7th Annual Research Symposium

Friday, April 19th, 2024

Vanderbilt Student Life Center

A Message from the Director

Thank you for joining us for the VI4 Annual Research Symposium. We are proud to host our 7th annual event and look forward to a day full of exciting talks, learning, and community, highlighting the outstanding research and training that happens within VI4. While you are enjoying the day's activities, we also invite you to learn more about our upcoming events and opportunities and encourage you to seek out new ways to connect with VI4's programming. Thank you to our Symposium partners, who helped us organize a world-class event!

Eric P. Skaar, Ph.D., M.P.H.
Director
Vanderbilt Institute for Infection, Immunology and Inflammation

Welcome to the 7th Annual VI4 Research Symposium

presented by the

Vanderbilt Institute for Infection, Immunology and Inflammation

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Division of Pediatric Infectious Diseases
Vanderbilt Microbiome Innovation Center
Division of Infectious Diseases
Vanderbilt Vaccine Research Program

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Center for Personalized Microbiology and microVU
Program in Computational Microbiology and Immunology
Program in Imaging of Infection and Immunity
Vanderbilt Microbiome Innovation Center

Poster Abstracts

Scan the QR code to view the poster abstracts.

Agenda

8:45 - 9:15 am	Registration
9:15 - 9:20 am	Welcome: Eric Skaar, PhD, MPH
9:20 - 9:55 am	**A. Sloan Devlin, PhD** "Host-produced, bacterially modified gut metabolites"
9:55 - 10:30 am	**Lionel Ivashkiv, MD** "Regulation of Macrophage Activation and Inflammatory Cytokine Production"
10:30 - 11:45 am	Poster Session #1
11:45 - 12:50 pm	Lunch
12:50 - 1:25 pm	**Nadine Rouphael, MD** "Journey in Translational Immunology"
1:25 - 2:00 pm	**Bo Shopsin, MD, PhD** "*Staphylococcus aureus* Adaptation During Clinical Infection"
2:00 - 3:15 pm	Poster Session #2
3:15 - 3:45 pm	Trainee Presentations
3:45 - 4:05 pm	Jr. Faculty Citation Award Presentation
4:05 - 4:40 pm	**Tiffany Reese, PhD** "Viral Exploitations of the Host"
4:40 - 4:45 pm	Closing Remarks
4:45 - 6:00 pm	Reception

Guest Speakers

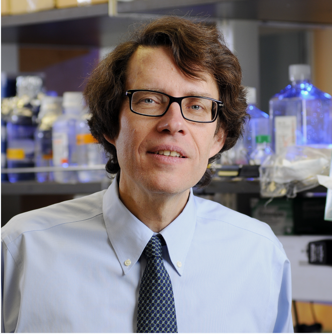
A. Sloan Devlin, PhD

Associate Professor

Harvard University

Dr. Sloan Devlin is currently an Associate Professor in the Department of Biological Chemistry and Molecular Pharmacology (BCMP) at Harvard Medical School. She received her A.B. degree in chemistry from Harvard College in 2006, where she conducted organic chemistry research in the laboratory of Andrew Myers. She earned her Ph.D. in 2012 from Stanford University under the direction of Justin Du Bois, where she performed research in total synthesis and organometallic methodology development. As a postdoc with Michael Fischbach at the University of California, San Francisco, she elucidated biosynthetic pathways and biological activities for small molecules produced by human gut bacteria. Sloan joined the Department of BCMP as an Assistant Professor in Fall 2016. The Devlin lab uses strategies from chemical biology to study the human microbiome. She is the recipient of a 2021 Alfred P. Sloan Research Fellowship in chemistry and a 2018 NIH Maximizing Investigators' Research Award (MIRA).

Guest Speakers



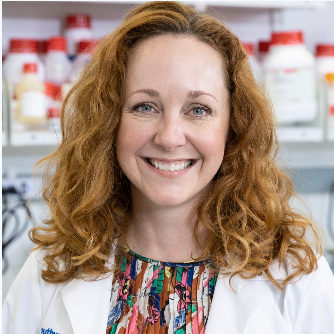
Lionel Ivashkiv, MD

Professor

Cornell University

Lionel B. Ivashkiv received his undergraduate degree from Columbia College and medical degree from Harvard Medical School. He completed an Internal Medicine residency at Bellevue Hospital-NYU Medical Center and a Rheumatology Fellowship at Brigham and Women's Hospital-Harvard Medical School. Dr. Ivashkiv joined the faculty of Hospital for Special Surgery and Weill Cornell Medical College in 1992, where he is currently Chief Scientific Officer (HSS), Richard L. Menschel Research Chair, David H. Koch Chair in Arthritis Research, Director of the David Z. Rosensweig Genomics Research Center, and Professor of Medicine and Immunology. Dr. Ivashkiv is an elected Member of the American Society for Clinical Investigation and the Association of American Physicians. He has authored more than 180 publications. Dr. Ivashkiv's laboratory studies genes and molecular pathways that are important in autoimmune, inflammatory and musculoskeletal diseases and can be therapeutically targeted. He is recognized as a leader in the fields of cytokines and epigenomic regulation of innate immune cells that drive pathogenesis of autoimmune and inflammatory diseases such as rheumatoid arthritis, and are important for repair of tissue injury.

Guest Speakers



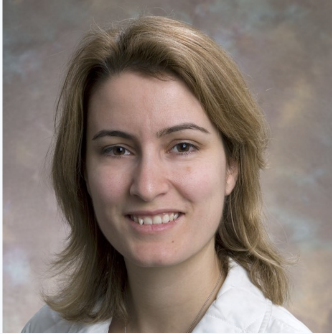
Tiffany Reese, PhD

Assistant Professor

UT Southwestern Medical Center

The Reese laboratory investigates the pathways exploited by viruses and parasites to reveal fundamental aspects of cell biology and immunology. Reese's lab is pioneering the use of mouse models of multiple infections to understand the complex interactions that occur during co-infection. They discovered that infections with parasites reactivate latent herpesvirus infections through manipulations of the immune system that depend on the timing of co-infection. These studies are some of the first to demonstrate a direct relationship between co-infection and effective immune responses to viruses. Harnessing the power of viruses to teach us cell biology, Dr. Reese's lab discovered that noroviruses encode their own death-inducing protein domain in NS3 to facilitate viral egress, which mimics a host protein involved in program cell death. This study illuminated a novel viral egress strategy and may reveal a new pathway of programmed cell death. In other studies, they revealed a new mechanism for the tuning of the cytoplasmic DNA sensing pathway. Herpesviruses are master manipulators of the host and thrive in cells with high levels of reactive oxygen species (ROS). They found that ROS oxidizes a key signaling molecule, STING, that controls the induction of the antiviral cytokine, interferon-β, leading to dampened anti-herpesvirus responses. Dr. Reese was recently promoted to Associate Professor at UT Southwestern. She is an active member of both the Immunology and Molecular Microbiology Graduate Programs at UT Southwestern, and trains both graduate students and postdoctoral fellows in her lab.

Guest Speakers



Nadine Rouphael, MD

Professor

Emory University

Dr. Nadine Rouphael (MD) is the Sumner E. Thompson, III Distinguished Professor of Vaccinology and Infectious Diseases at Emory University in Atlanta, USA. She serves as the executive director of the Hope Clinic, the clinical arm of the Emory Vaccine Center and the Emory principal investigator for the NIH funded Vaccine Treatment and Evaluation Unit (VTEU) and the co Clinical Core principal investigator for NIH funded Stanford Human Immunology Project Consortium (HIPC). She has served as the national chair/co-chair as well as overall PI/site PI of 75 clinical studies and an investigator on more than 200 studies. She has interest in antimicrobial resistance, vaccine clinical trials, vaccine delivery methods, translational research on innate immunity and systems biology, immune aging and correlates of protection. She is also the joint PI for the Emory Clinical Immunization Safety Assessment (CISA) funded by CDC. She has published more than 220 peer reviewed publications and has received many awards. She is currently the associate editor for Clinical Infectious Diseases for the Vaccine section. She is passionate about mentoring the next generation of physician scientists and serves as Co-Director of the Stimulating Access to Research in Residency (StARR) R38 Program and the Director of the T32 vaccinology grant.

Guest Speakers

Bo Shopsin, MD, PhD

Associate Professor

New York University

Dr. Shopsin received a BS in Biology from Indiana University and an MD and PhD from New York University School of Medicine. For his PhD, he developed single-locus sequence typing ("spa typing") for Staphylococcus aureus in Barry Kreiswirth's lab. After medical school, he completed an internal medicine residency and fellowship in Infectious Diseases at the New York University Langone Health (NYULH). During fellowship, he completed a postdoc in molecular genetics in Richard Novick's lab studying the molecular epidemiology of mutations in agr, a quorum-sensing regulator of virulence, in natural populations of S. aureus. He joined the faculty of NYULH in 2007, where he currently leads the Antimicrobial Resistance Program and runs a research lab studying the ways in which S. aureus adapts to human hosts during the course of clinical infection.

Poster Abstracts

Session #1

10:30 am - 11:45 am

Click the names to read their abstract.

Taseer Ahmad
Benjamin Bratton
Juan Carvajal-Garcia
Jesse Chen
Ziche Chen
Sarah Comer
Camila Brito
Martin Douglass
Kara Eichelberger
Lauren Emmerson
Kyle Enriquez
Emilia Fallman
Samantha Grimes
Clinton Holt
Shannon Kordus
Alexander Kwiatkowski
Haley Lewis
Tamara Machado
James McBride
Dudley McNitt
Jared Oakes
Ciamaka Okoye
Mary Oliver
Chris Peritore-Galve
Mark Petrovic
Allyson Ray
Seth Reasoner
Valeria Reyes-Ruiz
Abigail Rich
B. Ilkin Safa
Ana Salina
Catherine Shelton
Jennifer Shuman
Clara Si
Isabella Sirit
Alaina Skellett
Jacquelyn Spathies
Tegy Vadakkan
Paige Vega

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Poster Abstracts

Session #2

2:00 pm - 3:15 pm

Click the names to read their abstract.

- Danial Asgari
- Brandon Baer
- Lindsay Bass
- Tomas Bermudez
- Hannah Bhattacharya
- Kaeli Bryant
- Georgia Caso
- Jeanette Critchlow
- Alejandra Flores
- Jamisha Francis
- Azuah Gonzalez
- Emily Green
- Parker Jamieson
- Alyssa Jarabek
- Kat Jones
- Kyung Eun Kim
- Dillon Kunkle
- Sabina Leonard
- Kaitlin McKernan
- Jia Mei
- Caitlin Murdoch
- Kateryna Nabukhotna
- Bianca Nguyen
- Alexis Pope
- Sarah Price
- Dante Reyna
- Antiana Richardson
- Brynn Roman
- Nicolas Shealy
- Shajila Siricilla
- Rachael Smith
- Luisella Spiga
- Kacie Traina
- Logan Treat
- Jacqueline Van Ardenne
- Max Van Belkum
- Elias West
- Mandy Westland
- Christopher Wilson

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Antigen Presenting Cell activator protein 1 (AP-1) complex contributes to Salt-sensitive Blood Pressure

Taseer Ahmad, Mohammad Saleem, Ashley Pitzer Mutchler, Lale A. Ertuglu, and Annet Kirabo

Salt-sensitivity of blood pressure (SSBP) affects 50% of the hypertensive and 25% of the normotensive population and is a major independent risk factor for cardiovascular morbidity and mortality. We previously found that SSBP is associated with activation of the NLRP3 inflammasome in antigen presenting cells (APCs) via increased oxidated stress and isolevuglandin (IsoLG) formation, but the underlying mechanisms are unknown. The activator protein 1 (AP-1) (FOS/JUN) has been implicated in activation of the NLRP3 inflammasome but its role in SSBP is not known. We hypothesized that AP-1 transcription factor in APCs senses elevated sodium and contributes to SSBP. We further hypothesize that antioxidant flavonoid, diosmetin prevents AP-1 activation and SSBP. Using bulk RNA-sequencing in human monocytes, we found elevated sodium increases expression of the AP-1 gene family when compared to normal sodium concentration, including cFOS (2378.18 ± 480.7 vs 6494.09 ± 945.55, p= 0.0009), FOSB (53.63 ± 17. 55 vs 131.06 ± 10.30, p= 0.397), cJUN (7313.90 ± 984.93 vs 11370.09 ± 1286.35, p= 0.2563) JUNB (4218.4.36 ± 199.17 vs 570.45 ± 200.91, p= 0.0445) and JUND (3309. 63 ± 270.64 vs 8057.90 ± 1043.05, p= 0.00006). In additional experiments, we enrolled people with hypertension and phenotype them for SSBP using an established in-patient protocol of salt-loading/depletion and performed single-cell transcriptomic analyses in vivo on peripheral blood mononuclear cells (PBMCs). We found that expression of the FOSB and JUNB genes are more sensitive in concert with blood pressure in salt-sensitive (SS) but not salt-resistant (SR) humans. Moreover, diosmetin attenuated high salt-induced hypertension, 123.5 ± 4.1 vs 146.8 ± 2.5 mm Hg in controls, **p< 0.01). In addition, diosmetin also reduced high salt-induced inflammation including APC production of IsoLG-protein adducts, IL-1β, and NLRP3 inflammasome expression. These findings reveal a role for immune cell AP-1 (FOS/JUN) signaling in salt-sensitive hypertension and diosmetin may provide a potential therapeutic target for treatment of SSBP.

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Peering Inside the Spatiotemporal Organization of Bacteria

Robert Mann, Val Z. Jackson-Hundley, Grace E. Howard, Melissa Herring, Benjamin P. Bratton

From van Leeuwenhoek to artificial intelligence, the fields of microscopy and microbiology have been in lockstep, tying together technological advances and discovery of biological phenomena. In the Bratton lab, we continue this tradition to study the dynamic internal and collective organization of microbes, particularly in Gram-negative bacteria, in response to complex physicochemical environments. Here we present a few vignettes to showcase ongoing research questions in the lab. (1) Being alone in a stressful environment: single-cell bacterial responses to environmental stresses. (2) Building yourself up: cytoskeletal structures pattern bacterial cell shape. (3) Building a community with a trillion of your closest friends: emergent phenotypes of uropathogenic E. coli colony biofilms. (4) Dyeing dynamically: capturing dynamic bacterial growth processes through color specific pulse-chases. (5) Dynamically dying: quantifying the phenotypic phase space of bacterial death during antibiotic treatment.

The Role of Error-Prone DNA Polymerases in Bacterial Mutagenesis and Evolution

Juan Carvajal-Garcia, Haoxin Liu, Houra Merrikh

Mutations, coupled with natural selection, shape the natural world and are responsible for the biodiversity we enjoy today. In addition, mutations are crucial for human health and disease. Through mutations in their DNA and selection for increased fitness, bacteria become resistant to antibiotics and cancer cells to chemotherapy; de novo mutations are also responsible for a number of genetic diseases. However, what cellular processes promote spontaneous mutagenesis, and therefore drive evolution remains poorly understood.

We recently showed that transcription-coupled repair promotes spontaneous mutagenesis and the evolution of antibiotic resistance in highly divergent bacteria. We also showed that two DNA polymerases of the Y family are responsible for this mutagenesis. Importantly, this represents mutagenesis that is independent of replication, but is dependent on transcription. This mechanism of mutagenesis is highly relevant to bacteria that do not divide in their natural habitats but still mutate and adapt.

We have expanded our study of Y family DNA polymerase-dependent mutagenesis using a forward genetic assay that allows for detection of many different types of inactivating mutations. In addition, we are looking into finding genome wide spontaneous mutations using novel high throughput sequencing methods. Last, by looking at the evolutionary trajectory of Y family polymerases, we have uncovered a previously unappreciated diversity, that can greatly expand our understanding of how pro-mutagenic proteins evolved.

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Defining the functions of heme degradation products of *Staphylococcus aureus* IsdG family heme oxygenases

Jesse P.Y. Chen, Jeffrey A. Freiberg, Lisa J. Lojek, Eric P. Skaar

Staphylococcus aureus is the leading cause of global morbidity and mortality. *S. aureus* requires the acquisition of heme to colonize its host and cause disease. Heme, an iron-bound protoporphyrin IX molecule, is essential for numerous biochemical functions, including energy generation and detoxification of environmental stresses. Heme can be degraded by heme oxygenases into free iron, porphyrin moiety, and byproducts. In eukaryotic and Gram-negative organisms, the heme oxygenase 1 (HO-1) family of proteins degrade heme to free iron, biliverdin, and carbon monoxide. Conversely, *S. aureus* encodes two homologous heme oxygenases, IsdG and IsdI, which are structurally distinct from the HO-1 family of proteins. IsdG and IsdI release iron and generate a yellow chromophore, staphylobilin, and byproduct formaldehyde. While the functions of biliverdin as an antioxidant and anti-inflammatory molecule are well-documented, the functions of staphylobilin are yet to be elucidated. In this study, we identify the genes and cellular pathways that affect *S. aureus* fitness in strains that either cannot degrade heme, degrade heme to staphylobilin, or degrade heme to biliverdin, when heme is the sole iron source. To determine the fitness advantages and transcriptomic changes due to heme degradation, we performed transposon-sequencing (Tn-seq) and RNA sequencing (RNAseq), respectively. Notably, genes involved in the biosynthesis of cell membrane and wall are amongst the list of the most conditionally essential pathways, identified through Tn-seq. We also report that transcript abundance of genes involved in NADH-dependent nitrate and nitrite dissimilations significantly increased in *S. aureus* expressing endogenous heme oxygenase compared to strains expressing HO-1 family of proteins. Taken together, these Tn-seq and RNAseq screens establish a relation between heme catabolism and S. aureus cellular physiology. The findings of this work lay ground for further investigation of staphylobilin's contribution to bacterial survival and pathogenicity.

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Increased Renal Dysfunction and Kidney Neutrophil Infiltration in a Mouse Model of Psoriasis

Ziche Chen; Bianca A, Nguyen; Jason Meyer; Tzushan Sharon Yang; Nicole L. Ward; Matthew R. Alexander

Psoriasis, an autoimmune disorder, is marked by T cell-mediated hyperproliferation of epidermal keratinocytes. Most psoriasis-related morbidity and mortality stems from an elevated risk of cardiovascular and renal disease, but mechanisms remain unclear. Circulating neutrophils are increased in psoriasis, and plasma levels of neutrophil activation markers correlate with extra-cutaneous manifestations such as vascular inflammation. We hypothesize that increased granulopoiesis drives neutrophil migration to the kidneys leading to renal dysfunction in psoriasis. Employing a KC-Tie2 mouse model of psoriasis with keratinocyte-specific overexpression of the angiopoietin receptor Tie2, we observed significant elevations in urinary albumin, NGAL, and glomerulosclerosis in KC-Tie2 mice compared to their littermate controls, indicative of renal injury. KC-Tie2 mice also demonstrated modestly higher blood pressure, particularly during the inactive period, than their littermate controls. Investigating the cause of augmented renal dysfunction, we analyzed skin and renal immune cell populations using flow cytometry. KC-Tie2 mice displayed not only an increase in skin neutrophils but also an approximate tenfold increase in renal neutrophils. This was a selective increase in neutrophils, as we noted no significant differences in renal dendritic cells, lymphocytes, monocytes, and macrophages. Similar selective increases in neutrophils were noted in the circulation, aorta, and bone marrow, prompting an examination of granulopoiesis mediators. We detected substantial elevations in granulocyte colony-stimulating factor (G-CSF) in the plasma and skin of KC-Tie2 mice. To explore the impact of neutrophils on renal damage, we assessed neutrophil extracellular trap (NET) formation in the kidneys. We identified a significant increase in the numbers of renal neutrophils undergoing both vital and suicidal NETosis. In conclusion, our findings demonstrate increased renal dysfunction in a mouse model of psoriasis, concomitant with notable rises in total and NETotic renal neutrophils and G-CSF production, potentially unveiling a novel link between skin inflammation and renal dysfunction via enhanced granulopoiesis.

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Determining the mechanism through which cytochrome bd-I modulates motility in uropathogenic *Escherichia coli*

Sarah L. Comer, Connor J. Beebout, Matthew A.B. Baker, and Maria Hadjifrangiskou.

Several bacterial species rely on flagellar motility to respond quickly to stimuli, acquire nutrients, and colonize different niches in an environment. It is well understood that flagella are powered by the flow of a proton gradient, known as the proton motive force (PMF), through the flagellar motor. The energy complexes of the electron transport chain (ETC) generate the PMF. Precisely how the PMF is directed to the motor is unknown. Recent work from our lab suggests this mechanism involves components of the ETC. We found that uropathogenic *Escherichia coli* (UPEC) mutants lacking the ETC complex, cytochrome bd-I (bd-I), are non-motile yet still produce flagella. As a facultative anaerobe, *E. coli* possesses a branched respiratory chain that encodes multiple dehydrogenases, NADH I and II (NDH-I and II), and terminal oxidases, cytochrome bo3, bd-I, and bd-II. Loss of the other terminal oxidases has no effect, indicating that bd-I may have a unique role in motility. The bd-I mutant also cannot reduce triphenyl tetrazolium chloride (TTC), an indicator of proton flux and NDH-I/II activity. We hypothesize that the lack of motility in the bd-I mutant is due to a decrease in the PMF, possibly through the impairment of NDH-I/II. This is supported by a partial rescue of motility when evaluating motility at a pH of 5.5. In this work, we evaluated the motility of single deletion mutants lacking NDH-I and II and a double deletion mutant that lacks both. Surprisingly, single deletion mutants experienced an increase in motility while double deletion caused a moderate decrease in motility. This suggests absence of NDH-I/II alone does not fully explain the bd-I phenotype. To explore alternative explanations, we plan to evaluate the level of expression and function of the other ETC components in bd-I mutants and determine if bd-I makes direct contact with the flagellar motor.

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A diet composed of L-amino overrides host genetic susceptibility to *Salmonella* Typhimurium gastroenteritis

Camila B. Brito, Nicolas Shealy, Mariane Font, Marco Aurélio Vinolo, Mariana X. Byndloss.

Host extrinsic factors (e.g., dietary choices), like host intrinsic factors (e.g., Genetics), can alter the host's susceptibility to enteric infections, including *Salmonella* Typhimurium (*S.*Tm) induced gastroenteriti*S.* Interestingly, a recent study by our group has shown that the diet can overcome the host's genetic resistance to *S.*Tm infection. Feeding CBA/J mice (a genetically resistant strain that usually tolerates *S.*Tm intestinal colonization with a diet consisting only of L- amino acids (AA) instead of a diet with casein as a protein source (CA) for two days can make them susceptible to *S.*Tm-induced gastroenteriti*S.* Interestingly, this phenotype was dependent on the microbiota and independent of *S.*Tm virulence. Since immune education is part of colonization resistance, we hypothesized that the host immune response could be essential to increasing AA diet-fed CBA/J mice's susceptibility to *S.*Tm. To test our hypothesis, we fed the CBA/J mice with an AA or CA diet for two days, followed by *S.*Tm infection via oral gavage. Mice were euthanized 3 and 7 days after infection, and the samples were collected for analysi*S.* AA diet-fed mice showed an increased susceptibility to *S.*Tm gastroenteritis, with a significant reduction in body weight and an increased *S.*Tm burden in feces, ileal, and colon content compared to CA diet-fed mice. The increase in bacterial load of AA diet-fed mice was independent of bacterial respiration. However, we did not observe any difference in *S.* Tm burden in the cecum content 7 days after infection. Surprisingly, we observed increased intestinal damage in the ileum, colon, and cecum. Demonstrating that AA diet-fed CBA/J mice presented ileitis, a key feature of Salmonellosis in human*S.* Therefore, our next step was to compare the host response in the cecum and ileum of these mice. RNA-seq analysis data showed that the ileum of AA diet-fed mice presented a greater number of differentially expressed genes when compared to CA diet-fed mice 3 and 7 days after infection. We also observed an up-regulation in genes involved in the cell cycle and inflammatory response in both tissue*S.* However, 7 days after infection in the ileum, we observed an up-regulation of genes implicated in antimicrobial response mediated by antimicrobial peptide*S.* Interestingly, AA diet-fed showed a down-regulation in genes involved in several metabolic processes, including cholesterol and retinol metabolism. In conclusion, our data demonstrate that feeding mice with a short-term AA diet changes the host response, increasing the inflammatory process in the ileum and cecum in a tissue-specific manner. Our study has established a new mouse model of *S.* Tm gastroenteritis, which is highly relevant to human Salmonellosis and may aid in future treatment strategies against *S.* Tm-induced gastroenteritis.

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Clostridioides difficile increases undecaprenyl pyrophosphate recycling and drug efflux in response to iron starvation

Marty V. Douglass & Eric P. Skaar

Clostridioides difficile infection (CDI) is the leading nosocomial intestinal infection in the United States and an urgent threat to public health. CDI onset begins with *C. difficile* outcompeting both the host microbiota and the innate immune response for limited nutrients. A critical factor in the host immune response to CDI is the innate immune protein calprotectin (CP) that chelates essential nutrient metals from the pathogen through a process termed nutritional immunity. CP is essential for the host to combat CDI, yet how *C. difficile* overcomes CP to acquire nutrients is not well understood. To uncover how *C. difficile* responds to nutritional immunity, we evaluated the transcriptional changes that *C. difficile* undergoes when challenged with CP. We identified a putative two-component system (TCS), 2822 and 2823, to be transcriptionally increased in the presence of CP and iron chelators. 2822 and 2823 regulates three genes immediately downstream: 2821, 2820, and 2819. Based on bioinformatic predictions, 2820 and 2819 encode an ATP driven efflux pump, and 2821 encodes an undecaprenyl pyrophosphatase. Further experiments revealed that 2822/2823 is activated by the antibiotic bacitracin, and mutants lacking the TCS are extremely sensitive to the cell surface targeting molecules bacitracin and vancomycin, the latter of which is clinically relevant. Our results support a model in which *C. difficile* overcomes nutritional immunity by coordinating an increase in undecaprenyl pyrophosphate recycling and drug efflux to defend against external threats such as cell envelope targeting antimicrobials.

Candida albicans regulates *Staphylococcus aureus* virulence through cross-kingdom interactions

Kara R. Eichelberger, Brian M. Peters, James E. Cassat

The bacterial pathogen *Staphylococcus aureus* and opportunistic fungus Candida albicans are frequently co-isolated from colonization and infection sites. Because polymicrobial interactions at these sites may influence disease outcomes, our overarching research goal is to characterize how *C. albicans* impacts *S. aureus* pathogenesis. This study aims to determine mechanisms by which *C. albicans* enhances *S. aureus*-mediated immune cell death. Consistent with published data, we observed that *C. albicans* co-culture enhances *S. aureus* cytotoxicity towards human and murine monocytes through greater activation of the *S. aureus* accessory gene regulator (Agr) system, which regulates production of multiple cytolytic toxins. Unexpectedly, we also observed that *C. albicans* induced cytotoxicity of the typically non-toxic *S. aureus* agr mutant towards human, but not murine, monocytes. This is important because a significant proportion of *S. aureus* isolates that have lost Agr activity still cause disease, and therefore may be susceptible to virulence regulation by co-colonizing microbes. We hypothesized that *C. albicans* enhances both Agr-dependent and Agr-independent *S. aureus* virulence via distinct mechanisms. We determined that *C. albicans* induces activation of the *S. aureus* SaeRS virulence system, and SaeRS is required for Agr-independent human cell death. We observed that cytotoxicity towards human monocytes requires Panton-Valentine Leukocidin (PVL), a SaeRS-regulated toxin with greater affinity for the human C5aR1 receptor. Blockade of C5aR1 on human monocytes prevented cell death, further supporting a critical role for PVL. Using a panel of *C. albicans* clinical isolates, we tested if enhancement of *S. aureus* cytotoxicity is conserved among diverse strains. We identified *C. albicans* isolates that induce Agr-dependent, but not Agr-independent, cytotoxicity, suggesting that *C. albicans* enhances Agr and SaeRS activation through distinct mechanisms. Collectively, these data reveal that *C. albicans* activates at least two *S. aureus* virulence systems to augment host cell death. Therefore, cross-kingdom interactions may radically alter staphylococcal pathogenesis.

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High Specificity Mapping of Gangliosides in Staphylococcus aureus Infected Bone by MALDI TIMS Imaging Mass Spectrometry

Lauren N. Emmerson, Christopher J. Good, Katerina V. Djambazova, Lukasz G. Migas, Raf Van de Plas, James E. Cassat, Jeffrey M. Spraggins

Osteomyelitis, or inflammation of the bone and bone marrow, is a debilitating disease most often caused by *Staphylococcus aureus* infection. *S. aureus* colonizes the bone marrow forming abscesses, characterized by a central staphylococcal abscess region surrounded by both viable and necrotic neutrophils, and encapsulated in a fibrotic border. These tissue regions possess unique molecular profiles, with gangliosides being a distinct molecular class localized to the infection site. While the function of gangliosides is not entirely understood, they have been linked to infected tissues and host immune responses. The distribution of this lipid class can be interrogated using matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS), a powerful analytical tool capable of detecting a wide array of analytes with high sensitivity and specificity. MALDI IMS revealed the localization of gangliosides in the cell population bordering the necrotic bone marrow abscess, for example GM1(d42:2) (*m/z* 1626.948), GM1-NeuGc(d42:2) (*m/z* 1642.940), and HexNAc-GM1(d42:2) (*m/z* 1830.026). However, there could be multiple ganglioside isomers with unique distributions and differing biological functions with identical *m/z* values. The structural complexity and heterogeneity of gangliosides, being composed of "building blocks" that result in conformationally distinct structures with the same mass, stresses the need for additional separation methods to differentiate between these species. Trapped ion mobility spectrometry (TIMS) is a gas phase separation technique that can be implemented to characterize structural isomers, such as a- and b- series gangliosides that differ in the position of a sialic acid. MALDI TIMS imaging mass spectrometry revealed the distinct localization of GM1a and GM1b isomers near the abscess in bone marrow. These data emphasize the power of MALDI TIMS IMS in identifying the specific localizations of isomers in situ. Further studies are necessary to determine the biological function associated with each ganglioside isomer detected in the abscess border of *S. aureus*-induced osteomyelitis.

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Staphylococcus aureus ZigA functions as a COG0523 metallochaperone to aid in pathogenesis

Kyle T. Enriquez, Yasiru R. Perera, Tae Akizuki, Andy Weiss, Cait Murdoch, Sydney Drury, Walter J. Chazin, Eric P. Skaar

Staphylococcus aureus is a leading cause of infectious death worldwide and presents a growing threat due to increasing resistance to current antimicrobial strategies. Nutritional immunity, the process by which metals are sequestered by the host to prevent pathogen growth, presents a strategy to address current limitations in antimicrobial development. COG0523 proteins, like those discovered in eukaryotic (ZNG1) systems, have been shown to act as zinc (Zn) metallochaperones, aiding in the response to Zn limitation by establishing a hierarchical distribution of Zn. *S. aureus* encodes multiple COG0523 proteins that have conserved motifs for metal-binding and GTPase activity, including ZigA. Yeast-2-Hybrid interaction screening with ZigA suggests high-confidence interactions with UvrA and RpoC which work in tandem at sites of nucleotide excision repair. Similarly, results suggest that ZigA acts in concert with UvrA to promote DNA damage repair in the setting of zinc limitation in vitro. Biochemical experiments support the conclusion that metal-stripped ZigA acts as an intrinsic GTPase and that this activity is increased in the setting of exogenous Zn and/or UvrA. ZigA has been implicated in Zur regulatory networks which are critical to the response to low-Zn environment. In addition, zigA mutants exhibit significant defects in host colonization and infection of the lung in a systemic model of *S. aureus* infection. Taken together, these data address the hypothesis that ZigA acts as a Zn- and GTP-dependent Zn metallochaperone to promote *S. aureus* pathogenesis. In addition, this work is uniquely positioned to bolster current investigations of bacterial COG0523s as targets for antimicrobial development, as the *S. aureus* infectious environment is often metal-starved. Future work will focus on evaluating the biochemical mechanism by which ZigA impacts resistance to DNA damage through functional assays of UvrA, bio-structural investigation of ZigA-UvrA complexes, and co-immunoprecipitation to potentially implicate other clients in the response to Zn limitation.

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Role of Glutamic-oxaloacetic transaminase 1 (GOT1) in dendritic cell immune responses

Emilia Fallman, Kyungeun Kim, Denis A. Mogilenko

Conventional dendritic cells (cDCs) are antigen-presenting cells that act as mediators between the innate and adaptive immune system. Upon activation via toll-like receptors (TLRs) cDCs transfer antigens from tissues to lymph nodes to coordinate T cell-mediated immune responses. cDCs rely on intracellular metabolic pathways to support their immune functions. Glutamic-oxaloacetic transaminase 1 (GOT1) regulates energy production by linking metabolic reactions between the cytoplasm and mitochondria. Importantly, GOT1 has been shown to direct T cell immune functions, however how GOT1 affects cDCs is not entirely understood. We hypothesized that GOT1 has an impact on mitochondrial metabolism and DC immune functions. Here we show that GOT1 is differentially expressed between mouse cDC populations in vivo, and that GOT1 deficiency altered cytokine expression in activated bone-marrow derived DC (BMDCs) in vitro. Single-cell transcriptomics and flow cytometry analysis showed that cDCs resident in tissues express increased GOT1 mRNA and protein levels compared to cDCs in the spleen. Moreover, GOT1 expression was upregulated in CCR7+ migratory cDCs. Genetic knockdown of GOT1 in BMDCs changed inflammatory responses of these cells to TLR ligands. Specifically, activated GOT1-deficient BMDCs showed increased IL-12 secretion. These results suggest that GOT1 can affect migration and cytokine production in cDCs. Thus, GOT1 might play a key role in cDC immune responses to bacterial and viral infections.

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Mutations in the coronavirus nsp14-nsp10 interface impair replication

Samantha L. Grimes, Mark R. Denison, Jordan Anderson-Daniels

Coronaviruses (CoVs) encode nonstructural proteins (nsps) 1-16, which assemble to form replication-transcription complexes that function in viral RNA synthesis. All CoVs encode a proofreading 3'-5' exoribonuclease (ExoN) in nsp14 (nsp14-ExoN) that mediates high-fidelity replication as well as other critical roles in replication and pathogenesis. The ExoN activity of nsp14 is enhanced in the presence of the cofactor nsp10, and mutations in nsp10 have been shown to impair replication and increase sensitivity to nucleoside analogs. In this study, we tested the impact of alanine substitutions in nsp14 at the nsp14-nsp10 interface on viral replication and ExoN function in the betacoronavirus murine hepatitis virus (MHV). Some substitutions did not allow for viral recovery, suggesting critical roles in nsp14 function or interactions with nsp10. Two substitutions, K7A and D8A demonstrated impaired viral replication and decreased peak titer. Passage of the K7A and D8A mutant viruses selected for viruses with partially-compensated replication kinetics and additional mutations in nsp14. These results identify and support potential determinants of nsp14-nsp10 interaction and the potential for targeting novel protein interfaces for viral inhibition and attenuation.

Predicting Same-Site Antibodies Using Contrastive Learning

Clinton Holt, Perry Wasdin, Ivelin Georgiev

Antibodies are heteromeric immunoglobulin proteins with an immense diversity that allow them to bind nearly anything. With an estimated sequence diversity of 10^15, the task of predicting antibody binding specificity based on sequence alone is daunting. Typical machine-learning classifiers for antibody binding have a small number of fixed output classes which do not accommodate the large and ever-changing landscape of binding sites such as those on microbial targets. To create a method capable of scaling and handling classification for binding sites outside of our training set we focus on developing one-shot binding site prediction algorithms using contrastive learning. In this scenario, the binding site is defined by one antibody and the task is to predict if a second antibody binds the same site or a different site. We first applied contrastive learning to identify a simplistic definition for a public clonotype where the goal is to achieve a near 100% true-positive rate for antibodies targeting the same-site. Second, we used contrastive learning to fine-tune antibody-specific large language models for the task of predicting same-site antibodies. While this second model achieved greater overall accuracy, it lost simplicity over public clonotyping, showcasing a use for both techniques in the field moving forward.

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Understanding the roles of TcdE and TcdL in toxin release in *Clostridiodies difficile*

Shannon L. Kordus, Rubén Cano Rodríguez, Katerina Nabukhotna, Anna Smith, Kevin Childress, Natalie Loveridge, Evan Krystofiak, Kay Washington, and D. Borden Lacy

The nosocomial pathogen *Clostridioides difficile* produces two large toxins, TcdA (308 kDa) and TcdB (270 kDa). Although toxin effector functions in host cells have been extensively studied, little is known about how these toxins are released from the bacterium. We recently developed highly-sensitive reagents to detect TcdA and TcdB *in vitro* and *in vivo*. Using these reagents, we generated growth curves with a variety of different *C. difficile* strains and found toxin release varies greatly in log-phase, stationary phase, and death phase amongst strains. TcdA and TcdB are encoded on a pathogenicity locus which also encodes the holin-like protein, TcdE, and the remnants of a partial endolysin, TcdL. While bacteriophages use holin/endolysin systems to trigger bacterial cell lysis and escape, multiple reports now suggest that TcdE can promote lysis-independent toxin release. To further understand toxin secretion, we created deletions in tcdE and tcdL and found these strains released less toxin compared to the parent strain. We tested the relevance of these deletions *in vivo* and found both had significantly reduced disease severity when compared to infection with the wild-type strain. Next, we sought to investigate the mechanism of how TcdL contributes to toxin release. We found that strains lacking tcdL had thinner, more compact cell walls, and had less cell wall remodeling. In addition, these strains were more resistant to the beta-lactam, ampicillin. Our data indicate that both TcdE and TcdL are required for lysis-independent toxin release.

RIG-I Activating Nanoparticles for Treatment of Glioblastoma

Alexander J. Kwiatkowski, Christian R. Palmer, Asa A. Brockman, Rebecca A. Ihrie, and John T. Wilson

Glioblastoma (GBM) is a rare form of brain cancer with a dismal outlook for patients who face a mean survival time of 12-18 months post-diagnosis. Higher levels of the pattern recognition receptor retinoic acid-inducible gene-I receptor (RIG-I) are associated with improved survival outcomes in GBM, but RIG-I immunotherapy has not yet been explored for GBM. The clinical utility of 3pRNA RIG-I agonists is currently limited by significant drug delivery barriers, which we will seek to overcome using **RIG-I activating nanoparticles (RANs)**. Di-block polymer was formed with reversible addition–fragmentation chain transfer (polymerization using a first block of 10K polyethylene glycol and a second block of poly[(DMAEMA-c-butyl methacrylate. The di-block copolymer was dissolved in ethanol, diluted with sterile-filtered citrate buffer (pH=4.2), and complexed with RNA for 45 minutes to form micelles ~90 nm in diameter. Mouse Glioma261 (GL261) or CT2A cells were treated with RANs, then 24 hours later, supernatants were collected for ELISA, and cells were stained for flow cytometry. We show that GL261 and CT2A cells treated in vitro with RANs produced significantly more interferon beta (IFN-β) and had increased MHC I expression following RAN treatment compared to cells treated with control RNA-loaded RANs (cRANs). In addition to cancer cells, RANs also activate antigen presenting cells, with THP1-Dual (monocytes) and RAW-Dual (macrophages) producing more type one IFN in response to RAN treatment. Mice were inoculated with 1,000,000 GL261 or CT2A cells on the right flank and treated intratumorally with RANS at a tumor volume of ~50 mm3 and on days 3 and 6 post treatment initiation. Following intratumoral treatment of GL261 and CT2A flank tumors with RANs, mice had prolonged survival with cRANs providing no benefit compared to vehicle control. We demonstrate that RANs can activate multiple cell types and show efficacy against flank tumors.

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IFNγ-IL-17 Producing CD4+ Cells in Polyarticular Juvenile Idiopathic Arthritis

Haley K. Lewis, Maria C. Eckmann, Anna E. Patrick

Juvenile Idiopathic Arthritis (JIA) is the most common autoimmune arthritis that affects children. JIA is characterized by chronic inflammation in the joints, and patients with polyarticular JIA (pJIA) have five or more affected joints during the first six months of illness. Naïve CD4+ cells differentiate into T helper type 1 (Th1) cells in response to T cell receptor stimulation and the polarizing cytokine interleukin 12 (IL-12). Our prior studies found that in pJIA patients, Th1 differentiation results in abnormal inflammation and high levels of IFNγ, IL-17, and IFNγ-IL-17 dual-producing cells. IL-17 is not secreted in Th1 differentiation but is abnormally expressed in pJIA. Mechanisms underlying the production of high IFNy and IL-17 in pJIA Th1 differentiation have not been discovered. In this project, we investigate the role of IL-12, along with other cytokines associated with Th17 differentiation, in the production of IFNγ and IL-17 in pJIA Th1 differentiation. T cell culture experiments in pJIA and pediatric control peripheral blood mononuclear cells (PBMCs) were performed. Both PBMCs and isolated CD4+ cells underwent T cell activation with anti-CD3 and anti-CD28 in the presence of combinations of IL-2, IL-12, IL-6, IL-23, and IL-21. Cells were washed and stimulated on day 5 and analyzed on day 7 by flow cytometry and/or enzyme-linked immunosorbent assay (ELISA). Analysis revealed that pJIA cells have increased IFNy and IL-17 in CD4+ cells and that IL-12 contributes to the heightened production of IL-17. Heterogeneity was identified in individual patient responses to IL-23 and IL-21. We aim to identify novel targets for therapeutics by developing a greater understanding of the drivers causing inflammatory cells in pJIA.

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Prolonged high-fat diet exposure promoted colonic carcinogenesis via disruption of gut microbiota and intestinal epithelium interactions

Tamara R. Machado Ribeiro, Nicolas G. Shealy, Camila B. de Brito, Tiago F. Mota, Artur T. L. Queiroz, Mariana X. Byndloss

Colorectal cancer (CRC) is the third most diagnosed malignancy and the second leading cause of cancer death worldwide. Emerging evidence suggests that a Western-style high-fat diet, which is associated with obesity, is correlated with colorectal cancer (CRC), and it has been classified as an obesity-related disease. Moreover, the alarming rise in obesity rates across the United States has brought with it a concerning increase in CRC among young people. Numerous studies have investigated the correlation between gut microbiota and colorectal cancer (CRC). Due to the intimate connection and continual interaction between the colorectal epithelium and gut microbiota, it is believed that the gut microorganisms influence the development of CRC. To investigate the link between HFD, gut microbiota composition, and epithelium global gene expression, we used a diet-induced obesity (DIO) mouse model. Our findings showed that an HFD resulted in a significant increase in the population of Bacteroidota and Proteobacteria phyla. These phyla are known to harbor species of bacteria that correlate with colorectal cancer, such as *E. coli pks*+ and *B. fragilis*, respectively. The RNAseq from colonocytes has shown upregulation of different oncogenes under a HFD, such as *Tnfrsf10b*, *Fdft*, and *. Our research has shown that prolonged exposure to a HFD results in dysbiosis, which in turn allows cancer-causing bacteria to thrive in the colon. These findings also suggest that excessive intake of fatty acids, like those found in an HFD, could significantly contribute to the activation of oncogenes in the epithelial cells of the intestine.

VINSE: A Core Facility Not Just for Engineers

James R. McBride, Benjamin W. Schmidt, Dmitry S. Koktysh, Christina L. McGahan, Megan K. Dernberger, Jason G. Valentine, & Sharon M. Weiss

The Vanderbilt Institute for Nanoscale Science and Engineering is multidisciplinary core facility that supports research programs for Vanderbilt and the surrounding community. VINSE comprises of three facilities: a cleanroom, an analytical lab and an advanced imaging suite. The cleanroom is equipped to fabricate micro-to-nanoscale structures including microfluidics as well as the deposition of custom surface coatings. The analytical lab is equipped UV-Vis and fluorescence, a Raman microscope as well as a Zetasizer for DLS. The advanced imaging suite houses an advanced analytical scanning/transmission electron microscope (S/TEM), a conventional high-resolution scanning electron microscope and a focused ion beam scanning electron microscope (FIB-SEM) with cryo-EM and volume imaging abilities. We will highlight how users across Vanderbilt and VUMC are utilizing these tools to further their research.

CD4-driven loss of the key germinal center protein, BCL6, prevents type 1 diabetes development

Dudley H. McNitt, Jonathan M. Williams, Joseph G. Santitoro, Jacob Kim, James W. Thomas, and Rachel H. Bonami

High-affinity islet autoantibodies predict type 1 diabetes in mice and humans and implicate germinal centers (GCs) in type 1 diabetes pathogenesis. T follicular helper (Tfh) cells support GC responses and depend on the transcriptional repressor BCL6 for their maturation. Tfh cells are increased in type 1 diabetic individuals and alterations in Tfh-like cells in the peripheral blood predicted individual responses to abatacept. We therefore hypothesized that CD4+ cell-specific deletion of *Bcl6* would disrupt essential T-B lymphocyte interactions in autoimmune GCs to prevent type 1 diabetes. To test this hypothesis, we generated *Bcl6fl/fl-CD4*.Cre.NOD mice. CD4-driven loss of functional BCL6 resulted in significantly reduced GC Tfh cells in the pancreas and draining lymph nodes relative to *Bcl6fl/fl*.NOD controls, as expected, and led to a significant reduction in GC B cells, in line with their dependence on GC Tfh cells. CD4-driven loss of BCL6 completely prevented type 1 diabetes and blunted spontaneous anti-insulin autoantibody production relative to Bcl6fl/fl.NOD controls. Similar levels of insulitis and tertiary lymph structure formation were present in the pancreas of *Bcl6fl/fl-CD4*.Cre.NOD mice compared to *Bcl6fl/fl*.NOD controls and CD4+, CD8+, and B cell numbers were unchanged. These data highlight BCL6 as a novel therapeutic target in type 1 diabetes and suggest a critical role for GCs in promoting autoimmune islet attack.

Impact of aging on adaptive immune responses to influenza vaccination

Jared Oakes, Joshua Simmons, Cindy Hager-Nochowicz, Leslie Kirk, and Spyros A. Kalams

Influenza related illness is a significant cause of morbidity and mortality in older adults. With age comes decreases in antibody responses, reductions in naïve thymic output, and increases in T cell senescence. Recent vaccine formulations have improved the magnitude of antibody responses in older adults; however, older adults still remain at a significant risk for severe influenza-related illness. Antibodies are the primary correlate of protection after influenza vaccination, yet T cells play a crucial role in providing B cell help and viral clearance after infection. In our cohort of 15 older adults and 32 younger adults, we investigated vaccine-elicited B and CD4+ T cell responses in vitro and ex vivo. While older adults had lower pre-vaccination titers to particular vaccine antigens compared to younger adults, by day 28 post-vaccination both age groups mounted robust antibody responses. Prior to vaccination, younger adults had more H1N1-reactive CD4+ T cells. Post-vaccination, younger adults had increased frequencies of H1N1 and H3N2 antigen-reactive CD4+ T cells compared to older adults. Immune profiling data ex vivo revealed higher frequencies of circulating T follicular helper (cTfh) cells at baseline in younger adults, increased frequencies of activated cTfh cells after vaccination in younger adults, and no difference in senescent CD4+ CD28- CD57+ T cell frequencies between age groups. Single-cell sequencing data supported the lack of influenza-specific activated CD4+ T cells in older adults by reduced antiviral gene profiles. Furthermore, CMV serostatus had no impact on immune responses. Our data reveals despite productive antibody responses, older adults had poor CD4+ helper responses after vaccination. These data highlight the need to understand further the effects of aging on CD4+ T cell responses to improve future vaccine efforts.

Role of CagX (VirB9) and CagY (VirB10) domains in *Helicobacter pylori* Cag Type IV secretion system architecture and activity

Chiamaka D. Okoye, Sirena C. Tran, Mark S. McClain, and Timothy L. Cover

Helicobacter pylori strains that produce CagA and the Cag Type IV Secretion System (T4SS) are associated with an increased risk of stomach cancer. The Cag T4SS delivers CagA, a bacterial oncoprotein, into gastric epithelial cells. The Cag T4SS outer membrane core complex consists of an outer membrane cap (OMC), a periplasmic ring (PR) and a stalk. CagX and CagY are the only components localized to both the OMC and PR, and these two components are required for Cag T4SS activity. CagX and CagY are homologs of VirB9 and VirB10 from prototype T4SSs, but each contains large regions that lack homology to T4SS components in other bacterial species. To investigate how specific CagX and CagY domains contribute to the architecture and activity of the Cag T4SS, we generated H. pylori mutants in which specific CagX or CagY domains were deleted or strains that expressed two fragments of CagX or CagY instead of single intact proteins. Core complex assembly was assessed by immunopurification using epitope-tagged CagF (a putative CagA chaperone) as a bait, SDS-PAGE, mass spectrometry, and negative-stain electron microscopy. Core complexes were successfully isolated from a CagYΔAP mutant, which lacks a CagY antenna projection that inserts into the outer membrane, but not from mutants designed to produce CagX or CagY fragments instead of intact proteins. Immunopurification of an epitope-tagged CagX or CagY fragment resulted in co-purification of both the tagged fragment and the non-tagged fragment, suggesting that the two fragments retain the capacity to interact. Each of the described mutants lacked Cag T4SS activity in an H. pylori-gastric epithelial cell co-culture assay. These results provide new insights into CagX and CagY domains required for stable assembly of the Cag T4SS core complex and Cag T4SS activity.

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TLR-Mediated Trained Immunity Augments Leukocyte Metabolic Function after Burn

Mary Oliver, Xenia Davis PhD, Edward Sherwood MD PhD, Julia Bohannon PhD

Introduction: Nosocomial infections globally impact over 100 million people, with the US incurring 9.8 billion in inpatient care costs alone. Severe burn injury predisposes patients to the risk of infection by impairing innate and adaptative cell function. Previous research including ours, has highlighted the potential for innate immune training, or trained immunity, as a means to confer protection against infection post-burn. Pharmacological modulation (training) of the innate immune system with microbial ligands, including toll-like receptor (TLR) agonists induces sustained metabolic, antimicrobial, and epigenetic alterations in innate leukocytes. This immunomodulation primes innate cells for robust non-specific responses and offers heterologous protection against diverse pathogens. We hypothesize that TLR agonists can reverse burn-induced immunosuppression by enhancing metabolic reprogramming and antimicrobial function in monocytes and macrophages.
Methods: BALB/C mice with a 30% total body surface area scald burn received 20 µg of TLR4 agonist monophosphoryl lipid A (MPLA) intraperitoneally 6 days post-burn. On day 7, peritoneal cells were harvested for flow cytometry and Single Cell Energetic metabolism by profiling Translation Inhibition (SCENITH) assay. Additionally, human monocytes from healthy volunteers were trained with 40 µg/ml MPLA for 24 hours. Seahorse and SCENITH analyses were performed either immediately after MPLA or 3 days after removal of MPLA to assess their metabolic profiles.
Results: Burn-injured mice displayed impaired glucose and mitochondrial dependence in monocytes and macrophages, measured by SCENITH, compared to sham controls. MPLA training reversed this deficit. Flow cytometry revealed reduced expression of immunosuppressive markers CD38 and PD-L1 in MPLA treated cells, while vehicle-treated cells maintained elevated levels. Human monocytes treated with MPLA showed increased extracellular acidification rate and oxygen consumption rate in Seahorse analysis. SCENITH results aligned, indicating higher glucose and mitochondrial dependence in MPLA-treated cells.
Conclusions: Burn injury induced impaired glucose and mitochondrial dependence in macrophages and monocytes, along with elevated immunosuppressive markers, all of which were reversed with MPLA training. Human monocytes exposed to MPLA exhibited effective metabolic reprogramming. Future plans include evaluating the impact of MPLA training on burn patient monocytes. TLR innate immune training post-burn injury offers a promising approach for treating immunosuppressed patients, restoring immune function, and enhancing resistance against infections. Upregulating aerobic glycolysis and oxidative phosphorylation provides innate immune cells with increased energy, boosting antimicrobial function for rapid and efficient pathogen clearance.

HomiMap: Developing a multimodal imaging platform to uncover novel host-microbe interactions within infected tissue microenvironments.

F. Christopher Peritore-Galve, Angela R. S. Kruse, Juan P. Barraza, D. Borden Lacy, and Jeffery M. Spraggins

A bacterial infection initiates complex interactions between the host and the pathogen at the cellular and molecular level. Molecular biology and imaging tools have been essential to understanding how these interactions underlie disease. However, a significant limitation is the requirement for prior knowledge about the molecular targets before dissecting the mechanisms of host-microbial interactions. To address this issue, we developed HomiMap, which combines fluorescence *in situ* hybridization (FISH) and matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) to discover new molecules involved in host-pathogen interactions within the spatial context of infected tissue. We used the mouse model of *Clostridioides difficile* infection to develop HomiMap. We infected mice with PBS, wildtype *C. difficile*, or an isogenic mutant knockout of both genes that encode large glucosylating toxins, *tcdA*, and *tcdB*, that mediate pathogenesis. Colons were harvested during acute infection, fixed in Carnoy's, and then paraffin-embedded. We probed tissues with fluorophore-conjugated FISH probes specific to the 16s rRNA sequence of all Eubacteria or *C. difficile*. FISH microscopy images were acquired using a Zeiss Axioscan. Tissues were then subjected to epitope retrieval, *in situ* trypsin digestion, and matrix application. One tissue section per condition was subjected to MALDI on a timsTOF Flex, and the other was used for liquid chromatography-tandem mass spectrometry to create a paired peptide database. IMS characterized peptide distributions with a spatial resolution of 20 µm, and FISH images were overlaid to identify the spatial relationship of peptides to *C. difficile* cells. We are now working towards developing a computational pipeline to categorize the spatial relationships between peptides and *C. difficile* cells before identifying and validating the candidate proteins. By using HomiMap and rationally prioritizing protein candidates, we can elucidate novel molecular pathogen-host interactions in a spatial context as potential targets for intervention.

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Characterization of complement deposition in lungs recovered using human-xenogeneic cross-circulation after human blood re-exposure

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Human-xenogeneic cross-circulation (HX-XC) is an effective platform for functional rehabilitation of human lungs initially deemed unsuitable for transplant. Transient exposure of human lungs to circulating porcine blood during HX-XC results in deposition of porcine antibodies and leukocytes within the graft. These residual xenogeneic antigens may serve as a nidus for hyperacute rejection (HAR) upon re-exposure to the human immune system, which poses a significant hurdle to clinical translation of this technology. To investigate this risk, we added a period of ex-vivo lung perfusion (EVLP) using autologous donor blood after HX-XC. Human lungs declined for transplant were recovered as previously described, rehabilitated using HX-XC for up to 12 hours, transitioned to an EVLP platform primed with the donor's own whole blood, and evaluated for up to 6 hours. Autologous donor blood replete with antibodies, complement proteins, and other pre-formed immune components was used. Physiologic data and biopsies were collected during HX-XC and EVLP. Tissue evidence of HAR, diffuse graft injury mediated by pre-formed antibodies that typically occurs within hours of exposure, was evaluated using immunohistochemical staining for C4d and the membrane attack complex (MAC). All immunostaining was performed by the Translational Pathology Research Core at Vanderbilt University Medical Center. Re-exposure of human lungs to autologous blood after HX-XC led to no significant increase in capillary endothelial C4d deposition and minimal MAC formation throughout the lung microvasculature. Together these findings argue against acute antibody-mediated injury after HX-XC and provide evidence against a potential risk posed by HX-XC to potentiate hyperacute rejection in a clinical transplant scenario. Further work is needed to mitigate the intrinsic variability between human lung donors and discern other potential mechanisms of graft injury through human-porcine immune system interactions.

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Translation dynamics during the innate immune response in Tribolium castaneum flour beetles

Allyson M. Ray, Sangeevan Vellappan, Srujana Samhita Yadavalli, and Ann T. Tate

Immune dynamics during early acute infection drive disease outcomes. While a rapid, robust, and effective activation of the host immune response is necessary to quell infection, the expression of immune effectors is costly, and may result in auto-immune damage. Previous work has implicated differential translation as a possible immune regulator in innate immune memory (i.e. immune priming) in insects (Tate et al 2017). Selective translation of immune effectors after pathogen detection may act to rapidly suppress pathogen proliferation prior to the onset of severe infection, thereby increasing immune response efficiency while minimizing the cost and consequence of immune activation. We therefore investigated the role of differential translation and efficiency as an immune response modulator and mechanism for infection resistance in the red flour beetle (*Tribolium castaneum*). We first adapted recent techniques in ribosome profiling (Riboseq), which selectively purify ribosome-bound transcripts to assess differential expression at the level of protein synthesis. We then used Riboseq to examine translation dynamics during early immune response using time series data of acute septic infection in naïve beetles. Paired with full mRNA sequencing, this technique can be used to further assess variation in translational efficiency of the immune response across immune-primed and unprimed individuals. Here we report on methods optimization outcomes for the *T. castaneum*-specific Riboseq library preparation protocol, as well as present considerations for method adaptation to other species. We also share preliminary findings regarding translation dynamics in infected vs uninfected adults in early acute infection. Future work will apply these techniques in the context of immune priming, as well as incorporate gene expression knockdowns to functionally assess the role of differential translation in innate immune dynamics.

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Delineating the Pathogenesis of *Pseudomonas aeruginosa* Urinary Tract Infections

Seth A. Reasoner, Grace Morales, Gerald Van Horn, Tomas Bermudez, Jonathan Schmitz, Maria Hadjifrangiskou

Pseudomonas aeruginosa frequently causes severe infections in immunocompromised and hospitalized patients. The infectious biology of *P. aeruginosa* has been well-characterized in infections of multiple anatomic sites, including the respiratory system, skin and wound infections, and ophthalmic infections. The pathogenesis of *P. aeruginosa* in causing urinary tract infections (UTIs) has not received adequate research attention. We reviewed laboratory records at our medical center to determine the frequency of *P. aeruginosa* UTIs. We performed whole genome sequencing on a subset of *P. aeruginosa* UTI isolates. We compared the genomes of *P. aeruginosa* UTI and respiratory isolates. We utilized bladder epithelial cell tissue culture models and mouse models of UTI to interrogate the interactions of *P. aeruginosa* with the urinary tract. Over a five-year period, *P. aeruginosa* UTIs were as common as skin/wound infections and more common than lower respiratory infections at our medical center. Comparative genomics and genome-wide association testing identified the cytochrome c oxidase (ccoN) of *P. aeruginosa* as a genomic region with different sequences between urinary and respiratory isolates. Using tissue culture models, we identified that *P. aeruginosa* can invade bladder epithelial cells. In a mouse model of UTI, different mouse backgrounds are differentially susceptible to *P. aeruginosa* infection. The urinary tract is a common site of *P. aeruginosa* infection. *Pseudomonas* can invade urothelial cells. Ongoing research will delineate the role of bacterial oxygen utilization and intracellular epithelial infection to *P. aeruginosa* UTI pathogenesis.

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Exploiting *Staphylococcus aureus* signal transduction to identify novel antibacterial effectors of the macrophage

Valeria M. Reyes Ruiz, Juan P. Barraza, Callie B. Valtierra, Catherine S. Leasure, Sydney D. Miquelon, Anderson Miller, and Eric P. Skaar

Staphylococcus aureus is a leading cause of morbidity and mortality. While *S. aureus* is mainly considered an extracellular pathogen, a growing body of literature describes an intracellular reservoir of *S. aureus*, which is poorly accessed by antibiotics. Moreover, *S. aureus* that grows and persists inside macrophages can disseminate and cause disease in mouse models of infection. Studies examining the interaction of intracellular *S. aureus* with macrophages would provide insight into the development of therapeutics to treat this bacterial reservoir, which circumvents antibiotic efficacy. *S. aureus* has evolved intricate regulatory networks that allow the bacterium to produce a diverse array of virulence factors and defense mechanisms within the host environment. These include two-component signal transduction systems (TCSs), in which a histidine kinase (HK) responds to extracellular stimuli and transfers a signal to a response regulator (RR) that mediates regulation of gene expression. *S. aureus* contains 16 TCSs that respond to a diverse array of environmental signals. We have engineered reporter constructs for each of these sensing systems in *S. aureus* and identified TCS activated inside macrophages. We then developed an arrayed genome-wide CRISPR screen in macrophages with the use of robotics and high-content imaging to discover host factors responsible for the control of the intracellular reservoir of *S. aureus* and for the activation of these regulatory systems. Our data analysis pipeline relies on the use of machine learning with robust segmentation to measure both TCS-dependent reporter signal and constitutive bacterial signal on a per-cell basis for each well in our arrayed library. This screen has revealed host gene products that affect TCS activation and bacterial burdens and provide a foundation to identify novel innate immune factors required for the control of bacterial infections.

The SRSF2P95H splicing factor mutation exerts distinct splicing effects across myelopoiesis that converge upon dysregulated G-CSFR signaling

Abigail L. Rich, Sandra. S. Zinkel, M.D., Ph.D.

*SRSF2*P95H (P95H) splicing factor mutations are initiating genetic lesions in myeloid malignancies. These are associated with myeloid-biased hematopoiesis in both mouse models and human disease. Mechanistically, P95H substitutions induce transcriptome-wide alterations in RNA splicing that vary by cell type and genetic background, complicating efforts to understand how P95H drives disease. Efforts to understand P95H mutations focus on splicing in progenitor cell populations, however, to date no studies have investigated this mutation in the mature myeloid cells that comprise >40% of the marrow. We evaluated P95H-associated splicing across myelopoiesis. We subjected promyelocytes, myelocytes, and neutrophils from P95H mice to RNAseq and integrated these data with RNAseq from P95H hematopoietic stem cells and human MDS samples. A cluster-based splicing analysis approach identified cell-type specific splicing with overrepresentation of differentially spliced transcripts involved in myeloid effector function pathways in myelocytes and neutrophils. Missplicing of *Csf3r/CSF3R*, the granulocyte colony-stimulating factor receptor (GCSFR), was conserved. Notably, P95H mouse bone marrow exhibits impaired activation of GCSFR-dependent pathways (STAT3/5, ERK) that is unique to GCSFR. Overall, these results define P95H-driven alternative splicing as a differentiation-dependent phenomenon and implicate dysregulated GCSFR signaling as conserved feature of P95H-dependent myeloid hematopoiesis.

SGLT2 Inhibitors Modulate the Immune Cell Metabolic Profile

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Obesity is associated with chronic systemic and adipose inflammation which significantly increases the risk of cardiometabolic diseases. Sodium-glucose cotransporter-2 (SGLT2) inhibitors reduce blood glucose and weight by causing glucosuria. Beyond these effects, SGLT2 inhibitors also reduce the risks of heart, kidney, and liver disease through unknown mechanisms. SGLT2 inhibitors reduce inflammation in animals, but their effects on inflammation in humans are less understood. We hypothesize that SGLT2 inhibitors improve inflammation in humans by improving the immune metabolic profile, which is closely linked to immune cell activation and inflammation.

In this pilot study, sixteen women with obesity and pre-diabetes were treated with the SGLT2 inhibitor empagliflozin (25 mg/day). Peripheral blood mononuclear cells were collected at baseline and after 12-weeks of treatment. We used the SCENITH protocol (Single Cell ENergetic metabolism by profilIng Translation inHibition) to measure cellular metabolism before and after treatment *ex vivo*. Within-individual change from baseline was tested using Wilcoxon signed rank.

Baseline characteristics include age 55±11.5 years, 68.8% white, 12.5% black, weight 97.2±19.3 kg, and BMI 36.9±5.8 kg/m2. The change in weight was +0.6±2.7 kg at 12 weeks. We tested immune cell dependence on glucose using the glycolysis inhibitor 2-deoxyglucose. Glucose dependence increased in two T cell subsets: naive CD8+ T cells (+7.1±13.3%, p=0.02) and CD4+ central memory cells (+4.6±9.8%, p=0.02). By contrast, glucose dependence decreased in cytotoxic natural killer (NK) cells (-9.6±14.6%, p=0.03).

The SGLT2 inhibitor empagliflozin differentially modulates the metabolism of distinct immune cell populations in individuals with obesity and pre-diabetes. Empagliflozin reduces cytotoxic NK cell dependence on glucose as an energy source, which makes these cells more reliant on fatty acid and amino acid oxidation. This immune metabolic profile is associated with a resting/non-activated state and may be anti-inflammatory. Empagliflozin alters glucose dependence in an opposite manner in two T cell subsets, with an increase in glucose reliance and decrease in fatty acid and amino acid oxidation. We propose that SGLT2 inhibitors may promote a less inflammatory metabolic immune phenotype in certain subsets such as cytotoxic NK cells, while facilitating other immune responses (e.g. to vaccines or infections) in naive and memory T cells by increasing glucose dependence. This hypothesis will need to be tested in future studies. Further investigation of immune metabolic effects of SGLT2 inhibition in humans is ongoing in a randomized, controlled trial.

Skin resident macrophage-derived microRNA21 drives susceptibility to Methicillin-resistant *Staphylococcus aureus* infection.

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Staphylococcus aureus skin infections involve a complex interplay between resident and recruited immune cells, leading to abscess formation and bacterial clearance MicroRNA21 (miR-21) emerges as a crucial modulator, influencing macrophage polarization and the expression of pro- or anti-inflammatory mediators in vitro. Whether miR21 is an important component of skin host defense remains to be determined. Our hypothesis posits miR-21 as a central node connecting the regulation of the inflammatory response to antimicrobial effector functions in the infected skin. Our data show a significant increase in miR-21 levels, peaking at 24 hours post-infection. Depletion of skin resident macrophages, but not neutrophils decreased miR-21 expression during skin infection. MRSA infection in miR21Δmyel mice results in decreased lesion size and lower bacterial burden than in WT animals. Skin analysis in miR-21-deficient mice reveals increased IL-1ß and TNF-α, coupled with decreased IL-6 and IL-1α. Furthermore, one day after infection, pro-resolution genes (CD36 and collagen3a1) were enhanced. The balance between pro- and anti-inflammatory environment contributes to forming a highly organized abscess enriched with macrophages in the periphery of the abscess, leading to a thicker abscess capsule in miR21Δmyel than in infected WT mice. Topical treatment with a MiR-21 antagomir (antagomir-21) reduces lesion size and bacterial burden. In silico analysis, indicate that miR21 could target the TIR-adaptor MyD88. Indeed, 3'UTR analysis showed that miR21 mimic indeed decreases MyD88 expression. Blocking MyD88 with a blocking peptide prevents antagomir-21-mediated bacterial clearance in vivo. These findings show that blocking miR-21 actions leads to a well-controlled inflammatory response essential for effective skin bacterial host defense.

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The early-life microbiota regulates the differentiation and function of the intestinal epithelium

Catherine D. Shelton, Julia Lane, Camila Brito, Mariana X. Byndloss

Early-life antibiotic treatment is associated with an increased risk for several non-communicable disease, such as inflammatory bowel disease, asthma, and obesity. Although early-life antibiotic treatment is hypothesized to promote disease by perturbing the developing microbiota, the mechanism by which disease occurs remains largely unknown. We hypothesized that early-life disruption to the gut microbiota alters the differentiation and development of the intestinal epithelium. To investigate this hypothesis, we developed a mouse model in which mice were exposed to low doses of penicillin (LDP) from birth until weaning (21 days in total). At weaning, mice exposed to LDP weighed more and had more abdominal fat than mock-treated control pups. 16S rRNA sequencing of the microbiota revealed that LDP depleted segmented filamentous bacteria (SFB) from the small intestine. In addition to changes in the small intestine microbiota, RNA-sequencing of intestinal epithelial cells (IECs) in LDP-treated mice revealed a significant enrichment in the retinol metabolism pathway. Importantly, retinol metabolism plays an important role in the differentiation of the intestine and promotes the development of enterocytes, cells responsible for the uptake of nutrients from the intestinal lumen. Importantly, mice treated with LDP during the first three weeks of life and then switched onto a HF diet continued to gain more weight and accumulate more body fat than mock-treated controls, despite no longer receiving LDP. Together, these data suggest that perturbation to the small intestine microbiota alters retinol metabolism in IECs, increasing the abundance of nutrient-absorbing enterocytes. Significantly, this work provides new insight into mechanisms by which early-life antibiotics can promote disease by disrupting the differentiation of the intestine.

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Key roles of CagA and the Cag Type IV Secretion System in the gastric response to *Helicobacter pylori* infection

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H. pylori colonizes the stomach of about half of all humans, and its presence is the primary risk factor for development of gastric cancer. *H. pylori* can trigger a cascade of histologic alterations preceding cancer, but the corresponding molecular alterations are incompletely understood. We used a Mongolian gerbil model to evaluate the contributions of the Cag type IV secretion system (T4SS) and CagA (a bacterial oncoprotein) on *H. pylori*-induced gastric molecular alterations. Animals were infected with a Cag T4SS-positive wild-type (WT) *H. pylori* strain, one of two Cag T4SS mutant strains, or a cagA mutant for 12 weeks; all strains colonized the gerbil stomach. Histologic staining revealed a biphasic distribution of gastric inflammation severity in WT-infected animals and minimal inflammation in mutant-infected animals. Atrophic gastritis, dysplasia, or gastric adenocarcinoma were only detected in WT-infected animals with high inflammation scores. Consistent with the histological findings, transcriptional profiling revealed that tissues from infected animals with high inflammation scores exhibited increased levels of cytokines, chemokines, and markers of immune cells (including neutrophils, B cells, and Th1/Th17 cells). Further, these stomachs expressed decreased levels of transcript markers of specialized gastric cells (parietal cells and chief cells). LC-MS/MS analysis of micro-extracted tryptic peptides revealed hundreds of proteins with altered abundance in gastric tissues from WT-infected animals with severe inflammation compared to tissues from other groups, but few differences when comparing mutant-infected animals to uninfected animals. Correspondingly, imaging mass spectrometry revealed altered gastric protein abundance in gastric tissues from WT-infected animals with severe inflammation, including diffusely increased or decreased abundance of proteins or protein localization to lymphoid follicles. These results indicate that the development of severe gastric disease and accompanying molecular alterations (which could serve as markers of early carcinogenesis) are dependent on the presence of CagA and a functional Cag T4SS.

Receptor Activator of NF-κB Ligand signaling dampens monocyte innate immune responses against intracellular bacteria

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Monocytes and their effector mechanisms, such as inflammasome-mediated interleukin-1β (IL-1β) release, are critical for the host innate immune response to intracellular pathogens. Monocytes are unique amongst leukocytes in that they can differentiate into bone-resorbing osteoclasts upon stimulation with the cytokine Receptor Activator of Nuclear Factor kappaB Ligand (RANKL). However, bone is not the only milieu where cells encounter RANKL. Recently, RANKL signaling has been shown to be vital for homeostatic processes from secondary lymphoid organ development to mammary tumorigenesis. Although much is known about RANKL signaling during osteoclast differentiation, it is not known how this broadly expressed cytokine reprograms monocyte lineage cells outside of bone. Based on observations from the osteoimmunology field, we hypothesize that RANKL signaling inhibits key monocyte immune effector genes and renders cells more susceptible to intracellular infection. To study host-pathogen interactions, we use the bacteria *Salmonella enterica* serovar Typhimurium (STm), a model intracellular pathogen and clinically important microbe whose pathogenesis overlaps significantly with RANKL-mediated physiology. As the first line of defense against invading pathogens, elucidating how monocyte responses intersect with this homeostatic signaling axis is vital for understanding infection outcomes.

Using a combination of *in vitro* and *in vivo* approaches, we found that RANKL treatment resulted in a 50-to-100-fold increase in bacterial burdens during STm infections of both murine and human monocytes and macrophages. Paradoxically, RANKL-treated cells were significantly blunted in their ability to produce IL-1βduring live STm infection and exposure to PAMPs. RANKL-treated cells were found to have markedly diminished baseline pro-IL-1β levels. We postulate that RANKL downregulates the IL-1 signaling pathway as a protective response to inflammatory processes conventionally associated with RANKL signaling during osteoclast formation, which has deleterious effects on monocyte antibacterial functions. Future work will focus on the mechanism of IL-1β regulation and host intracellular cues that may increase STm virulence.

ADP-heptose-driven activation mediates STING suppression by *Helicobacter pylori*

Isabella Sirit, Judith Romero-Gallo, M. Blanca Piazuelo, Richard M. Peek, Jr

Helicobacter pylori infection and the gastric inflammatory response are the strongest known risk factors for gastric cancer. The cytotoxin-associated gene (cag) pathogenicity island, encodes a type IV secretion system (T4SS) and translocates the oncoprotein CagA, microbial DNA, and LPS precursors, such as ADP-heptose, into host epithelial cells. Microbial DNA classically activates the intracellular DNA sensor stimulator of interferon genes (STING); however, we previously demonstrated significant suppression of STING following *H. pylori* infection. RNA-seq data from murine gastric tissue revealed *H. pylori*-dependent upregulation of STING inhibitor, TRIM30a. Human studies identified additional TRIM proteins that were significantly increased in gastric premalignant lesions, including TRIM 47 and TRIM 15. ADP-heptose, triggers various signaling pathways, including *NF-κB* following translocation into host cells. Since TRIM proteins are induced by *NF-κB*, our aim was to define the role that *H. pylori* ADP-heptose exerts on induction of STING inhibiting TRIM proteins. In AGS cells transfected with an *NF-κB* specific reporter, expression of E3 ubiquitin ligases, TRIM 15, and TRIM 5 were significantly increased following infection with the cag+ *H. pylori* strain PMSS1 in tandem with increased *NF-κB* activation. Further, use of a specific *NF-κB* inhibitor, BAY-11-7082, decreased expression of these targets, implicating *NF-κB* in the production of potential STING inhibitors in response to *H. pylori*. We then utilized ADP-heptose over-expressing mutant strains generated in the wild-type (WT) *H. pylori* cag+ strain G27 that lack key components of ADP-heptose production, including ADP-L-*glycero*-*β*-D-manno-heptose (*hldD*), and D-*glycero*-β-D-*manno* 1-phosphate (*gmhB*). In AGS *NF-κB* reporter cells, *NF-κB* activity was significantly increased in cells infected with ADP-heptose mutants versus uninfected cells. Compared to WT G27 *H. pylori*, *hldD*- mutant strains increased expression of TRIM 47. These data suggest that deficiency of *hldD* leads to an overproduction of *NF-κB* downstream effectors, including TRIM 47, an E3 ubiquitin ligase that functions to suppress STING.

Investigating B cells reactive against Sjogren's syndrome-associated autoantigens in patients who develop autoimmune sicca following immune checkpoint inhibitor therapy for cancer

Alaina Skellett, Tommi Taylor, Doug Johnson, and Rachel Bonami

Cancer patients undergoing immune checkpoint inhibitor (ICI) therapy often develop unwanted autoimmunity, termed immune-related adverse events (irAEs). One such irAE that can develop, termed ICI-sicca, mimics the rheumatologic disease, Sjögren's syndrome, and is associated with inappropriate immune infiltration of salivary gland causing dry mouth which can be irreversible. An estimated 10-25% of ICI-sicca patients are seropositive for Sjogren's syndrome-associated autoantibodies, implicating autoreactive B cell breach of immune tolerance mechanisms in this disease. It is currently unknown how such B cell autoimmunity evolves in ICI-sicca patients. To address this knowledge gap, we deployed high-throughput screening and advanced human hybridoma technology to identify and characterize autoantigen-specific B lymphocytes reactive against the Sjogren's syndrome autoantigens, Ro52, Ro60, and La in the peripheral blood of ICI-sicca patients. We found that 2/12, 2/12, 3/12 ICI-sicca patients were seropositive for Ro52, Ro60, and La, respectively. Peripheral blood mononuclear cells (PBMCs) from n=2 patients were stimulated to drive antibody secretion and wells containing autoantigen-specific B cells for the indicated autoantigens were identified as follows: 11/192 (5.73%) Ro52, 8/192 (4.17%) Ro60, and 13/192 (6.77%) La. Positive wells were chosen and fused with a myeloma line to generate hybridoma lines. We generated n=34 candidate hybridoma lines, and studies are underway to isolate monoclonal hybridoma lines with the desired autoantigen reactivity and identify individual B cell receptor (BCR) sequences. Future studies will determine BCR attributes such as V(D)J gene usage and % somatic hypermutation to determine how autoantigen-specific B cells evolved in ICI-sicca. Our ultimate goal is to identify how such autoimmunity develops, to highlight new therapeutic strategies that could be deployed to limit such autoimmunity in the future.

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The development of a multimodal molecular imaging workflow for the study of intestinal mucus

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The gastrointestinal tract (GI) contains heterogeneous cell populations such as mucin-secreting goblet cells that creates a thick hydrogel like structure. This mucus barrier is the first line of defense against infection and interacts heavily with the residing microbial community by providing nutrients or attachment sites for bacteria. The ability to discover localized molecular alterations in critical tissue microenvironments including the mucus lining the GI tract can aid in our understanding of how damage to this region contributions to inflammatory bowel disease or colorectal cancer. Imaging mass spectrometry (IMS) offers an untargeted approach to metabolite, lipid, glycan, and protein identification and mapping in tissue. High spatial resolution IMS data can be co-registered with histological images to determine the molecular profiles of specific tissue features and cell types. In this study, we developed a matrix-assisted laser desorption ionization (MALDI) IMS workflow for the analysis of zebrafish and mouse intestine, two commonly used vertebrate model organisms. Sample processing and preparation were optimized to enable high spatial resolution IMS at 10 µm. Unsupervised machine learning was performed, revealing multivariate lipid profiles of sub-mucosal, mucosal, and luminal regions within the GI tract based on k-means clustering. Utilizing the genetic tractability of zebrafish, Claudin 15-like a (cldn15la), an intestinal-specific membrane protein, was fluorescently tagged. The microscopy images from the transgenic zebrafish intestine were used to validate the molecular localization data generated from IMS experiments. Post-IMS H&E stain and alcian blue staining for mucus was also collected to confirm IMS regions delineated using k-means clustering in both zebrafish and mouse. Smaller lipids, such as sterols or cholesterol classes, were found to localize to the secreted mucus, while the mucosal and submucosal regions exhibited a higher abundance of glycerophospholipids. The most abundant lipid found in the mucus was the bile salt 5α-cyprinol sulfate in zebrafish and taurocholate in mice, making this workflow a suitable model for studying bile salt metabolism. The methods developed here can be used to study shifts in the molecular composition along the GI tract during infection and inflammation in two different vertebrate model systems.

Vanderbilt University Cell Imaging and Shared Resource (CISR)

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The Vanderbilt Cell Imaging Shared Resource (CISR) is an institutional, fee-for-service, advanced microscopy resource. The CISR provides researchers with access to state-of-the-art imaging equipment and expert technical support for sophisticated microscopy and analysis of tissue and cellular anatomy and physiology. As of 2024, the CISR independently manages 19 advanced optical microscopes, one TEM, and one SEM. These instruments and the array of advanced capabilities offered by them are available to support any investigator with an appointment at Vanderbilt University or Vanderbilt University Medical Center. The CISR supports over 200 labs per year (over 400 individual users) for 12,000+ microscope hours per year and 100+ publications associated with these microscope hours. The CISR continuously strives to add new microscope technologies to support our wide variety of biomedical researchers. In the past year, we have added a new Nikon Spinning Disk Confocal with SoRa super-resolution, a new JEOL 2100+ TEM, a new Zeiss Crossbeam 550 Focused-Ion-Beam Scanning Electron Microscope for volume EM, and a new Nikon AX R MP confocal with multi-photon for intravital imaging (NIH S10, PI: Jim Cassat). The multi-photon microscope is built for fast high-resolution imaging at hundreds of microns deep within a sample as well as label free imaging via second harmonic generation. Ideal samples for this new two-photon system include tissue explants, organoids, and infectious tissue in live animals. For VI4 members, CISR staff offer microscope and image analysis training as well as support for optimizing imaging, image analysis, and sample preparation protocols such as tissue clearing for lightsheet and freeze substitution for EM.

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binduces innate immune function of intestinal epithelial cells to drive Crohn's-like colonic inflammation

Paige N. Vega, Jiawei Wang, Alan J. Simmons, Yanwen Xu, Yilin Yang, Nicholas O. Markham, Julia Drewes, Erin P. Smith, Luisella Spiga, Monica Brown, James Ro, William Kim, Amrita Banerjee, Mariah H. Harned, Joseph Roland, M. Kay Washington, Raphael H. Valdivia, Wenhan Zhu, Qi Liu, Lori A. Coburn, Keith T. Wilson, & Ken S. Lau

The majority of Crohn's disease patients present with ileal or colonic inflammation, however, the factors that drive the development of inflammation in different sites of the gastrointestinal tract remain elusive. To-date, the TnfΔARE model is published as a spontaneous intestinal inflammation model that is specific to the ileum, however, we observe severe colonic inflammation that is dependent on the murine housing facility. We used co-housing methods to expose non-colitic, "restrictive" facility TnfΔARE mice to those with colitis in the "permissive" facility and found these mice developed colonic inflammation, suggesting colitis is transmissible through the microbiota. Shotgun metagenomics was performed to assess intestinal microbiota differences between the two housing facilities. *Chlamydia muridarum* was unique to permissive facility mice and using immunofluorescence imaging, we found infection was localized to the colonic epithelium. We administered doxycycline to treat *C. muridarum* infection and found infection clearance was associated with a reduction in colonic inflammation. Moreover, restrictive facility TnfΔARE mice treated with *C. muridarum* isolate developed colonic inflammation, demonstrating this single microbe is sufficient to drive inflammation in the colon. We used single-cell RNA-sequencing to examine epithelial cells in the context of *C. muridarum* infection and identified upregulation of indoleamine 2,3-dioxygenase (IDO1), an immune regulator, in intestinal epithelial goblet cells. Doxycycline-treated mice downregulated IDO1 expression and perturbation of the IDO1 pathway resulted in a reduction of colonic inflammation in TnfΔARE mice. Analysis of Crohn's disease patient samples revealed a specific subpopulation of IDO1-expressing epithelial cells in the ascending colon and the presence of intracellular and epithelia-associated microbes. Here, we provide the field with the first model of Crohn's-like inflammation that is specific to the ascending colon and demonstrate the capacity of a specific microbe, *C. muridarum*, to drive Crohn's-like colonic inflammation by inducing the expression of IDO1 in intestinal epithelial goblet cells.

Signaling pathways depend on negative feedback loops (NFLs) to regulate internal noise.

Danial Asgari, Ann Thomas Tate

Across diverse organisms, signaling pathways are regulated by NFLs that function at different cellular locations. These range from NFLs functioning upstream near signal-receiving receptors to those downstream within the nucleus. Multi-level regulation of signaling pathways by NFLs is ubiquitous; however, we do not know how it influences noise regulation and ultimately host fitness. Here, we quantify noise in the expression of antimicrobial peptides (AMPs) upon induction of immune signaling using stochastic models. We hypothesize that noise regulation in the expression of immune genes is crucial for mounting nuanced responses to diverse environmental challenges. By altering the strength of NFLs that function at different cellular locations, we measured the effect of noise on fitness across various environmental conditions. We discovered that upstream NFLs reduce noise whereas downstream NFLs increase noise in the expression of AMPs. The noisy expression of AMPs by downstream NFLs increases host fitness during repeated exposure to pathogens. Conversely, upstream NFLs reduce fitness variation across genotypes possibly giving rise to bet-hedging. This study shows the significance of multi-level regulation by NFLs and contributes to our understanding of noise regulation in diverse signaling pathways.

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Tissue Factor Maintains Lung Barrier Integrity in Acute Lung Injury Through Increased β1 integrin-mediated Epithelial Cell Adhesion

Brandon Baer, Nathan D. Putz, Jason Lin, Lorraine B. Ware, & Julie Bastarache

Acute Respiratory Distress Syndrome (ARDS) is a common cause of respiratory failure. While loss of lung epithelial barrier integrity is a pathologic mechanism of ARDS, little is known about the factors that regulate epithelial barrier integrity in the lung. Our previous publication showed that loss of alveolar epithelial (ATII) tissue factor (TF) increased lung edema following LPS administration. As an integral membrane protein, TF both initiates extrinsic coagulation and serves several non-coagulant functions including cell adhesion through interactions with cell surface β1 integrin. As such, we hypothesize that epithelial TF is necessary for maintaining lung barrier integrity and that its overexpression is protective in ALI through increased cell adhesion. Transgenic mice with inducible ATII-specific TF overexpression (TFEpi+) and their wild-type littermates were intranasally instilled with Klebsiella pneumoniae or PBS. After 24 hours, mice were euthanized, and we collected bronchoalveolar lavage (BAL) and lung tissue. Lung barrier dysfunction was measured by lung wet-to-dry weight ratio and BAL protein. Lung inflammation and bacterial burden were assessed by BAL cell counts and lung bacterial counts. TF-knockout and -overexpressing A549 cells were created using CRISPR and cultured on β1 integrin-specific ligands. On-cell western blots and DAPI staining were used to assess cell surface β1 integrin expression and cell adhesion. TFEpi+ mice infected with KP had lower BAL protein and lung wet-to-dry weight ratios compared to WT. Bacterial burden and BAL inflammatory cell counts were higher in infected WT mice compared to PBS. These outcomes did not differ between infected TFEpi+ and WT mice. Loss of TF in A549 cells reduced cell surface β1 integrin expression and cell adhesion to all β1 integrin ligands tested, while TF overexpression increased cell surface β1 integrin and cell adhesion. Alveolar epithelial TF overexpression is protective for maintaining lung barrier integrity, potentially through increased β1 integrin-mediated epithelial cell adhesion.

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The complex role of somatic hypermutation in supporting insulin recognition by B lymphocytes in at-risk type 1 diabetes participants

Lindsay E. Bass, Tommi C. Taylor, Mason V. Forchetti, Steven R. Scaglione, Alaina Skellett, Anika Mahajan, Daniel J. Moore, Scott A. Smith, and Rachel H. Bonami

Insulin autoantibodies predict type 1 diabetes (T1D), yet the molecular basis for B cell receptor (BCR) recognition of insulin in humans has not been well-studied. We used single-cell RNA-seq/BCR-seq/CITE-seq and advanced hybridoma technology to identify insulin-binding B cells from the peripheral blood of n = 18 TrialNet Pathway to Prevention study participants who were positive for ≥ 2 islet autoantibodies and thus at high risk for diabetes. These participants were categorized as Stage 1 or Stage 2 based on normal or impaired glucose tolerance test results at the time of blood draw, respectively. We identified clonally expanded B cells in BCR-seq data based on n ≥ 3 BCRs per clonotype. Clonally expanded B cells expressed an autoreactive-prone CD21lo CD27+ phenotype. Recombinant expression of these clonally expanded BCRs revealed that ~50% recognized insulin. Sequence analysis of insulin-binding BCRs revealed they ranged from 0-11% somatic hypermutation. For one BCR, germline reversion of the single amino acid mutation eliminated insulin recognition, whereas in another, germline reversion of all 22 mutations resulted in only modest diminution of insulin binding. These data highlight clonally expanded, CD21lo memory B cells as a reservoir for insulin-binding B cells. Further, we show extensive somatic hypermutation of BCRs may not always substantially enhance insulin autoantigen recognition, and rather may simply be a byproduct of a chronic autoimmune response.

UPEC outcompetes commensal *E. coli* in the gut, only following a UTI.

Tomas Bermudez, Seth Reasoner, Tamara Machado, Mia Cecala, Michael DaCosta, Mariana Byndloss, Maria Hadjifrangiskou

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide, 25% of which result in recurrent infections appearing after the initial treatment of UTI. Uropathogenic *E. Coli*, the primary cause of UTIs, stably colonizes the gut after elimination from the host urinary tract through treatment. This stable UPEC reservoir in the host gut has been shown to seed recurrent infection in humans who suffer recurrent or chronic UTI. Utilizing a murine intestinal colonization model, I observed that UPEC that is orally gavaged encounters significant colonization resistance by resident *E. Coli*. However, the same strain stably colonizes the murine gut, outcompeting the resident *E. Coli*, if transurethrally inoculated. I hypothesize that mice chronically infected with UPEC overcome their colonization resistance phenotype in the gut due to consistent and repetitive seeding from the UPEC populations in their bladders. Similarly, perhaps the habitation of the host urinary tract, in which UPEC causes active infection, pre-conditions UPEC to be more competitive in context of the gut following transit from the host urinary tract. These observations provide a better understanding of UPEC infection dynamics and may demonstrate comparable trends to human patients that suffer from recurrent UTI. Future studies will compare the ability of UPEC to colonize the host gut following UTI in mice pre-colonized with either commensal or alternatively tagged UPEC strains. This will help determine if UPEC holds a competitive edge over non-pathogenic *E. Coli*, or if any UPEC population with an active urinary tract reservoir can overcome the competitive environment of the gut. Furthermore, we can evaluate how a common stressor, like a regimen of antibiotics, can prime an otherwise colonization-resistant host for UPEC gut colonization. This would also allow for assessment of UPEC gut to urinary tract transit events that our lab has previously observed in Enterobacteriaceae-free mice.

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Examining Transcriptional and Immune Repertoire B Cell Changes After Immune Checkpoint Therapy in Melanoma Cancer Patients

Hannah Bhattacharya, Tommi C. Taylor, Doug B. Johnson, and Rachel H. Bonami

Melanoma cancer patients often receive immune checkpoint inhibitor (ICI) therapy. Although this therapy saves lives, it can result in the onset of autoimmunity, termed immune related adverse events (irAEs), some of which cause lasting tissue damage. One such irAE (autoimmune sicca) resembles the rheumatic disease, Sjogren's syndrome, which causes immune-mediated damage of salivary glands. One of the diagnostic criteria for Sjogren's syndrome is autoantibodies, which are produced by B cells, a type of immune cell. Sjogren's-associated autoantibodies are also identified in a fraction of patients with ICI-sicca, signifying autoreactive B cell activity in this disease process. However, a gap lies in the knowledge regarding what B cell changes are associated with the development of this disease. To address this, we used single-cell technology to evaluate transcriptional and immune repertoire changes in B cells before and after ICI therapy in n=6 patients who developed ICI-sicca. Serum ELISA data confirmed the presence of Sjogren's-associated autoantibodies in a fraction of donors. Single-cell data were processed with CellRanger and analyzed with a Seurat-based pipeline that integrates IMGT/HighV-Quest outputs. High-quality cells were recovered post filtering that included both RNASeq and BCRSeq data. Continued analysis aims to identify phenotypic and immune repertoire attributes of B cells that change following ICI-sicca development. Our ultimate goal is to offer insight into predicting who might be at risk of developing this irAE in the future.

Type IV Secretion System-Mediated Delivery of a Bacterial Oncoprotein into Gastric Epithelial Cells

Kaeli N. Bryant, Mark S. McClain, Timothy L. Cover

Helicobacter pylori is the strongest risk factor for the development of gastric cancer. Pathogenesis is largely determined by the presence of CagA, designated as a bacterial oncoprotein, which is secreted into gastric cells by the *H. pylori* Cag type IV secretion system (T4SS). Previous studies suggested that CagA is secreted in an unfolded state. We hypothesized that GFP-CagA interacts with the Cag T4SS, inhibiting secretion of other effector molecules and allowing for probing of the process of CagA secretion. To test this hypothesis, we constructed an *H. pylori* strain producing a GFP-CagA fusion protein. The strain exhibited fluorescence, and Western blotting confirmed production of the GFP-CagA fusion. When this strain was co-cultured with gastric epithelial cells, GFP-CagA was not delivered into host cells. To determine if GFP-CagA was recruited to the Cag T4SS apparatus, we co-expressed GFP-CagA with HA-CagF, a bait protein used to isolate the Cag T4SS outer membrane core complex (OMCC). Immunopurification with an anti-HA antibody yielded GFP-CagA and large ~40 nm complexes visible by negative stain-electron microscopy, similar to OMCCs isolated from a wild-type strain, suggesting that the GFP-CagA protein retained its capacity for interactions with CagF and components of the OMCC. In addition to its role in CagA secretion, the Cag T4SS also mediates delivery of non-protein substrates into host cells. For example, Cag T4SS-mediated delivery of ADP-heptose (an LPS metabolite) into host cells results in IL-8 secretion. Interestingly, we found that the GFP-CagA-expressing *H. pylori* strain was defective in stimulating IL-8 production by gastric epithelial cells. Collectively, these results suggest that the GFP-CagA fusion protein is recruited to the T4SS apparatus, fails to undergo unfolding required for secretion, and blocks T4SS-mediated delivery of non-protein substrates into host cells. We hope to gain a better understanding of where and how CagA interacts with the Cag T4SS.

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Helicobacter pylori VacA alters cholesterol biosynthesis in human gastric epithelial cells.

Georgia Caso, Owen Burroughs, Mark McClain, Timothy Cover

Helicobacter pylori employs strain-specific virulence factors to establish long-term colonization in the human stomach. Among these virulence factors is vacuolating cytotoxin A (VacA), a pore-forming toxin that forms membrane channels and induces swelling of endosomal compartments (vacuolation). To further define the effects of VacA on host cells, we treated AGS human gastric epithelial cells with VacA or control buffer for varying time intervals and analyzed the transcriptional profiles of the cells by RNA-seq. We detected numerous genes that were differentially expressed in VacA-treated versus buffer-treated cells (573 and 5209 differentially expressed genes at 2- and 8-hour timepoints, respectively). Gene set enrichment analysis revealed cholesterol biosynthesis to be the most significantly enriched pathway in VacA-treated cells ($p < 0.005$); over a dozen genes in this pathway were upregulated in response to 8-hour VacA treatment. We further analyzed the expression of six genes in the cholesterol biosynthesis pathway via RT-qPCR and confirmed that they were all upregulated in response to treatment with wild-type VacA. In contrast, two VacA mutant proteins with defects in membrane channel formation did not stimulate increased expression of these genes. Lastly, biochemical assays showed that wild-type VacA increases cholesterol levels in AGS cells. We speculate that the observed increase in expression of cholesterol biosynthesis genes may be part of a mechanism by which cells repair membrane damage caused by VacA. *H. pylori* is a cholesterol auxotroph and can acquire cholesterol directly from host cells. Therefore, we speculate that VacA-induced upregulation of cholesterol biosynthesis might provide an additional pool of cholesterol for *H. pylori* to exploit for survival and growth. Further characterization of VacA-induced changes in cholesterol biosynthesis will lead to a better understanding of cellular responses to VacA and may advance our understanding of a potential tug-of-war for cholesterol between *H. pylori* and the host.

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An *Acinetobacter baumannii* zinc binding COG0523 protein interacts with MurD and is involved in cell wall biogenesis.

Jeanette Critchlow, Joey Rocchio, Melanie McKell, David Giedroc, Eric Skaar

Acinetobacter baumannii is an opportunistic human pathogen and a leading cause of ventilator-associated pneumonia. Multi-drug resistant *A. baumannii* infections have become increasingly prevalent, emphasizing the requirement for novel therapeutic development. To colonize and survive in the host during infection, *A. baumannii* must acquire nutrient metals like zinc (Zn). To cope with periods of Zn starvation, pathogens like *A. baumannii* upregulate the expression of COG0523 metallochaperones which are predicted to transfer Zn to cognate metalloprotein clients. Loss of the *A. baumannii* COG0523 protein, ZigA, results in a severe fitness defect during Zn deficiency, indicating the essentiality of COG0523 proteins under these conditions. To further assess the influence of Zn deficiency on the fitness of wildtype (WT) *A. baumannii*, we performed a transposon sequencing (Tn-seq) screen and identified migC (MurD-interacting GTPase COG0523) as having significantly reduced fitness in Zn-depleted media. To determine the cognate metalloprotein client of the predicted COG0523 protein, MigC, we performed a yeast-two-hybrid screen using MigC as bait and identified MurD, an essential muramyl ligase, as an interacting protein. We have demonstrated that MigC functions as a Zn-binding COG0523 GTPase and strains lacking migC (*ΔmigC*) are sensitized to Zn deficiency. Additionally, *ΔmigC* is less virulent in a murine pneumonia model of infection in mice fed a low Zn diet, further indicating the importance of MigC under these conditions. As MurD is an essential enzyme in de novo cell wall biogenesis, we hypothesized that MigC transfers Zn to Zn-requiring cell wall enzymes during periods of Zn perturbation. To test this hypothesis, we performed transmission electron microscopy and found that *ΔmigC* cells have a wider diameter and cell wall than WT cells. Further, murD overexpressed in both WT and *ΔmigC* backgrounds alleviates the reduced fitness during Zn deficiency indicating that the Zn deficient fitness burden may be due to MigC-MurD interactions. Indeed, the importance of this interaction is further demonstrated in the sensitivity of *ΔmigC* to ceftriaxone, a beta lactam antibiotic, supporting aberrant cell wall biogenesis in *ΔmigC* cells. Ongoing work focuses on determining how MurD and MigC interact to allay Zn dysregulation.

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Recombination Pattern and Frequency Vary between Reovirus Strains but Are Independent of the RNA Polymerase Complex.

Alejandra Flores, Julia R. Diller, Kristen M. Ogden

Defective viral genomes (DVGs) retain sequences that allow their packaging into viral particles but contain deletions or rearrangements that make them nonfunctional. DVGs might prevent a virus from self-replicating, so DVGs have been considered as potential vaccines or anti-viral therapies. For RNA viruses, recombination has been proposed as a driver of DVG formation. Previous studies indicate that mammalian orthoreovirus (reovirus), a segmented double-stranded RNA virus, generates DVGs during serial passage that result from sequence-directed recombination. Two reovirus strains, T1L and T3DI, exhibited distinct DVG profiles, but mechanisms underlying these differences are unknown. We hypothesized that the distinct T1L and T3DI polymerase complexes produce dissimilar junction recombination patterns. Using reverse genetics, we exchanged the polymerase and its cofactor, between T1L and T3DI. To identify recombination junctions in packaged DVGs for T1L, T3DI, and polymerase-exchanged viruses, I utilized short-read sequencing and Click-Seq library preparation to facilitate identification of recombination junctions and avoid artifactual recombination during library preparation. Sequencing data was analyzed with ViReMA, a viral recombination mapper, to identify recombination sites, which are defined as deletions greater than 5 bp flanked both upstream and downstream by a 25-bp high-quality alignment. Collectively, these analyses revealed the most common recombination sites within DVGs and their frequency for each virus. Results suggest that the T3DI and T1L strain are different from each other, and differences are independent of their polymerase complex. Other mechanisms might favor DVG synthesis such as a packaging preference through RNA-RNA or RNA-protein interactions.

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Getting to know a urobiome member and its role as a potential uropathogen

Jamisha D. Francis, Mollie G Gidney, Seth A. Reasoner, Jonathan Schmitz, & Maria Hadjifrangiskou

The advent of next generation sequencing led to the discovery of the urinary microbiome (urobiome), opening a new world for discovery, especially as it pertains to uropathogen-urobiome interactions. One urobiome member identified in all studies to date – including from our group - is *Actinotignum schaali*i. *A. schaali*i, a Gram-positive facultative anaerobe has also been associated with negative urinary tract clinical outcomes in older males. In order to begin to elucidate the roles of *A. schaali*i as a urobiome member and as a potential uropathogen, we sought to a) determine the prevalence of *Actinotignum species* in the community; b) develop methodologies to propagate Actinotignum and, c) understand metabolic interactions between *A. schaali*i and uropathogens, like uropathogenic *Escherichia coli* (UPEC). To determine prevalence, we combined an Expanded Urine Culture (EUC) protocol – that allows the propagation of low abundance bacteria – with 16s rRNA amplicon sequencing. Our data revealed *Actinotignum spp* detected in 21/100 urine specimens. Conversely, we detected 26 *Prevotella*, 17 *Corynebacterium*, and 13 *Peptoniphilus* ASV present within our samples. This is consistent with other urobiome studies as being a core microbiota of the urinary tract. To investigate the potential role that *A. schaali*i may exhibit within the urobiome, we developed protocols that have enabled us to define growth requirements of the bacteria. We found that growth of *A. schaali*i in BHI supplemented with myristic or arachindonic acid increases growth. This suggests that fatty acids may act as a nutritional requirement for *A. schaali*i. Our preliminary data indicates that *A. schaali*i may influence the uropathogen biofilm behavior To date, no study has investigated the prevelance of *Actinotignum* in the community or its role within the urobiome. This work is significant, because it will be the first to determine growth conditions of *Actinotignum* and its potential interactions with other uropathogens.

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CaMK4 is a Novel Regulator of Myeloid Phenotype and Function in Atherosclerosis

Azuah L. Gonzalez, Matthew M. Dungan, Kristin Fuller, Danielle Michell, Jamshed Rahman, Kasey C. Vickers, Jonathan D. Brown, & Amanda C. Doran

Chronic inflammation is a major driver of atherosclerotic cardiovascular disease, and therapeutics that target inflammation reduce clinical cardiac events beyond levels seen with conventional strategies targeting cholesterol alone. Recent work has demonstrated that advanced atherosclerosis is also characterized by the failure of an active repair process termed ‘inflammation resolution’. Our review of publicly available RNAseq data revealed an increase in expression of Calcium/calmodulin-dependent protein kinase 4 (CaMK4) in myeloid cells in advanced/unstable regions of plaque within human carotid arteries as well as in atherosclerotic murine aortas. Therefore, we hypothesized that CaMK4 promotes inflammation and impairs resolution. Control and *Camk4-/-* mice were injected with AAV-8 PCSK9 virus and fed a high-fat diet for 12 weeks. Cross-sectional analysis of aortic roots showed that *Camk4-/-* mice had significantly less plaque burden than Controls. Flow cytometric analysis revealed elevated monocyte numbers in *Camk4-/-* mice compared to Controls. Further analysis demonstrated increased skewing of *Camk4-/-* monocyte populations toward a Ly6clow subset, indicating a more pro-reparative phenotype. We considered whether CaMK4 may regulate a gene signature driving monocyte conversion and found that *Camk4-/-* monocytes expressed higher levels of *Nr4a1, Cebpb*, and *Klf2*, which have been shown to promote conversion to Ly6clow monocytes. As Ly6clow monocytes give rise to pro-reparative macrophages, we examined bone marrow-derived macrophages and found *Camk4-/-* macrophages elaborated higher levels of pro-reparative cytokines in response to LPS stimulation and cleared dead cells more efficiently than Controls. These findings suggest that CaMK4 regulates myeloid phenotype in vitro and in vivo and that loss of CaMK4 promotes pro-resolving macrophage function. Therefore, targeting CaMK4 may offer a unique way to target atheroprogression.

Deleted in malignant brain tumors 1 (DMBT1) glycoprotein is lost in colonic dysplasia

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Colorectal cancer (CRC) is the third most common cancer in the United States and is responsible for more than 50,000 deaths annually. Emerging evidence strongly supports a causal role for specific pro-carcinogenic driver bacteria within the colonic microbiota. Invasive bacterial biofilms may initiate or accelerate CRC through epithelium-autonomous or inflammation-dependent mechanisms. To better understand host-microbe interactions during colonic tumorigenesis, we combined single-cell RNA-sequencing (scRNA-seq), spatial transcriptomics, and immunofluorescence to define the molecular spatial organization of colonic tissue from germ-free ApcMin/+ mice colonized with bacteria from human biofilm-associated CRC. In absorptive colonocytes, differential gene expression analysis showed the gastric metaplasia-associated glycoprotein Deleted in Malignant Brain Tumors 1 (DMBT1) is highly upregulated by C. difficile. Surprisingly, our spatial transcriptomic analysis showed DMBT1 was dramatically downregulated in dysplastic foci compared with normal-adjacent colonic crypts. We show DMBT1 protein is downregulated in 100% of dysplastic foci across 3 different mouse models of colonic tumorigenesis: C. difficile-associated tumorigenesis in ApcMin/+, azoxymethane/dextran sodium sulfate, and Lrig1CreER/+;Apcfl/+ mice (n = 57 foci from 11 mice). Immunofluorescent staining of DMBT1 is markedly downregulated compared with normal-adjacent crypts. Using scRNA-seq data and tissue microscopy in human CRC, we confirmed the same pattern of downregulated DMBT1 expression in adenoma-specific cells compared with normal crypt-top cells. We present data from a human mucosal biofilm-associated colonic tumorigenesis murine model at single-cell resolution to reveal interesting cell type-specific transitions and generalizable mechanisms of tumorigenesis. We hypothesize DMBT1 is a component of the gut epithelial response to pathogens and a critical regulator of proliferation and differentiation during post-injury restitution. We are now functionally testing how the loss of DMBT1 impacts tumorigenesis using human organoids. Ultimately, these studies aim to reveal novel biomarkers and/or targets for therapeutic intervention in CRC.

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High-Throughput Mapping of the Antibody Response to Multivalent HIV-1 Vaccination

Parker J. Jamieson, Alexandra A. Abu-Shmais, Matthew J. Vukovich, Perry T. Wasdin, Yukthi P. Suresh, Shan Lu, Spyros A. Kalams, and Ivelin S. Georgiev

A challenge for HIV-1 vaccine design is eliciting effective HIV-1 antibodies that recognize a diverse range of viral variants. HVTN124 is a first-of-its-kind 5-valent prime-boost human vaccine clinical trial aiming to elicit cross-reactive neutralizing antibodies (NAbs) against HIV-1. HVTN124 utilized two different vaccination strategy groups. Group 1 received a DNA prime at day 0, followed by protein boost at day 168. Group 2 received both the DNA prime and protein boost at day 0. Both groups elicited polyclonal cross-reactive HIV-1 antibodies in serum; however, Group 1 displayed higher IgG titers than Group 2. To date, the monoclonal antibody response has not yet been characterized for either group. Monoclonal characterization is important for HIV-1 vaccine design as it informs features of antibodies that may help define their effectiveness. These include the ability of a vaccine to elicit antibodies that recognize diverse epitopes across inter- and intra-clade HIV-1 variants, or cross-react, and neutralize against virus. I propose to perform high-throughput antigen specificity mapping of B cell receptor (BCR) sequences from PBMCs isolated from vaccinated individuals using LIBRA-seq, which uses barcoded antigens to map BCR-antigen specificities. This study will be the first to look at the B cell responses to HIV-1 multivalent vaccination at such degree. Our overall goal is to use LIBRA-seq and antibody characterization assays to evaluate the antibody responses from the multivalent HVTN124 human vaccine clinical trial. Dissecting the response from this new strategy will improve upon what is currently known for HIV-1 vaccine design by providing novel insights into antibody responses to a multivalent vaccine candidate in humans, as a potential template for further vaccine engineering and optimization.

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Using trained immunity to model MDS progression by TET2-driven inflammation

Alyssa N. L. Jarabek, Xenia Davis, Mary Oliver, Julia K. Bohannon, & Sandra Zinkel

Myelodysplastic syndrome (MDS) is an age-related bone marrow failure disorder characterized by ineffective hematopoiesis, cytopenias, and frequent infections. Approximately 30% of MDS patients progress to acute myeloid leukemia (AML), which contributes to poor prognosis and high mortality. However, the factors that drive MDS to leukemia progression have not been elucidated. Ten-eleven-translocation protein 2 (*TET2*) is a cytosine demethylase that is frequently mutated in MDS patients. Importantly, *TET2* loss leads to impaired innate immune cell function and augmented inflammation, yet persistent inflammatory states favor the expansion of *TET2* mutant cells. Our group has also shown that inactivating receptor interacting serine/threonine kinase 1 (RIPK1) ameliorates these effects. Conversely, trained innate immunity enhances innate immune responses to repeated infections while minimizing inflammation. Thus, because inflammation cultivates a microenvironment conducive for *TET2* mutant cell survival but restricts pathogenic clearance, the paradoxical nature of trained immunity presents a unique model system to understand how inflammation impacts MDS progression during *TET2* loss. Thus, we utilized a mouse model of *TET2* deletion to investigate the pathological repercussions of repeated inflammatory stimuli. We injected mice with monophosphoryl lipid A (MPLA), a toll-like receptor 4 agonist that initiates a trained immune response, followed by infection with *Pseudomonas aeruginosa*. This revealed that untrained *TET2* deficient mice exhibit impaired bacterial clearance compared to WT controls, yet MPLA training can protect against infection. However, MPLA training with subsequent P. aeruginosa infection promotes bone marrow progenitor and stem cell depletion accompanied by worsened cytopenias. We also show that MPLA followed by LPS stimulus heightens cytokine production and inflammation *in vitro* during *TET2* loss. Additionally, inactivation of RIPK1 resolves these effects. These results demonstrate that recurrent inflammatory stimuli in *TET2* deficiency promotes worsening of MDS related pathologies, suggesting that targeting inflammation may serve as a novel therapeutic strategy for preventing MDS progression.

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Salmonella enterica **serovar Typhimurium metabolizes** ***Bacteroides*****-derived propionate to modulate intestinal invasion and overcome colonization resistance**

Katerina M Jones, Woongjae Yoo, Catherine D Shelton, Nicolas G Shealy, Mariana X Byndloss

Salmonella enterica serovar Typhimurium (*S.* Tm) is an important human pathogen that causes 60,000 deaths globally. Understanding how *S.* Tm establishes infection will allow for better therapeutic development to reduce the burden accompanying *S.* Tm infection. Propionate is a short-chain fatty acid produced by intestinal microbiota that proposedly mediates colonization resistance against *S.* Tm. Previously, our lab has shown that *S.* Tm can attenuate microbiota-mediated colonization resistance by utilizing propionate as a carbon source for anaerobic respiration using inflammation-derived nitrate as the terminal electron acceptor. However, the impact of propionate catabolism in regulation of *S.* Tm invasion of intestinal epithelial cells and pathogen expansion during infection is unknown. We hypothesize that *S.* Tm can transition from the invasion to the expansion phase by utilizing Bacteroides-derived propionate for respiration in the inflamed gut to downregulate its invasion machinery. Experiments were conducted under anaerobic conditions that facilitate propionate metabolism using wildtype *S.* Tm (WT) and an isogenic mutant unable to catabolize propionate (*ΔprpC*). We found that nitrate influences *S.* Tm invasion gene expression in response to propionate and that *ΔprpC* showed reduced regulation of invasion compared to WT *S.* Tm in vitro. We also performed competition experiments in mice infected with an equal mixture of WT and *ΔprpC*. Interestingly, WT outcompeted the *ΔprpC* mutant in colon content, suggesting it survived better in the intestinal lumen. However, the mutant outcompeted in samples collected from the Peyer's patch and cecal tip, indicating that it invaded tissue more. Combined, these results demonstrate that *S.* Tm can utilize propionate metabolism to downregulate invasion and shift towards the expansion phase of infection for survival. Along with illustrating that the effects of microbial metabolites on enteric pathogens may be context dependent, our findings reveal a novel mechanism used by *S.* Tm to evade commensal microbiota and cause infection.

Role of glutaminase (GLS) in inflammatory mouse dendritic cells to regulate allergic inflammation

Kyung Eun Kim, Emilia Fallman, Denis Mogilenko

Dendritic cells are the most potent antigen-presenting cells that regulate T cell differentiation and activity, which can drive disease progression. Particularly, dendritic cells promote skin inflammation in atopic dermatitis by activating effector type 2 helper CD4 cells.

Recently, it has been shown that glutamine promotes anti-tumor effects of cytotoxic T cells by enhancing dendritic cell immune functions and antigen presentation. Glutamine is the most abundant amino acid in circulation that is converted into glutamate inside the cell by a rate-limiting enzyme glutaminase (GLS). GLS is known to balance polarization of naïve CD4 T cells into Th1 and Th17 subsets. Macrophages also rely upon glutaminolysis to enhance pro-inflammatory cytokine production. However, the mechanisms of how glutamine metabolism dictates the immune functions of dendritic cells and controls disease progression have not been clearly elucidated.

To determine the role of glutaminase in dendritic cells, we employed pharmacological inhibitor and siRNA-induced genetic knockdown of GLS in bone marrow-derived mouse dendritic cells. This led us to find that GLS activity in dendritic cells supports antigen presentation and cytokine secretion in vitro. Aligning with this, inhibiting glutaminolysis in dendritic cells increases expression of GATA3 and FOXP3 proteins in antigen-activated CD4 T cells, key regulators of Th2 and Treg development and function related to skin inflammation. Intriguingly, inhibiting GLS activity in dendritic cells was associated with reduced autophagy and decreased H3 histone acetylation. These data suggest that GLS-mediated glutaminolysis in dendritic cells switches them to a pro-immunogenic state through a metabolism-dependent epigenetic regulation and supports antigen presentation and activation of CD4 T cells, which might drive the progression and exacerbation of chronic skin allergic inflammation.

Collectively, we propose new insights into the role and mechanism of GLS in dendritic cells in the development and progression of antigen-mediated inflammatory diseases and suggest that GLS might be a pharmacological target to modulate dendritic cell function.

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Acinetobacter baumannii utilizes acetylation-mediated metabolic modulation to overcome iron starvation.

Dillon E. Kunkle & Eric P. Skaar

Respiratory infections are the deadliest transmissible diseases in the world and are the fourth leading cause of deaths globally. *A. baumannii* is a leading cause of ventilator-associated pneumonia and the fifth leading cause of antibiotic-resistant associated deaths worldwide. Several *A. baumannii* strains that are resistant to all available antibiotics have been isolated from patients, leaving little to no therapeutic options to treat these infections. Collectively, these facts have led the World Health Organization to categorize *A. baumannii* as the number one organism for which the development of new antibiotics is urgently required. To meet this challenge, we must understand the systems *A. baumannii* utilizes to colonize and persist within the host environment. Iron is an essential nutrient to nearly all life forms, including pathogenic bacteria. Vertebrates exploit this requirement by sequestering iron from invading pathogens in a process known as nutritional immunity. Our laboratory has shown that the struggle for iron at the host-pathogen interface is a critical determinate of *A. baumannii* infection severity and outcome. However, the mechanisms that *A. baumannii* employs to respond to, and overcome, host-imposed iron starvation to colonize the lung and induce pneumonia remain incompletely understood. We have identified an uncharacterized *A. baumannii* acetyltransferase, 3410, that is induced during iron limitation, enables *A. baumannii* to survive iron starvation, and is required for virulence in a mouse model of pneumonia. However, 3410 does not impact iron uptake from the environment, but instead drives adaptation of *A. baumannii* metabolism and maintenance of ATP production. Specifically, 3410 impacts *A. baumannii* fitness during iron limitation through acetylation-mediated regulation of pyrimidine metabolism. Collectively, these findings indicate that metabolic modulation via protein acetylation induced by the iron-deplete environment of the lung is a key virulence strategy that allows *A. baumannii* to overcome nutritional immunity.

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Nanobody Discovery for Enhanced Protection Against Viral Antigens of Interest

Sabina E.W. Leonard & Ivelin Georgiev

Nanobodies are single-domain antibodies, produced naturally in camelids and sharks, and have demonstrated impressive clinical potential for a variety of indications in pre-clinical and clinical studies. Compared to canonical monoclonal antibodies, nanobodies possess greater antigen affinity, higher stability, increased tissue penetration and improved hydrophilicity—making them ideal biomolecules for therapeutic development. The identification of nanobody biologics for infectious disease indications has relied largely on the development of immune libraries—which are antigen-specific and slow to produce. Linking B cell Receptor to Antigen Specificity by Sequencing (LIBRA-Seq), a technology developed in our lab, enables the rapid identification of antigen-specific B cells. Briefly, the process involves mixing B cells with a pool of oligonucleotide conjugated antigens, sorting for antigen-positive B cells by fluorescence activated cell sorting and co-encapsulation of single B-cells with bead-delivered oligos using droplet microfluidics followed by next-generation sequencing. Essentially, this links the B-cell to its cognate antigen and results in the recovery of both antigen-specificity and endogenously paired heavy and light chain BCR sequences. LIBRA-Seq enables high-throughput antibody discovery as B-cells can be screened against a large panel of antigens simultaneously, allowing the identification of thousands of B-cells with a variety of antigen specificities. I will adapt LIBRA-Seq technology for nanobody discovery against antigens of interest using PBMC's from immunized alpacas. This technological advance will provide a rapid, high-throughput alternative method for identification of antigen-specific nanobodies.

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Male pups have increased allergen and RSV-induced airway inflammation after in utero allergen exposure

Kaitlin McKernan, Jacqueline Y. Cephus, Shelby Kuehnle, Emely Henriquez Pilier, Andrew Peebles, Allison E. Norlander, R. Stokes Peebles, Jr., Dawn C. Newcomb

As children, males having a higher incidence and prevalence of asthma compared to females at a 2:1 ratio. Maternal asthma is a risk factor for childhood asthma; however, it remains unclear whether maternal asthma contributes to the sex disparity in childhood asthma. We hypothesized that, following in utero exposure to allergen, male offspring would have decreased Treg stability and function, leading to increased allergic airway inflammation. To test our hypothesis, we intranasally administered house dust mite (HDM) allergen or vehicle to pregnant Foxp3 fate mapping females ($Foxp^{3EGFP\text{-}Cre}$ $Rosa^{26YFP/YFP}Il13^{TdTomato/TdTomato}$) that were mated with $Foxp^{3EGFP\text{-}Cre}Rosa^{26YFP/YFP}$ males. At 3 weeks of age, male and female pups ($Foxp^{3EGFP\text{-}Cre}Rosa26^{YFP/YFP}Il13^{WT/TdTomato}$) were intranasally challenged with mock or RSV 01/2-20 clinical isolate (3x107 PFU/ml). Lungs were harvested from pups 7 days post infection. Mock challenged males exposed in utero to HDM had increased IL-13+ CD4 T cells, increased ex-Tregs (Foxp3 GFP-YFP+), and decreased current Tregs (Foxp3 GFP+YFP+) compared to mock challenged, in utero HDM exposed female pups or in utero PBS exposed pups. Additionally, male pups infected with RSV had increased IL-13+ CD4 T cells, increased ex-Tregs (Foxp3 GFP-YFP+), and decreased current Tregs compared to in utero HDM exposed female pups. Bronchoalveolar lavage (BAL) fluid showed increased eosinophils in male pups exposed in utero to HDM following RSV infection compared to female challenged pups. Combined, these results show sex differences in airway inflammation and Treg numbers following in utero exposure to allergen, providing a potential mechanism for the sex disparity in childhood asthma prevalence and severity.

Investigating Antibiotic Tolerance in *Staphylococcus aureus*

Jia A. Mei, Kara R. Eichelberger, Andrew Beaudoin, Brittney D. Gimza, James E. Cassat

Staphylococcus aureus (*S. aureus*) is a leading cause of persistent, life-threatening bloodstream infections, or bacteremia, causing an annual death toll of 20,000 in the US in 2017. Although treatment failure is commonly attributed to antimicrobial resistance, the phenomenon of antibiotic tolerance, or the ability of the bacteria to survive lethal concentrations of antibiotics despite in vitro susceptibility, has been appreciated as a cause of treatment failure in recent years. The genetic and phenotypic signatures of tolerant *S. aureus* remain largely uncharacterized. To investigate the bacterial factors that contribute to clinical outcomes, we performed Illumina sequencing on 52 *S. aureus* strains from pediatric patients that had prolonged or non-prolonged bacteremia (2010-2020). To link a genotype to the phenotype of prolonged bacteremia, DBGWAS (**D**e **B**ruijn graphs **G**enome-**W**ide **A**ssociation **S**tudy), a k-mer-based method of associating genetic variants with phenotypes in prokaryotes, was performed. Preliminary results suggest a correlation between the gene scrB and prolonged bacteremia (p-value 3.84E-05) and the gene fnbA and prolonged bacteremia in beta-lactam treated patients (p-value 9.96E-4). However, a larger number of isolates are needed to draw more significant conclusions. In characterizing the virulence of these isolates, supernatant cytotoxicity screening on murine macrophages revealed that these strains, despite causing severe infections, had a broad range of cytotoxicity, ranging from non-toxic to highly toxic. Notably, 81% (21/26) of the prolonged bacteremia group were nontoxic. Highly toxic strains are from diverse pulse-field gel electrophoresis type (USA300, 400, 600, 1000 1200), highlighting the presence and circulation of non-USA300 hypervirulent strains. Studies are underway to further define the tolerance phenotypes of these isolates to clinically relevant antibiotics in order to find patterns of in vitro traits that would predict tolerant outcomes in vivo. Understanding the bacterial mechanisms that drive tolerance may lead to the development of more effective therapies to combat *S. aureus* infections.

Maintenance of brain homeostasis by an ancient zinc metallochaperone

Caitlin C. Murdoch, Andy Weiss, Katherine A. Edmonds, David P. Giedroc, & Eric P. Skaar

Metals are essential micronutrients for all kingdoms of life. Zinc (Zn) serves many roles in human health and disease, functioning as a cofactor for an estimated 10% of proteins. Dysregulated Zn homeostasis underlies diseases including neurodegeneration, yet the mechanisms by which Zn promotes these pathologies remain largely unexplored. Zn delivery to target proteins is critical as intracellular concentrations of free Zn are virtually non-existent. We have discovered that specialized proteins called metallochaperones traffic intracellular Zn to client metalloproteins in vertebrates. However, the contribution of Zn metallochaperones to animal health and disease remains completely unknown. We have identified a vertebrate Zn metallochaperone, which we named Zn regulated GTPase metalloprotein activator 1 (ZNG1). We have demonstrated that vertebrate ZNG1 binds to and transfers Zn to metalloproteins to promote their enzymatic activity. Further, we have identified numerous candidate ZNG1 interacting proteins that are associated with brain development and function, including the Zn-finger transcription factors (TF), ZFHX3 and ZFHX4. To determine the in vivo role of ZNG1 proteins in brain homeostasis, we generated animal and cell culture models deficient in Zng1. Preliminary data demonstrate abnormal development of the central nervous system (CNS) in Zn starved Zng1 mutant animals and a sensitivity of cultured neurons to perturbations in Zn levels. Collectively these findings raise the possibility that ZNG1 is a regulator of signaling in the brain via interactions with specific TFs that modulate neurogenesis and neuronal homeostasis. To further elucidate the role of Zn and ZNG1 on brain development, we are coupling spatial transcriptomic analysis of the brain with in vivo imaging and molecular characterization of neurons in zebrafish, cell culture, and mouse model systems. Collectively, this work will define the role of ZNG1 in cellular metal homeostasis in the brain while dissecting how Zn dysregulation contributes to human neurodegenerative disorders.

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The *Clostridioides Difficile* Binary Toxin CDT Activates the NLRP3 Inflammasome

Kateryna Nabukhotna, Shannon L. Kordus, John A. Shupe, Anna Smith, Julia K. Bohannon, M. Kay Washington, & D. Borden Lacy

Clostridioides difficile is a spore-forming pathogen and the most common cause of healthcare-associated diarrhea and colitis in the United States. Besides producing the main virulence factors, toxin A (TcdA) and toxin B (TcdB), many of the common clinical strains encode the *C. difficile* transferase (CDT) binary toxin. The role of CDT in the context of *C. difficile* infection (CDI) is poorly understood. We show that CDT activates the inflammasome in bone marrow-derived dendritic cells (BMDCs). In bone marrow-derived macrophages (BMDMs), the cell binding and pore-forming component of the toxin, CDTb, alone activates the inflammasome and is dependent on K+ efflux. The presence of the enzymatic component, CDTa, inhibits this activation. The activation was not observed in BMDCs and BMDMs derived from Nlrp3 deficient mice suggesting the involvement of the NLRP3 inflammasome. To evaluate the physiological role of the NLRP3 inflammasome during CDI and to test if CDT contributes to *NLRP3*-dependent phenotypes in vivo, wildtype (WT) and Nlrp3-/- C57BL/6J mice were infected with R20291 and R20291 Δ*cdtB* strains of *C. difficile*. We show that at the acute timepoint of 2 days post-infection, CDT contributes to increased weight loss and cecal edema, and an increase in the total number of immune cells recruited to the site of the infection. Additional effects of CDT-mediated NLRP3 inflammasome activation in vivo, particularly further post-infection, remain to be investigated. Overall, this study indicates that CDT might manipulate the host immune responses during CDI.

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Role of Bacteroides in driving colonic hyperproliferation in response to dietary inulin in mice

Pollyana R. Castro, Nicolas G. Shealy, Renan O. Corrêa, Marco Aurélio R, Vinolo, & Mariana X. Byndloss

Dietary soluble fibers, such as inulin, are known for their ability to impact the gut bacterial community, and their consumption has usually been associated with health improvement in mice and humans. Still, in specific circumstances, inulin can promote inflammatory states. How interactions between dietary inulin and the gut microbiota determine a beneficial vs. a detrimental outcome during inulin supplementation is a critical but poorly understood question. Therefore, we proposed to investigate the influence of inulin diet on epithelium homeostasis and how it influences the composition and function of the colon microbiota. For this, mice were fed for 30 days with a diet containing 5% of the insoluble fiber cellulose or the same diet enriched with 10% of the soluble fiber inulin. Using a combination of histochemistry, host cell transcriptomics, 16S microbiome analysis, germ-free, and gnotobiotic mouse models, we analyzed the impact of inulin intake on the colonic epithelium and colon bacteria. A fiber-rich diet containing inulin altered the colon epithelium by increasing the proliferation of intestinal stem cells leading to deeper crypts and longer colons. These effects were dependent on the inulin-modified gut microbiota, as no modulations were observed in animals deprived of microbiota (GF model and antibiotics treatment). Moreover, inulin supplementation caused an expansion of commensal *Bacteroidales* spp. We also demonstrated that *B. ovatus* but not *B. thetaiotaomicron* could grow in inulin-enriched media. These data indicate that *B ovatus* and its metabolites may be involved in inulin-induced hyperproliferation in the colon of mice. Additional analyses will be carried out using gnotobiotic models, untargeted metabolomics and streamlined genetic manipulation of diverse *Bacteroides* to confirm this hypothesis.

CCR10 Promotes Blood Pressure Elevations, End-Organ Damage, and Cutaneous Infiltration of Regulatory T Cells in a Murine Model of Hypertension

Bianca Nguyen, Ziche Chen, Xue Zhong, Na Xiong, Daniel Fehrenbach, Meena Madhur, Matthew Alexander

Inflammation plays a key role in the pathogenesis of hypertension and its associated end-organ damage. While regulatory T cells (Tregs) primarily function to limit inflammation, Treg subsets can play pathogenic roles in inflammatory diseases through mechanisms such as limiting angiogenesis or promoting apoptosis of vascular smooth muscle and endothelial cells. Thus, the role of Tregs and its subsets in hypertension remain unclear. Recently, we demonstrated that CCR10+ Tregs are selectively decreased in the circulation of hypertensive patients. CCR10 is a key chemokine receptor that recruits immune cells to the skin. Although skin has not been well studied in hypertension, reports in humans demonstrate that skin microvascular rarefaction is a putative mechanism for blood pressure (BP) elevations in hypertension. Herein, we demonstrate that, compared to normotensive littermates, mice with angiotensin II (Ang II)-induced hypertension have selectively increased abundance of CCR10+ Tregs in the skin with a corresponding decrease in circulation. This increase in Treg abundance corresponded to increased cutaneous levels of the CCR10 ligand CCL27. To test the role of CCR10 in hypertension, CCR10-deficient (CCR10-/-) and CCR10+/+ control mice were infused with Ang II. Compared to CCR10+/+ mice, the CCR10-/- mice had blunted responses to Ang II, as exhibited by significant reductions in BP, albuminuria, and cardiac fibrosis. Furthermore, CCR10-/- mice had a selective decrease in Tregs in the skin without change in abundance of conventional CD4+ or CD8+ T cells. CCR10-/- mice also exhibited greater cutaneous arteriolar density after Ang II, consistent with reduced skin microvascular rarefaction. Finally, using PrediXcan, we demonstrate that higher genetically predicted levels of CCR10 are associated with increased risk of hypertension in humans. Taken together, our data suggest that CCR10 promotes Ang II-induced BP elevations and associated end-organ injury, at least in part by promoting Treg recruitment to the skin leading to microvascular rarefaction.

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Mapping the lipid landscape of microbial biofilms by MALDI IMS

Alexis P. Pope, Jeffrey A. Freiberg, Lukasz G. Migas, Madeline E. Colley, Raf Van de Plas, Eric P. Skaar, Jeffrey M. Spraggins

Staphylococcus aureus and *Pseudomonas aeruginosa* are opportunistic pathogens that generate bacterial biofilms to infect and persist in host tissue. Biofilms are adhesive bacterial communities encased in an extracellular matrix that confer host immune evasion and antibiotic tolerance. Uncovering the molecular signatures expressed within these communities may yield insight into their emergent virulent properties. To investigate this, matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS) can be used to map the molecular landscape within monocultured *S. aureus*, P. aeruginosa, and co-cultured biofilms. Herein, high spatial resolution MALDI IMS and fluorescence microscopy were used to reveal lipid and metabolite distributions within biofilm sections.
Monomicrobial biofilms yielded differences in detected lipid classes, degrees of unsaturation, and spatial localization between *S. aureus* and P. aeruginosa. Saturated lipids, such as, phosphatidylglycerols (PGs), Lysyl-PGs, and cardiolipins (CLs) were observed in *S. aureus*, whereas unsaturated PGs, phosphatidylethanolamines (PEs), and longer chained CLs were observed in P. aeruginosa. These lipid signatures exhibited distinct layering within the biofilm architecture whereby *S. aureus* biofilms exhibit three layers organized by lipid species; PGs localize to the towards the top layer whereas CLs localizes to middle and lower layers. However, *P. aeruginosa* displays two layers separated based on lipid saturation; unsaturated lipids are observed in the top layer and saturated lipid are localized to the bottom layer. These patterns were further evaluated in mixed biofilms containing both species. Dual-species biofilms displayed a consistent cellular organization where *S. aureus* occupies the bottom layer of the biofilm and *P. aeruginosa* is oriented on top. These mixed biofilms showed loss of lipid stratification and reduced lipid peak intensity in both *S. aureus* and *P. aeruginosa* regions. Ultimately, this study defined unique lipid profiles observed within single-species biofilms and how they are changed within the dual-species environment.

An *in vivo* genetic selection identifies Clostridioides difficile gene products targeted by the host immune response

Sarah L. Price, Matthew J. Munneke, Elias X. West, Martin V. Douglass, Tess P. McNeely, Emma B. Irbe, Ivelin S. Georgiev, Eric P. Skaar

Clostridioides difficile is a Gram-positive, spore-forming bacterium responsible for severe colitis and is the predominant cause of hospital-acquired infection. Despite the robust inflammatory response triggered during *C. difficile* infection (CDI), a chronic recurrent disease can develop that is challenging to eradicate with antibiotics. The prolonged timeline of chronic CDI's impact on the host adaptive immune response remains largely uncharacterized. We have established a model of protective immunity, where mice exposed to an avirulent strain of *C. difficile* show decreased inflammation and increased survival compared to naïve mice upon a secondary CDI challenge. These data suggest that the immune system recognizes *C. difficile* and mounts an adaptive response to thwart future infections. These results also support a model whereby *C. difficile* expresses antigens that are recognized by the host immune system. To identify *C. difficile* antigens targeted by the adaptive immune response, we employed a genetic approach by transposon sequencing where a transposon library was subjected to selection in mice vaccinated with *C. difficile* compared to naïve mice. Antigens will be validated by generating mutants in candidate genes and infecting vaccinated mice. Mutants that exhibit a fitness advantage in vaccinated mice will represent strains that lack an immunogenic factor that is recognized by the adaptive immune system. Further, potential antigens will be evaluated by a high throughput, next generation sequencing approach called Linking B cell Receptor to Antigen Specificity through Sequencing (LIBRA-seq), where candidate proteins will be assessed for antibody interactions. LIBRA-seq allows mapping of single-cell antibody-antigen interactions and provides sequences for recombinant antibody production against target antigens. Concurrently, these results will define the host's adaptive immune response to chronic CDI. Importantly, our research will identify immunogenic factors expressed by *C. difficile* to unveil novel vaccine antigens and mAb targets and support our understanding of recurrent infections caused by *C. difficile*.

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Candida albicans skin infection is more severe in a murine model of hyperglycemia.

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People with chronic hyperglycemia exhibit a heightened susceptibility to *Candida albicans* skin infections in comparison to euglycemic individuals. The immunological interactions and underlying pathogenic mechanisms that drive poor skin infection outcomes are poorly understood. Our study explores the link between metabolic dysfunction and the host's antifungal defense using both *in vivo* and *in vitro* infection models. To evaluate the effects of hyperglycemia on *C. albicans* infections, mice were injected with streptozotocin to induce hyperglycemia, followed by s.c. infection with *C. albicans*. Hyperglycemic mice were more susceptible to *C. albicans* skin infection than their euglycemic counterparts, as evidenced by larger lesions and higher fungal loads. Histology analysis showed larger abscesses and increased numbers of hyphae in diabetic mice. When we determined the expression of genes involved in innate immune response in the skin, we observed decreased Dectin-1 expression, while levels of other important antifungal receptors, such as the Mannose Receptor and TLR2, were elevated in our diabetic mouse model. Likewise, the expression of downstream signaling molecules of TLRs, such as MyD88 and SOCS-3, significantly increased in diabetic mice, indicating changes in innate immune signaling induced by hyperglycemia. In vitro, cultured dermal macrophage cell lines with high glucose inhibited *C. albicans* phagocytosis compared to cells kept in low glucose. These findings highlight the importance of glucose control in the outcome of *C. albicans* skin infections.

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Mechanisms of RNA Cap-Proximal Methylation During Stress

Antiana Richardson and John Karijolich

RNA modifications play important roles in regulating gene expression. Cap-proximal methyltransferase Phosphorylated CTD Interacting Factor 1 (PCIF1) catalyzes m6Am on RNAs whose first nucleotide is adenosine. PCIF1 has been implicated in RNA stability and translation, but its mechanism of action remains enigmatic. The modification, m6Am, has shown clinical significance through its association with various pathologies including diabetes, cancer, and viral infection. PCIF1-mediated m6Am deposition is best characterized as a nuclear co-transcriptional process; however, PCIF1 also localizes to the cytoplasm where its function is unknown. The overarching hypothesis is that PCIF1 localization is dynamically regulated during stress, and that cytoplasmic localization functions to ensure efficient cap-proximal m6Am formation during stress enabling translation to proceed. To determine the biological function of cytoplasmic PCIF1 I have determined its localization in a panel of primary and transformed cells. In addition, preliminary work has identified a nuclear/cytoplasmic export pathway responsible for PCIF1 cytoplasmic localization. Ongoing as well as future work will be directed at further defining the mechanism of PCIF1 nuclear/cytoplasmic transport as well as identification of its cytoplasmic substrates. Completion of this work will broaden the understanding of RNA cap-proximal methylation and its integration into cellular stress responses.

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Manipulation of host translation machinery by coronavirus nonstructural protein nsp2

Brynn Roman, Lars Plate

Nonstructural proteins (nsps) play a crucial role setting up RNA viral replication in the infected host cell. Exerting control over host translation machinery is necessary during the viral life cycle to slow endogenous host protein synthesis while promoting viral protein translation. However, the underlying mechanisms by which viral nsps regulate protein translation are not well-understood. SARS-CoV-1 nsp2 has been identified as a viral protein that interacts with host translation repressors 4EHP and GIGYF2. SARS-CoV-2 nsp2 does not strongly interact with 4EHP or GIGYF2, despite the nsp2 homologs having a sequence similarity of 79.1%. As 4EHP and GIGYF2 are translational repressors, it is important to determine the effects of the nsp2 homologs on the host proteome and host translation. To this end, we utilized tandem mass spectrometry to analyze differences in the host proteome in the presence of SARS-CoV-1 and SARS-CoV-2 nsp2. Biorthogonal noncanonical amino acid tagging (BONCAT) was implemented to investigate and compare the effects of SARS-CoV-1 and SARS-CoV-2 nsp2 on host translation rates. Significant differences in both host protein abundances and translation rates affected by the presence of each homolog were observed, indicating the SARS-CoV-1 and SARS-CoV-2 nsp2 are targeting different host proteins. Specifically, a subset of interferon-stimulated genes (ISGs) were significantly downregulated in the presence of the SARS-CoV-1 homolog compared to the SARS-CoV-2 homolog. This suggests nsp2 exhibits a strain-specific effect on ISG translation. In addition to characterizing effects of nsp2 on the host proteome, we have identified the general region of nsp2 responsible for the difference in 4EHP/GIGYF2 interaction strength using SARS-CoV-1 nsp2 truncations and SARS-CoV and SARS-CoV-2 chimeras. Upon identifying the 4EHP/GIGYF2 binding region of nsp2, nsp2 chimeras with a mutated binding region were utilized to determine effects of SARS-CoV-1 and SARS-CoV-2 nsp2 that can be attributed to the nsp2:4EHP/GIGYF2 interaction.

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Short-term dietary changes enable pathogen access to microbiota-sequestered nutrients before disease onset

Nicolas G. Shealy, Madi Baltagulov, Pollyana Castro, Camila de Brito, Tamara Machado, Scott Beiting, John McClean, Mariana X. Byndloss

Successful enteric pathogens must overcome microbiota-mediated colonization resistance to expand and cause disease. The bacterial pathogen *Salmonella enterica* sv. Typhimurium (*S.* Tm) elicits gut inflammation to gain access to nutrients, enabling *S.* Tm to outcompete the gut microbiota. However, little is known about how short-term dietary changes (e.g., protein sources) affect the nutrient pool in the gut and the host's susceptibility to *S.* Tm infection. To address this knowledge gap, we exposed CBA/J mice to one of two diets, including either whole protein casein (CA) or free L-amino acids (AA) prior to infection. AA diet-fed mice demonstrated significantly accelerated disease kinetics and severity, such as gastroenteritis onset within 3-5 days faster than CA diet-fed mice. Ileal tissue of AA diet-fed mice showed edema, epithelial damage, and immune cell influx to the submucosa, all consistent with *S.* Tm-induced gastroenteriti*S.* Interestingly, the *S.* Tm-induced ileitis phenotype observed only in AA-fed mice resembles features of *S.* Tm infection in humans and thus may allow interrogation into a poorly understood gut geography. 16S rDNA sequencing of AA diet-fed mice revealed a significant decrease in species richness and was found to be significantly different from that of CA-fed mice. Metabolomics and host RNAseq demonstrated significant alterations in glycosylated amino acids, bile acids, and cholesterol homeostasis in the terminal ileum of uninfected mice. *In vivo*, RNAseq of *S.* Tm in intestinal content from AA diet-fed mice showed increased use of fructosyl-asparagine and ascorbate, microbiota-sequestered nutrients, prior to inflammation. Taken together, this data suggests that diet-driven modifications in the metabolic landscape of the small intestine enable *S.* Tm to cause ileitis by gaining access to nutrient sources normally sequestered by the small intestine microbiota. This project will aid in understanding enteric bacterial pathogens by demonstrating how environmental factors overcome genetic resistance to infection.

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Development of a High-throughput Screening Assay for Drug Discovery Against Infection-induced Inflammation Leading to Preterm Birth

Shajila Siricilla, Rohita Kotyada, Alexus J. Brown, Madeline C. Jones, Devansh Nigam, & Jennifer L. Herington

Preterm birth (PTB) remains a significant global health problem affecting ~10% of pregnancies and is associated with 75% of infant mortality and 50% neonatal morbidities. While there are several pathologic causes of PTB, intrauterine infection and/or inflammation (chorioamnionitis) is present in 40–60% of PTB cases and is the most firmly established trigger of early preterm labor (PTL). Multiple microorganisms (Ureaplasma urealyticum, Streptococcus agalactiae, Escherichia coli, etc.) are implicated in infection-induced inflammation leading to preterm labor (III-PTL) by provoking an inflammatory reaction (mediated by IL1b, IL6, TNFa and others) in the fetal membranes (FMs). The goal of the current study was to develop a high-throughput screening assay (in 96-well format) using human FM explants stimulated with pathogen-associated molecular patterns (PAMPs) of common bacteria associated with PTL. An HTS assay was developed and optimized for the assay sensitivity (viability of FMs, time-course, concentration-response), robustness (z`-factor), and reproducibility (3 patients, 3 different days). Multiple PAMPs that are agonists of different pathogen recognition receptors such as toll-like receptor (TLR) 1/2/4 and 6, representing gram-negative, gram-positive and Mycoplasma spp. were used: lipopolysaccharide (LPS), peptidoglycan (PG), triacylated lipopeptide (PAM3CSK4), and lipoprotein (FSL-1). This represents the polymicrobial nature of III-PTL. Released proinflammatory cytokines (IL1b, IL6 and TNFa) were quantified using Luminex/Lumit technology. This assay was validated by testing inhibitors (TPCA1 and SB239063) of two targets in the inflammatory pathway, IKK and p38 MAPK, respectively. Both molecules were identified to inhibit PAMP-induced cytokine release from FM explants. Collectively, these studies demonstrate that we possess a sensitive and robust assay to measure changes in proinflammatory cytokine release from human FMs in 96-well format. Future studies will be directed towards high-throughput screening against small-molecule libraries to identify novel inhibitors of III-PTL.

Heat can STING: Defining the relationship between temperature and STING activation in regulatory and effector T cells.

Rachael C. Smith, Darren R. Heintzman, Emilie L. Fisher, Xiang Ye, Yasmine T. Toudji, Alex J. Kwiatkowski, Erin Q. Jennings, Neil Chada, Jacob A. Schulman, Kelsey C. Voss, Channing Chi, Jeff C. Rathmell, John T. Wilson

The cGAS-STING pathway plays a critical role in immune surveillance through its capacity to sense aberrantly localized DNA in the cytosol caused by infection, cancer cell death, or cell stress-induced self-DNA leakage. STING pathway activation ultimately results in the production of Type I Interferons (IFN-Is) and other inflammatory cytokines that stimulate T cell immunity. However, T cell-intrinsic effects of STING agonists have been underexplored, especially in regulatory T cells (Tregs) which are crucial for suppressing excessive inflammation but can also suppress wanted anti-tumor T cell responses. Further, inflammation can also coincide with heat (e.g. fever-range temperature, FRT, 39°C), which can cause nuclear or mitochondrial self-DNA leakage, yet the effects of FRT on STING in Tregs are unknown. Unexpectedly, we have found that STING activation repolarizes Tregs to IFN-I producing Th1/Th9-like cells with enhanced inflammatory function accompanied by decreased oxidative metabolism and suppressive capacity. We surprisingly observed differential effects of FRT in Tregs and Th1 cells, finding that Tregs have decreased suppressive capacity but are uniquely resilient to cell stress at FRT and that FRT appears to induce DNA damage and subsequent cGAS/STING activation only in Th1 cells. Upon investigation of pharmacological STING activation at FRT, we found that FRT strongly potentiates the inflammatory effects of STING activation in Tregs and Th1 cells but only Th1 cells experienced significant cell death in response to FRT-potentiated STING activation. Together, these initial data suggest a novel axis linking FRT to STING signaling in T cells and that this can be leveraged to inhibit Treg immunosuppressive function and promote Treg transdifferentiation to inflammatory effector-like T cells without inducing cell death as found in Th1 cells. These findings motivate further investigation to dissect the mechanisms intertwining STING and FRT in Tregs and suggest potential synergy between heat and Treg-targeted STING agonists for applications in immunotherapies.

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Salmonella exploits commensal iron acquisition to evade nutritional immunity

Luisella Spiga, Ryan Fansler, Yasiru R. Perera, Matthew Munneke, Katrina Richardson, Holly David, Eric Skaar, Walter Chazin, Wenhan Zhu

During *Salmonella* infection, essential nutrients such as iron decrease significantly due to host processes known as nutritional immunity. It is well established that *Salmonella* overcomes host nutritional immunity by producing high-affinity iron-chelating molecules termed siderophores. However, how commensal bacteria acquire iron to remain resilient, and how commensal iron acquisition modulates host-*Salmonella* competition for iron remain incompletely understood. Here, we demonstrate *Bacteroides thetaiotaomicron* (*B. theta*) captures siderophores from *Salmonella* by using a specialized system. By secreting lipoprotein XusB into outer membrane vesicles (OMV), *B. theta* binds enterobactin with high affinity, creating a pool of siderophores with reduced accessibility to host nutritional immunity. We further showed that *Salmonella* can “re-capture” siderophores bound to XusB to further evade nutritional immunity. This work suggests that commensal iron acquisition modifies host nutritional immunity in the context of intestinal inflammation.

A putative RNA pyrophosphohydrolase impacts zinc homeostasis in *Acinetobacter baumannii*.

Kacie A. Traina, Jeanette M. Critchlow, Erin R. Green, & Eric P. Skaar

Acinetobacter baumannii is a Gram-negative, opportunistic human pathogen and a leading cause of ventilator-associated pneumonia. Multi-drug resistant *A. baumannii* infections are becoming increasingly prevalent, reinforcing the need for new therapeutic development. To colonize and survive in the host, *A. baumannii* must acquire essential nutrient metals, including zinc (Zn). The vertebrate host actively sequesters these nutrient metals to defend against infection in a process called "nutritional immunity." *A. baumannii* employs specific mechanisms to combat Zn dysregulation. To better understand Zn stress in *A. baumannii*, a transposon-sequencing (Tn-seq) screen was performed to profile the fitness of a WT *A. baumannii* ATCC 17978 library in Zn deplete, replete, and excess environments. A mutant in A1S_*0414* (Δ*0414*) was selected against in high Zn, suggesting A1S_*0414* plays an important role in maintaining Zn homeostasis. A1S_*0414* is a predicted RNA pyrophosphohydrolase (RppH), an important enzyme in bacterial mRNA degradation. While RppHs have been preliminary studied in other bacterial species, no RppH has been discovered yet in *A. baumannii*. The role of RppH in Zn homeostasis in any bacterial species also remains unknown. Through experiments with a marked deletion strain (Δ*0414*), we confirmed sensitivity to Zn toxic conditions, as seen in the Tn-seq. Complementation experiments of Δ*0414* mirrored WT growth curves, demonstrating that A1S_*0414* is important in Zn toxic environments. In a vertebrate host, bacterial pathogens can encounter both Zn deplete and Zn toxic environments. To assess the role of A1S_*0414* in pathogenicity, a murine pneumonia model was utilized to show that infections with a Δ*0414* strain resulted in a significant decrease in bacterial burden in the lungs as compared to WT *A. baumannii*. Ongoing work focuses on determining the role of A14_*0414* in maintaining proper Zn regulation. Defining mechanisms by which *A. baumannii* maintains Zn homeostasis, possibly through A1S_*0414*, could identify new antimicrobial targets.

Aspartate family amino acid metabolism by *Staphylococcus aureus*: Implications for osteomyelitis

Logan Treat, Aimee Potter, & Jim Cassat

Staphylococcus aureus (*S.a.*) is the leading cause of infectious osteomyelitis (OM), a debilitating bone infection typically treated with extensive antibiotics and surgical debridement but can result in amputation. Outcomes remain poor despite therapeutic intensity, with treatment failure ranging from 30-40%. To establish and maintain infection, *S.a.* leverages its metabolic flexibility to compete with the host for nutrients. Our group made the curious observations that while endogenous aspartate (Asp) is essential for purine biosynthesis and survival during OM, endogenous synthesis of Asp family amino acids (lysine (Lys), methionine, threonine, and isoleucine) is dispensable, indicating that *S.a.* has alternative means of acquiring these amino acids. The goal of this project is to investigate means of *S.a.* Asp family amino acid acquisition and to determine its relevance during OM.

We first focused on Lys, which *S.a.* proficiently consumes *in vitro* and uses to modify peptidoglycan to resist antimicrobial peptides, making it a potential therapeutic target. To determine *S.a.* Lys requirements in vitro, growth curves were conducted in defined minimal media. Lack of exogenous Lys slows the growth rate of WT *S.a.*. Transposon mutagenesis revealed aspartokinase *thrD*, involved in converting Asp to Lys, was essential for growth without exogenous Lys, whereas the paralog *lysC* is dispensable. Using MIC assays with the toxic analog of Lys, thialysine, I demonstrated that thialysine effectively inhibited *S.a.* growth in the absence of exogenous Lys, however media replete with Lys increased the MIC over 1,000x, indicating that *S.a.* can preferentially import or utilize Lys over thialysine.

Taken together, these data show that endogenous Lys synthesis is *thrD*-mediated, and that acquisition of exogenous Lys by *S.a.* is specific and advantageous for the bacteria under our conditions. Future studies will identify *S.a.* Lys transporters, and we will determine metabolic requirements for *S.a.* to cause OM.

Uncovering the Cellular and Molecular Organization of Murine Kidney Abscesses

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Staphylococcus aureus (S. aureus) infections are a leading cause of death globally, with high mortality rates and frequent reoccurrence. Mouse models of S. aureus bacteremia demonstrate the formation of characteristic abscesses in tissues throughout the body after infection. These unique cellular microenvironments are created from interactions between the host immune system and bacteria. By leveraging multimodal imaging strategies, including matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS), immunofluorescence, and histological stains, we can characterize the cellular and molecular architecture of mouse kidney abscesses.

Preliminary data revealed heterogeneity in the lipid profiles both within and between abscesses. Phosphatidylinositol species, such as PI(38:4) and PI(36:2) were present primarily around the periphery of some abscesses and were uniformly distributed throughout others. Conversely, sphingomyelin, SM(d34:1), was present in the middle of abscesses, around bacterial colonies. MALDI IMS data were also used to identify lipids associated with bacteria in the tissue sections, including cardiolipins, phosphatidylglycerols, and lysyl-phosphatidylglycerols which have heterogeneous distributions in and between bacterial colonies. These are just a few examples of numerous lipids in the infected tissues identified using MALDI IMS. Immunofluorescence experiments and histology stains on serial sections have confirmed that these abscesses are primarily comprised of neutrophils. Further analysis will seek to understand how these different distributions of lipids in the abscess relate to the progression of the immune response to bacterial infection by integrating spatial transcriptomics into multimodal imaging experiments. This will allow the differentiation of neutrophil populations based on gene expression levels, such as those involved in migration to the site of infection, secretion of pro-inflammatory cytokines, and bacterial phagocytosis, to understand the organization of immune cells in the abscess.

Early-Life Antibiotic Exposure and High-Fat Diet Drive Gut Dysbiosis and Colorectal Carcinogenesis in Mice

Max Van Belkum, Tamara Machado, Nicolas Shealy, Catherine Shelton, Savanna Telaak, Madi Baltagulov, Mariana Byndloss

Early-onset colorectal cancer (EOCRC) incidence is rapidly increasing, with antibiotic use and high-fat diet (HFD) emerging as potential risk factors. These factors may contribute to colorectal carcinogenesis by altering the gut microbiota composition, particularly favoring the expansion of pro-carcinogenic bacteria. However, the mechanisms by which antibiotics and HFD synergistically influence the gut microbiome and promote EOCRC remain unclear. To investigate these effects, male C57BL/6J and C57BL/6NCrl mice were colonized with *pks+ E. coli*, which are known to produce the genotoxin colibactin linked to colorectal cancer. Mice were administered multiple clinical oral doses of penicillin, one of several narrow spectrum β-lactam antibiotics commonly prescribed to children, during early life (weeks 3-8) and fed a HFD. Our data show that early-life penicillin exposure caused significant blooms of *pks+ E. coli*, depletion of beneficial *Lactobacillus* species in the gut microbiome, and morphological changes in the colon and cecum, including reduced colon length and increased cecum size, which may be a result of intestinal inflammation. Thus, early-life antibiotic exposure drives expansion of carcinogenic bacteria, gut dysbiosis, and potentially colorectal carcinogenesis in mice. Moreover, HFD has been shown to directly induce enterocyte dysfunction, which may exacerbate the impact of *pks+ E. coli* expansion on tumorigenesis in colorectal cancer models. Future directions will focus on elucidating the synergistic effects of antibiotics and HFD on epithelial damage, inflammation, carcinogenesis, and overall shifts in the gut microbiota using multi-omics approaches and colorectal cancer mouse models. This work highlights the potential role of modifiable early-life factors in the etiology of EOCRC and underscores the need for further research to inform personalized, microbiome-aware clinical practices for cancer prevention.

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Identification of Novel Heme Detoxification Mechanisms in Clostridioides difficile

Elias X. West, Matthew J. Munneke, Tess P. McNeely, Jeffrey A. Freiberg, & Eric P. Skaar

Clostridioides difficile is a Gram-positive, spore forming enteric pathogen, and the leading cause of antibiotic-associated nosocomial disease in the United States. *C. difficile* infection (CDI) causes gastrointestinal damage, inflammation, and perturbs nutrient dynamics in the lumen. The bacterial toxins produced by *C. difficile* during CDI cause lysis of erythrocytes and a release of host heme at the sites of infection. Heme is cytotoxic at high concentrations causing DNA damage, protein oxidation, and membrane damage. Two systems have previously been described in *C. difficile* that sense heme and prevent toxicity to maintain homeostasis; the *hatRT* and *HsmR*A operons. HatR binds heme relieving the suppression of *hatRT* and HatT is a heme efflux pump. *HsmR* is a transcriptional regulator that binds heme and increases expression of *HsmR*A and HsmA is membrane protein that directly binds heme sequestering it from the rest of the cell and exploiting the reactivity of heme to reduce redox damage. We hypothesized that *C. difficile* expresses additional, as-yet-unidentified systems involved in heme metabolism. To identify additional genes that protect against heme we performed a suppressor screen in an inactive hsmA strain. We isolated 51 suppressor mutants and will sequence them to identify where in the genome mutations occurred. Additionally, we generated a *C. difficile* transposon library and subjected it to a subinhibitory concentration of heme. Our results identify two previously undescribed genes essential for *C. difficile* to persist in the presence of heme. We will use targeted mutagenesis to uncover the role these genes play in preventing heme toxicity and mouse models to determine their importance during CDI. This work will contribute to our understanding of how *C. difficile* survives nutrient restructuring of the microenvironment of the inflamed gut during infection.

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Helicobacter pylori VacA toxin alters lysophospholipid and arachidonic acid metabolism in host cells

Mandy D. Westland, Georgia C. Caso, Alexandra C. Schrimpe-Rutledge, Simona G. Codreanu, Stacey D. Sherrod, John McLean, Mark S. McClain, Timothy L. Cover

Colonization of the human stomach with *Helicobacter pylori* strains producing active forms of a secreted toxin (VacA) is associated with an increased risk of peptic ulcer disease and gastric cancer compared to colonization with strains producing hypoactive forms of VacA. To gain a better understanding of host cell metabolic alterations caused by VacA, we conducted untargeted metabolomic analyses of VacA-treated AGS gastric epithelial cells compared to buffer-treated control cells and identified several hundred differentially abundant metabolites. These included 84 lysophospholipids that were increased in VacA-treated cells, accounting for 56% of the metabolites exhibiting significant increases at an 8-hour time point. We also detected increased levels of lysophospholipids in supernatants from VacA-treated cells. Levels of lysophospholipids were significantly higher in the supernatants of cells treated with wild-type VacA than in supernatants from cells treated with mutant VacA proteins exhibiting defects in membrane channel-forming activity. In addition to increased abundance of lysophospholipids in VacA-treated cells, we detected increased concentrations of fatty acids, such as arachidonic acid (another product of glycerophospholipid hydrolysis), which is a precursor for prostaglandin synthesis. Therefore, we hypothesized that VacA might alter expression of phospholipase 2 (PLA2), the phospholipase responsible for the hydrolysis of glycerophospholipids into lysophospholipids. RNAseq revealed increased transcript levels of two genes that encode for members of the PLA2 family (PLA2G3 and PLA2G4C) in VacA-treated cells. We propose a model in which VacA stimulates increased PLA2 activity, leading to an increased hydrolysis of glycerophospholipids and increased levels of lysophospholipids and arachidonic acid. Both prostaglandins and lysophospholipids can act as signaling molecules during cell stress, inflammation, and infection. These results provide new insights into the wide range of VacA-induced cellular alterations and are relevant to mechanisms by which VacA actions may contribute to gastric inflammation and gastric disease.

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Inside out: Cytometry-based glycosylation analysis to reveal metabolic function and responsiveness to immune therapy in autoimmune disease.

Christopher S. Wilson, Alexander C. Falk, August Estabrook, Justin M. Gregory, & Daniel J. Moore

Glycosylation is the most abundant form of post-translational modification, yet little is understood about the nature of extracellular glycosylation in development of the adaptive immune system in health and autoimmunity. Additionally, glycosylation is one of the initial markers of self and non-self-discrimination utilized by the immune system. In this study we employ a robust multi-color lectin panel to dissect the glycosylation status of developing and mature B and T lymphocytes. Combining these lectin panels with traditional markers of B and T lymp4hocyte subsets provides a comprehensive view of the dynamic changes in glycosylation status within traditionally defined subsets of developing lymphocytes that indicates that marker expression as well as glycosylation status drives the function of immune cells. We then apply this technology to autoreactive B cells to determine whether glycosylation status reveals a glycolytic marker of autoreactive B cells in autoimmunity. Finally, we present the use of glycosylation as a marker for predicting immune response to newly FDA-approved TZIELD (anti-CD3) in the prevention of Stage 3 Diabetes, demonstrating the interplay between glycosylation, cell metabolism, and immune therapy responsiveness.

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