infected IL-21^{-/-} mice exhibited limited Th1 and Th17 responses in their gastric mucosa. It was reported previously that IL-21 can inhibit impact dendritic cell function, but much of the data was based on dendritic cell differentiation assays. The major goal of this study is to investigate whether IL-21 modulates DC responses during *H. pylori* infection. We tested the hypothesis that IL-21 regulates DC function through controlling cytokine production and altering DC-mediated antigen specific T cell responses. IL-21 did reduce the ability of dendritic cells to produce pro-inflammatory cytokines in response to *H. pylori*, but did not impact unstimulated DC cytokine production. *H. pylori* increased expression of B7.1, B7.2 and MHC Class II on DCs, but IL-21 did not impact the expression of these markers in the presence of *IL-21*. Ongoing experiments will determine if IL-21 impacts the ability of DCs to induce antigen specific proliferation. These data suggest that IL-21, while pro-inflammatory in most settings, may downregulate the inflammatory phenotype of dendritic cells in the presence of *H. pylori*.

5 Mechanism of Dominant-Negative Inhibition of HIV-1 Maturation by Uncleaved Gag

Jordan Anderson-Daniels and Christopher Aiken

Maturation is a critical step in the HIV-1 replication cycle and occurs during budding of a nascent particle from a host cell. During maturation the Gag polyprotein is specifically processed by the viral protease into its constitutive proteins: matrix (MA), capsid (CA), nucleocapsid (NC), and p6. The proper processing of Gag is essential for infectivity. A recent study demonstrated that while mutations which disrupt cleavage of any sites in Gag impair HIV-1 infectivity, cleavage disruption at MA-CA is highly transdominant when incorporated into HIV-1 particles. Inclusion of 10% of the MA-CA cleavage mutant (Y132I) is sufficient to inhibit HIV-1 infectivity by ~95%. While the resulting viral particles exhibited aberrant capsids, the precise antiviral mechanism of MA-CA inclusion remains unknown. Biochemical analysis of WT/MA-CA mixed cores revealed no loss in core protein or genomic content and WT levels of core recovery after membrane removal, suggesting that mixed cores do not have a stability defect. In a complementary experiment, we observed that WT/MA-CA mixed particles abrogate restriction of TRIMCyp in target cells at WT levels, providing further evidence that the mixed cores do not exhibit an instability phenotype. Using a disulfide cross-linking approach, the effect of MA-CA inclusion on CA-CA interface formation was monitored. MA-CA co-transfection did not result in aberrant cross-linking at any of the CA-CA interfaces, which supports the abrogation data that MA-CA mixed cores form hexameric structures that can be recognized by TRIMCyp. Surprisingly, we did not observe an impairment to reverse transcription from MA-CA mixed particles, in contrast with a previous report. Thus, MA-CA impairs infection at nuclear entry and/ or integration. We plan to map the genetic determinants in MA and CA required for transdominant activity. A detailed understanding of the MA-CA antiviral mechanism may reveal novel insights into the role of maturation post cellular entry.

6 MiR-22 as a regulator of immune homeostasis and its pathogenic role in systemic lupus erythematosus

Brenna D. Appleton, Ashley W. Faust, Danielle L. Mitchell, Jared L. Moore, Kasey C. Vickers & Amy S. Major

Systemic lupus erythematosus (SLE) is a devastating autoimmune disease affecting over 1.5 million Americans and at least 5 million individuals worldwide. There is evidence to demonstrate that both autoantibody producing B cells as well as dysfunctional CD4⁺ T cells contribute to SLE pathology, however a lack of understanding surrounding underlying mechanisms of disease pathogenesis have prevented therapeutic advancement. Studies indicate one mechanism for dysregulated immune homeostasis in autoimmunity is through intra- and intercellular communication via microRNAs (miRNAs). MiRNAs are small (~22 nucleotides), endogenous post-transcriptional regulators of gene expression which act by degradation or translational repression of target mRNAs. In preliminary studies from our group, miR-22 was enriched on high density lipoprotein (HDL) from SLE patients compared to healthy controls. These findings were validated by qPCR of miR-22 in plasma RNA from a separate cohort of patients and controls. MiR-22 is highly conserved in vertebrates and has been studied in the disease processes of cancer, emphysema, and cardiovascular disease. Several lines of evidence suggest miR-22 could also play a role in autoimmune disease. Studies from our laboratory determined that treatment of lupus-prone B6.SLE.1.2.3 mice with locked nucleic acid-22 (LNA-22), a specific miR-22 inhibitor, decreased early anti-dsDNA antibody titers, CD4⁺ T cell activation, and frequency of IFN-y⁺ CD4⁺ T cells. Data suggest blunted autoimmune responses in LNA-22 treated B6.SLE1.2.3 mice ultimately led to a decrease in antibody deposition in kidney glomeruli and significant reductions in kidney pathology. Additionally, when naïve CD4⁺ T cells from B6 mice were skewed in vitro to a Th1 phenotype, miR-22 was increased compared to non-skewed controls. However, preliminary data suggest inhibition of miR-22 is not sufficient to prevent Th1 polarization. Together, these data lead us to conclude that, while miR-22 is not necessary for initiation of Th1 responses, it may influence immune homeostasis promoting inflammation and autoimmunity.

7 N6-methyladenosine-dependent regulation of the pre-replicated Chikungunya viral genome

Sarah Arcos, Byungil Kim, Katherine Rothamel, Yuqi Bian, Seth Reasoner, and Manuel Ascano

The battle between viral RNA and host factors begins the instant the viral genome enters a cell. Prior to transcriptional upregulation of interferons, cytokines, and antiviral genes, cells must rely on mRNAs and proteins that already exist in their cytoplasmic arsenal. Viruses also benefit from any mechanism they can utilize to co-opt host processes before the onslaught of new defenses from transcription arrives. However, the molecular events that comprise these primary interactions are not well understood. We developed a novel method to identify proteins that directly bind to pre-replicated, primary, viral RNA genomes that we term VIR-CLASP for Viral Cross-Linking And Solid-phase Purification. Our approach is amenable to essentially any RNA virus, and captures interactions that occur within minutes of viral entry. We used this approach to identify hundreds of host RBPs that interact with the primary Chikungunya virus (CHIKV) RNA genome, including the YTHDF family of N6methyladenosine (m6A) binding proteins. We established that m6A-modifications are abundant on CHIKV genomic RNA. m6A is involved in regulating many aspects of cellular RNA metabolism, and can modulate the pathogenicity of several RNA viruses including Zika, HIV, and Influenza. However, the biological impact of interaction between YTHDF proteins and primary viral genomes is unknown. We discovered that the effect of YTHDFs on CHIKV viral replication is subject to combinatorial regulation: knockdown and over-expression studies revealed that YTHDF1 strongly restricts viral replication, while YTHDF2 and YTHDF3 have the opposite effect. Our data indicate that YTHDF proteins have distinct regulatory effects on CHIKV replication. There are currently no direct therapeutic options available to those at risk for CHIKV infection and the debilitating, long-lasting joint pain that follow. VIR-CLASP aims to increase the known repertoire of drug targets for CHIKV, and other RNA viruses, in order to advance current efforts to design vaccines and anti-viral compounds.

8 HIV-1 Capsid Protein Inhibits Viral DNA Integration

Muthukumar Balasubramaniam^{1,2}, Jing Zhou⁴, Jui Pandhare^{1,3}, Chris Aiken⁴, Chandravanu Dash^{1,2}

¹Meharry Medical College, Center for AIDS Health Disparities Research , Nashville, TN, ²Meharry Medical College, Biochemistry and Cancer Biology, Nashville, TN, ³Meharry Medical College, School of Graduate Studies and Research, Nashville, TN, ⁴Vanderbilt University Medical Center, Pathology, Microbiology and Immunology, Nashville, TN

The HIV-1 capsid protein (CA) is a multifunctional viral protein and an attractive target for antiviral therapy. While it is well established that CA is involved in reverse transcription and nuclear entry, whether and how CA affects integration is not well understood. We employed the CA-targeting antiviral compound PF74 as a probe for capsid function in target cells. At a low concentration (0.2 µM). PF74 effectively inhibits HIV-1 infection without reducing reverse transcription. Quantitative analysis of 2-LTR circle formation revealed a 50% reduction in nuclear entry by PF74, an effect that did not quantitatively account for the observed inhibition of infection. Accordingly, PCR analysis of integration in target cells revealed a 95% reduction of integrated HIV-1 DNA following infection with PF74, suggesting that PF74 also inhibits integration in target cells. To determine whether PF74 directly affects the formation of functional preintegration complexes (PICs), we isolated PICs from cytoplasmic and nuclear compartments and assayed their integration activity in vitro. Surprisingly, HIV-1 infection in the presence of PF74 resulted in PICs that exhibited increased integration activity relative to the no-drug control. Enhancement of PIC activity was attributed to an effect of PF74 on CA, as the compound did not increase the activity of PICs produced from a PF74-resistant CA mutant. Since PF74 has been reported to affect capsid stability, we hypothesized that PF74 affects integration activity by altering CA levels in the PICs. Gradient purification of PICs revealed that PICs assembled in the presence of PF74 contained lower levels of CA compared to untreated PICs, suggesting a negative correlation between CA and PIC-associated integration activity. To test for a link between levels of PIC-associated CA and integration activity, we measured the effects of added recombinant CA protein on integration activity. Addition of wild type CA to PICs inhibited integration activity, while addition of an oligomerization-defective CA mutant (W184A/M185A) protein showed no inhibitory effect. These results demonstrate that CA can inhibit HIV-1 integration and are consistent with a model in which efficient HIV-1 integration requires a final uncoating step occurring prior or subsequent to nuclear entry of the PIC.

9 Fetal sex modifies placental gene expression in response to metabolic and inflammatory stress

Theresa L. Barke, Kelli Money, Ana Serezani, Liping Du, Karoly Mirnics, David M. Aronoff

Problem: Metabolic stress (e.g., gestational diabetes mellitus (GDM) and obesity) and infections are common problems during pregnancy, impacting fetal development and the lifelong health of offspring. Such perturbations can co-exist within the same pregnant mother. Male fetuses appear to be more susceptible to many pregnancy complications than female.

Methods of Study: Female C57BL/6 mice were fed either a high fat diet (HFD), to induce a "diabesity"-like state (obesity + GDM), or a normal chow diet for 6 weeks prior to mating. Dams within each diet group at embryo (E) day 12.5 were then either given an intraperitoneal injection of Poly(I:C), a synthetic dsRNA analogue that stimulates TLR3, provoking a state of maternal immune activation (MIA), or saline. 3hr after Poly(I:C) injection (MIA) dams were euthanized and the placentae were collected and analyzed via Nanostring® transcriptomics assessing expression of a panel of 254 inflammatory genes. Fetal sex was included as a variable in multivariable logistic regression.

Results: Principal coordinate analysis (PCA) of gene expression profiles revealed strong separation of transcript profiles based on exposure to Poly(I:C) (MIA) compared to saline. Metabolic stress (HFD) was less influential. For 38 genes, the impact of MIA on expression was significantly associated with sex. The response to HFD was impacted by fetal/placental sex for 18 genes. Within mice who all received HFD, Poly(I:C) modulated 8 genes in a sex-dependent manner. Within mice exposed to MIA, the diet (HFD vs. saline) impacted the expression of 24 genes in a sex-dependent fashion. For mice exposed to both HFD and MIA vs. normal diet, saline-injected controls 8 genes were differentially expressed between male and female.

Conclusion: We observed sex-specific placental gene regulation within mice exposed to metabolic and/or inflammatory immune activation, which might underpin sexual dimorphism in adverse pregnancy outcomes in human offspring exposed to similar stressors. Supported by NIH grant F31 DK108652.

10 The PathLink Acquired Gestational Tissue Bank: Feasibility of Project PLACENTA

<u>Kisha Batey</u>, Jodell E. Linder, Rebecca Johnston, Ethan M. Cohen, Yu Wang, Xiaoming Wang, Lisa M. Rogers, William Hayes McDonald, Michelle L. Reyzer, Audra Judd, Jeffery Goldstein, Hernán Correa, Jill Pulley, David M. Aronoff

Background: The Vanderbilt Institute for Clinical and Translational Research piloted the development of Project PLACENTA (<u>PathLink Acquired gEstatioNal Tissue bAnk</u>). This project investigated the feasibility of a fresh gestational tissue biobank, which provides tissue linked to electronic medical records for investigators interested in maternal-fetal health.

Methods: We developed a pipeline for collection of placental tissue from Labor & Delivery within approximately 30 minutes of delivery. An email alert was developed, to signal delivery, with the ability to specifically flag patients with certain phenotypic traits. Once collected, 4 to 8 mm punch biopsy cores were snap frozen and subsequently used for RNA, DNA and protein extraction. Tissue was also collected for Formalin Fixed Paraffin Embedded (FFPE) histology, flow cytometry, and quality control measures.

Results: Of 60 deliveries using the email notification system, 25 (42%) were sent to Pathology or assigned to other research protocols and were not available for collection, 10 (16%) were discarded prior to arrival at L&D, and 25 (42%) were available for collection. Twenty placentas were collected and averaged 38 minutes per collection. DNA extraction yielded an average of 53 µg/µl per sample and RNA extraction yielded 679 ng/µl on average per sample. Proteomic studies showed no degradation of protein, abundant and similar quantities of protein across samples and differentiation between the amnion, decidua, and villi. Histological studies showed good quality for interpretation and occasional pathology including multifocal chronic villitis, meconium laden macrophages, and Stage 2 acute chorioamnionitis. Flow cytometry demonstrated good cell viability after isolation.

Conclusions: Collection of fresh gestational tissue is feasible. After successful piloting of collection techniques, Project PLACENTA received IRB approval to consent patients and link tissue to electronic health records. We are currently planning to enroll participants and shorten the arrival time after the email alert to allow for a greater number of placentas to be collected.

11 Bactericidal Mechanisms of Arachidonic Acid against Staphylococcus aureus

William N. Beavers, Venkataraman Amarnath, Raymond L. Mernaugh, Sean S. Davies, L. Jackson Roberts II, Eric P. Skaar

Staphylococcus aureus is a pathogen capable of infecting nearly every organ in the vertebrate host. The pervasiveness of this pathogen and the widespread overuse of antibiotics have heralded the emergence of antimicrobial resistant strains, including methicillin resistant S. aureus (MRSA). This presents the desperate need to identify and validate targets for the development of new therapies. Arachidonic acid (AA) is a polyunsaturated fatty acid produced by humans, but not by bacteria. In response to bacterial infections, AA serves as a precursor to both pro- and anti-inflammatory bioactive lipid metabolites. Additionally, AA is bactericidal to S. aureus independent of its role in signaling. We discovered that AA is bactericidal to S. aureus through a lipid peroxidation mechanism, where AA is oxidized to isolevuglandin (IsoLG). IsoLGs elicit toxicity in eukaryotic cells by reacting irreversibly with the ε-amine of lysine, often compromising protein function; however, this mechanism has not been defined in bacteria. AA toxicity is alleviated in S. aureus with the administration of antioxidants as well as IsoLG specific scavengers. Further, this toxicity is exacerbated through the increased generation of reactive oxygen species. To further understand the mechanisms by which S. aureus avoids AA toxicity, we selected for resistant mutants. Whole genome sequencing identified a non-sense mutation in LytR-Cpsa-Psr A (IcpA); and deletion of IcpA confers S. aureus with resistance to AA toxicity. LcpA ligates wall teichoic acids (WTA) to the cell wall, indicating that WTA may sensitize S. aureus to AA toxicity. However, blocking the synthesis of WTA through tarO deletion or inhibition sensitizes S. aureus to AA toxicity, indicating a more nuanced role for *ΔlcpA* and WTA in resisting polyunsaturated fatty acid stress. Future experiments will define the molecular mechanisms by which *ΔlcpA* alleviates AA toxicity in S. aureus.

12 Loss of cytochrome bd-I disrupts UPEC biofilms and increases penetration by antibiotics

Connor Beebout, Allison Eberly, John Brannon, Shuvro De, Madison Fitzgerald, Douglass Clayton, and Maria Hadjifrangiskou

Uropathogenic *Escherichia coli* (UPEC), the most common cause of urinary tract infections, forms complex bacterial communities known as biofilms during infection. To form a biofilm, bacteria attach to a surface, replicate, and secrete a matrix of exopolysaccharides and proteins. This matrix serves as a physical barrier that protects against complement, phagocytes, and bacteriophages. This barrier also impairs diffusion which, in conjunction with metabolic activity of biofilm bacteria, establishes a multitude of chemical gradients within the biofilm. As a result of environmental gradients within the biofilm, subpopulations arise with distinct gene expression and phenotypic patterns. In previous studies we established the presence of an oxygen gradient that directs subpopulation emergence in UPEC biofilms, suggesting that at least a fraction of the UPEC biofilm inhabitants must rely on high-affinity cytochrome oxidases to respire in the biofilm regions with the lowest amount of oxygen. To test this hypothesis, we deleted the *cydAB* genes that code of two of the components of the high-affinity quinol oxidase, cytochrome *bd*-I. The resulting mutants formed equal levels of total biomass as the parental strain, but biofilm architecture was significantly altered, characterized by a flattened topology, loss of structural heterogeneity, and an increased surface area to volume ratio. Consequently, biofilms formed by the *cydAB* mutant were substantially more susceptible to antibiotic penetration, presumably, due to the loss of extracellular matrix integrity. We conclude that cytochrome *bd*-I is essential for biofilm architecture and physiology, and that targeting cytochrome *bd*-I is a potential strategy to aid in the treatment of urinary tract infections.

13 Human mAbs to Staphylococcus aureus lsd proteins provide protection through Fc-mediated mechanisms

Monique R. Bennett, Robin Bombardi, Nurgun Kose, Erica Parrish, Isaac P. Thomsen, Eric P. Skaar, and James E. Crowe Jr.

Nutrient acquisition is important for the survival of microorganisms. Nutrient metals, including iron, play key roles in cellular metabolism, respiration, and redox catalysis. One particularly important nutrient acquisition system is the iron-regulated surface determinant (Isd) system that scavenges heme-iron from the human host. *Staphylococcus aureus* uses the cell surface receptors IsdB and IsdH to bind hemoproteins and transfer heme to IsdA, ultimately leading to the transfer of heme across the cell surface for use as an iron source. The development of a bacterial system to sequester iron fills a fundamental metabolic requirement for the bacterial pathogen, enabling the acquisition of iron in iron-deplete conditions. The importance of this system in both nutrient acquisition and virulence to *S. aureus* has been validated in the laboratory, establishing the Isd system as a promising therapeutic target. To define the human B cell response to Isd proteins, panels of cross-reactive human monoclonal antibodies (mAbs) specific to the surface proteins of the staphylococcal Isd system were isolated and used to investigate whether antibodies to Isd proteins function solely by blocking heme-iron uptake. This paper describes the first isolation of a panel of fully human IsdA and IsdH mAbs, as well as three cross-specific mAbs each capable of binding to IsdA, IsdB, and IsdH. Two of the identified IsdA-specific mAbs worked cooperatively in a murine septic model of infection to reduce bacterial burden during staphylococcal infection. This protection is largely dependent on Fc-mediated antibody effector functions with additional protection imparted from partial heme blocking. This study suggests that the Isd system is a potential target for therapeutic antibodies, which mediate antistaphylococcal effects using diverse functions as shown here.

14 Defining the American Poopulation: Gut Microbiota Diversity across Ethnicities in the United States

Andrew W. Brooks, Sambhawa Priya, Ran Blekhman, Seth R. Bordenstein

In the advancing age of personalized medicine, the human gut microbiome offers a potentially malleable target for treating or preventing diseases. To this end, variation in diet, introduction of xenobiotics and probiotics, and a wide-range of environmental and lifestyle factors could be used to rapidly shift gut community composition. However, microbiome variation within an individual is frequently dwarfed by interpersonal distinctions, suggesting that individualized patterns of community assembly for subsets of the population could serve as the basis for more personalized treatments. Using the American Gut Project (N=1,375) and Human Microbiome Project (N=298), we searched for reproducible microbiome variation that associates with ethnicity, age, gender, and body mass index. Notably, we identified a set of 12 microbial genera and families which recurrently vary in abundance by human ethnicity, form the core of a reproducible cluster of co-occurring families, and share overlapping sets of predicted, metabolic processes. All except for one of these taxa are heritable, and seven of them replicate significant heritability across three or more large studies, including Christensenellaceae which has been christened the "most heritable taxon". Examining 49 single nucleotide polymorphisms (SNPs) in the human genome that associate with the abundance of one of the 12 taxa, we find that 21 SNPs have high rates of population differentiation (F_{ST}>95% of chromosomal and genome-wide SNPs) between 1,000 genomes continental superpopulations. As an example, we examined possible connections between Clostridiales abundance and a gut tissue eQTL variant for the HECW2 gene with very high differentiation rates between three pairs of populations, and link HECW2 to enteric nervous system function and Hirschsprung's disease. We propose additional hypotheses concerning interpersonal microbiome variation, shared metabolic functions, associated human genetic variation, and connected diseases. Further investigation of these novel patterns is warranted and could serve as the basis of microbiome interventions tailored to population subsets and their health disparities.

15 HIV-1 Engages the Dynein-Dynactin-BICD2 Complex for Infection and Transport to the Nucleus

Stephanie K. Carnes, Jing Zhou, and Christopher Aiken

HIV-1 infection depends on efficient intracytoplasmic transport of the incoming viral core to the target cell nucleus. Evidence suggests that this movement is facilitated by the microtubule motor dynein, a large multi-protein complex that interacts with dynactin and cargo-specific adaptor proteins for retrograde movement via microtubules. Dynein adaptor proteins are necessary for activating dynein movement and for linking specific cargoes to dynein. We hypothesized that HIV-1 engages the dynein motor complex via an adaptor for intracellular transport. Here, we show that siRNA depletion of the dynein heavy chain, components of the dynactin complex, and the dynein adaptor BICD2, reduced cell permissiveness to HIV-1 infection. Depletion of dynein heavy chain and BICD2 resulted in impaired HIV-1 DNA accumulation in the nucleus and decreased retrograde movement of the virus. Biochemical studies revealed that dynein components and BICD2 associate with capsid-like assemblies of the HIV-1 CA protein in cell extracts and that purified recombinant BICD2 binds to CA assemblies *in vitro*. Our findings indicate that BICD2 is a capsid-associated dynein adaptor utilized by HIV-1 for transport to the nucleus.

16 Functional consequences of sequence diversity in the Helicobacter pylori VacA toxin p55 domain

Rhonda R. Caston, Anne M. Campbell, Nora J. Foegeding, Arwen E. Frick-Cheng, Aung S. Lin, Mark S. McClain, Melanie D. Ohi, and Timothy L. Cover

Background: *Helicobacter pylori* colonizes the gastric mucosa of more than 50% of the world's population and can cause peptic ulcer disease or gastric cancer. *H. pylori* secretes a pore-forming toxin (VacA), which can cause a wide range of cellular alterations. The *vacA* alleles in different strains of *H. pylori* are genetically diverse in three main regions: 5' end (s-region), middle region (m-region), and intermediate region (i-region). Epidemiological studies indicate that *H. pylori* strains that secrete certain forms of VacA (s1, i1, m1) are associated with more severe disease outcomes than strains that secrete other forms of VacA (e.g., s2, i2, m2). The m-region is located within the p55 domain of the toxin. The goal of this study is to compare the activities of type m1 and m2 forms of VacA and map the specific residues that account for differences in activity.

Methods: We generated a panel of 11 *H. pylori* strains producing chimeric m1/m2 VacA proteins, each engineered with a streptag to facilitate purification.

Results: Each of the strains secreted VacA into the extracellular space, but several chimeric VacA proteins were secreted less efficiently than the wild-type m1 protein. Electron microscopy of 3 chimeric proteins, in which large regions of m1 sequence were replaced by corresponding m2 sequence, revealed that these proteins assemble into oligomeric structures, similar to wild-type m1 VacA. In comparison to an m1 VacA protein, a VacA chimeric protein containing m2 sequences in the N-terminal portion of the m-region was less potent in causing vacuolation and cell death when added to cultured cells. This chimeric toxin retained the ability enter AGS human gastric epithelial cells.

Conclusion: These results help to define sequences in the VacA m-region that account for differences in activity of m1 and m2 VacA proteins.

17 Human neutralizing antibody response to Rift Valley Fever Virus

Nathaniel Chapman, Nurgun Kose, Pavlo Gilchuk, Robin Bombardi, Mattie Jensen, Rachel Stecker, Matt Goff, Morgan Scarlett-Jones, Masha Majedi, Amrit Patra, & James E. Crowe Jr.

Rift Valley Fever Virus (RVFV) is a virus endemic in regions of Africa and the Middle East that typically causes mild symptoms but can result in hemorrhagic fever, meningoencephalitis, and/or blindness. National Institute of Allergy and Infectious Diseases has classified it as a Category A priority pathogen and the World Health Organization has listed it as a top 10 priority pathogen in recent reports given its ease in dissemination and potential to cause a pandemic. To date, there are no vaccines available for general human use, although attenuated candidate MP-12 and glycoprotein subunit vaccines have given hope in animal models. We still have major gaps in knowledge of the human immune response to RVFV, and in this study we aim to characterize the antibody response in naturally and vaccinated patients. Using hybridoma technology, supplemented with unique screening methods that enrich for neutralizing antibodies, we have begun to isolate antibodies that react to RVFV glycoproteins Gc and Gn. These antibodies will help us 1) characterize the neutralizing antibody response to RVFV, 2) understand the point these antibodies function in the viral replication cycle and the structural mechanism of neutralization, and 3) identify potential cross-reactive epitopes against other pathogenic *Bunyaviruses* 4) give understanding to the protective capacity these antibodies have *in vivo* both in terms of neutralization and Fc-mediated protection. Here we report the initial findings of this study; the first human monoclonal antibodies against Rift Valley Fever Virus.

18 Hypoxia-Inducible Factors regulate metabolism and germinal center response

Sung Hoon Cho, Ariel Raybuck, Edna Kemboi, & Mark Boothby

Germinal centers (GCs) are micronantomic structures that B cells proliferate, are selected for affinity maturation, undergo antibody (Ab) class-switch recombination and differentiate into Ab-secreting plasma cells and memory B cells. Both cell-autonomous and extrinsic metabolic reprogramming have emerged as modulators of T cell-mediated immunity. We have shown that GC light zone (LZ) are hypoxic, and the low oxygen tension or persistent stabilization of Hypoxia-Inducible Factor (HIF)-1 α and 2 α alters B cell physiology and function. Depletion of HIF-1a and HIF-2a in CD4 T cells caused deficiencies of the antibody response due to HIF functions in both B lineage cells and the follicular CD4 subset. Among CD4 T cells, HIF restrained the FoxP3-positive T follicular regulatory (T_{FR}) population while promoting CD40L expression. In the B lineage, HIF promoted humoral responses both through their capacity to promote effective proliferation and via enhancement of plasma cell differentiation. Collectively, these results provide evidence that HIF transcription factors regulate humoral responses via vital functions of B and Tfh cells. We propose that restriction of oxygen in lymphoid structure and organs, which can be altered in pathophysiological status, modulates humoral immune response and memory.

19 Metabolic differences between Th2 and Th17 cells in airway inflammation

Diana C. Contreras, Jacqueline Cephus, Dawn Newcomb, & Jeffrey Rathmell

Asthma is an airway inflammatory disease that is mediated by T effector (Teff) cells, specifically Th2 and Th17 cells. As disease increases in severity, there is a shift towards a higher Th17 response. Treatments of asthmatic patients include the use of glucocorticoid (GC) steroids. These drugs are effective at controlling inflammation in mild cases but as disease severity increases towards neutrophilic disease there is resistance by Th17 cells to effects of the drugs. A key therapeutic objective is to identify either alternative treatments for asthma or targets that make Th17 cells more susceptible to GCs. Studies have shown that cells that have increased glycolysis and oxidative phosphorylation are more resistant to the effects of GCs. In this study we show, that in the murine model of airway inflammation there are differences in the expression of metabolic proteins between Th2 and Th17 cells that are present in the lung. We also show that reducing oxidative phosphorylation has less of an impact in Th17 cells compared to Th2 cells. This has been seen in cells that have higher amounts glucose uptake. These results imply that the higher glucose utilization of Th17 cells and their metabolic flexibility may contribute to their resistance to the effects of GCs.

20 Elucidation of the Structural Domains Important for the Function of the Translocase of the Mitochondrial Inner Membrane 17 (Tim17) in *Trypanosoma brucei*

Chauncey Darden, Ujjal K. Singha*, Joseph T. Smith*, Minu Chaudhuri*

Department of Biochemistry, Cancer Biology, Neuroscience, and Pharmacology, Meharry Medical College, Nashville, TN *Department of Microbiology and Immunology, Meharry Medical College, Nashville, TN

African Trypanosomiasis, a fatal disease in humans and domestic animals in sub-Saharan Africa, is caused by the parasitic protozoan Trypanosoma brucei. Like other eukaryotes, T. brucei, imports hundreds of nuclear encoded proteins into its single mitochondrion. Despite this similarity, the mitochondrial protein import machinery is guite divergent in this group of parasites with only a single homologue of the Tim17 family of proteins (TbTim17) being expressed. TbTim17 is essential for parasite survival and is involved in import of different targeting signal-containing mitochondrial proteins as well as tRNAs into mitochondria. How this single protein performs all these functions remains elusive. To elucidate the structural domains of TbTim17 critical for its function; we generated a series of TbTim17 deletion mutants with 2X-myc tags at the C-terminal: N-terminal deletion mutants, Δ 10-, Δ 20-, Δ 30-, Δ 50-, Δ 100-, Δ 120-TbTim17 and C-terminal deletion mutants, Δ C12-, and Δ C31-TbTim17. We found that Δ 10-, Δ 20-, Δ 30-TbTim17 mutants were expressed and targeted to mitochondria in *T. brucei*. However, expression of the Δ 50-, Δ 100-, ∆120-, and both C-terminal deletion mutants were not detectable by anti-myc antibodies, suggesting that these mutants are likely unstable while expressed in T. brucei. Further studies revealed that Δ20- and Δ30-TbTim17 mutants although targeted to mitochondria, were not assembled properly in the TbTim17 protein complex of the mitochondrial inner membrane therefore, failing to functionally complement TbTim17 RNAi cells. Contrastingly, knock-in of the full-length copy of TbTim17-2X-myc complemented the function of TbTim17. As an alternative to Myc-tagging we are now attempting to express Δ 50-, Δ 100-, Δ 120mutants as GFP-fusion proteins in T. brucei to determine the location of the internal targeting signal of TbTim17. Using Yeast two -hybrid and co-immunoprecipitation analysis we also found that the first 50 amino acid residues of TbTim17 is critical for homotypic association of TbTim17, indicating an essential function of the N-terminal domain of TbTim17.

21 TREX1 Enhances HIV-1 PIC Function

Benem-Orom Davids, Muthukumar Balasubramaniam, Thomas Hollis, Jui Pandhare, and Chandravanu (CV) Dash

The HIV pandemic has resulted in approximately 72 million infections worldwide. Although there is no cure for HIV, antiretroviral therapy (ART) have been highly effective in controlling the virus. However, the current ART regimen is expensive, has significant side effects, and face viral resistance. Thus, there is a continuous need for the identification of novel targets for the development of next generation antiviral drugs. One such target is TREX1, which has been shown to play a critical role in HIV-1 infection. TREX1 is the most abundant 3'–5' exonuclease in mammalian cells and assists HIV-1 to evade innate DNA sensing pathways. Even though the exact mechanism is unclear, depletion of TREX1 causes accumulation of cytosolic viral DNA. Therefore, it has been proposed that TREX1 degrades the reverse transcribed viral DNA to aid the virus in evading immune detection. In initial studies, we observed that HIV-1 infection resulted in the induction of TREX1 in T cells lines. Interestingly, cells treated with interferon gamma which is elevated in HIV-1 infected individuals, also show higher levels of TREX1. Since TREX1 degrades the viral DNA (vDNA) and vDNA is an integral component of the preintegration complex (PIC), we are studying the effects of TREX1 overexpression on PIC function. Using stably expressing TREX1 T cell lines, we extracted PICs from acutely infected cells. Results from in vitro integration assay show that activity of PICs isolated from TREX1 exonuclease activity and can be efficiently integrated into a target. To better understand the effects of TREX1 on PIC function, currently we are quantifying reverse

22 Glutamate receptors provide costimulatory signals to improve T cell immune response

Maria Teresa P. de Aquino¹, Thomas Hodo^{1,2}, Roman Uzhachenko¹, Anil Shanker^{1,2,3,4}

¹Department of Biochemistry, Cancer Biology, Neurosciences and Pharmacology School of Medicine, ²School of Graduate Studies and Research, Meharry Medical College, Nashville, TN; ³Vanderbilt-Ingram Cancer Center, ⁴Vanderbilt Institute for Infection, Immunology and Inflammation, Vanderbilt University, Nashville, TN

The role of neurotransmitters in lymphocyte function has sparked interest in recent years. They have been shown to impact cytokine secretion, integrin expression and chemotaxis. We found that naive lymphocytes expressed various neurotransmitter receptors. Further, following TCR stimulation, glutamate receptors (GluR) were significantly upregulated on both $CD4^+$ and $CD8^+T$ cells, with a peak expression at 48 h. We also observed an upregulated expression of glutamate receptors on tumor specific T cells that infiltrated into orthotopic 4T1 mammary tumors that express a low avidity epitope of a model antigen hemagglutinin (4T1HA). We found that HA-reactive $CD8^+T$ cells in the tumor-infiltrate had an upregulated expression of glutamate receptors when compared with the control group. Concomitant with the upregulation of T cell activation molecules CD69, CD25 and CD44, proliferating CD8⁺ T cells presented higher levels of GluR3 and GluR1, as well as a higher production of IFN-gamma and granzyme B, when compared with non-proliferating cells. The blockade of GluR signaling by antagonists down-regulated T cell activation proliferation and impaired with cytolytic activity without any effect on T cell viability. TCR-activation pathways such as Lck/Akt as well as global cytosolic calcium were down-regulated by GluR antagonists, suggesting a signaling interaction between GluR and TCR pathways. Overall, data suggest that glutamate receptors may have costimulatory effects on T cell function and their agonists could be explored as a novel class of therapeutics for enhancing T cell immunity in an immunosuppressive setting such as cancer.

23 Endogenous retrovirus expression is associated with response to immune checkpoint inhibition in clear-cell renal cell carcinoma

Anshuman Panda^{1,2}, <u>Aguirre A. de Cubas</u>³, Mark Stein^{1,4}, Gregory Riedlinger¹, Christof C. Smith⁵, Benjamin G. Vincent⁵, Kathryn E. Beckermann^{3,6}, Shridar Ganesan^{1,4*}, Gyan Bhanot^{1,2,7*}, W. Kimryn Rathmell^{3,6*}

¹Rutgers Cancer Institute of New Jersey, 195 Little Albany Street, New Brunswick, NJ 08903, USA; ²Department of Physics and Astronomy, Rutgers University, 136 Frelinghuysen Road, Piscataway, NJ 08854, USA; ³Vanderbilt-Ingram Cancer Center, 691 Preston Building, Nashville, TN 37232, USA; ⁴Rutgers Robert Wood Johnson Medical School, 125 Paterson Street, New Brunswick, NJ 08901, USA; ⁵Department of Medicine, Lineberger Comprehensive Cancer Center, University of North Carolina, 450 West Drive, Chapel Hill, NC 27514, USA; ⁶Department of Medicine, Division of Hematology and Oncology, Vanderbilt University Medical Center, 1211 Medical Center Drive, Nashville, TN 37232, USA; ⁷Department of Molecular Biology and Biochemistry, Rutgers University, 604 Allison Road, Piscataway, NJ 08854, USA

A subset of clear-cell renal-cell carcinoma (ccRCC) patients respond to immune checkpoint blockade (ICB), but predictors of response remain uncertain. Here we investigated the hypothesis that abnormal expression of endogenous retroviruses (ERVs) in tumors may be associated with local immune checkpoint activation (ICA), and may predict response to ICB. RNAseq data from The Cancer Genome Atlas (N=472 for ccRCC, N=4,438 for 20 other solid cancers) was analyzed to identify potentially immunogenic ERVs (π ERVs), defined as ERVs whose expressions correlate with RNAseq-based markers of ICA. The validation cohort consisted of metastatic ccRCC patients treated with single-agent PD-1/PD-L1 blockade (N=13).

In total, twenty π ERVs (N=20) were identified in ccRCC, and tumors stratified into three groups based on the expression of these π ERVs. The π ERV-high group showed increased immune infiltration, up-regulation of checkpoint pathway genes, and higher CD8⁺ T-cell fraction in infiltrating leukocytes compared to the π ERV-low group. Similar results were observed in ER+/HER2– breast, colon, and head-neck squamous-cell cancer. ERV expression was correlated with expression of genes associated with histone methylation and chromatin regulation, and enrichment of *BAP1* mutations were observed in π ERV-high ccRCC. *ERV3-2* expression correlated with ICA in 11 solid cancers, including the above four cancers. In the validation cohort, *ERV3-2* expression was significantly higher (P<0.05, two-sided Wilcoxon Rank-sum test) in tumors from responders compared to tumors from non-responders and was an excellent predictor of response to ICB (ROC AUC 0.88-0.98). *ERV3-2* expression was also associated with enrichment of specific B-cell receptor clones. Abnormal expression of π ERVs are associated with ICA in ccRCC and several other solid cancers. *ERV3-2* expression was validated as a predictor of response to ICB in ccRCC.

24 The impact of the TRPM2 ion channel on host inflammation and macrophage metabolism

<u>Beverly R.E.A. Dixon</u>, Sharia Yasmin, M. Kay Washington, Yasmin Saba, Frank Mason, Dina Polosukhina, Danyvid Olivares-Villagómez, Lori A. Coburn and Holly M. Scott Algood

Macrophages play a vital role in regulating pro-inflammatory pathways that drive chronic inflammation and impact carcinogenesis. Thus, imperative for combatting these detrimental pathways is an understanding of how macrophage function is regulated. One cellular regulatory mechanism in macrophages is the control of cytosolic levels of Ca²⁺ through the transient receptor potential melastatin 2 (TRPM2) channel. This project was designed to examine how TRPM2 regulates oxidative stress and metabolism and how its activation impacts the development of inflammation. Three different in vivo mouse models of inflammation have been used to investigate the role of TRPM2: the Helicobacter pylori (Hp) infection model, the dextran sulfate sodium (DSS) colitis model, and the azoxymethane (AOM)/DSS colitis-associated cancer (CAC) model. During Hp infection, TRPM2 deficient mice (TRPM2^{-/-}) developed more acute inflammation resulting from a hyper pro-inflammatory macrophage phenotype and exacerbated ROS production. Moreover, TRPM2^{-/-} mice were not protected against DSS-induced colitis as previously reported. Finally, using the AOM/DSS CAC model, we found that TRPM2^{-/-} mice develop more tumors, but their tumors had more cellular infiltrate and were smaller; therefore, the overall tumor burden was reduced in the TRPM2^{-/-}. This was further associated with decreased histologic injury in the TRPM2^{-/-} mice. The impact of the TRPM2 channel on macrophage metabolism, using bone marrow derived macrophages, was investigated with Seahorse Extracellular Flux Assays, gene expression analysis, and mitotracker assays. The data demonstrated an unfavorable shift in mitochondrial content in TRPM2^{-/-} macrophages after co-culture with Hp. Consistent with altered metabolism, the oxygen consumption rate was reduced in TRPM2^{-/-} macrophages, and the extracellular acidification rate at baseline and glycolytic reserve were higher in TRPM2^{-/-} macrophages following Hp exposure. These data indicate that TRPM2 may impact metabolic function and control of pro-inflammatory gene expression in macrophages through the regulation of ROS.

25 Streptococcus agalactiae induces placental macrophages to release macrophage extracellular traps loaded with tissue remodeling enzymes via an oxidative-burst-dependent mechanism

Ryan S Doster, Jessica Sutton, Lisa M. Rogers, David M. Aronoff, Jennifer A Gaddy

Background: Group B *Streptococcus* (GBS) infections are important causes of preterm birth and neonatal sepsis. Placental macrophages (PMs) are resident leukocytes present in gestational tissues. We sought to identify PM functions during GBS infection.

Methods: PMs were incubated *ex vivo* with GBS cells to evaluate bacterial killing and host-pathogen interactions with scanning electron or confocal microscopy. Extracellular traps (ETs) were identified by staining with Sytox Green for extracellular DNA, and ET protein contents were identified via immunofluorescence staining. Intracellular reactive oxygen species (ROS) generation was measured using a ROS reactive fluorescent dye. Human fetal membrane tissues were infected *ex vivo* with GBS and tissues were evaluated for macrophage ET release by immunofluorescence staining.

Results: PMs release ETs in response to GBS infection, and DNase treatment dissolved these structures. ET structures contained proteins including myeloperoxidase, elastase, histones, and several matrix metalloproteinases. PMs treated with DNase demonstrated impaired bactericidal activity. Conditions that inhibited cellular ROS generation, including treatment with diphenyleneiodonium chloride, inhibited ET release. MMP-8 and 9 were significantly elevated in the supernatants of co-culture experiments compared to uninfected controls, and infected cell supernatants demonstrated a significant increase in MMP activity. Finally, macrophages appearing to release ETs were identified in human fetal membrane tissues infected with GBS.

Conclusion: GBS infection of PMs results in release of ETs containing histones and proteases, and ETs are capable of killing GBS cells. PM ET release requires ROS generation. Additionally, PMs release significantly more active MMPs in response to GBS infection. MMPs catalyze the breakdown of fibronectin, laminin, and collagens important for fetal membrane structural integrity. Thus ET release by these cells, an immune strategy thought to trap and kill invading microbes, may also result in localized damage to fetal membranes during infection, potentially contributing to premature membrane rupture.

26 FUS Restricts Kaposi's Sarcoma-Associated Herpesvirus Reactivation by Negatively Affecting Viral Gene Expression

William Dunker, Yu Song, Yang Zhao, John Karijolich

Kaposi's sarcoma-associated herpesvirus (KSHV) is a human gamma-herpesvirus that is the etiological agent of Kaposi's sarcoma and is associated with the development of several lymphoproliferative diseases, including primary effusion lymphoma (PEL). KSHV reactivation from latency requires robust transcription of over 80 lytic cycle viral genes. The KSHV lytic cycle plays an integral role in the progression to KSHV-associated disease as several lytic proteins have angiogenic and anti-apoptotic functions essential to the tumor microenvironment. Thus, restriction of KSHV reactivation represents an attractive therapeutic target. Here, we demonstrate that the cellular protein Fused-in-sarcoma (FUS) robustly restricts KSHV lytic reactivation in PEL and in an epithelial cell-based model. Depletion of FUS significantly enhances viral mRNA and protein expression, resulting in an increase in viral replication and production of infectious virions. Chromatin immunoprecipitation analyses demonstrates that FUS is present at several KSHV lytic cycle genes during the latent stage of infection. KSHV reactivation drives eviction of FUS from the KSHV genome. We demonstrate that FUS interacts with RNAP II and negatively affects Serine-2 and -5 phosphorylation on its C-terminal domain (CTD), which are required for efficient gene transcription. Collectively, these data reveal a novel role for FUS in regulating viral gene expression and are the first to demonstrate its role as a viral restriction factor.

27 Role of a Direct Interaction with the Viral Env protein gp120 in the Antiviral Mechanism of the Host Cell Restriction Factor SERINC5

Austin Featherstone, Jing Zhou and Christopher Aiken

Human Immunodeficiency Virus (HIV) is a human retrovirus that infects cells within our immune system. HIV is the sole cause of the severe human disease known as Acquired Immune Deficiency Syndrome (AIDS). Infection by HIV requires binding of the viral envelope (Env) glycoprotein gp120 with the major cell-surface receptor CD4 and its co-receptors CXCR4/CCR5. Subsequently, HIV-1's transmembrane Env glycoprotein, gp41, undergoes a conformational change which promotes fusion between the viral membrane and the host membrane. In 2015, the SERINC (serine incorporator) family of proteins was identified as host cell inhibitors of HIV-1 infection. SERINC3 and SERINC5 were the only two proteins in the family to show restriction of HIV-1 infection. SERINC3 is incorporated into budding HIV-1 particles and inhibit HIV-1 infectivity in a manner that is counteracted by the HIV-1 accessory protein Nef (Negative Regulatory Factor). In the presence of Nef, the SERINC proteins are removed from the plasma membrane and transported into late endosomes to be degraded. These findings suggest that the SERINC3/5 proteins account for the infectivity impairment that has long been seen in Nef-defective HIV-1.

Our initial studies provide evidence that SERINC5 inhibits HIV-1 infectivity at a 1:1 stoichiometry with Env on the surface of the virion. Thus, I hypothesize that SERINC5's antiviral activity is linked to a direct association of the protein with HIV-1 gp120. To test this hypothesis, I propose to answer several key questions directed at elucidating the antiviral determinants of SERINC5. Specifically, I will: (1) determine whether SERINC5 has a direct association with HIV-1 Env, and (2) identify the specific structural domains of HIV-1 gp120 required for SERINC5's antiviral activity and incorporation into budding HIV-1 particles.

28 Host Cells Resist Helicobacter pylori VacA Intoxication by Degrading VacA

Nora J. Foegeding, Krishnan Raghunathan, Timothy L. Cover, Melanie D. Ohi

Helicobacter pylori persistently colonizes the gastric mucosa of more than half of the world's population. Infection with *H. pylori* causes chronic gastric inflammation and is the leading cause of stomach ulcers and gastric cancer. An important *H. pylori* virulence factor is the pore-forming toxin known as vacuolating cytotoxin A (VacA). VacA enhances the ability of *H. pylori* to colonize the stomach and contributes to the pathogenesis of peptic ulcer disease and gastric cancer. VacA has been reported to trigger a wide range of cellular responses including cellular vacuolation, increased plasma membrane permeability, disruption of mitochondrial membrane permeability, and apoptosis. VacA activity is potentiated by the presence of weak bases, but the mechanistic basis for this phenomenon is not completely understood. We observed that VacA-treated cells in the absence of weak bases recover from VacA-induced vacuolation. Furthermore, we found that VacA-induced cell death is dependent on the presence of supplemental ammonium chloride (NH₄CI). We show that host cells degrade VacA through lysosomal degradation and that inhibiting lysosomes before colocalizing with autophagosomes. Finally, we determined that inhibiting autophagy does not inhibit VacA degradation. We propose that intracellular degradation of VacA in the lysosome is a defense mechanism that allows host cells to resist VacA-induced cell death, and weak bases like NH₄CI enhance VacA activity by inhibiting its degradation.

29 Osteoblast Innate Immune Responses During Staphylococcus aureus Osteomyelitis

Caleb Ford, Nicole Putnam, Jacob Curry, James E. Cassat

Osteomyelitis is a devastating invasive infection of bone and is most commonly caused by the Gram-positive *Staphylococcus aureus*. Osteomyelitis causes severe bone loss through the direct effects of bacterial factors and indirect effects modulated by the host immune response. Osteoblasts, the cells responsible for the production of the extracellular matrix of bone, have multiple pattern recognition receptors. Moreover, osteoblasts have previously been demonstrated to permit the intracellular survival of *S. aureus*, which may function as a protective reservoir for relapsing, chronic infection. In addition to its role in bone formation, the osteoblast controls osteoclast-mediated bone resorption through the production of receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG). Following exposure to concentrated supernatants of toxin-deficient strains of *S. aureus* in vitro, osteoblasts are shown to decrease the production of mineral through alizarin red staining and to decrease alkaline phosphatase activity in osteoblast cells at high doses of supernatant. Further, qRT-PCR was used to discover that osteoblasts in potentiating osteoclast formation is further demonstrated through co-culture of primary precursors of osteoblast and osteoclast lineages with subsequent TRAP-staining for osteoclast formation. The results indicate that osteoblasts are an important regulator of bone mineral density during osteomyelitis and that modulation of osteoblast responses to *S. aureus* may serve as an important target of pharmaceuticals aimed at preventing bone loss and subsequent fracture risk following osteomyelitis.

30 Testosterone decreases while ovarian hormones increase house dust mite-induced dual type 2 and IL-17A-mediated airway inflammation

<u>Hubaida Fuseini</u>, Jacqueline-Yvonne Cephus, Jeffrey. A. Yung, Jian Zhang, Kasia Goleniewska, Ray. S. Peebles Jr., Dawn. C. Newcomb

Severe asthma prevalence is greater in women compared to men, suggesting sex hormones regulate severe asthma pathogenesis. Patients with severe asthma have increased airway inflammation mediated by type 2 cytokines (IL-4, IL-13, and IL-5) and/or IL-17A. We hypothesize that testosterone decreases and ovarian hormones increase type 2 and IL-17A-mediated airway inflammation. To test this hypothesis, house dust mite (HDM) or vehicle was administered to gonadectomized and sham-operated male and female adult BALB/c mice 4 times per week for 3 weeks. Lungs and bronchoalveolar lavage fluid (BALF) were harvested 24 hours after the last challenge for analysis. Digested lungs cells were restimulated with PMA, ionomycin, and golgi stop. IL-13+ and/or IL-17A+ cells were quantified by flow cytometry. Our results showed that eosinophils and neutrophils in the BALF and lung IL-13 and IL-17A protein expression were increased in HDM challenged intact female mice compared to HDM challenged intact male mice. CD4 T cells accounted for >50% of IL-13+ or IL-17A+ cells in HDM challenged mice The IL-13+ and IL-17A+ $\gamma\delta$ T cells were increased in HDM challenged gonadectomized male mice and shamoperated female mice compared to HDM challenged sham-operated female mice and gonadectomized male mice and shamoperated female mice and gonadectomized male mice and shamoperated female mice and gonadectomized male mice had increased IL-13+ ILC2 compared to HDM challenged shamoperated male. Combined, testosterone decreased and ovarian hormones increased HDM-induced eosinophil and neutrophilic inflammation and IL-13 and IL-17A production. These results provide a potential explanation for the increased prevalence of women with severe asthma.

A workflow to support metabolomics analysis of a cell's response to an unidentified toxin in 30 days

Randi L. Gant-Branum, Stacy D. Sherrod, Simona Codreanu, Alexandra Schrimpe-Rutledge, James M. Poland, Jerry Holman, Danielle Gutierrez, Melissa Farrow, Jeremy Norris, Nicole Muszynski, Richard Caprioli, John A. McLean

We have demonstrated a method whereby metabolomic analysis and preliminary MOA construction, in conjunction with proteomic and transcriptomic analyses, may be completed for a model human cell line upon exposure to an unidentified biological agent and reported in a 30-day turnaround time while maintaining high standards of data quality and extraction efficiency. Bendamustine, a chemotherapy drug that has little canonical knowledge known about it, was selected to test our rapid threat analysis platform. Human promyelocytic leukemia cells (HL-60) were extracted using a unified sample preparation described previously and analyzed by LC-IM-MS analysis (Synapt G2, Waters) in HILIC (+) and RPLC (+) ion modes and LC-MS/MS analysis (Orbitrap, Thermo) in HILIC (+) and (-) ion modes. Data was processed by Progenesis QI (Nonlinear Dynamics) to align, peak pick, normalize, deconvolute, and determine significant compounds on the basis of p-value and fold change. Mummichog, an open-source network activity analysis software, was implemented to prioritize metabolites on the basis of potential biological significance. Compound Discoverer 2.0 (Thermo) was used to annotate metabolites on the basis of fragmentation spectra matching to the mzCloud library. Of 24993 features detected across twelve exposure times in LC-IM-MS analysis, 2573 were prioritized by significance (<0.1) and fold change (≥|1.5|) and 2174 were tentatively annotated on the basis of accurate precursor mass. Network activity analysis (mummichog) was then utilized to provide a framework for additional targeting and analysis of metabolites of interest in parallel with compounds prioritized by p- value and fold change. This facilitated an approach for targeting specific metabolites of interest for further investigation. Prioritized metabolites were added to an inclusion list for semitargeted analysis. Using this strategy, 91 metabolites were putatively annotated, which allowed for further investigation, biological interpretation and ultimately MOA generation.

32 IFN-y Regulation through Enhancer Associated LncRNAs

Hunter Gibbons, Henry Garcia, Thomas Aune

The IFN-γ cytokine is a primary defense against many different cellular infections. Despite being expressed in TH1 cells and silenced in other T-helper subsets, IFNG is expressed in by CD8+ T cells, NK cells, and NKT cells. The *IFNG* gene is regulated via a large network of transcription factors, multiple enhancers, and antisense lncRNAs. The genomic locus contains enhancers specific to both *IFNG* and *IFNG-AS1*, a local antisense lncRNA that stimulates *IFNG* expression. These enhancers produce novel lncRNAs that are highly regulated during Th cell development. However, in resting T Effector Memory cells where *IFNG* is not produced, enhancer-associated lncRNAs are still highly produced. In this study, we show that the expression of *IFNG*, *IFNG-AS1*, and the enhancer-associated lncRNAs (IFNG-Rs) all inter-regulate to create a dynamic transcriptional network dependent upon T -cell polarization. Further, IFNG-Rs bind NF-kB, which helps regulate the expression of *IFNG*. Thus, novel enhancer-associated lncRNAs represent a major regulatory force in the expression of *IFNG*.

33 Analysis of adaptive pathways in a proofreading-deficient coronavirus

Kevin W. Graepel, Thayer L. Taft, and Mark R. Denison

Coronaviruses (CoVs) encode a 3'-to-5' exoribonuclease (ExoN) in nonstructural protein 14 (nsp14) that proofreads during genome replication and antagonizes the innate immune response. Alanine mutagenesis of conserved ExoN catalytic residues (3 motifs: DE-E-D) dramatically reduces CoV fitness. Interestingly, double amino acid ExoN(-) mutants in motif I (AA-E-D; 4 nucleotide changes) of both MHV and SARS have not reverted in diverse selective environments, suggesting that two amino acid substitutions represent a high genetic barrier to reversion. Recently, we demonstrated that MHV-ExoN(-) can adapt for wild-type-like level replication and compensate for defective proofreading without reverting ExoN Motif I. However, only a portion of the proofreading compensation could be attributed to the evolution of a high-fidelity RNA-dependent RNA polymerase (nsp12-RdRp), with little to no contribution from mutations in nsp14-ExoN. In this report, we have used reverse genetics to probe genetic pathways that can lead to wild-type-like phenotypes and to search for determinants outside of the nsp12-RdRp and nsp14-ExoN that regulate CoV fidelity and fitness. Our results indicate that single- and double-nucleotide substitutions to engineered Motif I alanines do not increase viral fitness, suggesting that step-wise accumulation of motif I mutations is unlikely to result in selection for primary ExoN(-) reversion. We also determined that passage-acquired mutations in the replicase proteins nsp8, nsp9, and nsp13 did not affect nucleoside analogue sensitivity, suggesting that proteins outside of replication complex can partially compensate for defective proofreading. Our results are consistent with a model in which complex, multiprotein evolution drives fitness adaptation in ExoN(-) CoVs, for which a high genetic barrier limits the probability of primary ExoN(-) reversion.

34 A metal-responsive transcriptional regulator protects *Acinetobacter baumannii* from manganese limitation and oxidative stress

Erin R. Green, Lillian J. Juttukonda, Eric P. Skaar

Acinetobacter baumannii is an important opportunistic pathogen that commonly infects critically ill patients in hospital settings. Because of its rapid acquisition of antibiotic resistance, infections caused by A. baumannii have become extremely difficult to treat, underlying the importance of identifying new antimicrobial targets for this pathogen. Neutrophils inhibit growth of A. baumannii through a variety of mechanisms, including the production of ROS and the release of calprotectin, an immune protein that chelates zinc (Zn) and manganese (Mn). We previously identified an NRAMP-family Mn transporter, MumT, which is essential for growth of A. baumannii during Mn starvation. MumT is encoded within an operon adjacent to a LysR-family transcriptional regulator, MumR. Transcription of mumT is dependent on MumR, and is heightened in the presence of calprotectin. Because Mn import is a common defense against oxidative stress, we hypothesized that MumT may play an important role in defense against H₂O₂. Surprisingly, $\Delta m um R$, but not $\Delta m um T$, was defective for growth in the presence of H₂O₂, leading us to hypothesize that MumR regulates other genes that promote resistance to H₂O₂ stress. To determine whether MumR acts through the canonical redox-sensitive regulator OxyR, a $\Delta m u m R \Delta oxyR$ mutant was constructed. This mutant displayed heightened sensitivity to H_2O_2 relative to $\Delta mumR$ and $\Delta oxyR$, indicating that both proteins activate distinct regulons to promote H₂O₂ resistance. RNA-sequencing was performed to define the regulon of MumR, which revealed a role for MumR in regulating several catabolic pathways. Finally, *AmumR* exhibited reduced fitness in a murine model of pneumonia that was restored in the absence of neutrophils. In summary, these results suggest that MumR facilitates resistance to neutrophil killing by activating a transcriptional program that is critical for surviving Mn starvation and oxidative stress. Future directions will be aimed at further characterizing the functions of genes in the MumR regulon under these conditions.

35 Characterizing the impact of osteopontin on IEL survival and microbiome composition

Michael Greer, Qi Liu, Bing Zhang, and Danyvid Olivares-Villagomez

Intraepithelial lymphocytes (IELs) comprise distinct subpopulations of lymphocytes positioned between the epithelial cells of mucosal surfaces. Intestinal IELs are in close proximity to luminal antigen and function as a first line of defense against invading pathogens. IELs have been implicated in many inflammatory disorders of the gut and understanding their biology is imperative to developing effective therapies. An important open question in IEL biology is deciphering the molecular signals that support IEL survival. Recent work suggests that osteopontin (Opn), a multifunctional acidic phosphoprotein involved in tissue remodeling, contributes to IEL survival, however, the mechanism by which Opn affect IEL survival and the broader impact of Opn on the microbiome remains unknown. In this study we will address these questions by performing 16s sequencing and RNA-Seq analysis of WT and Opn knockout B6 mice to determine Opn induced changes in microbiome composition and gene expression signature. The results of this analysis will guide further efforts to determine precisely how osteopontin supports IEL survival.

36 Diversity and Evolution of Coronavirus Defective RNAs

Jennifer Gribble, Xiaotao Lu, Laura Stevens, Mark R. Denison, and James Chappell

Coronaviruses (CoVs) are a genetically diverse group of positive-sense single-stranded RNA viruses containing the largest known genome of any RNA virus, >30 kb. The CoV replication strategy is driven by discontinuous RNA synthesis and long-range movement of the replication-transcription complex, predisposing toward recombination events and the production of short defective RNAs (DRNAs) derived from distant regions of the parental viral genome. To better understand the diversity, evolution, and biologic functions of CoV DRNAs, we have undertaken studies to define the DRNA spectrum arising during adaptation of wild -type and mutant viruses to serial passage in cell culture. RT-PCR screening for murine hepatitis virus (MHV) and Middle East respiratory syndrome (MERS)-CoV DRNAs that retain 5'- and 3'- terminal-region sequences. Internal organization of CoV DRNAs is influenced by the parental genetic background and suggests that replication-mutant viruses aberrantly produce and select DRNAs with unique structural and/or functional properties. Further studies combining deep- and long-read next-generation sequencing are in progress to comprehensively profile the range of sequence architectures, population structures and dynamics, and generative pathways that define CoV DRNAs and guide their emergence. This work demonstrates the complex quasispecies of satelliting DRNAs in CoV genetic space and points toward probable functional roles for these partial genomes in the replication programs of diverse CoVs.

37 MntE is essential for maintaining intracellular manganese homeostasis in Staphylococcus aureus

Caroline M. Grunenwald, Jacob E. Choby, William N. Beavers, Eric P. Skaar

Staphylococcus aureus is a significant cause of human morbidity and mortality. Manganese (Mn), is an essential micronutrient and plays an important role in S. aureus physiology and metabolism; thus acquisition of Mn is critical to pathogenesis. Paradoxically, high concentrations of Mn are toxic, therefore maintaining intracellular homeostasis through coordinated regulation of Mn export and import is essential for bacterial survival. Here we describe a putative Mn exporter, MntE, which is a member of the Cation Diffusion Facilitator protein family and highly conserved among other Gram-positive pathogens. Upregulation of mntE transcription in response to excess Mn is dependent on the presence of mntR, a DtxR-like protein and transcriptional repressor of the mntABC Mn uptake system. This suggests MntR acts as a positive regulator of mntE and serves to co-regulate the uptake and efflux of intracellular Mn. Inactivation of mntE or mntR leads to reduced growth in media supplemented with Mn, but not other metals, which is complemented by constitutive expression of *mntE* in trans. This demonstrates MntE is required for resistance to excess Mn and MntE activity is highly specific for Mn. Inactivation of MntE results in intracellular accumulation of Mn, further supporting the hypothesis that MntE functions as a Mn exporter. Additionally, mntE mutants are more sensitive to exposure to HOCI and paraquat, indicating intracellular Mn homeostasis is critical for resisting oxidative stress. Mutants lacking either mntR or both mntE and mntR show reduced burdens in murine hearts and spleens following systemic infection, suggesting Mn efflux is important for survival within the host. Combined, these data support a model where MntR regulates Mn uptake through MntABC and Mn export through MntE and suggests Mn efflux contributes to bacterial survival during infection. Future work will further define MntR regulation of *mntE* and the contribution of Mn homeostasis to S. aureus pathogenesis.

38 Characterizing human helminth-specific IgE antibodies and their potential cross-reactivity towards known allergens

Azadeh Hadaddianpour, Jacob Daniel, Olivia Bogdan, Thomas B. Nutman,, Scott A. Smith

According to WHO estimates, more than 1.5 billion people (>24% worldwide) are living with some type of soil-transmitted helminth infection and there is no FDA-approved anti-helminth vaccine available. The type 2 immune response is understood to be involved in our defense against helminth infection with the IgE antibody molecule acting as the master controller, linking innate and adaptive arms together. Epidemiologic studies show that helminth-infected patients harbor an elevated level of IgE in their blood but no correlation exists between the level of IgE and the severity of their disease. Therefore, it is not clear if the IgE antibody response is capable of protecting against helminth infection. Helminths are large complicated multicellular parasites encoding more than 11,000 proteins. Many aspects of the human immune response to helminth infection are still unknown, including which immunogenic proteins are responsible for stimulating IgE production. As IgE also plays a critical role in type I hypersensitivities and the pathophysiology of allergic diseases, we suggest that homology exists between helminth immunogenic proteins and common known allergens. In this study, we have generated first ever panels of naturally occurring human antihelminth IgE monoclonal antibodies (mAbs) from helminth infected patients using human hybridoma technology. Both ELISA and Western blot techniques were used to test binding of purified IgE mAbs to helminth lysate. Afterward, Immunoprecipitation and mass spectrometry were used to identify the helminth antigen proteins. We observed homology between helminth proteins targeted by IgE and common known allergens, suggesting that the immune system mistakenly attacks innocuous allergens by producing IgE due to their resemblance to helminth proteins. The information obtained regarding IgE epitope targets and their immune dominance will assist in our basic understanding of the role IgE plays in defending us against helminth infection and will ultimately aid in the rational design of anti-helminth vaccines.

39 Analysis of Helicobacter pylori Hop outer membrane proteins

M. Lorena Harvey¹, Mark S. McClain², and Timothy L. Cover^{1,2,3}

¹Department of Pathology, Microbiology and Immunology, ²Department of Medicine, Vanderbilt University, Nashville, TN; ³Veterans Affairs Tennessee Valley Healthcare System, Nashville, TN

Background: Gastric colonization by *Helicobacter pylori* results in gastritis and can lead to more severe diseases, including peptic ulcer disease or adenocarcinoma. In order to successfully colonize the human host, *H. pylori*, like many other Gram-negative bacterial pathogens, uses specific outer membrane proteins (OMPs) to adhere to host cells, obtain nutrients, or evade the immune system. The *H. pylori* genome encodes more than 60 predicted OMPs, but the functions of most of these OMPs are unknown. Several proteins in the largest family of *H. pylori* OMPs (Hop OMPs) are known to act as adhesins. We hypothesize that the presence of a large repertoire of Hop proteins allows *H. pylori* to colonize the stomach, and that specific Hops play critical roles during various phases of colonization. To facilitate further study of Hop proteins, we have generated a panel of barcod *H. pylori* mutant strains.

Methods: To generate the panel of barcoded *H. pylori* mutants, individual hop genes were subjected to insertional mutagenesis with an antibiotic cassette and a unique 21-nucleotide barcode (specific to each *hop* gene). Pools of these mutants were analyzed by high-throughput sequencing (HTS).

Results: We successfully mutated 16 *hop* genes and 8 additional control genes, which indicates that these genes are not required for *H. pylori* viability in vitro. Mutant strains were pooled together (1) at equal concentrations, (2) with one mutant reduced by 100-fold, or (3) with one mutant left out of the pool. HTS analysis successfully detected these changes in barcode composition within the pool of mutants. The barcoded mutant pool was then cultured with gastric epithelial cells (AGS), and the population of adherent bacteria was analyzed by HTS. The adherent population exhibited changes in the proportional abundance of several mutants, compared to the starting population.

Conclusion: These results indicate that most Hop proteins are not required for *H. pylori* viability in vitro, and that changes in the composition of a pool of barcoded *hop* mutants can be detected and quantified by HTS. This approach can be utilized to broaden our understanding of how individual Hop proteins promote *H. pylori* interactions with gastric epithelial cells in vitro and *H. pylori* colonization of the stomach.

40 The Role of Granzymes in Intestinal inflammation and Disease

Kristen L. Hoek, Ali Nazmi, M. Blanca Piazuelo, Danyvid Olivares-Villagomez

The intestinal intraepithelial lymphocyte (IEL) compartment represents a first line of defense against invading pathogens while providing tolerance to commensal microorganisms, which allows for intestinal homeostasis. IEL are comprised of TCR⁺ and TCR⁻ cells belonging to both the innate and adaptive arms of the immune system. We recently discovered that granzyme A and B are the two most highly expressed genes in a subset of TCR, known as iCD8 α cells, suggesting a role for these proteases in intestinal immunity. To determine the role that granzymes play in IEL functions and mucosal immune responses, we first investigated granzyme (Grz) A and B expression in IEL during steady state conditions by intracellular flow cytometric staining. Both GrzA and GrzB were expressed in TCR $\gamma\delta^{\dagger}$, TCR β^{\dagger} CD8 α^{\dagger} , and TCR-CD8 α^{\dagger} IEL cells in the small intestine. GrzA was expressed in the same cell populations within the colon, while GrzB was virtually undetectable in colon IEL. Phenotypic analysis of the IEL compartment in granzyme-deficient mice revealed no gross abnormalities, although GrzB^{-/-} animals showed a slight decrease in the numbers of TCR $\gamma\delta^{+}$ and TCR β^{+} CD4⁺CD8 α^{+} cells in the small intestine and TCR β^{+} CD8 α^{+} cells in the colon. To monitor the role of granzymes in pathogen-induced intestinal inflammation and disease, wild-type and granzyme-deficient animals were infected with Citrobacter rodentium, an inflammatory-inducing bacterium with colon-specific colonization in mice. WT mice displayed mild disease and cleared bacteria by 14d post-infection. In contrast, C. rodentium colonization was observed in the colon of both GrzA^{-/-} and GrzB^{-/-} animals at 14d post-infection. GrzB^{-/-} demonstrated severe weight loss and diseaseassociated changes in appearance 10-14d post-infection; however, GrZA^{-/-} animals appeared resistant to C.rodentium-induced disease. Together, these data suggest that granzyme A and B play different roles in intestinal inflammation and disease.

41 Glutaminase enhances Th17 and inhibits Th1 T cells through chromatin modifications and the mTOR pathway

Marc Johnson

T cells require metabolic reprogramming to enact host defense. Effector T cells (Teff) increase glutamine and glucose uptake during activation to support proliferation and metabolic demands. Subset effector Th1 and Th17 cells are known to be important in viral immunity, cancer immunity, and autoimmune disease. The role of glutamine in T cells is relatively unexplored and may offer new avenues of treatment for autoimmune disease and cancer. Glutamine is required for T cell activation and the glutamine transporter, SLC2A5, is upregulated in activated Teff cells. Glutaminase, the first enzymatic step of glutamine metabolism, is also induced during Teff activation. A key role of glutaminase (GLS) is to convert glutamine into glutamate, which can be further processed to α-KG, used for protein synthesis, or exported via xCT to transport cystine into the cell. Pharmacologic and genetic GLS deficiency reduced T cell activation, proliferation, and IL-17 production in Th17 cells, but enhanced Th1 and CD8 cytotoxic lymphocytes IFNy and TBET production. This was reflected in increased accessibility of chromatin in specific loci of Th1 but decreased chromatin accessibility in Th17 cells. In vivo, GLS deficient T cells failed to induce Graft vs Host Disease in mice, and GLS deficient CAR T cells were unable to maintain B cell aplasia. However, temporary inhibition of GLS during CAR T cell generation resulted in enhanced T cell survival over the course of several weeks. This implies important distinctions in the timing of GLS inhibition that can be harnessed to either prevent autoimmunity or enhance tumor elimination in vivo.

42 Development of a new cell line to explore the role of the dermal macrophage in host defense

Allison K. Judge, Stephanie L. Brandt, C. Henrique Serezani

Skin host defense is initiated by keratinocytes and the resident phagocytes, Langerhans cells and dermal macrophages (DMs). Studies focusing specifically on DM biology are rather limited due to the low cell number and difficulties in isolating these cells. Here, we generated a new resident DM cell line (SB89) with a retrovirus that expresses a p50 oncogene (J2). DMs from C57BL/6 were isolated by sorting of F4/80+ cd68+ cells and treating with MCSF1 and J2 retrovirus. Proliferative cells were cloned and expanded for further characterization. We confirmed that our clones were indeed DMs by comparing the expression pattern of specific markers for both macrophages and dendritic cells. Macrophage effector functions were studied by comparing cytokine production and phagocytosis among SB89 and macrophage cell lines from the peritoneal cavity (PMs) and alveolar space (AMs). Our data show that DMs are more responsive to Staphylococcus aureus than PMs and AMs as evidenced by higher phagocytic capability and production of TNF-a, IL-6, and IL-1β. Interestingly, DMs showed poor ingestion of fungal particle zymosan when compared to the other cell lines. Conversely, seahorse experiments showed that DMs were more glycolytic than AMs and PMs when challenged with S. aureus, but not LPS or zymosan. Together, our data indicate that DMs SB89 can be a useful tool to study skin macrophage biology in different disease settings.

43 Dietary manganese promotes staphylococcal infection of the heart

<u>Lillian J. Juttukonda</u>^{1,2}, Matthew T. Stier^{1,2}, Joseph P. Zackular¹, Jessica L. Moore^{3,4}, Evelien T. M. Berends⁵, Yaofang Zhang⁴, Jonathan E. Schmitz¹, William N. Beavers¹, Christiaan D. Wijers^{1,2}, Thomas Kehl-Fie⁶, James Atkinson¹, R. Stokes Peebles^{1,7}, Victor J. Torres⁵, Richard M. Caprioli^{3,4,8}, and Eric P. Skaar^{1,9}

¹Department of Pathology, Microbiology, and Immunology; Vanderbilt University Medical Center; Nashville TN 37232; USA. ²Medical Scientist Training Program; Vanderbilt University School of Medicine; Nashville TN 37232; USA. ³Department of Chemistry; Vanderbilt University; Nashville TN 37232; USA. ⁴Mass Spectrometry Research Center; Vanderbilt University; Nashville TN 37232; USA. ⁵Department of Microbiology, Microbial Pathogenesis Program; New York University School of Medicine; New York NY 10016; USA. ⁶Department of Microbiology; University of Illinois at Urbana-Champaign; Urbana IL 61801; USA. ⁷Division of Allergy, Pulmonary and Critical Care Medicine, Department of Medicine; Vanderbilt University Medical Center; Nashville TN 37232; USA. ⁸Department of Biochemistry; Vanderbilt University; Nashville TN 37232; USA. ⁹Tennessee Valley Healthcare System; U.S. Department of Veterans Affairs; Nashville TN 37212; USA

During infection, pathogens obtain essential nutrient metals from the host. Alterations in metal homeostasis are associated with increased infectious disease risk, but the current understanding of how dietary metals influence the host-pathogen relationship remains limited. Manganese (Mn) is a common dietary supplement, and tissue levels of Mn vary based on exposures and clinical status, with contaminated groundwater, intravenous drug use, and parenteral nutrition increasing tissue Mn concentrations up to 20-fold. Furthermore, Mn is required by many bacterial pathogens for virulence. Despite this, the impact of dietary variation in Mn levels on infection outcomes has not been studied. Staphylococcus aureus is the leading cause of bacterial endocarditis and the second most frequent agent of bloodstream infections. S. aureus relies on high-affinity Mn import systems for virulence in mice, suggesting that manipulation of Mn could alter the risk of S. aureus infection. However, the impact of dietary Mn on S. aureus infection is unknown. Therefore, we set out to investigate the contribution of dietary Mn to S. aureus pathogenesis. Here we report that dietary Mn levels dictate the outcome of systemic infections caused by S. aureus. Mice fed a high Mn diet and infected with S. aureus displayed alterations in Mn levels and localization within tissues. When mice were provided a high Mn diet, S. aureus exhibited enhanced infection of the heart and augmented virulence. Although the canonical mammalian Mn-chelating protein calprotectin did accumulate within staphylococcal abscesses, calprotectin surprisingly did not sequester Mn in the heart. Indeed, excess Mn acquired from the high Mn diet was bioavailable to S. aureus in the heart. Bioavailable Mn was utilized by S. aureus to detoxify reactive oxygen species and protect against neutrophil killing, enhancing fitness within the heart. Therefore, excess dietary Mn is a weapon that S. aureus wields to overcome host antimicrobial strategies.

44 RNA Processing As A Gate Keeper To Cell Intrinsic Immunity

Yang Zhao, Xiang Ye, William Dunker, Yu Song, John Karijolich

The RIG-I like receptors (RLRs) RIG-I and MDA5 are cytosolic RNA helicases best characterized as restriction factors for RNA viruses. However, evidence suggests RLRs participate in innate immune recognition of other pathogens, including DNA viruses. Kaposi's sarcoma-associated herpesvirus (KSHV) is an oncogenic human gammaherpesvirus and the etiological agent of Kaposi's sarcoma and primary effusion lymphoma (PEL). Here, we demonstrate that RIG-I and MDA5 restrict KSHV lytic reactivation in PEL. Furthermore, we define the in vivo RLR substrates and demonstrate that RIG-I and MDA5-mediated restriction is facilitated exclusively by the recognition of host-derived RNAs. Misprocessed noncoding RNAs are prominent RIG-I bound RNAs, and biochemical characterizations reveal that an infection-dependent reduction in the cellular triphosphatase DUSP11 results in an accumulation of triphosphorylated noncoding RNAs, enabling their recognition by RIG-I. These finds reveal an intricate relationship between RNA processing and innate immunity, and demonstrate that an antiviral innate immune response can be elicited by the sensing of misprocessed cellular RNAs.

45 Prostaglandin E2 improves abscess formation and resolution in diabetic skin infection

Nathan Klopfenstein, Stephanie L. Brandt, Allison Judge, C. Henrique Serezani

Methicillin-resistant Staphylococcus aureus (MRSA) is the primary cause of skin infection in people with diabetes. MRSA skin infection is characterized by the migration of phagocytes and abscess formation to prevent deeper tissue infections. However, the events that drive poor host defense in diabetes is poorly understood. Here, we examined the role of the lipid mediator prostaglandin E2 (PGE2) and its impact on host defense during MRSA skin infection in diabetes. We have shown that diabetic mice are unable to control MRSA skin infection, along with excessive cytokine production and neutrophil migration. Our data indicate that PGE2 production is lower in diabetic mice than nondiabetic mice at both days 1 and 9. Daily topical treatment with a PGE2 analog decreased lesion size and improved bacterial clearance in the skin of infected diabetic mice. Reduced lesion size in PGE2-treated diabetic mice correlated with decreased production of the cytokines TNF-, IL-1, and IL-6 and neutrophil migration. While we did observe a well-defined abscess in infected nondiabetic mice, infection in diabetic mice also correlated with increased numbers of dead cells at days 1 and 9 post-infection suggesting that dead cells are not eliminated. When we studied the expression of molecules involved in dead cell ingestion, we observed an increased abundance of inhibitory receptor SIRPa in infected diabetic mice and PGE2 ontiment restored SIRPa levels back to those observed in infected nondiabetic mice. These data indicate that the threshold of PGE2 drives the inflammatory milieu during infection and effects skin homeostasis in diabetes.

46 Mucosal Nanoparticle Vaccine Generates Protective Tissue-Resident Memory CD8 T Cells in the Lungs

<u>Frances C. Knight</u>, Pavlo Gilchuk, Amrendra Kumar, Kyle W. Becker, Sema Sevimli, Max Jacobson, Sebastian Joyce, & John T. Wilson

Stimulating immunity through vaccination at mucosal surfaces mimics natural routes of pathogen entry and can generate tissueresident memory T cells (T_{RM}), which respond more quickly to subsequent pathogen encounter than memory T cells located in the bloodstream or secondary lymphoid organs. In order to generate long-lasting protective immunity against respiratory viruses or mucosal cancers, CD8 TRM are particularly important. We have developed a nanoparticle (NP) vaccine that can be loaded with protein antigen and nucleic acid adjuvant. The system utilizes a ApH-triggered endosomal escape mechanism to release cargo intracellularly, biasing the immune response toward generation of CD8 T cells. To test the ability of this vaccine to elicit a long-lasting cellular immune response with a CD8 TRM phenotype and protect against viral challenge, NP loaded with ovalbumin protein and CpG DNA (OVA-NP/CpG) were administered intranasally to C57BL/6 mice. Numbers of OVA-specific CD8 T cells expressing characteristic pulmonary TRM markers (CD103, CD69) were quantified at 30 and 60 days post-immunization and compared to the response from control formulations. The percentages of polyfunctional (IFNy⁺TNF α^+) CD8 T cells in the lungs and spleen were also determined at day 13 post-immunization. Protection against weight loss and reduction in viral burden after intranasal challenge with recombinant OVA-epitope-expressing vaccinia virus were assessed at 30 and 60 days postimmunization. On day 13, there are significantly higher levels of IFN γ^{+} TNF α^{+} cells in the lungs and spleens of mice immunized with OVA-NP/CpG relative to control groups. Despite a reduction in OVA-specific CD8 T cells of up to 15-fold by day 60, protection against viral challenge at 30 and 60 days is significantly better in mice immunized with OVA-NP/CpG, and there are also significantly more cells expressing CD103 and CD69 at these timepoints. This work has implications for developing vaccines against respiratory infections and lung cancer.

47 Mechanism and function of heme sensing and detoxification in *Clostridium difficile*

Reece J. Knippel^{1,2}, Joseph P. Zackular^{1,2}, Jessica L. Moore³, Arianna I. Celis⁴, Jennifer L. DuBois⁴, and Eric P. Skaar^{1,2}

¹Department of Pathology, Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN, USA, ²Vanderbilt Institute for Infection, Immunology and Inflammation, Vanderbilt University, Nashville, TN, USA, ³Department of Chemistry, Vanderbilt University, Nashville, TN, USA, ⁴Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT, USA

Clostridium difficile is a Gram-positive, spore-forming anaerobic bacterium that infects the colon, causing a number of symptoms ranging from infectious diarrhea to fulminant colitis. In the last decade, the number of C. difficile infections has dramatically risen making it the leading cause of reported hospital acquired infection in the United States. Surprisingly, little is known about the mechanisms by which C. difficile survives the harsh environment of the host during infection. During C. difficile infection (CDI), host heme is present in the gastrointestinal lumen due to bacterial toxin-mediated epithelial damage. The reactive nature of heme can lead to toxicity through membrane disruption, membrane protein and lipid oxidation, and DNA damage. We hypothesize that C. difficile detoxifies excess heme to survive within the lumen during infection. The heme responsive up-regulation of an operon was discovered through an RNA-seg analysis of C. difficile R20291 grown in the presence of a sub-toxic concentration of heme. This operon contains a candidate TetR family transcriptional regulator, which we have named heme activated transporter regulator (hatR), and a major facilitator superfamily transporter, which we have named heme activated transporter (hatA). Strains inactivated for hatR and hatA are more sensitive to heme toxicity. Further analysis determined HatR binds heme, which relieves the repression of the hatRA operon and HatA functions as a heme efflux pump. In a mouse model of CDI, a strain inactivated for hatA displayed lower pathogenicity in a toxin independent manner. Taken together, these data suggest that HatR senses intracellular heme concentrations leading to increased expression of the hatRA operon and subsequent heme efflux by HatA during infection. This system is unique to C. difficile as no homologs to HatR or HatA have been identified in other bacterial species. These results describe a novel mechanism to employed by C. difficile to relieve heme toxicity during infection, and set the stage for therapeutic interventions that target this bacterial specific system.

48 Using Multidimensional Approaches to Define the Risk and Immunopathogenesis of Serious Cutaneous Adverse Drug Reactions Associated with Nevirapine in South Africa

<u>Katherine Konvinse</u>¹, Jonathan Peter², Katie White¹, Louise Barnett¹, Alan Boyd¹, Rebecca Pavlos³, Julie Esterhuizen², Ramesh Ram³, Wyatt McDonnell¹, Shay Leary³, Alec Redwood³, Simon Mallal^{1,3}, Abha Chopra³, Rannakoe Lehloenya² and Elizabeth Phillips^{1,3}

¹Vanderbilt University Medical Center, Nashville, Tennessee; ²University of Cape Town, Cape Town, South Africa; ³The Institute for Immunology & Infectious Diseases, Murdoch University, Western Australia

Nevirapine use in combination antiretroviral therapy is limited by T-cell-mediated adverse reactions such as Stevens-Johnson Syndrome/toxic epidermal necrolysis (SJS/TEN) and Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS). SJS/ TEN is a human leukocyte antigen (HLA) class I restricted, CD8+ T-cell dependent hypersensitivity, which presents as a blistering skin rash that can lead to life-threatening epidermal necrosis, DRESS is characterized by fever, rash, hepatitis and eosinophilia. To study the immunopathogenesis of nevirapine-associated SJS/TEN and DRESS, a biobank of samples including PBMCs, plasma, buffy coat, saliva, blister fluid and skin was accrued from >80 nevirapine-exposed HIV+ patients that included motherchild nevirapine-exposed pairs where only one relative developed SJS/TEN. HLA, killer-cell immunoglobulin-like receptor (KIR), endoplasmic reticulum aminopeptidase (ERAP) and CYP2B6 typing was performed on all subjects. 100% of SJS/TEN cases (n=15) carried HLA-C*04:01 compared to 22.5% of unrelated individuals tolerating nevirapine (n=7/31) (p<0.00001). Novel associations with HLA-B*44:03 and HLA-B*45:01, which share peptide binding specificities, were identified for nevirapine DRESS. CYP2B6 genotyping revealed that hypersensitive patients had slower nevirapine metabolizing phenotypes than tolerant controls. Immunohistochemistry was used to stain formalin-fixed, paraffin-embedded skin from nevirapine SJS/TEN patients with antibodies against SJS/TEN biomarker granulysin as well as general T cell (CD3, CD4, CD8), skin homing (CCR4, CLA), natural killer cell (CD56), regulatory T cell (FoxP3), and tissue-resident (CD103) markers. Single T cell receptor sequencing (scTCRseq) revealed a dominant TCR $\alpha\beta$ CDR3 in the activated (CD137+) CD8+ T cells in the blister fluid that was found in only 1.0% of the CD8+ T cells in the peripheral blood. In a South African population, I identified a strong association between HLA-C*04:01 and SJS/TEN suggesting that this allele is necessary but not sufficient for SJS/TEN development. Results from the immunohistochemistry and sc-TCR studies provide important insights into why not all patients carrying an HLA risk allele might develop a reaction.

49 The inhibitory checkpoint molecule NKG2A is upregulated on tumor infiltrating NK cells and CD8 T cells in human head and neck tumors

Michael Korrer and Young J. Kim

Immunotherapy has revolutionized cancer therapy by targeting checkpoint molecules found on endogenous immune cells. Presently, the most commonly targeted checkpoint molecules are CTLA-4 and PD-1, which results in response rates of approximately 25% in head and neck squamous cell carcinomas (HNSCC). To improve treatment, additional immune checkpoint molecules expressed in the tumor microenvironment must be identified. Natural Killer Group 2 A (NKG2A) is an inhibitory receptor found on NK cells and CD8 T cells, the receptor for which is HLA-E, a non-classical MHC molecule often overexpressed in solid tumors. This study aimed to identify if NKG2A is expressed on tumor infiltrating NK cells and CD8 T cells from human HNSCC tumors as it is a potential therapeutic target.

We analyzed and compared tumor infiltrating NK cells and CD8 T cells from HNSCC patients with matched PBMC by flow cytometry for the expression of activating and inhibitory receptors. We found a unique population of effector memory PD-1+ NKG2A+ CD8 T cells which was absent from the blood. NKG2A+ PD-1+ CD8 T cells expressed higher levels of CTLA-4 and LAG3 as well as produced lower IFN γ than NKG2A- PD-1+ CD8 T cells. Interestingly, NKG2A+ PD-1+ CD8 T cells expressed higher levels of both Perforin and Granzyme B, suggesting that these cells are cytotoxically potent. In addition, we determined that the ligand for NKG2A, HLA-E, was abundantly expressed on CD45+ monocytes and T cells, but not on CD45- cells in the tumor.

We believe that this study provides the first characterization of human tumor-infiltrating NKG2A+ PD-1+ CD8 T cells. As we have also shown that NKG2A is upregulated in tumor infiltrating NK cells, we believe this makes NKG2A an ideal therapeutic target to improve anti-tumor cytotoxic responses. Ongoing studies are underway to determine the effect of inhibiting NKG2A on tumor immune responses.

50 Nur77 has a cell intrinsic role in natural killer T cell development and function

<u>Amrendra Kumar</u>^{1,2}, Naveenchandra Suryadevara², Timothy M. Hill², Laura E. Gordy², Jelena S. Bezbradica², Lan Wu², Pankaj Acharya³, Andrew I. Flyak², Scott W Hiebert³, Luc Van Kaer² & Sebastian Joyce^{1,2}

¹Veterans Administration Tennessee Valley Healthcare System, Nashville; ²Pathology, Microbiology & Immunology; ³Department of Biochemistry; Vanderbilt University School of Medicine, Nashville, TN

Natural killer T (NKT) cells are self reactive, innate-like lymphocytes that respond to self glycolipids under inflammatory conditions. Nur77, an orphan nuclear receptor family transcription factor, is induced upon self glycolipid recognition by the T cell receptor very early during NKT cell ontogeny. Nur77 function in NKT cell has not been explored, however. Nur77 promotes negative selection of conventional T cell. It transcriptionally controls the development of regulatory T cells (Tregs) and effector CD8 T cells. Thus, we hypothesized that Nur77 initiates an NKT cell lineage-specific gene expression program and sculpts the semi-invariant NKT cell T cell receptor repertoire. We found that double positive cells in transgenic mice that overexpressed wild type Nur77 (wtNur77tg) in T lineage only underwent apoptosis. Consequently, wtNur77tg mice poorly developed mature T and NKT cells. Introgression of the rearranged Va14i a-chain gene into wtNur77tg mice (wtNur77tg;Va14tg mice) rescued NKT cell ontogeny but arrested development at a precursor stage immediately after positive selection. Residual NKT cells in wtNur77tg;Va 14tg mice do not respond to glycolipid agonists. These finding are consistent with new global gene expression and *in-silico* analyses. Collectively, our data suggest that Nur77 induction initiates a gene expression program in developing NKT cells that are critical for developmental progression and function of NKT cells.

51 Two component system crosstalk: cooperative response to host stressors in Bacillus anthracis

Clare L. Laut, Laura A. Mike, Devin L. Stauff, & Eric P. Skaar

The rapidly increasing resistance of pathogenic bacteria to all relevant antimicrobials is a tremendous threat to global public health. Targeting the elegant systems possessed by bacterial pathogens used to cope with the onslaught of toxic molecules present within the hostile host environment is a promising strategy for antibacterial development. While in the host, Bacillus anthracis, the causative agent of anthrax, grows to extreme densities in the blood resulting in erythrocyte death and heme release. Accordingly, B. anthracis is extraordinarily resistant the toxic heme stress. In bacteria, gene expression changes in response to stress can occur via signal transduction systems known as two component systems (TCS). Though these systems most often function linearly with cognate partners, there is emerging recognition for interactions of signaling systems after stimulus that have physiologic importance, termed cross regulation. B. anthracis, encodes two signaling systems, HssRS and HitRS, which demonstrate the rarely observed occurrence of TCS cross regulation . HssRS (Heme sensor system) responds to high levels of heme, the iron-containing cofactor of hemoglobin, and interacts with HitRS (HssRS interfacing TCS) that is activated by cell envelope stress. Initial stages of anthrax infection involve germination of spores in host macrophages. We propose that HitRS is required for survival in the phagocyte because of the response to cell wall damage. The vegetative bacilli escape the macrophage and proceed to intoxicate the host while circulating in the bloodstream, presumably resulting in strong HssRS activation. We propose a model whereby (i) HssRS and HitRS sense heme and cell envelope stress to enable a coordinated response to these distinct stressors, (ii) this response results in the appropriate response to the stressor to maintain bacterial fitness, and (iii) the integrity of this synchronized stress response is required for priming during the B. anthracis pathogenic cycle between macrophages and the bloodstream. This model will be tested by defining the functions of HssRS and HitRS using growth analysis and in vitro cell culture infections, and by determining if crosstalk signaling in vivo using fluorescent reporters. Quantification of the ability of B. anthracis to escape from cultured macrophages confirmed that HitRS is required for this process. HssRS and HitRS signal integration was observed after specific activation using genetic reporters imaged with super resolution microscopy, solidifying the occurrence of cross-regulation. Future work will identify the agonists of HitRS and HssRS, determine signaling kinetics of crosstalk, and query the contribution of crosstalk to B. anthracis infection. Reacting to host factors in a cooperative manner may provide B. anthracis with the foundation to be a professional pathogen, partially explaining how this organism causes such severe infectious diseases.

52 How a bacteriophage helps a tiny bacterium combat arbovirus transmission

Brittany A. Leigh¹, J. Dylan Shropshire¹, Edward van Opstal¹, Katrina Ngo¹, Seth Bordenstein^{1,2}

¹Vanderbilt University, Department of Biological Sciences, Nashville, TN, USA; ²Vanderbilt University Medical Center, Department of Pathology, Microbiology & Immunology, Nashville, TN, USA

Mosquito-borne viruses impact hundreds of millions of people worldwide, and many of the diseases they cause lack effective therapeutics. Consequently, many governments and institutions are using novel biocontrol strategies that curb virus transmission based on genetic or symbiont modification. The World Health Organization recently endorsed pilot releases of mosquitoes harboring the endosymbiotic bacteria *Wolbachia* because it confers insect resistance to a number of RNA viruses and encodes a natural drive system that allows it to spread through mosquito populations, a process known as cytoplasmic incompatibility (CI). CI is characterized by an unknown sperm genomic modification set in the testes that results in early embryonic death. Two genes in prophage WO of *Wolbachia* - cytoplasmic incompatibility factors A and B (*cifA* and *cifB*) – can recapitulate CI. Building upon these results, we now show that prophage WO induces particles in the released *Wolbachia* strain used for vector control, raising the hypothesis that phage WO particles transport the Cif proteins to sperm to cause CI. These viral particles are ~30 nm and occur inside and outside the *Wolbachia* cells inhabiting the testes. To test the hypothesis that phage WO particles transport the Cif proteins to sperm to cause CI. These viral particles transport the Cif proteins, injection of uninfected male insects with viral particles from their respective *Wolbachia* infections resulted in recapitulation of CI. Ongoing experiments seek to characterize localization patterns of CifA and CifB in the reproductive tissues through the generation of specific antibodies for each protein. This emerging project on the mechanistic basis of CI aims to (i) provide insight into the functions of the two CI proteins, (ii) determine how they interact with sperm to confer CI, and (iii) inform ongoing and future vector control efforts enabled by *Wolbachia* and prophage WO genes.

53 Functions and Protein-Protein Interactions of the Helicobacter pylori cag Type IV Secretion System ATPases

Aung S. Lin, Arwen E. Frick-Cheng, Mark S. McClain, and Timothy L. Cover

Background: *Helicobacter pylori* persistently colonizes the gastric mucosa of about half of the world's population. Chronic infection with this bacterium can lead to the development of peptic ulcer disease or gastric adenocarcinoma. The *cag* type IV secretion system (*cag* T4SS) is utilized by *H. pylori* to translocate the oncogenic effector protein CagA and other bacterial constituents into gastric epithelial cells. The ATPases of the *cag* T4SS (Cag α , Cag β and CagE) are predicted to power assembly of the secretion system and/or effector translocation. The goals of this study are to dissect the specific functions of each of the ATPases and define their interactions with other components of the *cag* T4SS.

Methods: Genes encoding each of the *cag* ATPases were individually mutated. *H. pylori* strains were co-cultured with gastric epithelial cells and the activity of the *cag* T4SS was assessed by monitoring NF-kB activation or IL-8 production. Strains producing epitope-tagged *cag* ATPases were constructed and the tagged proteins were immunopurified. Proteins co-purified with the tagged proteins were identified using mass spectrometry.

Results: cagE::kan and $\Delta cag\alpha$ mutants were unable to activate NF-kB or induce IL-8 secretion in AGS cells, indicating loss of cag T4SS activity. In contrast, a $\Delta cag\beta$ mutant strain retained the ability to activate NF-kB and stimulate IL-8 secretion. Strains producing epitope-tagged forms of Cag α , Cag β and CagE were generated and the tagged proteins were immunopurified in the presence of multiple detergent conditions. CagZ was consistently co-purified with Cag β , which suggests that these proteins physically interact. Co-purification of CagZ with Cag β was also detected when Cag β was immunopurified from lysates of *H. pylori* co-cultured with gastric epithelial cells.

Conclusions: These results provide evidence that multiple ATPases associated with the *cag* T4SS are required for *cag* T4SS activities, and provide insight into protein-protein interactions involving these ATPases.

54 Acinetobacter baumannii employs a zinc binding carboxypeptidase to overcome host-imposed nutrient and envelope stress

Zachery R. Lonergan^{1,2}, Brittany L. Nairn¹, Walter J. Chazin^{3,4,5}, and Eric P. Skaar¹

¹Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN; ²Microbiology and Immunology Training Program, Vanderbilt University School of Medicine, Nashville, TN; ³Center for Structural Biology, Vanderbilt University School of Medicine, Nashville, TN; ⁴Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN; 5Department of Chemistry, Vanderbilt University, Nashville, TN

Acinetobacter baumannii has emerged as an important nosocomial pathogen that is capable of causing a range of diseases, including respiratory infections and bacteremia. Treatment for these infections is limited due to increasing rates of antibiotic resistance, underscoring the importance of identifying new targets for antimicrobial drug development. During infection, *A. baumannii* must acquire nutrient metals in order to survive and colonize the host. Vertebrates have developed mechanisms to sequester these metals from invading pathogens by a process termed "nutritional immunity." The vertebrate protein calprotectin (CP) inhibits *A. baumannii* growth through zinc (Zn) sequestration. We have found that one of the most upregulated genes during Zn starvation encodes a Zn-binding lipoprotein that localizes to the *A. baumannii* inner membrane and is a putative carboxypeptidase involved in peptidoglycan remodeling. Based on these findings, we have named this gene *zrlA* (Zur-regulated Lipoprotein A). Genetic inactivation of *zrlA* results in diminished growth in the presence of CP. The *zrlA* mutant also displays increased cellular envelope permeability and decreased outer membrane barrier function, which results in increased antibiotic susceptibility. Finally, a mouse model of *A. baumannii* pneumonia revealed the *zrlA* mutant is defective in colonizing the lungs and disseminating to the liver. Taken together, these results show that *A. baumannii* utilizes ZrlA to overcome Zn limitation and maintain cell envelope integrity and suggests that ZrlA is an important bacterial component to survive the host immune response.

55 A Host Immune Protein Guides Clostridium difficile Niche Selection in the Gut

Christopher A. Lopez, Joseph P. Zackular, Reece J. Knippel, and Eric P. Skaar

The large intestines house a diverse microbiota that collectively protect against invasion by pathogenic bacteria, termed colonization resistance. *Clostridium difficile*, an anaerobic spore-forming bacterium, takes advantage of antibiotic-induced disruption of colonization resistance to initiate gut colonization. However, subsequent to initial colonization *C. difficile* must establish a niche where the pathogen can compete with the resident microbes for nutrients while resisting host-mediated changes to the nutrient environment. The limiting nutrients governing *C. difficile* interactions with the microbiota and the role of the host in manipulating access to those nutrients during *C. difficile* infections (CDI) are unclear. One arm of the host response during CDI that impacts the gut environment is the release of the protein calprotectin, which binds and sequesters the nutrient metals zinc, manganese, nickel, and iron. In a mouse model of CDI we found that the presence of calprotectin was associated with shifts in *C. difficile* gene expression, particularly in genes required for proline fermentation. We thus hypothesize that proline metabolism is regulated in part through the availability of nutrient metals and that this metabolic shift allows *C. difficile* to grow in the presence of competing microbes. Future work to uncover the role of proline metabolism in the context of infection will provide insight into how the host immune response drives pathogen adaptation to alternate nutritional niches as well highlight strategies used by *C. difficile* to overcome barriers to gut survival.

56 Oxidized Low-density Lipoprotein Immune Complexes Modulate Adaptive Immune Responses and Contribute to Atherosclerotic Outcomes *in vivo*

Jennifer Marvin, Jillian P. Rhoads, Yanice Mendez-Fernandez, and Amy S. Major

Atherosclerosis is a chronic inflammatory disease that underlies two of the top five leading causes of death - heart attack and stroke. Oxidized lipoproteins (oxLDL) have long been known to contribute to plaque formation, however, much of the oxLDL in circulation complexes with specific antibodies to form oxLDL immune complexes (oxLDL-ICs). While oxLDL-ICs have been used clinically as a biomarker of disease severity, recent evidence from our laboratory and others suggest that oxLDL-ICs may directly impact disease pathogenesis. We previously reported that oxLDL-ICs are more potent primers of the NLRP3 inflammasome and generate higher levels of IL-1ß in dendritic cells (DCs) compared to oxLDL alone. In turn, oxLDL-IC mediated IL-1ß promoted greater IL-17 production from OTII T cells. Here, we report that oxLDL-IC not only increased IL-17 production, but also subsequently decreased IFN γ levels compared to oxLDL. We hypothesized that the decrease in IFN γ could be due to increased IL-23. When we treated BMDCs with oxLDL-IC, we indeed found that they expressed 4-fold more il23 compared to treatment with oxLDL. Furthermore, performing the T-cell co-culture experiment in the presence of an IL-23 neutralizing antibody restored IFN γ levels but had no effect on IL-17 production. These results demonstrate that oxLDL-ICs have the ability to modulate not only innate but also adaptive immunity. To test the relevance of these findings in an *in vivo* model, we injected mice with vehicle, oxLDL, or oxLDL-ICs biweekly for 8 weeks. We found that the mice injected with oxLDL-IC had a 40% higher plague burden compared to control groups, and these changes were independent of serum cholesterol and triglyceride levels. Collectively, these data demonstrate that not only are oxLDL-ICs are important modulators of both the innate and adaptive immune system, but they actually contribute to atherosclerosis progression in vivo.

57 Manganese and calprotectin alter neutrophil function in the heart during Staphylococcus aureus infection

Andrew J. Monteith, Lillian J. Juttukonda, & Eric P. Skaar

Staphylococcus aureus is responsible for approximately 40,000 annual deaths in the United States, and is the leading cause of bacterial endocarditis. Further, the increasing prevalence of antibiotic resistant S. aureus has heightened the risk of infection while limiting therapeutic options. Combined, this makes S. aureus one of the most important infectious threats to public health. We previously demonstrated that increased dietary manganese (Mn) renders the heart hyper-susceptible to S. aureus infection. Mn is required for S. aureus to achieve full pathogenicity, and as such, the host restricts Mn from the pathogen through a process termed 'nutritional immunity'. Calprotectin (CP) is a heterodimer consisting of S100A8 and S100A9, and is critical to nutritional immunity since it is the only identified Mn-sequestering immune protein in vertebrates. Surprisingly, CP-deficient (\$100a9^{-/-}) mice, which fail to restrict Mn from the pathogen, have a multi-log decrease in S. aureus burdens in the heart. This suggests alternative roles for Mn and CP within the heart. Herein, we show that increased concentrations of Mn reduces mitochondrial production of superoxide (O_2^{-}), while s100a9^{-/-} immune cells produce heightened levels of mitochondrial O_2^{-} . We demonstrate that this is due to an alteration in mitochondrial function. In neutrophils, O2- production is necessary for neutrophil extracellular trap (NET) activation and release (NETosis). Consistent with a role for O2⁻ in neutrophil function, increased concentrations of Mn enhances neutrophil degranulation and restricts NETosis. In contrast, s100a9^{-/-} neutrophils fail to degranulate but have heightened NETosis; a phenotype that is enhanced in the heart during S. aureus infection. Overall, these findings offer insight into the host-pathogen interface revealing that nutrient metals, such as Mn, and CP are not only important to the pathogen and nutritional immunity, but may also play a direct and organ-specific role in modulating immune cell function during infection.

58 Zika virus infects human vaginal epithelial cells: Implications of sexual transmission

James W. Mungin Jr., Bindong Liu

Department of Microbiology, Immunology, and Physiology, Meharry Medical College, Center for AIDS Health Disparity Research, Meharry Medical College, Nashville, TN

Zika virus (ZIKV), a RNA virus in the Flavivirus genus, have been declared a public health concern. Since the 2015 ZIKV outbreak in Brazil, Centers for Disease Control, and Prevention reports over 35,000 cases ZIKV disease cases within the United States (US) and US territories. As of 2018, more than 6,000 pregnant women with laboratory evidence of possible ZIKV infection were documented in the US. Out of these women, the CDC revealed over 200 cases of live and dead infants with birth defects including but not limited to structural eye abnormalities, calcification, and microcephaly. In addition to these defects, clinical evidence show ZIKV-infected adult patients suffering from Gullian-Barré syndrome, an autoimmune neuropathy in which damages neurons result in muscle weakness and limb paralysis. While ZIKV is primarily transmitted through an infected Aedes mosquito, studies have emerged showing ZIKV can be acquired via sexual contact with an infected partner. The CDC confirmed 52 ZIKV human cases have been acquired through sexual transmission within the US. Mounting evidence from animal and cell studies demonstrate that the female reproductive tract supports ZIKV replication. Furthermore, one study shows ZIKV vaginal infection resulted in fetal brain infection and growth restriction in pregnant mice. Despite the growing evidence associating ZIKV to sexual transmission, the underlying molecular mechanism involving vaginal transmission remains elusive. In this study, we investigated the role of the vaginal epithelium in ZIKV sexual transmission using the VK2/E6E7 cell model system. Using gRT-PCR analysis and immunofluorescent assay, we showed that human vaginal epithelial cells (hVECs) are permissive to ZIKV infection. In addition, our findings indicate hVECs persistently produce infectious particles, while exhibiting no cytopathic effect following exposure. Collectively, these data suggest that the vaginal epithelium may serve as an initial site for ZIKV infection and replication during sexual transmission.

59 Development of DIMAGEN and Fingerprinting Technologies for the Design and Evaluation of Next-Generation HIV-1 Immunogens

Amyn Murji

HIV-1 continues to impose a large 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 simultaneous presentation of multiple diverse Envs to the immune system. Here, we present progress toward the design and validation of a number of these technologies, which we call diverse multi-antigen immunogens (DIMAGENs). To allow for efficient and accurate evaluation of antibody responses to the designed DIMAGENs, we are developing a binding fingerprinting technology, which applies computational algorithms for predicting the epitope specificities of antibody responses to infection and to vaccination. The technologies that we have developed will be generalizable to vaccine design for other viruses that exhibit high levels of sequence diversity.

60 Osteopontin provides a survival signal for intestinal intraepithelial lymphocytes in mice

Ali Nazmi, M. Blanca Piazuelo and Danyvid Olivares-Villagomez

Intraepithelial lymphocytes (IEL) are a diverse immune population residing in between intestinal epithelial cells, which possess important roles during mucosal immune responses. The IEL family comprises cells such as $TCR\alpha\beta^+CD4^+$, $TCR\alpha\beta^+CD8^+$, $TCR\alpha\beta^+CD8\alpha\alpha^+$, $iCD8\alpha\alpha^+$ and $iCD3^+$, among others. Osteopontin (OPN), a pleiotropic cytokine encoded by the Spp-1 gene, is well known for its function in tissue remodeling, modulation of Th1 responses development, maintenance of Th17 function, and homeostasis of NK cells. Herein, we investigated the impact of OPN in the IEL compartment.

We compared the cell numbers of IEL, lamina propria (LP) lymphocytes and splenocytes from Spp-1^{-/-} and wild-type (WT) mice. OPN deficiency reduced the number of TCR $\gamma\delta^+$, TCR β^+ CD4⁺, TCR β^+ CD8 α^+ and TCR β^+ CD4⁺CD8 α^+ IEL, but had no effect on LP and spleen lymphocytes. In in vitro studies, IEL from WT mice cultured in the presence of rOPN showed better survival rates than IEL incubated alone. The increased survival was blunted to control levels when CD44, a receptor for OPN, was blocked with anti-CD44 antibody.

When total T cells (including Tregs) from WT splenocytes were transferred into Spp-1^{-/-}Rag-2^{-/-} and Rag2^{-/-} mice, we observed that although OPN deficiency did not affect donor-derived IEL reconstitution at day 7 post-transfer, it decreased the number of CD4⁺ and CD4⁺CD8a⁺ IEL at day 28 post-transfer. As expected, Rag-2^{-/-} mice remained healthy through the experiment; however, Spp-1^{-/-}Rag-2^{-/-} recipient mice presented a remarkably loss of body weight and colon inflammation.

Together, our results indicate that OPN is a critical survival signal for IEL via CD44, and that OPN-deficiency promotes development of colitis even in the presence of T_{regs} .

61 Mature anti-insulin B cells survive and present antigen in the absence of Bruton's tyrosine kinase

Lindsay E. Nyhoff^{1,2}, Emily S. Clark³, Wasif N. Khan³, and Peggy L. Kendall^{1,2}

¹Department of Pathology, Microbiology, and Immunology, Vanderbilt University School of Medicine, Nashville, TN 37232 USA; ²Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN 37232 USA; ³Department of Microbiology and Immunology, University of Miami Miller School of Medicine, Miami, FL 33136 USA

Bruton's tyrosine kinase (Btk) is a tec-family kinase present in B lymphocytes and innate immune cells. Btk is an important regulator of autoreactive B cells. In the non-obese diabetic (NOD) mouse model of type 1 diabetes (T1D), *Btk*-deficiency is protective against disease development and results in significant loss of anti-insulin IgG, even as total IgG is preserved. Anti-insulin B cells drive T1D by presenting antigen to autoreactive T cells. In a transgenic model, conventional *Btk*-deficiency reduces anti-insulin B cells by 95%, effectively blocking their development. However, the ability of mature anti-insulin B cells to survive or present antigen without Btk was unknown. We induced deletion of *Btk* using a loxP-flanked *Btk* mouse model paired with tamoxifen-inducible Cre. Surprisingly, these anergic anti-insulin B cells survive without Btk, as normal numbers of mature B cells were maintained in transgenic *Btk*^{flox}/*Cre-ER*^{T2} animals after tamoxifen treatment. *Btk*-negative anti-insulin B cells also remained able to internalize and present antigen to cognate T cells and to phosphorylate phospholipase C γ 2 in response to anti-IgM. These findings show that though anti-insulin B cells require Btk for their development, it is not required for mature anti-insulin B cell survival. In addition, our finding that Btk-negative anti-insulin B cells can present antigen may have implications for the use of Btk-inhibition in autoimmunity driven by this mechanism.

62 Circulating microbial small RNAs are altered in patients with rheumatoid arthritis

<u>Michelle J Ormseth</u>, Shilin Zhao, Ryan Allen, Joseph F Solus, Quanhu Sheng, Yan Guo, Fei Ye, Marisol A Ramirez, Qiong Wu, Kasey C Vickers, C Michael Stein

Bacteria and fungi produce small RNAs (sRNAs) with capacity to alter human cellular processes. Interest surrounds microbial influence on rheumatoid arthritis (RA), but mechanisms remain unclear. Our objective was to determine if microbial plasma sRNAs are altered in RA versus control subjects and changed by disease modifying anti-rheumatic drugs (DMARDs). Small RNA sequencing was performed on plasma from 165 RA patients and 91 matched controls and a separate cohort of 70 RA patients before and after starting a DMARD. Genome alignments for RA-associated bacteria, representative bacterial and fungal human microbiome genomes and environmental bacteria were performed. Microbial genome counts and individual sRNAs were compared by DESeq2 with multiple test correction. Genome counts of Lactobacillus salivarius, Anaerobaculum hydrogeniformans, Staphylococcus epidermidis, Sphingobacterium spiritivorum, and Staphylococcus aureus were decreased in RA versus controls. Three individual microbial sRNAs derived from a microbial transfer RNA were increased in RA versus controls, inversely associated with disease activity, and bioinformatically predicted to dampen RA-associated inflammatory response and promote bacterial survival. Higher total microbial sRNA reads were associated with lower RA disease activity. Baseline total microbial sRNAs were 3-fold higher among RA patients who improved with DMARD versus those who did not, but did not change significantly after 6 months of treatment. These data show that microbial plasma sRNAs are altered RA vs control subjects, inversely associated with disease activity, and increased in those who improved on DMARD. Bioinformatics predictions suggest individual sRNAs enriched in RA have the capacity to act as virulence factors by promoting microbial survival while dampening RA disease activity.

63 Examining the role of Cyclophilin A in HIV-1 pre-integration complex function

Adrian Padron, Muthukumar Balasubramaniam, Jiong Shi, Jui Pandhare, Christopher Aiken, and Chandravanu Dash

Center for AIDS Health Disparities Research, School of Graduate Studies and Research, Meharry Medical College, Nashville, TN; Department of Microbiology, Immunology, and Pathology, Vanderbilt University School of Medicine, Nashville, TN

The ongoing global HIV/AIDS epidemic has accounted for 35 million deaths and additional 37 million people that are living with the virus. Although there is no cure for HIV, antiretroviral therapies (ART) have been highly effective in controlling and managing the disease. However, the drugs used in the ART regimen are toxic, face viral resistance and costly. Therefore, there is a continuous need to identify novel targets for new antiretroviral drugs. The goal of this study is to understand the role of Cyclophilin A (CypA) in HIV-1 infection, with a specific focus on testing the effects of CypA on HIV-1 Preintegration complex (PIC) function. CypA is a cellular prolyl isomerase that promotes HIV-1 infectivity. CypA binds to HIV-1 capsid and knockdown, deletion and inhibition of CypA blocks infection. However, the mechanism by which CypA affects these early steps of infection is poorly understood. In initial studies, we infected the T cell lines Jurkat.WT and Jurkat.CypA^{-/-} and isolated the cytoplasmis PICs. Integration activity measurements by qPCR show that PICs from CypA^{-/-} cells exhibit lower integration activity relative to the PICs from Jurkat.WT cells. These results suggest a regulatory role for CypA-CA interaction in PIC function. To better understand the role of CypA, currently we are probing the effects of CypA on PIC function in several CD4+ T cell lines that support HIV-1 infection. Isolation of PICs and activity measurements from the T cells (both WT and CypA^{-/-}) will provide biochemical evidence for a role of CypA on the early events of HIV-1 infection. Finally, probing the composition of PICs isolated from WT and CypA^{-/-} cells will reveal the effects of CypA on PIC function. Collectively, these studies will generate new knowledge on host-pathogen interaction during early steps of HIV-1 infection that is critical for future antiviral therapy.

64 Environmentally Triggerable Retinoic Acid-Inducible Gene I Agonists Using Synthetic Polymer Overhangs

Christian R. Palmer, Max E. Jacobson, Olga Fedorova, Anna M. Pyle, and John T. Wilson

Retinoic acid-inducible gene I (RIG-I) is a cytosolic pattern recognition receptor that potently activates antiviral innate immunity upon recognition of 5' triphosphorylated double-stranded RNA (pppRNA). Accordingly, RNA ligands of the RIG-I pathway have recently emerged as promising antiviral agents, vaccine adjuvants, and cancer immunotherapeutics. However, RIG-I is expressed constitutively in virtually all cell types, and therefore administration of RIG-I agonists causes risk for systemic inflammation and possible dose-limiting toxicities. Here, we establish proof-of-concept and initial design criteria for pppRNA prodrugs capable of activating the RIG-I pathway in response to specific environmental stimuli. We show that covalent conjugation of poly(ethylene glycol) (PEG) to the 3' end of the complementary strand, i.e., on the same side but opposite strand as the 5' triphosphate group, can generate a synthetic overhang that prevents RIG-I activation. Additionally, conjugation of PEG through a cleavable linker—here, a reducible disulfide bond—allows for removal of the synthetic overhang and restoration of immunostimulatory activity. Furthermore, we demonstrate that blockade of RIG-I activation via synthetic overhangs is dependent on PEG molecular weight, with a critical molecular weight between 550 and 1000 Da required to inhibit activity. Additionally, we demonstrate that blockade of RIG-I activity is conjugation of PEG to the opposite end of the RNA did not influence ligand activity. Collectively, this work demonstrates that conjugation of synthetic polymer overhangs to pppRNA through cleavable linkers is a viable strategy for the development of environmentally triggerable RIG-I-targeting prodrugs.

65 Dietary zinc restriction compromises host immunity to Acinetobacter baumannii lung infection

Lauren D. Palmer, Lillian J. Juttukonda, Kelli L. Boyd, Eric P. Skaar

Zinc deficiency affects one third of the global population, and the World Health Organization estimated that zinc deficiency contributes to up to 16% of lower respiratory infections globally. However, the molecular mechanisms linking zinc deficiency and pneumonia remain uncharacterized. In the United States, intensive care unit patients are at increased risk for zinc deficiency and infection by the opportunistic pathogen *Acinetobacter baumannii*. Because zinc acquisition is critical to *A. baumannii* during lung infection, we established a murine model of zinc deficiency and *A. baumannii* pneumonia. In this model, zinc deficient mice suffer significantly higher mortality within 24 h of infection, establishing a system to probe host and bacterial response to dietary zinc restriction. At 24 h post infection, zinc deficient mice have higher bacterial burdens, higher production of pro-inflammatory cytokines, lower production of anti-inflammatory cytokines, and higher rates of inflammation and immune cell apoptosis. In order to understand how dietary zinc alters the host environment, *A. baumannii* genes important for survival in zinc deficient mice were identified using transposon sequencing (Tn-seq). This analysis identified key *A. baumannii* metabolic pathways as important during infection of the zinc deficient host including metal acquisition. Further experimentation revealed that zinc deficient mice have a defect in nutritional immunity, the innate immune response that restricts pathogen access to nutrient metals. Together, these results indicate that dietary zinc restriction causes host immune dysregulation and failure to control bacterial replication, which demand additional metabolic requirements of the bacterial pathogen.

66 Juvenile Idiopathic Arthritis Mediated by Defective GATA-binding Protein 3

Anna E. Patrick, T. Brent Graham, Thomas M. Aune, Jessica B. Duis

Juvenile inflammatory arthritis (JIA) is the most common inflammatory joint disease in childhood, yet its etiology and pathogenesis are poorly understood. Both genetics and environmental influences play an important role in development of JIA. Many of the identified genes that confer risk have recognized roles in T helper (Th) cell function. Importantly, patterns of Th cell function including augmentation of Th1 and Th17 pathways and decrease of the Th2 pathway have been implicated in various models of arthritis. In genome wide associated studies, *GATA3* has an association with rheumatoid arthritis and displays altered levels of transcription in JIA. This gene encodes GATA-binding protein 3 (GATA-3), which is a key transcription factor that drives the differentiation of T cells along the Th2 pathway and suppresses the Th1 and Th17 pathways. Mutations in GATA3 cause a syndrome of hypoparathyroidism, sensorineural deafness, and renal dysplasia (HDR syndrome) due to loss of GATA-3 function. We identified a child with HDR syndrome and JIA who has a novel mutation in GATA-3. This mutation results in a frame shift causing stop-gain and elongation of the C-terminus of the protein. The mutant GATA-3 is expressed at both the RNA and protein levels. It has decreased function as indicated by a reduced ability to drive GATA-dependent luciferase expression. In an *ex vivo* assay, peripheral blood mononuclear cells from the index patient were activated without polarizing cytokines to become Th0 cells, or polarized with cytokines to become effector Th1, Th2, and Th17 cells. In the index patient, the activity of effector Th1 and Th17 cells is increased as compared to effector Th2 cell activity. Surprisingly, the Th0 cells have a Th1 response. This suggests a previously undescribed mechanism of JIA and extends the spectrum of GATA3 associated disease to include JIA.

67 The Impact of Dysbiosis and Cytokine Changes on Inflammatory Bowel Disease-Associated Bone Loss

Christopher Peek, Nicole Putnam, Caleb Ford, Jacob Curry, & Jim Cassat

Inflammatory bowel disease (IBD) affects over 3.6 million individuals and is marked by severe gastrointestinal inflammation. Yet, up to 40% of patients suffering from IBD experience extra-intestinal disease manifestations, of which alterations in skeletal homeostasis are the most common and increase osteoporotic fracture risk by 40% compared to the general population. While many factors, including malabsorptive malnutrition and glucocorticoid use, contribute to pathologic bone remodeling, nutritionally replete and glucocorticoid naïve patients persistently experience bone loss. Emerging evidence supports a role for aberrantly elevated inflammatory cytokines and perhaps undiscovered factors as drivers of IBD-associated bone loss. Here we established a model of IBD-associated bone loss using dextran sulfate sodium (DSS) to induce gastrointestinal inflammation. We demonstrated rapid, significant reductions in trabecular bone volume prior to weight loss during DSS administration via micro-CT. These changes persisted following DSS withdrawal. Additionally, we identified several cytokines, including IL-12, IL-23, TNF- α , CXC5, and CXCL10 as potential drivers of IBD-associated bone loss. In addition to cytokine changes, patients with IBD undergo significant alterations to their microbiome. Recent studies highlight the ability for the intestinal microbiome to regulate skeletal homeostasis. Therefore, we hypothesized that in the context of gastrointestinal inflammation, circulating microbial components are translocated across the gut epithelium, sensed by skeletal cells, and contribute to IBD-associated bone loss. We have set up powerful *in vitro* assays to test these hypotheses. Ultimately, this work will help more precisely define the drivers of IBD-associated bone loss and clarify the effects IBD-associated cytokines on bone remodeling during gastrointestinal inflammation.

68 *wmk* is a Wolbachia prophage gene that kills male Drosophila

<u>Jessamyn I. Perlmutter</u>, Sarah R. Bordenstein, Daniel P. LePage, Jason A. Metcalf, Tom Hill, Julien Martinez, Robert L. Unckless, Francis M. Jiggins, & Seth R. Bordenstein

Wolbachia are maternally-transmitted bacteria that infect almost half of all arthropods and many nematode species worldwide. In arthropods, these bacteria selfishly manipulate host reproduction to enhance the fitness of infected, transmitting females, thereby facilitating their own spread through the host population. One such phenotype is male killing, where the sons of infected females are selectively killed. This reduces competition among their surviving, *Wolbachia*-transmitting sisters. Despite significant impact of *Wolbachia* on animal reproduction, evolution, and vector control, the microbial genes underlying most of these reproductive manipulations remain elusive. Here, we demonstrate the discovery of a single gene in the eukaryotic association module of *Wolbachia*'s prophage, WO, which kills male *Drosophila* embryos. The gene, hereafter denoted WO male killing (*wmk*), causes male lethality when transgenically expressed in uninfected *Drosophila* melanogaster. Specifically, transgenic expression of *wmk* results in a female-biased sex ratio, reduced hatching of male embryos, and several male-biased cytological defects during early embryonic development that are typical of *Wolbachia*-induced male killing. The discovery of *wmk* pioneers genetic studies of microbial-induced male killing. It also continues to highlight the significance of prophage WO genes in shaping selfish symbiont phenotypes and informs their potential in suppression or modification of pest and vector populations.

69 Molecular Insight into the Lipidome of the *Staphylococcal Infectious* Interface Using Multi-modal MALDI Imaging Mass Spectrometry

William J. Perry, N. Heath Patterson, Caroline Grunenwald, Jessica L. Moore, Boone M. Prentice, James E. Cassat, Raf Van de Plas, Eric P. Skaar, Jeffrey M. Spraggins, and Richard M. Caprioli

Antibiotic-resistant Staphylococcus aureus is a public health threat causing an estimated 20,000 deaths per year in the United States alone. Infections caused by S. aureus have the ability to cause a broad range of life-threatening illnesses in a vertebrate host, ranging from soft tissue infections to systemic infections such as sepsis. Characterization of the host-pathogen interface by spatially-targeted molecular analysis can enable a deeper understanding of the mechanisms of bacterial pathogenesis and host defense against infection. Matrix-assisted laser desorption/ionization (MALDI) Imaging Mass Spectrometry (IMS) is one such molecular imaging technology that can be used to map the distribution of thousands of chemical species directly from tissue surfaces. Previous studies in our laboratory determined calprotectin, an innate immune response protein, played a pivotal role in the formation and resolution of infectious foci. However, much remains unknown about the molecular species important for bacterial lesion growth and the host response. Here, we expand this investigation to IMS of lipids from abscessed tissue. Furthermore, the use of fluorescent bacterial reporters allows for spatially targeted IMS approaches as well as downstream analyses and interrogation. Using a modified 9.4Tesla Fourier transform ion cyclotron resonance (FTICR) mass spectrometer (MS), we have increased our achievable IMS spatial resolution to <10 µm per pixel while maximizing molecular specificity. Coupling bright field microscopy, fluorescence microscopy, untargeted LC-MS/MS, and MALDI IMS, this multi-modal analysis aids in isolating molecular signals of interest in the infection environment. Identification of such signals can provide insight into the mechanisms of bacterial pathogenesis in the host environment subsequently providing future targets for antimicrobial therapeutics.

70 Modulation of host innate immunity by pilus-expressing A. baumannii

Ly Huong T. Pham, M. Indriati Hood- Pishchany, William J. Burns, Yvonne Latour, Jennifer A. Gaddy, Eric P. Skaar, Michael J. Noto

Acinetobacter baumannii has emerged as an important nosocomial pathogen associated with significant morbidity and mortality. The threat of A. baumannii to public health is compounded by its ability to develop resistance to a broad range of antibiotics. During our investigations into A. baumannii pathogenesis, we discovered that transposon mutagenesis of this organism leads to expression of a pilus on the cell surface. In a murine pneumonia model, intranasal infection with pilus-expressing A. baumannii results in a greater than 7-log₁₀ reduction in bacterial burdens in the lungs compared to the WT strain. In co-infection studies, pilus-expressing A. baumannii alters host defense responses in the lung to enhance the clearance of the parental A. baumannii strain, A. baumannii 307, P. aeruginosa, and K. pneumonia. The protective immune response triggered by pilus-expressing A. baumannii is dependent upon MyD88 signaling, as MyD88^{-/-} mice infected with WT bacteria and treated with chemically killed pilus-expressing A. baumannii do not exhibit the enhanced pathogen clearance characteristic of MyD88^{+/+} mice. Pilus-expressing A. baumannii are differentially opsonized compared to the WT strain as determined by human and mouse total IgG ELISA. Furthermore, pilus-expressing A. baumannii alters the activation state of bone-marrow derived macrophages (BMDM) and dendritic cells (BMDDC) in an Fc dependent manner, as in-vitro blockade of Fcy-receptors partially reverses this shift. In this alternatively activated state, BMDM and BMDDC take on an intermediate phenotype in which both pro and anti-inflammatory mediators are upregulated, specifically IL-10 and IL-1^β. The increased production of IL-1^β upon exposure to pilus-expressing A. baumannii is suggestive of inflammasome activation. Taken together, these results indicate that pilus-expressing A. baumannii – mediated enhanced antibacterial lung immunity involves activation of TLR-MyD88, FcyR, and inflammasome pathways. The simultaneous activation of these three pathways leads to altered innate immune cell activation that is capable of orchestrating an effective immune response against a broad range of pneumonia-causing Gram-negative bacteria.

71 Population dynamics of tumor-specific CD8 T cell differentiation from plastic to fixed dysfunctional states

Wyatt J. McDonnell¹, Mark A. Pilkinton², Ram Ramesh⁴, Rama D. Gangula², Gulnara Anzarova¹, Steven Camara⁵, Abha Chopra⁴, Andrea Schietinger⁵, Simon A. Mallal^{2,4}, <u>Mary Philip^{1,4}</u>

¹Department of Pathology, Microbiology, and Immunology, Department of Medicine, ²Division of Infectious Diseases, ³Division of Hematology-Oncology, Vanderbilt University School of Medicine, Nashville, Tennessee, USA; ⁴Institute for Immunology & Infectious Diseases, Murdoch University, Perth, Australia; ⁵Immunology Program, Memorial Sloan Kettering Cancer Center, New York, New York, USA

Tumor-specific CD8 T cells (TST) encountering cognate antigen in tumors differentiate to a dysfunctional state characterized by expression of multiple inhibitory receptors and failure to make effector cytokines. We demonstrated that TST differentiate through two discrete chromatin states; TST were initially in a plastic chromatin state and could be functionally rescued but then transitioned to a fixed chromatin state resistant to therapeutic reprogramming. Importantly, human tumor-infiltrating PD1-high CD8 T cells were in a similar chromatin accessibility state as murine fixed dysfunctional T cells. Based on our studies, two models could describe TST differentiation: (i) TST progress en-masse from the plastic to fixed state, potentially through a transitional state or (ii) individual TST within a population exist in either the plastic or fixed dysfunctional state; initially, most TST are in the plastic state, however over time plastic T cells fail to proliferate or die while the fixed dysfunctional TST preferentially proliferate and/or survive. To test these models, we carried out single-cell RNA-Sequencing on TST at various time points during tumor progression. Determining the population dynamics of TST differentiation through dysfunctional states in tumors could inform our strategies for TST rescue, for example, selecting rare plastic TST from bulk populations versus novel strategies to reprogram the epigenome of fixed dysfunctional TST.

72 Myeloid-specific LTB4R1 control homeostatic production of pro- and anti-inflammatory programs during *Staphylococcus aureus* skin infection

Marco Pires-Lapa, Stephanie Brandt, Sonia Jancar & Henrique Serezani

The outcome of methicillin-resistant *Staphylococcus aureus* (MRSA) skin infection is dictated by first actions of both structural and phagocytic cells. Initial cell recruitment is controlled by chemokines and lipids produced by keratinocytes and resident macrophages. However, the signals that initially drive neutrophil and monocyte migration to the site of infection is poorly understood. The lipid mediator leukotriene B₄ via its cognate receptor BLT1 is quickly produced by the enzyme 5-Lipoxygenase (5-LO) potent neutrophil chemoattractant and its role as a homeostatic component of host defense are begging to be unraveled. Both whole body LTB4R1-/- and 5-LO-/- mice are more susceptible to a myriad of infectious agents, as observed by poor phagocytic and microbial killing capabilities. However, these studies were done using whole body knockout mice, and the cell-specific contribution of LTB₄ in host defense is unknown. We generated a BLT1fl/f mouse that were crossed with LysMcre mice to delete BLT1 specifically in myeloid cells. BLT1fl_LysMcre study the role of LTB₄ in neutrophil/macrophage-specific MRSA skin infection. Our data show that BLT1fl/fl_lysMcre mice showed increased lesion size and bacterial loads than its counterpart BLT1fl/fl at day 1 post-infection. Interestingly, while IL10 levels were enhanced in BLT1fl_lysMcre mice and IL1b were decreased, we did not observe significant changes in TNF- α levels. These data indicate that LTB₄ actions in phagocytes are essential to maintaining a homeostatic balance between pro- and anti-inflammatory molecules, necessary for the control of MRSA skin infection.

73 Cross-reactive and potently neutralizing antibody response in donors naturally infected with Ross River virus

Laura A. Powell, Nurgun Kose, Julie M. Fox, Andrew Miller, Thomas Klose, Michael S. Diamond, Richard Kuhn, Michael Rossmann, Thomas Morrison, and James. E. Crowe, Jr.

Ross River fever is a mosquito-transmitted viral disease that is endemic to Australia and the surrounding South Pacific Islands. Ross River virus (RRV) belongs to the "Old World" alphavirus group, which includes viruses causing disease characterized by debilitating joint pain, rash, and fever. An estimated 5,000-8,000 cases of Ross River fever occur every year in Australia and have an economic impact of ~10 million dollars annually. There is no specific treatment or licensed vaccine available, and mechanisms of protective immunity are poorly understood. In this study, we isolated a panel of nineteen human monoclonal antibodies (mAbs) against RRV from two donors. Thirteen of these antibodies neutralized RRV with half maximal inhibitory concentrations (IC₅₀ values) <100 ng/mL, and several exhibited ultra-potent neutralization activity (IC₅₀ values <10 ng/mL). One antibody was found to cross-neutralize four other alphaviruses, including Mayaro (MAYV), chikungunya (CHIKV), Sagiyama (SAGV), and Getah (GETV) viruses. This antibody binds to the B domain of the E2 structural protein, as evidenced by cryo-EM reconstructions of RRV, MAYV, and CHIKV in complex with Fab. Results from competition binding studies and neutralization assays using mutant viruses suggest that other antibodies were administered therapeutically in an RRV mouse model, mice survival rates increased up to 80%. As the first human mAbs isolated with neutralization activity against RRV, these antibodies could be considered for prevention or treatment of Ross River disease, and could contribute to the design of a universal alphavirus vaccine.

74 Staphylococcus aureus modulates bone remodeling and antibacterial defenses during osteomyelitis via MyD88- and IL-1R-dependent mechanisms

Nicole E. Putnam, Laura E. Fulbright, Jacob M. Curry, Andrew S. Hendrix, James E. Cassat

Staphylococcus aureus is the primary cause of invasive bone infection (osteomyelitis), which is notoriously refractory to antimicrobial therapy and carries significant morbidity. In a murine model of S. aureus osteomyelitis, we have previously observed profoundly dysregulated bone remodeling, suggesting alterations in the balance between bone-forming osteoblasts and boneresorbing osteoclasts. In this work, we hypothesized that S. aureus or its secreted products can modulate osteoclast differentiation through ligation of osteoclast pattern recognition receptors (PRRs) and the concomitant induction of inflammation. Consistent with this hypothesis, we observed increased osteoclastogenesis during S. aureus infection in vivo at sites distant to the infectious focus. To further test the role of PRR signaling pathways in osteoclastogenesis, we used primary osteoclast progenitor cells lacking the critical PRR and IL-1R signaling adapter MyD88. We discovered that S. aureus culture supernatants enhance osteoclastogenesis in a MyD88-dependent manner. Given that MyD88 signaling is a critical component of innate immune responses to bacterial pathogens, we next sought to determine how MyD88 signaling impacts staphylococcal proliferation and survival during osteomyelitis. MyD88-null mice not only had significantly elevated bacterial burdens in infected bones, but also experienced significantly increased mortality due to secondary bacterial dissemination. Since IL-1R is known to be a critical antibacterial determinant in skin and is well characterized to support osteoclast differentiation and activation, we hypothesized that IL-1 signaling might be a critical mediator of both MyD88-dependent antibacterial defenses and pathologic bone remodeling during S. aureus osteomyelitis. Indeed, IL-1R-null mice had dramatically higher bacterial burdens in the infected bone, and the loss of IL-1R signaling abrogated the *in vivo* increase in osteoclast numbers at sites distant to the infectious focus. Together, our data demonstrate that S. aureus osteomyelitis triggers MyD88- and IL-1R-dependent alterations in osteoclastogenesis, and that innate immune activation through IL-1R is critical for limiting bacterial survival during osteomyelitis.

75 Structural characterization of the small Tim chaperones of the intermembrane space in Trypanosoma brucei

Linda Quiñones¹, Joseph T. Smith Jr.¹, Jamaine Davis², Ujjal K. Singha¹, and Minu Chaudhuri¹

¹Department of Microbiology, Immunology and Physiology, ²Department of Biochemistry, Cancer Biology, Neuroscience and Pharmacology, Meharry Medical College, 1005 Dr D.B. Todd Jr Blvd, Nashville, TN 37208

Trypanosoma brucei is a parasitic protozoan that causes a fatal disease known as African sleeping sickness or African trypanosomiasis in human and domestic animals in sub-Saharan Africa. T. brucei possesses a single reticular mitochondrion that imports ~1000 proteins from the cytosol for mitochondrial function. Discoveries from our laboratory and others showed that T. brucei mitochondrion harbors divergent protein translocases of the mitochondrial outer and inner membranes. We also have characterized recently three out of five small translocase proteins in the mitochondrial intermembrane space, known as small Tims (Tim9, Tim10 and Tim8/13). Analysis of the primary sequences revealed that T. brucei small Tims are not the true homologues of the fungal and human small Tims, although they have similar predicted secondary structures. Small TbTims are found to be associated with the major translocase protein 17 (Tim17) of the mitochondrial inner membrane and play a crucial role for mitochondrial protein biogenesis thus are essential for the parasite growth. As shown in fungi, small Tims are good targets for small molecule inhibitors to block mitochondrial protein import. Therefore, characterization of the structure of the unique small TbTims may provide key information to design inhibitors for mitochondrial protein import in T. brucei. Here, we report that among these three small TbTims, TbTim10 was successfully expressed in Escherichia coli as a His6-tagged protein. The recombinant TbTim10 was solubilized ~90% in denaturing buffer containing urea (8M), however, under native conditions only 5% of the expressed protein was soluble. Purification of the solubilized recombinant TbTim10 by nickel-chelation chromatography under native conditions gave us a yield of ~5 mg of protein of 50 % purity from 500 ml bacterial culture. Further purification by ionexchange and gel-filtration chromatography is on-going to increase the purity of the recombinant protein, which will be utilized to determine the crystal structure of TbTim10.

76 NFPws: A web server for delineating eptiope-specific broadly neutralizing antibody responses from serum neutralization data

Nagarajan Raju, Ivelin Georgiev

Vanderbilt Vaccine Center, Vanderbilt University Medical Center, Nashville, TN

A better understanding of antibody responses to HIV-1 infection in humans can provide novel insights for the development of an effective HIV-1 vaccine. Neutralization fingerprinting (NFP) is an efficient and accurate algorithm for delineating the epitope specificities found in polyclonal antibody responses to HIV-1 infection¹⁻². Here, we report the development of NFPws, a webserver implementation of the NFP algorithm. The server takes as input serum neutralization data for a set of diverse viral strains as input, and uses a mathematical model to identify similarities between the serum neutralization pattern and the patterns for known broadly neutralizing monoclonal antibodies (bNAbs), in order to predict the prevalence of bNAb epitope specificities in the given serum. In addition, NFPws also computes and displays a number of prediction quality estimators, including a normalized residues score, a median delineation score and a frequency of random signals. For a given serum, these values can be used to estimate the confidence in the predicted antibody specificities, as well as the likelihood of presence of novel, previously uncharacterized, antibody specificities. Overall, the NFPws server will be an important tool for the identification and analysis of epitope specificities of broadly neutralizing antibodies responses against HIV-1.

77 Bortezomib Impacts Notch—miR-155 Mediated Augmentation of CD8⁺T Cell Antitumor Immunity

Ariana N. Renrick, Menaka C. Thounaojam, Portia L. Thomas, and Anil Shanker

The immunosuppressive tumor microenvironment dampens host antitumor immunity by multiple mechanisms including interference with the Notch system, which is important for various cell fate decisions and hematopoietic cell differentiation and function. We observed that treatment with bortezomib, a proteasome inhibitor, in mice bearing subcutaneous tumors resulted in an upregulated expression of various Notch signaling components in lymphoid tissues and increased CD8⁺T lymphocyte IFN_Y secretion and expression of effector molecules, perforin and granzyme-B, as well as the T-box transcription factor eomesodermin. Of note, bortezomib reversed tumor-induced downregulation of Notch receptors, Notch1 and Notch2, as well as increased the levels of cleaved Notch intracellular domain (NICD) and downstream targets Hes1 and Hey1 in tumor-draining CD8⁺T cells. These data suggest that bortezomib can reverse tumor-induced dysfunction of CD8⁺T cells by its intrinsic stimulatory effects. Our preliminary data also suggest that bortezomib can positively regulate miR-155 expression in CD8⁺ T cells from mice bearing tumor. Further, miR-155 suppression was found to downregulate bortezomib-induced increase in Notch target genes in T cells. We are currently elucidating how bortezomib affects the expression of miR-155 and its target genes, such as suppressor of cytokine signaling 1 (SOCS1) and inositol polyphosphate-5-phosphatase (SHIP1) that are associated with T cell function. These data provide novel insights on using bortezomib not only as an agent to sensitize tumors to cell death, but also to provide lymphocyte-stimulatory effects, thereby overcoming immunosuppressive actions of tumor on antitumor T cell functions.

78 Modeling diabesity *in vitro* activates the inflammasome in human placental macrophages: the importance of saturated fat

Lisa M. Rogers, Linda Englund Ögge, Bo Jacobsson, Carlos H. Serezani, Kasey Vickers, David M. Aronoff

Introduction: Macrophages are important defenders of maternal & fetal tissues. Metabolic dysfunction is related to obesity and linked to insulin resistance, and is associated with chronic inflammation. Gestational diabetes mellitus (GDM), often associated with obesity, is the most common metabolic disorder observed during pregnancy. We hypothesized that combined stress of GDM (high glucose and insulin) and obesity (high saturated fat), what we refer to as diabesity, would trigger inflammatory responses in placental macrophages (PMs).

Methods: PMs from healthy donors undergoing term scheduled C-sections were isolated and used for these studies. PMs were exposed to metabolic cocktail (MetaC) comprised of 30mM glucose, 0.4mM palmitate, and 10nM human insulin for 4 or 24h. Euglycemia (5mM glucose) was our control, and PMs were also stimulated with palmitate, insulin, or 30mM glucose (hyperglycemia) alone. Supernatants were collected for ELISA analysis (TNF- α , IL-6, MCP-1, IL1- β , caspase-1, LTB₄), and cells were harvested for flow (cellular death; Annexin V and PI). PMs were also exposed for 30m to a BLT-1 receptor antagonist before being exposed to 5mM glucose, MetaC or palmitate alone. Supernatants were harvested for IL1- β ELISA.

Results: PMs exposed to metabolic stress underwent cellular death (necrosis and apoptosis) and secreted active caspase-1 at 4h and IL-1 β at 24h. Euglycemia or insulin alone did not contribute to this phenotype, but palmitate alone resulted in the same phenotype as MetaC. Blocking the LTB₄ receptor BLT-1 resulted in suppression of IL1- β at 24h. At 24h, we also observed secretion of LTB₄ and TNF- α and a suppression of MCP-1 compared to euglycemia.

Conclusions: These studies demonstrate significant shifts in macrophage activation and viability due to the presence of metabolic dysfunction, mostly driven by exposure to palmitate. The effect of palmitate on caspase-1 activation and IL-1b secretion may be dependent on autocrine LTB₄-BLT1 signaling. The extent to which these changes contribute to disease pathogenesis remain to be determined.

79 The polyamine spermidine is an immunomodulatory molecule produced by *Batrachochytrium dendrobatidis* to evade immune destruction

Louise A. Rollins-Smith, Laura K. Reinert, Vishvaas I. Ravikumar, Megan Huebner, Audrey Aka, Marilyn Ayisi-Ahwireng, Bryan A. Joosse, Emily M. Hall, Thomas P. Umile, Kevin P.C. Minbiole, Antonio C. Ruzzini, and Jon Clardy

Amphibians have been declining around the world for more than four decades. One recognized contributor to these declines is the chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), which causes the disease termed chytridiomycosis. Amphibians have complex and varied immune defenses against *Bd*, but the fungus has a number of equally important counter-defenses. Previously, we isolated two small immunomodulatory molecules produced in significant amounts by *Bd* that inhibit frog lymphocyte proliferation; kynurenine (Kyn), methylthioadenosine (MTA). Here we report on the identification of the polyamine spermidine (SPD) as another significant immunomodulatory molecule for amphibian and mammalian lymphocytes. SPD is required for growth of *Bd*, but it is also released into cell-free culture supernantants that have significant lymphocyte inhibitory activity. Using inhibitors of spermidine synthesis pathways (a-difluoromethylornithine, DFMO; a-difluoromethylarginine, DFMA), we show that the major synthesis pathway for spermidine in *Bd* seems to be from ornithine via activity of ornithine decarboxylase to putrescine. A secondary pathway involving arginine decarboxylase through agamtine and putrescine may not be present in this primitive fungus. We hypothesize that spermidine is produced as a defense against immune recognition and clearance. SPD is inhibitory at concentrations $\geq 10 \ \mu$ M and appears to be present at this concentration in inhibitory supernatants. Our results show that a suboptimal concentration of MTA (10 μ M) enhances the inhibition of SPD at 1 and 10 μ M. Taken together, these results suggest that *Bd* has an "armamentarium" of small molecules that alone or in concert enable it to evade clearance by the amphibian immune system.

80 Examining the roll of chromatin in HIV-1 preintegration complex associated viral DNA integration

Nicklas E. Sapp, Nathan Burge, Muthukumar Balasubramaniam, Jui Pandhare, Michael Poirier and Chandravanu Dash

HIV-1 infection depends on integration of the viral DNA into the host genome. Accordingly, HIV-1 integration is a major target for anti-retroviral (ART) drug discovery. A better understanding of the molecular mechanism underlying HIV-1 integration will aid the development of novel ART. After entry into the cell, the HIV-1 RNA genome is converted into a DNA copy by the viral encoded reverse transcriptase. A preintegration complex (PIC) is formed by the viral DNA in conjunction with viral and host factors which protect the PIC and facilitate nuclear entry. In the nucleus, the HIV-1 PIC binds to the nucleosome, the organizing unit of chromatin, and integrates the viral DNA into the genome. Despite understanding the biochemical details of HIV-1 integration, how the PIC interacts with the human chromatin remains unclear. To better understand the molecular mechanism of HIV-1 integration, we developed a model that utilizes human chromatin and HIV-1 PICs isolated from acutely infected cells. With our model, quantification of integration activity using Alu-based nested quantitative PCR analysis shows that the integration activity of crude HIV-1 PICs is markedly higher with human chromatin as the substrate than with naked human genomic DNA. Chromatin remains the preferred substrate when purified PICs are the source of integration activity. To further probe the nucleosome features underlying the enhanced integration activity, we utilized recombinant human nucleosomes assembled on the Widom 601 sequence. Surprisingly, compared to the naked 601 DNA, integration activity was decreased into the 601 mono-nucleosome substrates alluding to nucleosomes as a potential barrier to HIV-1 integration. Ongoing experiments will test PIC integration activity into chromatin in the presence of host factors involved with integration. A clear understanding of the biochemical interactions between nucleosomes and specific HIV-1 PIC factors will increase our knowledge of DNA integration and facilitate the identification of novel antiretroviral therapeutics.

81 Multi-Donor Longitudinal Antibody Repertoire Sequencing Reveals the Existence of Public Antibody Clonotypes in HIV-1 Infection

<u>Ian Setliff</u>, Wyatt J. McDonnell, Nagarajan Raju, Robin G. Bombardi, Amyn A. Murji, Cathrine Scheepers, Rutendo Ziki, Charissa Mynhardt, Bryan E. Shepherd, Alusha Mamchak, Nigel Garrett, Salim Abdool Karim, Simon A. Mallal, James E. Crowe, Jr., Lynn Morris, Ivelin S. Georgiev

A thorough definition of antibody responses during natural infection can inform strategies for the design of an effective HIV-1 vaccine. While recent advances in next-generation sequencing of B-cell receptor transcripts enable large-scale interrogation of the functional antibody repertoire, these technologies primarily have been applied in the context of HIV-1 to study individual antibody lineages. Here, we sequenced the antibody variable heavy chain repertoires of six South African donors prior to infection and through both acute and chronic HIV-1 infection. Analysis of 97202 unique clonotypes revealed high turnover during the course of infection, with <3% of clonotypes persisting through chronic infection. Despite this turnover, overall repertoire features, such as antibody germline gene usage, appeared to be relatively stable over time within each donor. While the antibody repertoire of each donor was mostly unique, we identified public antibody clonotypes that were shared between multiple donors during infection. Representative antibodies from an IGHV1-69 public clonotype identified from paired heavy-light chain sequencing data, and shared by three donors, were produced and confirmed to be HIV-reactive. More generally, a search over published next-generation sequencing datasets from infection, vaccination and autoimmunity studies, as well as from healthy individuals, identified public antibody clonotypes with high sequence identity to known HIV-reactive antibodies. Taken together, these results provide novel insights into how antibody repertoires evolve over the course of HIV-1 infection and suggest new, previously unexplored directions for HIV-1 vaccine design.

82 Histamine at the host-pathogen interface during Acinetobacter baumannii infection

Jessica R. Sheldon and Eric P. Skaar

Acinetobacter baumannii is an emerging opportunistic pathogen, that poses a global health threat due to a lack of viable therapeutic options in treating extensively drug resistant strains. In addition to the acquisition of resistance to last resort antibiotics, the success of A. baumannii is thought to be due to its ability to successfully compete with the host for essential nutrients. As a facet of innate immunity, the host restricts the availability of essential metals to curtail bacterial proliferation. To counter this restriction, bacteria possess numerous mechanisms to obtain these metals, including through the production of small secreted siderophores, which bind and deliver iron to the bacterium. A. baumannii is elaborates up to ten structurally distinct siderophores; acinetobactin and pre-acinetobactin, baumanoferrins A and B, and fimsbactins A-F. Here we demonstrate that A. baumannii synthesizes histamine, a key precursor molecule to the production of acinetobactin, through the activity of a putative iron-regulated histidine decarboxylase, basG. While functional redundancy by the other siderophores largely masks the role of basG in vitro, we demonstrate that basG strongly influences survival of A. baumannii in vivo. Further, we show that histamine detection is increased in mice infected with wild-type A. baumannii versus those mock-infected or infected with a basG-deficient strain. Using nanoString technology, we reveal host histidine decarboxylase (hHDC) expression is also upregulated in A. baumannii infected hosts, suggesting it may also contribute to the presence of histamine at the host-pathogen interface. Given that histamine is an important immunomodulator, these results suggest that histamine production may play an important role not only in iron acquisition by A. baumannii, but in the overall pathophysiology of infection. Lastly, we also use nanoString to help uncover tissue-specific gene expression changes in host metal homeostasis pathways that may play important but unappreciated roles in nutritional immunity against A. baumannii.

83 A single prophage WO gene rescues cytoplasmic incompatibility in Drosophila melanogaster

J. Dylan Shropshire, Jungmin On, Emily M. Layton, Sarah R. Bordenstein, Helen Zhou, & Seth R. Bordenstein

Wolbachia are maternally-inherited, intracellular bacteria at the forefront of vector control efforts to curb arbovirus transmission. In international field trials, the cytoplasmic incompatibility (CI) drive system of *w*Mel *Wolbachia* is deployed to replace target vector populations, whereby a *Wolbachia*–induced modification of the sperm genome kills embryos. However, *Wolbachia* in the embryo rescue the sperm genome impairment, and therefore CI results in a strong fitness advantage for infected females that transmit the bacteria to offspring. The two genes responsible for the sperm modification of CI, *cifA* and *cifB*, were recently identified in the eukaryotic association module of prophage WO in *w*Mel *Wolbachia*, but the genetic basis of rescue is unresolved. Here we use transgenic and cytological approaches to demonstrate that *cifA* single-handedly rescues CI and nullifies embryonic death caused by *w*Mel *Wolbachia* in *Drosophila melanogaster*. Discovery of *cifA* as the rescue gene and previously one of two CI induction genes establishes a new 'two-by-one' model that underpins the genetic basis of CI. Results highlight the central role of prophage WO in shaping *Wolbachia* phenotypes that are significant to arthropod evolution and vector control.

84 Divergent small Tim homologues are critical for the assembly and stability of TbTim17 protein complexes in *Trypanosoma brucei*

Joseph T. Smith Jr., Ujjal K. Singha, Smita Misra, and Minu Chaudhuri

Department of Microbiology, Immunology, and Physiology, Meharry Medical College, Nashville, TN 37208

The small Tim proteins belong to a group of mitochondrial intermembrane space chaperones that aid in the import of mitochondrial inner membrane proteins with internal targeting signals. *Trypanosoma brucei*, the protozoan parasite that causes African trypanosomiasis, possesses multiple small Tim proteins that include homologues of Tim9 (TbTim9), Tim10 (TbTim10), and a unique small Tim that shares homology with both Tim8 and Tim13 (TbTim8/13). Here, we found that these three small TbTims are expressed as soluble mitochondrial intermembrane space proteins. Co-immunoprecipitation and mass spectrometry analysis showed that the small TbTims stably associated with each other and with TbTim17, the major component of the mitochondrial inner membrane translocase in *T. brucei*. Yeast two-hybrid analysis indicated direct interactions among the small TbTims, however their interaction patterns could be different than their counterparts in yeast and humans. Knockdown of the small TbTims reduced cell growth and decreased the steady-state level of TbTim17 and TbAAC, two polytopic mitochondrial inner membrane proteins. Small TbTims reduced TbTim17 import and greatly hampered the stability and assembly of the TbTim17 complexes in *T. brucei*. Altogether, our results revealed that TbTim9, TbTim10, and TbTim8/13 interact with each other, associate with TbTim17, and play a crucial role in the formation and maintenance of the TbTim17 complexes.

85 mTOR Signaling Regulates the Vascular Response to LPS

Cody Stothers, LiMing Luan, Julia Bohannon, Katherine Travisano, & Edward Sherwood

Infections trigger endothelial cell (EC) inflammation characterized by secretion of chemokines, expression of surface adhesion molecules, and relaxation of the vascular permeability barrier. While this response is necessary to combat pathogens, excessive inflammation and vascular permeability lead to hypotension and end-organ damage, which characterizes septic shock. Our lab has shown that modulation of cellular metabolism is essential for antimicrobial responses. Lipopolysaccharide (LPS) activates the mechanistic target of rapamycin (mTOR) signaling cascade, which facilitates aerobic glycolysis. The role of cellular metabolism in the vascular response to infection is unknown. We hypothesized that hEC immune activation requires mTOR-driven immune activation. To test our hypothesis, primary human microvascular ECs (hEC) were cultured with or without 100nM rapamycin, an inhibitor of mTOR. hECs were stimulated with 100ng/mL LPS for up to 24hrs. LPS-stimulated hEC secreted proinflammatory cytokines, such as IL-6, G-CSF, and MIP-1 β , which was inhibited by rapamycin pretreatment, as measured by multiplex assay. LPS rapidly induced translocation of FITC-dextran across an hEC monolayer grown on 0.2µm transwells. Importantly, rapamycin protected the barrier and decreased permeability. Stimulated cells were assayed using the Seahorse extracellular flux analyzer to assess glycolysis (extracellular acidification rate, ECAR) and oxidative metabolism (oxygen consumption rate, OCR). We found that LPS increased glycolysis in hECs while oxidative metabolism was unchanged. Surprisingly, the combination of LPS and rapamycin significantly diminished both glycolytic and oxidative metabolism. Cell death was measured by extracellular LDH activity. Neither LPS, rapamycin, nor the combination induced more cell death than unstimulated cells. Inhibition of mTOR signaling by rapamycin prevents LPS-induced metabolic reprogramming, vessel permeability, and chemokine secretion in hECs. These studies provide a novel therapeutic target to prevent the onset of septic shock.

86 Amino Acid Transport of T cell Subsets in Lung Inflammation

Ayaka Sugiura and Jeff Rathmell

Asthma is characterized by an imbalance between pro-inflammatory effector T (Teff) cells and anti-inflammatory regulatory T (Treg) cells within the respiratory tree microenvironment that leads to dysregulated responses to airborne antigens. This chronic inflammatory-lung disease affects approximately 30 million people globally and incurs direct and indirect costs totaling 56 billion dollars and growing to the nation. Moreover 38.4% of children and 50.0% of adults continue to have uncontrolled asthma with currently available therapies. Thus, new types of treatments are in high demand to reduce the increasing and disproportionate disease burden. Previously, our lab has shown that Teff and Treg cells can be distinguished by their distinct metabolic programs and that this exposes a new potential way to preferentially target specific T cell subsets. Our work has shown that Teff cells rely heavily on glycolysis and glutaminolysis for energy, whereas Treg cells are more reliant on mitochondrial oxidative phosphorylation and lipid oxidation. Here, I propose a model in which the Th17 cells that distinguish severe asthma have a survival advantage over Treg cells within the asthmatic respiratory tree microenvironment afforded by its unique metabolic program. I further propose that this metabolic program is dependent on a robust capacity to manage the level of Reactive Oxygen Species (ROS). ROS management in turn requires availability of the major antioxidant glutathione, whose synthesis is limited by cystine uptake. This is supported by data from our preliminary studies and the literature that show cytosolic ROS is decreased in T cells from asthmatic lungs, increased ROS is detrimental to Th17 effector function but favorable for Treg stability, and cystine deprivation leads to differential changes in glutathione, cytosolic ROS, and mitochondrial ROS levels in Th17 and Treg cells. Extending from this, I hypothesize that T cell subsets have distinct ROS-sensitivities and cystine-transport requirements to fuel their metabolic programs, and that interference of the cystine transporter may provide a new means to specifically target Th17 cells. This study is aimed at providing new targets for therapeutic intervention with the goal of developing a novel method for restoring immunological homeostasis in the lungs.

87 Selective autophagy: A mechanism for cytoplasmic antigen presentation by MCH class II molecules

Naveen Chandra Suryadevara¹, Amrendra Kumar^{1,2}, Pavlo Gilchuk¹, Timothy M. Hill¹, & Sebastian Joyce^{1,2}

¹Department of Pathology Microbiology & Immunology, Vanderbilt University Medical Center, ²Department of Veterans Affairs, Tennessee Valley Healthcare System, Nashville, Tennessee

The prevailing view suggests that macroautophagy delivers cytoplasmic antigens to the endo/lysosomes for presentation by MHC class II molecules. Contrary to the prevailing view, we found that the male HY alloantigen and to some extend the listerolysin O antigen of *Listeria monocytogenes*, were intact in mice deficient in Atg5-dependent macroautophagy. This finding suggested that another mechanism is operative for delivering certain cytoplasmic antigens to the endo/lysosomes. Chaperone mediated autophagy (CMA) is a cellular stress response, which selectively delivers cytoplasmic proteins to lysosomes for degradation, and is known to deliver cytoplasmic antigens to the endo/lysosomes. CMA is initiated by the recognition of a KFERQ motif by heat shock cognate chaperone of 70kD (Hsc70), which tethers to LAMP2 and induces its oligomerization into a transmembrane pore. Curiously, however, neither LAMP2 nor Hsc70 are NTP hydrolases, which leaves open the source of energy for peptide translocation across the endo/lysosomal membrane. Recent studies have shown that an orphan TAP (transporter-associated with antigen processing)-like protein and a member of the ABC family can transport cytoplasmic peptides into model vesicles. Hence, we postulated that TAP-L may participate in CMA especially of substrates lacking the KFERQ motif. As the first step in testing this postulate, we found that mouse TAP-L associates with mouse and human LAMP2 as does human TAP-L with human LAMP2 and Hsc70. This finding foretells a potential role for TAP-L in CMA, the mechanism of which is currently under investigation.

88 Group B Streptococcus Induces Human Placental Macrophage Activation in PKD-dependent manner

Jessica A. Sutton^{1,2}, Lisa Rogers², and David M. Aronoff^{1,2}

¹Department of Microbiology and Immunology, Meharry Medical College, Nashville, TN; ²Department of Infectious Diseases, Vanderbilt University, Nashville, TN

Group B *Streptococcus* (GBS), a vaginal colonizing gram-positive bacterium, is a leading cause of neonatal death and sepsis. How GBS evades innate immune defenses of the macrophage in the gravid uterus is not fully elucidated. It was recently discovered that GBS utilizes protein kinase D (PKD) 1 to induce pro-inflammatory macrophage activation in mouse macrophages and THP-1 cells. This study aims to define changes in placental macrophage (PM) activation through the lens of inflammation and polarization in response to GBS infection and the contribution of PKD in mediating this activation. Primary human PMs were infected with GBS (MOI 0.1:1) in the presence or absence of the pharmacological PKD inhibitor CRT. Resulting phenotypes were characterized by evaluating gene expression, cytokine release, inflammasome activation, and protein expression. We found that PMs display a pro-inflammatory cytokine profile in response to GBS infection upregulates the expression inflammatory mediators previously associated with M1 macrophage activation. The PKD-inhibitor CRT was able to inhibit the release of pro-inflammatory cytokines TNF α , IL-1 β , and IL-6 from GBS infected PMs indicating that PKD is necessary for inflammatory activation of PMs. Furthermore, inflammasome activation in response to GBS infection was also inhibited by CRT. These results suggest that GBS induces M1-like activation in PMs in a PKD-dependent manner. Taken together, our studies provide new insights into the role of placental macrophage activation in GBS pathogenesis.

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89 Microbe density dependence, inducible immune dynamics, and the evolution of optimal immune responses

Abby Perry, Derrick Jent, Ann Tate

Immune responses evolve to balance the benefits of microbial killing against the costs of autoimmunity and energetic resource use. Mathematical models that explore the evolution of optimal immune responses generally include a term for constitutive immunity, or the level of immunological investment prior to microbial exposure, and for inducible immunity, or investment in immune function after microbial challenge. However, studies rarely consider the functional form of inducible immune responses with respect to microbial density, despite the theoretical dependence of immune system evolution on microbe- versus immune-mediated damage to the host. In this study, we employ mRNA-seq on flour beetles (*Tribolium castaneum*) infected with a virulent bacterium to demonstrate that inducible immune responses mediated by humoral immune pathways exhibit both microbe density dependent and independent temporal dynamics. Further investigation of these dynamics in beetles derived from six wild-caught populations revealed substantial natural variation among the microbe-independent induction dynamics but remarkable consistency in the slope of immune gene expression over microbe density. To understand why these two inducible immune dynamics might be under different modes of selection, we built a mathematical model to explore the evolution of optimal inducible immune responses under pressure from diverse parasites and variable levels of immunopathology. This project provides new insight into the contribution of non-equilibrium host-microbe dynamics to immune system evolution and natural variation.

90 Notch signaling in NK cell activation and antitumor lymphocyte crosstalk

Portia L. Thomas, Roman Uzhachenko, & Anil Shanker

A large majority of immune-oncology research efforts focus on T-cell immunotherapy. However, limited clinical efficacy has been achieved with adoptive T-cell effectors alone. In addition, research has shown that the sole use of adoptive T-cells leads to the development of tumor escape variants and "occult cancer". Conversely, tumor-infiltrating natural killer (NK) cells have been shown to cause elimination of these escape variants. These observations indicate that the interaction between the innate and adaptive immune effectors is indispensable for effective tumor immunity. Notch is a major signaling system important for communication between neighboring cells. Our previous studies show that it plays a crucial role in the differentiation and antitumor function of CD8⁺T cells. However, its role in NK cell activation and antitumor function is not clear. Thus, we analyzed Notch receptor-ligand system on NK cells and in the context of their crosstalk with CD8⁺T cells. Our data indicate that inhibiting Notch signaling in NK cells decreases NK cell activation. In addition, Notch intracellular domain (NICD) expression is increased upon NK activation, suggesting that the Notch signaling pathway may be involved in NK activation. Our data also show that activated CD8⁺T cells activate naïve NK cells through direct physical contact. Transmembrane notch receptor expression is decreased during this interaction suggesting that the Notch receptors may be involved in CD8⁺T cell – NK cell crosstalk. Experiments are underway to further understand the relevance of Notch signaling pathway in innate-adaptive immune crosstalk. The findings will help develop novel strategies for adoptive cell immunotherapies, specifically against solid tumors.

91 Mitochondrial Ca²⁺ transport-dependent intercellular crosstalk between CD8 T and natural killer cells provides immunosurveillance against tumor escape

<u>Roman V Uzhachenko</u>¹, Michel Buferne², Claude Boyer², J. Shawn Goodwin¹, Lee Leserman², Anne-Marie Schmitt-Verhulst², Alla V Ivanova³, Anil Shanker^{1,4,5}

¹Department of Biochemistry and Cancer Biology, Meharry Medical College School of Medicine, Nashville, TN; ²Centre d'Immunologie de Marseille-Luminy, Aix Marseille Université UM2, Institut National de la Santé et de la Recherche Médicale U1104, Centre National de la Recherche Scientifique UMR7280, Marseille, France; ³Department of Surgery, Section of Otolaryngology, Yale University School of Medicine, New Haven, Connecticut, USA; 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

Unraveling the complexity of immune network is key to understanding lymphocyte functions and improving cancer immunotherapy. We explored the functional dynamics between T and NK cells in various adoptive cell transfer protocols in RAG^{-/-}, RAG^{-/-} α c^{-/-} and RAG^{-/-}GzmB-Tom mice established with solid tumors by tracing lymphocytes specific to a self-tumor antigen P1A encoded by a cancer-germline gene *Trap1a*. Data show that activated antitumor CD8⁺T cells augmented NK cell effector function by mechanisms involving physical interaction through membranous pseudopodia-like outgrowths and exchange. This interaction forged a functional teamwork necessary for efficient immunosurveillance against the development of antigen-escape tumor variants. We also found that the interaction between CD69^{high}CD25^{high} CD8⁺T cells and NK cells led to dynamic changes in mitochondria-related parameters and crossregulation of mitochondrial Ca²⁺ (mitoCa²⁺) transport in both cells. This crosstalk induced differentiation of NK cell effector phenotype and, in turn, NK cells polarized CD8⁺T cells towards a central memory phenotype. Intracellularly, CD8⁺T cells increased JAK1, JAK3, TYK2, STAT2 and STAT6 phosphorylation and oxidative signaling in NK cells, whereas NK cells restrained IL-2 signaling in CD8⁺T cells by dampening activation-induced STAT5-dependent signaling. These lymphocyte changes were abrogated following the blockade of mitoCa²⁺ uptake. Moreover, mice deficient in mitoCa²⁺ handling-regulatory gene *Fus1* showed increased incidence of a range of spontaneous sarcomas, lymphomas and leukemia. These findings suggest that mitoCa²⁺ transport-guided intercellular crosstalk between CD8⁺T and NK cells is critical for efficient immunosurveillance against tumor escape.

92 The Maternal Effect Gene Wds Controls Wolbachia Titers in Nasonia

Lisa J. Funkhouser-Jones*, Edward J. van Opstal*, Ananya Sharma, & Seth Bordenstein

Maternal transmission of bacterial symbionts is widespread in animal hosts despite profound host fitness consequences should symbiont titers go awry. However, the genetic basis of balancing heritable symbiont densities through multiple host generations remains poorly resolved. Here, we deploy the first forward genetic approach to map loci underlying an extreme interspecific difference in densities of a heritable endosymbiont, *Wolbachia*, in the evolutionary model of *Nasonia* parasitoid wasps. Utilizing an 80-fold *Wolbachia* density difference between *N. vitripennis* (low) and *N. giraulti* (high), we performed interspecific introgressions and RNA-Seq analysis to determine a *N. vitripennis* genomic region responsible for *Wolbachia* density suppression and validated *Nasonia* gene candidates with RNAi gene knockdown. We report four key findings: i) a maternal genetic effect acts dominantly to suppress *Wolbachia* densities in offspring, ii) a genomic region composed of 32 genes on chromosome three explains ~70% of the density suppression trait, iii) RNA-Seq of *Nasonia* ovaries from each *Nasonia* species identified 7 differentially-expressed genes in the candidate region, and iv) the partial knockdown of a candidate suppressor gene, *Wolbachia density suppressor* (*Wds*), resulted in a significant increase of *Wolbachia* titers in offspring. Together, these results imply that variation in maternal regulation of inherited symbiont titers can have a simple genetic basis governed by maternal effects. Repeated evolution of maternal microbial transmission across diverse animal systems may be enabled by relatively few host genetic changes.

93 The Human Antibody Response to Enterovirus-D68 Infection

Matthew R. Vogt, Lauren E. Williamson, Nurgun Kose, & James E. Crowe, Jr.

Enterovirus D-68 (EV-D68) causes sporadic worldwide outbreaks of human respiratory illness, especially in children with asthma. Spacio-temporally related outbreaks of acute flaccid myelitis (AFM), a polio-like illness, have implicated EV-D68 as a cause of AFM. We aim to use our laboratory's expertise in producing human monoclonal antibodies (mAbs) from the B cells in peripheral blood mononuclear cells (PBMCs) to investigate the poorly understood human humoral immune response to EV-D68. The key to identifying mAbs is developing an appropriate screen for antibody-producing B cells. We have focused on developing an ELISA that uses native virus particles in order to preserve tertiary and quaternary epitopes that are not present on recombinant proteins. We hypothesize that this approach will help identify mAbs that are most likely to have a protective effect during natural infection. It is difficult to achieve high titers of EV-D68 in virus suspensions in sufficient quantity to screen hundreds of 384-well plates of human B cell supernatants by ELISA. We overcame this obstacle by optimizing production of virus stocks using sonication to disrupt cellular membranes followed by ultracentrifugation of this lysate through a sucrose cushion. Further, we optimized ELISA conditions for screening using quantification based on signal from a fluorescent reporter molecule. Additionally, collaborators at the Universities of Wisconsin and Colorado have provided PBMCs from children known to have had either respiratory illness or AFM associated with EV-D68 during the 2014 US outbreak. With this, we now have the tools to create a large panel of human mAbs against EV-D68, which will be the first human mAbs against this virus. We plan to characterize the binding and neutralization characteristics of the resulting mAbs in order to provide preclinical support for their future development as therapeutics.

94 Autoreactive-prone CD21^{lo} B cells increased in scleroderma patients with interstitial lung disease

Katherine N. Vowell¹, Erin M. Wilfong¹, Leslie J. Crofford², and Peggy L. Kendall¹

¹Division of Allergy, Pulmonary and Critical Care, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN; ²Division of Rheumatology and Immunology, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN

Systemic sclerosis (SSc) is a devastating autoimmune disease characterized by fibrosis of the skin and vital organs. The pathogenesis of SSc is unknown, but detection of autoantibodies in >90% of SSc patients suggests that B lymphocytes play a role. Therefore, we explored B cell phenotypes in SSc patients using fluorescence cytometry and the multi-dimensional unsupervised analysis tool viSNE. We found that compared to healthy controls (n=5), SSc patients (n=11) had significantly higher proportions of an autoreactive-prone B cell subset identified by low expression of CD21 (CD21^{lo}). Previously, circulating CD21^{lo} B cells were shown to express autoantibodies and were increased in other autoimmune diseases. When considering the clinical phenotypes of the SSc patients, we found that the frequency of CD21^{lo} B cells was only increased in SSc patients with interstitial lung disease (ILD). ILD is a complication of SSc that is associated with increased morbidity and mortality. To our knowledge, this is the first time that a peripheral blood B cell subset has been identified in association with ILD in patients with a systemic autoimmune disease. We investigated the signaling properties of these anergic cells and found lower expression of Bruton's tyrosine kinase (BTK) in CD21^{lo} B cells compared to normal B cells. This was surprising, as BTK is thought to be overexpressed in autoimmunity, but may indicate that the anergic state is due in part to suppression of BTK-mediated signaling. Our data suggest that CD21^{lo} B cells should be investigated for their role in SSc pathogenesis, and for their utility as a biomarker of ILD. In addition, these findings demonstrate a potential mechanism by which B cell depleting therapy may specifically benefit SSc patients with ILD.

95 The role of COG0523 family proteins during staphylococcal infection: Intracellular metal trafficking at the host-pathogen interface

Andy Weiss, William N. Beavers, Eric P. Skaar

During infection, bacterial pathogens are exposed to immune factors that sequester essential metals to restrict bacterial proliferation. Consequently, intracellular metal is a valuable resource for the pathogen and must be hierarchically allocated. For example, members of the COG0523 family of GE3 GTPases function as metallochaperones that bind and distribute intracellular metals. Notably, COG0523 proteins are found in *Staphylococcus aureus* and human host alike and represent a conserved mechanism proposed to shape the struggle for essential elements at the host-pathogen interface. Here, we evaluated this possibility by characterizing the five human and three bacterial COG0523 proteins to determine their contribution to metal homeostasis and infection.

We found two *S. aureus* COG0523 proteins to be induced in the presence of the metal-sequestering immune factor calprotectin *in vitro*, implying a role for these proteins during infection. The hypothesis that COG0523 family members function in metal homeostasis is further supported by the finding that these proteins bind metals that are known to be sequestered by the immune system. Additionally, a combination of Co-IP and client stability assays identified COG0523 clients that are preferentially supplied with sparse metal cofactors. These experiments will not only elucidate biological roles of these metallochaperones but also identify metal-dependent cellular processes important for infection.

Experiments to investigate the role of human COG0523 proteins during bacterial infection have not been reported. To fill this gap in knowledge, we utilized a gene-based association method (PredixVU) developed at Vanderbilt. Our results revealed the exciting finding that decreased expression of the COG0523 protein CBWD5 is linked to a drastically augmented occurrence of human staphylococcal infections. We are currently in the process of generating a mouse model to expand on this work.

Overall, these findings support a role for COG0523 proteins as important factors in the competition for essential micronutrients between bacterial pathogens and their hosts.

96 "Bone Appétit": Skeletal nutrient availability drives host-pathogen interactions during S. aureus osteomyelitis

Aimee Wilde, Jacob Curry, Casey Butrico, Andrew Hendrix, Srivarun Tummarakota, Jim Cassat

Staphylococcus aureus is capable of infecting most organ systems in the human body, an ability that requires flexible metabolic programs in order to proliferate within diverse environments. The skeleton is an exceptionally common site of invasive staphylococcal infection that is recalcitrant to antibiotic therapy due to limited antibiotic penetrance of damaged bone and widespread antimicrobial resistance in staphylococci. The success of S. aureus in bone is intriguing considering the physiologic properties of skeletal tissue; bone is a continuously remodeling organ characterized by dynamic substrate accessibility, intrinsic hypoxia, and a glycolytic metabolism. We therefore sought to interrogate the mechanisms used by S. aureus to survive within bone. Given that all microbes rely on central metabolism to procure the 13 essential intermediates that fuel proliferation, we used a combination of transposon sequencing and targeted mutagenesis to develop an understanding of the metabolic pathways driving S. aureus replication in bone. Using a murine osteomyelitis model, we examined survival of S. aureus mutants in major metabolic hubs, including glycolysis, gluconeogenesis, the TCA cycle, and amino acid synthesis. Interestingly, our data suggests that despite high glucose uptake and glycolytic demand of skeletal cells, S. aureus can successfully compete for glucose and requires glycolysis for survival during osteomyelitis. Furthermore, amino acid biosynthesis is particularly important for survival, suggesting specific substrate limitations in the skeletal environment. To determine these substrate limitations, we have developed a system to examine the nutritional potential of skeletal tissue ex vivo using a chemically defined media supplemented with bone homogenate. Using this assay, we discovered that homogenized bone can rescue most wild-type S. aureus amino acid auxotrophies, whereas specific biosynthetic mutants cannot be rescued, revealing nutritional deficiencies within skeletal tissues. Together, our data elucidate metabolic programs required for invasive infection and demonstrate specific metabolic pathways as critical determinants of the host-pathogen interaction.

97 Neutralization of eastern equine encephalitis virus by human monoclonal antibodies

Lauren E. Williamson¹, Nurgun Kose³, Robin Bombardi³, Pavlo Gilchuk³, Erica Parrish³, Mattie Jensen³, Rachel Stecker³, Julie Fox⁴, Arthur Kim⁴, Michael Diamond⁴, James E. Crowe, Jr.^{1,2,3}

Departments of ¹Pathology, Microbiology and Immunology, ²Pediatrics, and the ³Vanderbilt Vaccine Center, Vanderbilt University Medical Center, Nashville, TN, ⁴Washington University in St. Louis, St. Louis, MO, USA

Eastern equine encephalitis virus (EEEV) is a mosquito-transmitted virus that is primarily associated with encephalitis in larger mammals, such as horses and humans. The North American lineage of EEEV is a category B priority pathogen and select agent that has a human mortality rate of up to 70%. Of those that survive infection, approximately 80% have neurological sequelae. Fortunately, on average there are only eight human cases in the United States per year. However, the threat as a potential bioterrorism agent and the increase in detection of the virus in circulating mosquito populations raises concern. In addition, there are no approved human vaccines or antiviral drugs available. The human antibody response to EEEV has not been studied. In particular, how antibodies neutralize and interact with EEEV is not understood. This leaves a significant gap in knowledge in understanding the pathogenesis of EEEV and for the rationale design of vaccines and therapeutics for EEEV. In this study, we have isolated a panel of 70 human monoclonal antibodies from a naturally infected EEEV donor. These antibodies recognize diverse epitopes amongst the E2 glycoprotein, are virus-specific, or recognize the E1 glycoprotein. From this panel, 14 exhibit neutralization activity, 6 of which have potent sub 10 ng/mL IC₅₀ values and recognize two neutralizing antigenic determinants on the E2 glycoprotein. Multiple mechanisms of neutralization appear to be involved, such as the prevention of receptor attachment and/or entry. From initial characterization of the panel of human antibodies to EEEV, the human antibody response appears to be diverse both in respect to epitope recognition and in the mechanism of neutralization.

98 CD4 T cell metabolism is a modifiable barrier to tolerance induction in islet transplantation

Christopher Scott Wilson, Blair Taylor Stocks, Andrew Frank Marshall, Daniel Jensen Moore

Metabolic pathways regulate T cell function including the differentiation of effector and regulatory T cells. However, whether there are specific metabolic requirements for tolerance induction have not been defined. The B6.SLE123 mouse is an animal model of lupus in which the autoimmune disease can be dampened by treatment with metabolic modulators, including metformin and 2-deoxyglucose. We have recently established that this animal model is completely resistant to tolerance induction in allogeneic islet transplantation even though it has no underlying anti-islet autoimmunity. We hypothesized that tolerance-inducing therapy with anti-CD45RB acts on key metabolic pathways to promote tolerance and that these pathways are resistant to therapy in B6.SLE123. Treatment with anti-CD45RB induced metabolic changes by downregulating glucose uptake and modulating mitochondrial function in B6 CD4 T cells, changes that favor CD4 Treg action. SLE123 mice resisted these metabolic changes. We determined the mechanism of anti-CD45RB's action was related to calcium signaling in CD4 T cells. No changes were identified in prototypical TCR signaling (no change in phospho-CD3). We finally demonstrated that tolerance to allogeneic beta cells is improved in SLE123 mice by targeting both glycolysis and mitochondrial activity with 2-deoxyglucose and metformin in combination with anti-CD45RB. This study highlights necessary metabolic conditions to permit allogeneic tolerance induction for islet transplantation.

99 ATP Citrate Lyase and T Cell Fates

<u>Melissa M. Wolf</u>, Gabriela Andrejeva, Marc O. Johnson, Diana A. Contreras, Matt Z. Madden, Ayaka Sugiura, & Jeffrey C. Rathmell

Metabolic reprogramming is crucial for T cell activation and differentiation into distinct T cell subsets. Effector T cells rely heavily on aerobic glycolysis while naïve, memory, and regulatory T cells employ oxidative phosphorylation. Understanding the role of specific metabolic enzymes during T cell differentiation and function could prove important for targeted immunomodulation in autoimmune disease and cancer immunotherapy. ATP Citrate Lyase (ACLY) is a cytosolic enzyme that converts mitochondrial derived citrate into acetyl-CoA. Acetyl-CoA is an essential substrate for fatty acid biosynthesis as well as acetylation reactions that modify proteins, including histones. Here, we sought to characterize ACLY fl/fl CD4 Cre+ T cell fates *in vitro* compared to WT T cells, and determine if differences are rescued by exogenous acetate supplementation to replenish acetyl-CoA. We found that ACLY fl/fl CD4 Cre+ T cells exhibit an overall decrease in proliferation. However, IFN-y expression was increased in Th1 cells. Interestingly, other effector CD4 T cell subsets with ACLY knocked out exhibited a decrease in cytokine production. Overall, our results suggest that ACLY deficiency favors CD4 T cell differentiation into Th1 cells.

100 Circulating transfer RNA-derived small RNAs are altered in patients with rheumatoid arthritis

<u>Qiong Wu</u>, Quanhu Sheng, Joseph F Solus, Kasey C Vickers, Ryan Allen, Shilin Zhao, Yan Guo, Fei Ye, C Michael Stein, Michelle J Ormseth

Small RNAs (sRNAs) are important gene regulators and markers of disease. Transfer RNA (tRNA)-derived sRNAs (tDRs), including tRNA fragments and halves, are novel regulatory sRNAs, often upregulated in the setting of cellular stress to downregulate metabolic processes. Rheumatoid arthritis (RA), a common autoimmune disease, is associated with excessive cellular stress due to immune activation. We hypothesized that circulating tDRs are altered in RA patients and serve as novel markers for RA and disease activity. To test this hypothesis sRNA sequencing was performed on archived plasma samples from 167 RA patients and 91 matched controls using Illumina NextSeg500. tDRs were quantified by TIGER pipeline, permitting one mismatch. Total tDRs, individual tDR sequences and tDRs based on amino acid of the parent tRNA normalized to total reads were compared between RA and control subjects by DESeq2 with adjustment for age, race, sex, and batch with 5% false discovery rate adjusted by Benjamini and Hochberg method. RA patients had 1.16-fold higher proportion of total plasma tDRs compared to controls. Among RA patients a higher proportion of total plasma tDR reads was associated with higher disease activity by DAS28 score (Rho=0.17, p=0.03), erythrocyte sedimentation rate (Rho=0.21, p=0.007) and swollen joint count (Rho=0.18, p=0.02). Seven individual tDR sequences were increased (3.7-fold to 1.5-fold), and one individual tDR sequence was decreased 2.2-fold among RA patients. tDRs from a suppressor tRNA were increased 1.7-fold, and tDRs from tRNAs encoding for asparagine, isoleucine, and aspartic acid were decreased (1.8-fold to 1.5-fold) among RA patients. These data show that RA patients have a greater proportion of plasma tDRs than controls, and total tDRs were correlated with disease activity. Several individual tDR sequences, and tDRs from a suppressor tRNA and several other parent tRNAs were altered in RA patients. Circulating tDRs may be novel markers of RA and disease activity.

101 Interspecies cross-talk between Enterococcus and Clostridium difficile promotes virulence

Joseph P. Zackular, Maribeth R. Nicholson, Nikesh Dahal, Jennifer A. Gaddy, Nichole M. Lareau, M. Kay Washington, Richard M. Caprioli, and Eric P. Skaar

Clostridium difficile is a spore-forming bacterium that causes a wide range of gastrointestinal (GI) disorders varying in severity from mild diarrhea to fulminant colitis and/or death. Over the past decade, incidence, severity, and costs associated with C. difficile infection (CDI) have increased dramatically; however, the factors that govern this broad spectrum of disease still remain largely unknown. Difficulties in treating infections with conventional antibiotics and increasing rates of recurrent infection underscore the need for the development of new therapeutic strategies and the investigation of the molecular correlates of disease. The primary risk factor for CDI is antibiotic treatment, which reduces colonization resistance to C. difficile by disrupting the resident microbial community inhabiting the GI tract. Surprisingly, there is a paucity of data describing the molecular interactions between C. difficile and commensal microbiota during infection and the impact of co-infection with other pathogens has been largely understudied. The major nosocomial pathogen vancomycin-resistant Enterococcus (VRE) shares numerous risk factors with C. difficile and colonization with VRE is correlated with more severe C. difficile-associated disease and increased rates of recurrence. Conversely, in patients with acute leukemia, CDI increases the risk of bacteremia due to VRE. Despite these associations, little work has been done to explore the interspecies interactions between Enterococci and C. difficile during infection and the impact co-occurrence of these two emerging pathogens on disease is unclear. Here, we show that Enterococcus significantly alters the metabolism of C. difficile and enhances toxin and virulence factor production in vitro. When grown in close proximity or in mixed-cultures, Enterococcus also dramatically changes C. difficile morphology and promotes the formation of integrate interspecies biofilms. In mice, Enterococcus abundance is associated with increased susceptibility to and severity of CDI, and during co-infection these two pathogens closely interact in biofilm-like structures on the host-epithelium. Finally, we observe that both adult and pediatric patients with CDI harbor significantly higher levels of Enterococci compared to uninfected controls. Together, these data suggest that co-occurrence of Enterococcus and C. difficile in the GI tract may have a profound and previously unappreciated impact on colonization, persistence, and recurrence of CDI.

Notes



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