Vanderbilt Institute
for
Infection, Immunology & Inflammation

virtually presents

3rd Annual VI4 Symposium

Tuesday, June 9th, 2020

VANDERBILT UNIVERSITY
MEDICAL CENTER

Welcome to the 3rd

VI4 Annual Symposium

virtually presented by

Vanderbilt Institute
for
Infection, Immunology & Inflammation

and our co-hosts:

Center for Structural Biology

Division of Infectious Diseases, Dept of Medicine

Division of Molecular Pathogenesis,
Dept of Pathology, Microbiology, and Immunology

Division of Pediatric Infectious Diseases, Dept of Pediatrics

Division of Rheumatology & Immunology, Dept of Medicine

Vanderbilt Center for Immunobiology (VCI)

Vanderbilt Digestive Disease Research Center (DDRC)

Vanderbilt Microbiome Initiative (VMI)

The Vanderbilt Pre3 Initiative

Vanderbilt Vaccine Center

Agenda

10 am - 5 pm Central Daylight Time

10:00 - 10:50 am
Password: 684340

Welcome: **Eric Skaar, PhD, MPH**

Emily Davenport, PhD

@emo_davenport

Assistant Professor - Pennsylvania State University

"Using human genetics to understand the physiological basis of host microbiome interactions"

11:00 - 12:00 pm — Virtual Poster Session #1

Break

12:15 - 1:00 pm
Password: 684340

Ansuman Satpathy, MD, PhD

@Satpathology

Assistant Professor - Stanford University

"Single-cell genomics in cancer immunotherapy"

1:10 - 2:10 pm — Virtual Poster Session #2

Break

2:15 - 3:15 pm — Virtual Poster Session #3

3:20 - 5:00 pm
Password: 121663

Award Announcements: **Eric Skaar, PhD, MPH**

Graduate Student Presentation

Postdoctoral Fellow Presentation

Maayan Levy, PhD

@MaayanLevy_Lab

Assistant Professor - University of Pennsylvania

"Intestinal Epithelial Cell Function in Host-Microbiome Interactions"

Closing Remarks: **Eric Skaar, PhD, MPH**

Guest Speakers

Emily Davenport, PhD

Pennsylvania State University

Dr. Emily Davenport is an Assistant Professor in the Department of Biology at Penn State University who is interested in understanding the relationship between humans and our microbiomes.

Having long been interested in microbes, Dr. Davenport earned a Bachelor of Science degree with comprehensive honors in Bacteriology from the University of Wisconsin-Madison in 2007. She became familiar with genomic techniques while working at the microarray company Roche NimbleGen between 2007 and 2009. She merged her interests in bacterial and eukaryotic genomics during her PhD in Human Genetics, which she earned from the University of Chicago in 2014. She continued to explore the role between host genetics, the microbiome, and phenotype during a postdoc at Cornell University between 2014 and 2019, which included a year as a visiting postdoc at the Max Planck Institute for Developmental Biology between 2018 - 2019. Since fall 2019, she has led a lab at Penn State interested in understanding how human gut microbiomes are determined and what role they have on human health and evolution.

Maayan Levy, PhD

University of Pennsylvania

Dr. Maayan Levy is an Assistant Professor in the Microbiology Department of the Perelman School of Medicine at the University of Pennsylvania.

She received her B.Sc. from Tel Aviv University and her M.Sc. and Ph.D. from the Weizmann Institute of Science.

During her Ph.D studies in the laboratory of Prof. Eran Elinav in the Immunology Department at the Weizmann Institute of Science, she elucidated several new mechanisms of host-microbial crosstalk at the intestinal mucosal surface. After completion of her graduate work, she established her research group at the University of Pennsylvania in 2018.

The research in the Levy lab is focused on understanding the biology of intestinal epithelial cells and on harnessing their function to treat multi-factorial human disease. The critical impact that the intestinal microbiota exerts on health and disease makes a detailed knowledge of the nature of host-microbiota interactions essential for the rational design of therapeutic interventions.

Ansuman Satpathy, MD, PhD

Stanford University

Dr. Ansuman Satpathy is an Assistant Professor in the Department of Pathology at Stanford University School of Medicine. He is a member of the Stanford Cancer Institute, the Parker Institute for Cancer Immunotherapy, the Immunology, Cancer Biology, and Biomedical Informatics Programs, Bio-X, and a faculty fellow in ChEM-H.

Dr. Satpathy completed an M.D. and Ph.D. in immunology at Washington University in St. Louis, clinical residency in pathology at Stanford Hospital and Clinics, and postdoctoral training in genetics at Stanford University.

Dr. Satpathy's research group focuses on developing and applying genome-scale technologies to study fundamental properties of the immune system in health, infection, and cancer.

Poster Abstracts

There are 3 sessions with times noted below. Each room will be accessible via the link and password provided. Clink the trainees name to read their full abstract.

Session 1 : 11:00 - 12:00 PM

Room A

Join Room | Password: 685793

Katherine Almasy
Connor Beebout
Sam Dooyema
Eric Huseman
Clare Laut
Catherine Leasure
Grace Morales
Amyn Murji
Michelle Wiebe

Room B

Join Room | Password: 228568

Ali Abdelnabi
Brenna Appleton
Paulo De Melo
Kristen Hoek
Julia Miranda Ribeiro Bazzano
Andrew Monteith
Anderson Sa-Nunes
Ayaka Sugiura
Celestine Wanjalla

Room C

Join Room | Password: 001919

Taylor Engdahl
Emma Guiberson
Jennifer Noto
Helen Parrington
Jenna Petronglo
Valeria M. Reyes Ruiz
Matthew Vogt

Session 2 : 1:10 - 2:10 PM

Room A

Join Room | Password: 647957

William Beavers
Austin Featherstone
Jessica Sheldon
Jacob Steenwyk
Christiaan Wijers
Woongjae Yoo
Jacob Zieba

Room B

Join Room | Password: 128531

Casey Butrico
Michael Doyle
Nora Foegeding
Dalton Greenwood
Kelsey Pilewski
Catherine Shelton
Sydni Smith

Room C

Join Room | Password: 122267

Justin Jacobse
Eunice Kim
Nicholas Markham
Dudley McNitt
Nayara Pereira
Bradley Richmond
Rana Smalling

Session 3 : 2:15 - 3:15 PM

Room A

Join Room | Password: 422847

Erin Green
Lorena Harvey
Laura Hesse
Jaime Jensen
Michael Sheedlo
Timothy Thoner
Aaron Wexler

Room B

Join Room | Password: 626856

Jonathan Davies
Caleb Ford
Nathan Klopfenstein
Caitlin Murdoch
Lauren Palmer
Andrea Shiakolas
Thomas Spoonmore
Andy Weiss

Room C

Join Room | Password: 392779

Todd Bartkowiak
Zerick Dunbar
William Dunker
Cara Lang
Mona Mashayekhi
Chris Peek
Jennifer Shuman

Poster Abstracts

Session 1 - Room A

Selective modulation of ER proteostasis to inhibit flavivirus and SARS-CoV-2 propagation

Katherine M. Almasy, Jonathan P. Davies, Alexander J. Mansueto, Samantha M. Lisy, Reyhaneh Tirgar, Sirena C. Tran, and Lars Plate

Flaviviruses are a genus of arthropod-borne viruses that encompass Dengue (DENV), Zika (ZIKV), West Nile (WNV), and Yellow Fever (YFV). We currently lack effective therapies against many flaviviruses in large part due to the development of antiviral resistance. This motivates efforts to find alternative therapeutic approaches. Our focus is on identifying and targeting host processes essential to the viral life cycle. Flaviviruses, coronaviruses, and many other RNA viruses replicate and assemble around the endoplasmic reticulum (ER) membrane, and thereby interact with several host protein folding (or proteostasis) factors. Our hypothesis is that by modulating the ER proteostasis network using pharmacologic methods, we can discover and target host factors crucial to viral propagation. We found that the small molecule proteostasis regulators **147** and **263** impair flavivirus replication. The compounds were developed as preferential activators of the ATF6 branch of the ER unfolded protein response and are generally non-toxic to infected host cells. Using a chemoproteomics target identification strategy, we showed compound **147** covalently modifies ER resident proteins, including protein disulfide isomerases (PDIs), which are involved in the activation of ATF6. However, we determined that the antiviral action of these molecules is independent of ATF6 activation and PDI targeting. Instead, we discovered alternative protein targets and are currently testing their underlying role in the reduction of viral propagation. Our results suggest preferential pharmacologic targeting of ER proteostasis processes provides routes through which viral propagation can be perturbed. Lastly, we show that the antiviral effects of this molecule span across multiple strains of both Dengue and Zika virus, and that compound 263 reduces SARS-CoV-2 infection without inducing cell toxicity in a HeLa cell infection model. These results indicate that the antiviral activity extends beyond flaviviruses and highlight that the ER proteostasis regulators could represent a broadly applicable strategy for host-targeted antiviral agents.

Cytochrome bd spatially organizes extracellular matrix production in Escherichia coli biofilms

Connor J. Beebout, Levy A. Sominsky, Allison R. Eberly, and Maria Hadjifrangiskou

Biofilms are multicellular bacterial communities commonly encountered in the environment and during infection. By secreting a highly structured extracellular matrix, bacteria in biofilms are able to organize into robust and metabolically versatile communities capable of withstanding threats from external agents including bacteriophages, phagocytes, and antibiotics. Accordingly, the spatiotemporal development of the extracellular matrix is critical for community development and resilience. Here we demonstrate that aerobic respiration is spatially stratified in *Escherichia coli* biofilms, and this spatial organization of respiration is necessary for community stress tolerance. In particular, the high affinity quinol oxidase cytochrome bd spatially organizes of extracellular matrix synthesis by facilitating respiration in hypoxic regions of the biofilm. Consequently, loss of cytochrome *bd* disrupts biofilm development, reduces community antibiotic tolerance, and impairs virulence of uropathogenic *E. coli* during acute urinary tract infection. These results demonstrate that oxygen availability and redox state stratifies extracellular matrix production and provide insights into mechanisms by which bacteria spatially organize community development in order to structure biofilm growth and enhance resilience against external attack.

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Poster Abstracts

Session 1 - Room A

Helicobacter pylori Actively Suppresses Innate Immune Nucleic Acid Receptors

Samuel D.R. Dooyema, Judith Romero-Gallo, M. Blanca Piazuelo, Alberto Delgado, Giovanni Suarez, and Richard M. Peek, Jr.

Helicobacter pylori incurs the highest risk for gastric cancer, and one microbial determinant that augments cancer risk is the *cag* type IV secretion system (T4SS). This system translocates several effectors into host cells including DNA, activating the innate immune receptor TLR9. Additional immune receptors that respond to foreign nucleic acids include STING, which can respond to microbial DNA, and RIG-I, which recognizes foreign RNA. However, chronic pathogens can not only activate but also suppress host immune responses to facilitate long-term survival. Thus, the goal of this study was to elucidate the effects of *H. pylori* strain-specific constituents on STING and RIG-I signaling. Utilizing HEK293 cells transfected with a STING-specific reporter, levels of STING activation increased 17-fold in cells co-cultured with the STING agonist 2'3'-cGAMP compared to controls, while *H. pylori* alone did not activate STING. However, pre-incubation of cells with *H. pylori* prior to the addition of 2'3'-cGAMP, or simultaneous co-culture of *H. pylori* and agonist significantly reduced STING activation by >50%, independent of *cag* functional status. Using HEK293 cells transfected with a RIG-I-specific reporter, RIG-I activation increased 18-fold in cells co-cultured with 3pHp-RNA, a RIG-I agonist, compared to controls. Similar to STING, no activation was seen in cells co-cultured with *H. pylori* alone, while pre-incubation with *H. pylori* prior to addition of 3pHp-RNA or co-culture of agonist and bacteria significantly reduced RIG-I activation. To analyze the role of STING on *H. pylori*-induced injury in vivo, C57Bl/6 WT and STING-deficient mice (Stinggt/gt) were infected with the *cag+ H. pylori* strain PMSS1. *H. pylori* significantly decreased STING expression in gastric epithelium compared to uninfected controls. In conclusion, *H. pylori* has evolved mechanisms to suppress innate immune nucleotide sensor signaling in host cells in a *cag*-independent mechanism, which may contribute to its long-term persistence in the stomach.

Synthesis of a Daunorubicin-Arimetamycin A Anthracycline Hybrid

Eric Huseman and Steven D. Townsend

Arimetamycin A (AMA) is a recently isolated anthracycline natural product that demonstrated potent activity against various cancer cell lines, including the multidrug resistant (MDR) lung cancer cell line H69AR. Interestingly, Arimetamycins B and C, two anthracyclines isolated alongside AMA, did not mirror this strong activity. As these three molecules differ only in the composition of their glycans, this indicates the critical importance of the molecules' carbohydrate functionalities in determining biological activity. This, in combination with AMA's superiority to daunorubicin and doxorubicin against both sensitive and MDR cancer cell lines, imparts urgency to the study of Arimetamycin A, in particular to its glycan. To determine if the potency imparted to the arimetamycin aglycone by the AMA glycan is unique to this one aglycone or is generally applicable, we have undertaken the synthesis of a hybrid anthracycline that merges the AMA glycan with the aglycone of daunorubicin, a clinically relevant anthracycline chemotherapeutic. We began with the synthesis of the AMA glycan, a disaccharide composed of two rare branched deoxy-amino sugars, L-brasiliose and L-lemonose. Starting from D-threonine, we developed a gram scale route to an L-lemonose thioglycoside donor. Activation under Hirama-like conditions in the presence of a brasiliose glycosyl acceptor, derived semisynthetically in gram scale from vancomycin, afforded the protected AMA disaccharide in good yield and α selectivity. Protecting group adjustment and amine dimethylation gave a silyl glycoside donor that underwent a Lewis acid mediated glycosylation with the daunorubicin aglycone to give the targeted hybrid anthracycline. Future efforts will focus on determining the compound's cytotoxicity.

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Session 1 - Room A

Bacillus anthracis responds to targocil-induced envelope damage through EdsRS activation of cardiolipin synthesis

Clare L. Laut, William J. Perry, Alexander L. Metzger, Andy Weiss, Devin L. Stauff, Suzanne Walker, Richard M. Caprioli, and Eric P. Skaar

Bacillus anthracis is a spore-forming bacterium that causes devastating infections and has been used as a bioterror agent. This pathogen can survive hostile environments through the signaling activity of two-component systems, which couple environmental sensing with transcriptional activation to initiate a coordinated response to stress. In this work, we describe the identification of a two-component system, EdsRS, which mediates the *B. anthracis* response to the antimicrobial compound targocil. Targocil is a cell envelope-targeting compound that is toxic to *B. anthracis* at high concentrations. Exposure to targocil causes damage to the cellular barrier and activates EdsRS to induce expression of a previously uncharacterized cardiolipin synthase, which we have named ClsT. Both EdsRS and ClsT are required for protection against targocil-dependent damage. Induction of *clsT* by EdsRS during targocil treatment results in an increase in cardiolipin levels, which protects *B. anthracis* from envelope damage. Together, these results reveal a two-component system signaling response to an envelope-targeting antimicrobial induces production of a phospholipid associated with stabilization of the membrane. Cardiolipin is then used to repair envelope damage and promote *B. anthracis* viability.

Maintenance of heme homeostasis in *Staphylococcus aureus*

Catherine Leasure, Jacob Choby, and Eric Skaar

Staphylococcus aureus is a formidable pathogen capable of colonizing a wide variety of niches within the human host. With the incidence of drug resistant *S. aureus* strains on the rise, the development of new antimicrobials is becoming increasingly important. One *S. aureus* pathway that represents a potential therapeutic target is heme synthesis, because *S. aureus* requires the heme as a cofactor for proteins involved in important cellular processes such as electron transport and stress response. *S. aureus* can acquire heme from the host or synthesize heme endogenously. High levels of heme can be toxic, however, so *S. aureus* has evolved mechanisms to balance heme uptake, efflux, and synthesis. One mechanism of maintaining heme homeostasis is to regulate heme synthesis. Glutamyl-tRNA reductase (GtrR) catalyzes the first dedicated step in heme biosynthesis and is post-translationally regulated through unknown mechanisms by heme and HemX, a conserved membrane protein. Systematic site-directed mutagenesis of GtrR identified several amino acids whose mutation abrogates heme binding. Additionally, a genetic selection was leveraged to identify residues in HemX and as-yet-unknown gene products required for the regulation of heme synthesis. This genetic selection identified multiple genes whose products may function in the regulation of heme homeostasis. Characterization of the *in vivo* effects of GtrR incapable of binding heme and validation of hits from the genetic selection are ongoing. An improved understanding of how *S. aureus* regulates heme biosynthesis could uncover universal mechanisms employed by bacteria to adapt to various environmental niches and reveal new targets for the development of antimicrobials designed to perturb heme homeostasis.

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Session 1 - Room A

Genomic Analysis of Clinical Uropathogenic *Escherichia coli* Isolates

Grace Morales, Gerald Van Horn, Alison Eberly, Maria Hadjifrangiskou, and Jonathan Schmitz

While significant insights can be gleaned from model strains, often these same insights do not translate to clinical isolates. Clinical isolates exhibit great diversity and frequently behave differently than their laboratory counterparts. To overcome this limitation, microVU was recently launched as a microbial biobank at Vanderbilt University Medical Center that links clinical isolates to the deidentified electronic medical records of source-patients. As such, microVU provides a unique infrastructure for personalized medicine. To test the utility of microVU, I sought to determine the genomic and multi-omic differences between urocolonizing and uropathogenic bacteria, focusing on *Escherichia coli.* As *E. coli* accounts for >80% of urinary tract infection, several thousand urinary isolates are banked by microVU. The overarching hypothesis to be tested is through a strength in numbers approach, I will be able to identify genomic and other -omic features associated with uropathogenic *E. coli*, but not urocolonizing. The objective of the proposal will be to 1. assemble a pangenome of all genes present of the sequenced isolates, 2. identify variants within core genes, and 3. utilize a microbial Genome Wide Association Study to correlate genomic features with clinical presentations. This will provide key information about the diverse nature of uropathogenic *E. coli*, help define a molecular signature for the pathogen, and provide a pipeline for use on other isolates in microVU.

Cross-clade Response to Multivalent HIV-1 Nanoparticle Immunogens

Amyn A. Murji, Juliana S. Qin, Tandile Hermanus, Lynn Morris, and Ivelin S. Georgiev

HIV-1 continues to impose a global health burden. Candidate vaccines using HIV-derived antigens have not proven effective to date, so efforts toward protection against new infections remain a high priority in HIV-1 research. One strategy for developing a prophylactic HIV-1 vaccine is to elicit broadly neutralizing antibodies, which can neutralize a large fraction of circulating HIV-1 variants. The sole target of these neutralizing antibodies is the envelope protein (Env) of HIV-1. However, due to the extensive global diversity of HIV-1, Env-based vaccine candidates have only led to the elicitation of antibodies with limited neutralization breadth. To address this challenge, we designed nanoparticle immunogens that simultaneously presented multiple, diverse Envs to the immune system. These immunogens were developed by genetically fusing Envs of BG505 and CZA97, viruses from clade A and clade C, respectively onto the N-termini of bacterial ferritin. Monovalent BG505 nanoparticle and CZA97 nanoparticle cocktails as well as multivalent nanoparticles bearing both Env trimers elicited cross-clade neutralization in mice and in guinea pigs. This nanoparticle-based platform is not limited to HIV-1 vaccine design but can be generalizable to vaccine design for other viruses that exhibit high levels of sequence diversity.

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Session 1 - Room A

Cross-regulation of the *E. coli* BtsSR and YpdAB two-component systems in response to low pH

Michelle Wiebe, John Brannon, Bradley Steiner, and Maria Hadjifrangiskou

The ability to withstand pH stress is of particular importance for *Escherichia coli* to establish infection. All *E. coli* harbored in the gut are thought to be acquired via ingestion. *E. coli* thus must be able to survive the acidic pH of the stomach. Upon exit from the gut, uropathogenic *E. coli* strains that cause urinary tract infection transit and oftentimes persist within the acidic environment of the vagina. While *E. coli* has developed strategies to persist in these various niches, the mechanisms behind acid resistance within the host remain unknown. We discovered a non-cognate signal transduction pair comprised of the membrane-bound sensor histidine kinase BtsS and the response regulator YpdB that becomes activated in response to acidic pH in the range of 4-5, as well as in the presence of serine or pyruvate in the culture media. In our vaginal epithelial cell infection model and our mouse models of infection, we saw that deletion of the *btsS* and *ypdB* reduced vaginal colonization. Induction of signaling results in the upregulation of *yhjX*, a gene that codes for a putative pyruvate exporter. Given that serine import leads to production of ammonia and pyruvate, I hypothesize that UPEC use BtsS-YpdB to sense decreases in pH and upregulate transcription of *yhjX* to export pyruvate. To test this hypothesis, I have investigated the effects of serine, pyruvate and pH on BtsSR and YpdAB target gene expression in mutants lacking all known and putative pyruvate transporters. Additionally, in the context of microbe-microbe interactions, I have investigated how BtsSR-YpdAB signaling affects UPEC interactions with acid-producing members of the vaginal microbiota. Overall, these studies will probe the role of BtsS-YpdB cross-regulation in pH homeostasis in *E. coli* and begin to probe the importance of acid sensing for UPEC fitness among members of the vaginal microbiome.

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Session 1 - Room B

Innate CD8 cells maintain intraepithelial lymphocyte homeostasis and protect against colitis

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

Innate CD8αα^{+} cells (iCD8α) are TCR^{neg} intraepithelial lymphocytes (IEL) that possess immune functions such as phagocytosis, bacteria killing, and antigen processing and presentation. Recently our group and others reported that iCD8α cells promote IEL survival via osteopontin. Herein, we investigate whether iCD8α cells sustain intestinal homeostasis through maintaining IEL balance.

We compared the number of IEL in the colon of WT and iCD8α-deficient mice ($E8_I^{-/-}$). $E8_I$ is an enhancer required for CD8α homodimer expression in IEL. Despite the dramatic reduction of CD8αα expression on TCRαβ^{+} and TCRγδ^{+} IEL in $E8_I^{-/-}$ mice, the total cell numbers were comparable to WT mice. $E8_I^{-/-}$ mice had higher number of TCRβ^{+}CD4^{+}, and lower number of TCRβ^{+}CD4^{+}CD8α^{+} and TCRβ^{+}CD8αα^{+} IEL. There were no differences in TCRβ^{+}CD8αβ^{+} and TCR^{neg} IEL.

Then, we tested the role of iCD8α cells during intestinal inflammation in different colitis models. iCD8α-deficient mice had higher susceptibility to *Citrobacter rodentium* infection as indicated by increased weight loss, disease index, and bacterial load in the colon compared to WT mice. Similar results were obtained using DSS-induced colitis: $E8_I^{-/-}$ mice presented increased shortening of colon and pathological scores. Finally, we performed adoptive transfer of naïve CD4 T cells into Rag-2$^{-/-}$ and $E8_I^{-/-}$Rag-2$^{-/-}$ mice. iCD8α-deficient mice rapidly developed chronic colitis, manifested by sever weight loss and bloody diarrhea. Further analyses showed that iCD8α-deficient mice are not able to sustain proper Foxp3 expression in T regulatory cells in the intestines and therefore the mice develop intestinal inflammation. These findings indicate that iCD8α cells have a critical role in the maintenance of IEL homeostasis and healthy intestines.

Oxidized Low-Density Lipoprotein Immune Complexes Drive Signaling Changes in Dendritic Cells which Alter CD4+ T Cell Differentiation

Brenna D. Appleton, Jillian P. Rhoads, Jennifer Marvin-Peek, and Amy S. Major

Atherosclerosis is a disease of sterile inflammation characterized by the accumulation of plaque in the arteries. This is thought to be initiated by the entry and sequestration of low-density lipoprotein (LDL) in the vasculature where it becomes oxidized (oxLDL). Studies show that much of the oxLDL in circulation is bound to specific antibody to form oxLDL immune complexes (oxLDL-ICs) and there is a positive correlation between titers of circulating oxLDL-ICs and atherosclerosis disease severity. However, it is not known if oxLDL-ICs contribute to atherosclerosis-associated immunity and inflammation. Our group has shown that oxLDL-ICs cooperatively signal through Fc gamma receptors (FcγRs), Toll-like Receptor 4, and CD36 in murine bone marrow-derived dendritic cells (BMDCs) *in vitro* to enhance the production of proatherogenic cytokine IL-1β. Data indicate oxLDL-IC pretreated BMDCs enhance Th17 and suppress Th1 responses, relative to oxLDL pretreated BMDCs. Collectively, these data lead to the hypothesis oxLDL-IC signaling alters DC function resulting in changes in T cell activation and differentiation that are proatherogenic. Interestingly, results indicate the two T cell responses observed rely on separate signaling mechanisms within oxLDL-IC treated BMDCS. The Th17 enhancement phenotype is mediated by FcγR signaling, TLR signaling, CARD9, Nlrp3, and IL-1β, whereas Th1 suppression is FcγR signaling and IL-23 dependent. Overall, results highlight how oxLDL-ICs not only directly impact dendritic cells, but also indirectly influence T cell responses.

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Session 1 - Room B

Macrophage-derived microRNA-21 drives proinflammatory and glycolytic program during sepsis

Paulo Henrique de Melo, Annie Roccio Pineiros, Amondrea Blackman, and Carlos Henrique Serezani

Background: Sepsis is associated with a hyper inflammatory state, switch of metabolic profile of immune cells and impaired innate immune functions of phagocytes, collectively leading to organ damage and lethality. Identification and regulation of molecules with pleiotropic can both prevent exaggerated inflammatory response and decreases morbidities associated with sepsis Although the microRNA-21 (miR-21) is abundantly expressed in macrophages, the role of miR-21 in sepsis is controversial Aim: Here, we aimed to identify the targets and mechanisms by which miR-21 influences glycolytic metabolism and inflammatory profile of macrophage during sepsis. We hypothesized that myeloid-miR-21 inhibits the production of anti-inflammatory mediators, leading to aberrant glycolysis, inflammatory response and animal lethality. Results and conclusion: $miR21^{fl/fl}$ and $miR21^{\Delta myel}$ mice were subjected to cecal ligation and puncture (CLP) or LPS-induced septic shock. Peritoneal lavage, serum, bronchi alveolar lavage fluid (BALF) were collected within 18h and the animal survival determined over time. We found that both, macrophages and neutrophils, from septic mice had an increase of miR-21 expression in the peritoneal and lung. $miR21^{\Delta myel}$ septic mice had an increase of survival associated with enhance of bacterial control and reduction of liver and heart damage markers (ALT and Ck-MB). Myeloid-derived miR-21 leads to aberrant mRNA expression and released of proinflammatory cytokines (TNF-α, IL-1β and IL-6) in CLP and LPS-induced septic shock mice. Furthermore, miR21Δmyel bone-marrow derived macrophages (BMDM) and peritoneal macrophages (PM) failed in the production of TNF-α, IL-1β and IL-6 in response to LPS in different time points (6-24h). Aerobic glycolysis is the critical event preceded the proinflammatory activity of macrophages. RNA sequencing analysis indicated the top 50 regulated genes in $miR21^{\Delta myel}$ BMDM, we observed a global decreased expression of critical genes involved in cell homeostasis, tissue homeostasis and repair and metabolisms. Indeed, myeloid-miR-21 deficient septic mice had a decrease of the expression of the main glycolytic associated genes. Flow cytometer and western blot analysis confirmed a reduction of HIF-1α, HK-1 and GLUT-1 in $CD11b^{+}$ peritoneal cells from $miR21^{\Delta myel}$ septic mice. Also, $miR21^{\Delta myel}$ BMDMs had an impairment of expression of mRNA and proteins associated with aerobic glycolysis and decrease of ECAR (extracellular acidification rate) in response to LPS compared to WT macrophages. The impairment of macrophage-miR-21 deficient in developed the proinflammatory and glycolytic response to LPS was associated to increase of PGE_2 and IL-10 production. *In vivo* and *in vitro* experiments showed an increase of these immunomodulators in the absence of miR-21. The mPGES1 inhibitor (CAY10526) abrogated the production of PGE_2 and IL-10 in macrophages leaded to increase of TNF-α and IL-6. Next, we blocked IL-10R (anti-IL10R) *in vitro* and observed the rescue of glycolysis (ECAR) and production of proinflammatory cytokines in $miR21^{\Delta myel}$ BMDM. Finally, we showed the IL-10R blockade in $miR21^{\Delta myel}$ LPS-septic shock mice reverted completely the protection to develop cytokines storm and survive. In conclusion, the miR-21 genetic locus disruption in macrophages results in the increase of PGE_2/IL-10 responsible for control the development of proinflammatory and glycolytic program during sepsis.

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Session 1 - Room B

Role of Granzyme B in mucosal immune responses

Kristen L. Hoek, Ali Nazmi, Michael Greer, Kshipra Singh, M. Blanca Piazuelo, Keith T Wilson, and Danyvid Olivares-Villagómez

Granzyme B is a serine protease initially described in cell-mediated cytotoxicity. Recently, granzyme B has been found to be produced by non-cytotoxic cells, and elevated levels of granzyme B or cells expressing it have been implicated in many disease states. To investigate the role of granzyme B in mucosal immune responses, we utilized two models of intestinal inflammation: infection with *Citrobacter rodentium*, a colon-specific bacterium that induces intestinal inflammation in mice; and a T cell transfer model of colitis. Following infection, *C. rodentium* colonization was observed in the colon of both *wt* and *grzB*$^{-/-}$ animals at 14d post-infection. While *wt* mice displayed no overt signs of infection, severe weight loss as well as disease-associated changes in appearance and colon pathology were observed in *grzB*$^{-/-}$ mice at 10-14d post-infection. Similarly, adoptive transfer of *grzB*$^{-/-}$ CD4^{+}CD45RB^{hi} naïve effector T cells into *rag2*$^{-/-}$ recipients led to severe weight loss and disease-associated changes in appearance by 21d post-transfer, while *rag2*$^{-/-}$ recipients of *wt* CD4^{+}CD45RB^{hi} T cells were relatively unaffected at this early time point. Interestingly, disease in recipients of *grzB*$^{-/-}$ T cells corresponded with increased IL-17^{+} CD4^{+} T cells in the mesenteric lymph nodes and colon lamina propria relative to recipients that received *wt* T cells. Additionally, RNAseq analysis of CD4^{+} cells purified from mesenteric lymph nodes three weeks post-transfer revealed significant transcriptomic differences between T cells generated from wt or *grzB*$^{-/-}$ donors. These data suggest that granzyme B functions in a protective manner during intestinal inflammation, possibly playing a role in T cell differentiation by holding generation of IL-17-producing T cells in check.

Greasing up the way to EAE: a novel role for PGE2 in the Th17 to Th1 plasticity

Júlia Miranda Ribeiro Bazzano, Letícia de Aquino Penteado, Ana Carolina Guerta Salina, Ludmilla Silva Pereira, Bruna Bueno de Campos, Ana Maria Marques, Alessandro do Santos Farias and Alexandra Ivo Medeiros

Prostaglandin E2 (PGE2) plays an important role in the differentiation and expansion of T helper (Th) Th1 and Th17 cells. This prostanoid has been described as a critical role in the progression of Encephalomyelitis Autoimmune Experimental (EAE), and the inhibition of PGE2 or selective antagonists of EP2/4 receptors decreases IL-17A and IFN-g levels as well as attenuates disease progression. The coexistence of Th17 and Th1 cells and the presence of IFN-γ-producing Th17 cells at the central nervous system (CNS), suggests possible plasticity between those lymphocytes in EAE. Since PGE2 are involved in the severity of inflammation in EAE and also plays a pivotal role in Th17/Th1 differentiation, we examined the role of PGE2 in the Th1-like cells conversion during the plasticity of Th17. EAE was induced by immunization of Il17crexROSAmTmG mice with MOG35-55 peptide and treated with indomethacin (5mg/kg, i.p.), every other day. PGE2 inhibition by indomethacin impaired IFN-γ production by Th17 cells isolated from the CNS and also decreased the level of IL-12 and TNF-a. To address the intrinsic effect of PGE2 on plasticity of Th17 to Th1 cells, naïve CD4+ T cells were cultured in Th17-skewing conditions for 3 days, and then Th1-polarizing conditions (IL-2 and IL-12) and presence or absence of PGE2, EP2 or EP4 antagonist, for 6 days. The presence of PGE2 in Th1-polarizing conditions increases the percentage of IFN-g-producing Th17 cells compared to Th1-polarizing conditions without PGE2. Moreover, this effect was blocked in the presence of EP4 antagonist. Taken together, these findings elucidate a new role of PGE2 during EAE development, increasing IFN-g producing-Th17 cells and Th1-like cells by intrinsic signaling through EP4 receptor on Th17 cells and also favoring the milieu of cytokines to a Th1-polarization condition.

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Calprotectin modulates neutrophil function and enhances macrophage mediate killing of *Staphylococcus aureus*

Andrew J. Monteith, Jeanette M. Miller, William N. Beavers, Andy Weiss, C. Noel Maxwell, Jonathon M. Williams, Leslie J. Crofford, Walter J. Chazin, and Eric P. Skaar

Neutrophils are the major antimicrobial phagocytes of the innate immune system. As such, they are equipped with an arsenal of antimicrobial processes including the production of reactive oxygen species, release of highly reactive antimicrobial proteins by degranulation, and the formation of web-like structures termed 'neutrophil extracellular traps' (NETs). While neutrophil activation plays a critical role in protecting the host from infections, a delicate balance is required to ensure pathogen clearance while limiting damage to host tissues. How neutrophils balance these antimicrobial processes during infection is unclear. Calprotectin (CP) is the most abundant cytosolic protein in neutrophils and an important component of the innate immune response through its role in restricting nutrient metals from pathogens during infection through extracellular sequestration. Surprisingly, CP-deficient ($A9^{-/-}$) mice are protected from *Staphylococcus aureus* infection with lower bacterial burdens in the heart. We have uncovered an intracellular function for CP in neutrophils, whereby CP alters the production of mitochondrial superoxide (O2-), which triggers a higher propensity for suicidal NETosis rather than degranulation. Elevated suicidal NETosis by $A9^{-/-}$ neutrophils required the presence of macrophages to enhance *S. aureus* killing, suggesting the NETosis may act as a conduit to enhancing the antimicrobial capacity of macrophages. Consistent with a role for CP in regulating mitochondrial O2-, neutrophils from murine models and patients with systemic lupus erythematosus (SLE) accumulate increased levels of intracellular CP, produce lower levels of mitochondrial O2-, and fail to undergo suicidal NETosis in response to *S. aureus*. Consequently, SLE-prone mice are more susceptible to *S. aureus* infections, suggesting that SLE-prone neutrophils are ineffective at combatting *S. aureus* and offering a partial explanation for the increased incidence of bacterial infections in patients with SLE. Based on these results, we propose a model whereby CP alters mitochondrial homeostasis and acts as a critical rheostat for neutrophil function during infection.

AeMOPE-1, a novel salivary peptide from Aedes aegypti, selectively modulates macrophage activation and improves experimental colitis

Priscila G. Lara, Helioswilton Sales-Campos, Eliane Esteves, Maressa O. Henrique, Michele S. Barros, Leila S. Neto, Pedro I. Silva Jr., Joilson O. Martins, Cristina C. B. Ribeiro, José M. C. Ribeiro, Anderson Sá-Nunes

The sialotranscriptome of Aedes aegypti revealed a transcript coding a 7.8 kDa peptide overexpressed in female salivary glands. The peptide is specific for the Aedes genus, presents putative secretory nature and its function is unknown. Here, we confirmed that the peptide is highly expressed in the salivary glands of female mosquitoes when compared to salivary glands of males, and its secretion in mosquito saliva is able to sensitize the vertebrate host and induce the production of specific antibodies. The synthetic version of the peptide downmodulated nitric oxide production by activated peritoneal macrophages, and this activity was reproduced by a salivary fraction recognized by antibodies against the peptide. The peptide selectively interfered with cytokine production by macrophages, inhibiting the production of IL-6, IL-12p40 and CCL2 without affecting TNF-α or IL-10 production. Intracellular proteins associated with macrophage activation were also selectively modulated in the presence of the peptide: while iNOS and NF-κB p65 expression were diminished, COX-2 and p38 MAPK expression had not changed. The *in vitro* anti-inflammatory property of the peptide was confirmed in vivo on a dextran sulfate sodium (DSS)-induced colitis model. The therapeutic use of the Ae. aegypti peptide reduced the inflammation and cytokines associated with the disease, resulting in improvement of its clinical signs. Given its biological properties *in vitro* and *in vivo*, the molecule was termed Aedes-specific MOdulatory PEptide (AeMOPE-1).

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Targeted CRISPR-Cas9 based *in vitro* and *in vivo* screening of metabolic pathways in primary T cells

Ayaka Sugiura, Katherine Beier, and Jeffrey C. Rathmell

Chronic inflammation and immunosuppression are characterized by a dysregulation in the balance between pro-inflammatory effector T (Teff) cells and anti-inflammatory regulatory T (Treg) cells. While many currently available therapies broadly target the immune compartment, selectively targeting the specific T cell subsets that contribute to disease may provide a new avenue for development of improved immunotherapies. We have previously shown that Teff and Treg cells can be distinguished by their reliance on distinct metabolic programs, and that this exposes a way to preferentially target specific T cell subsets. We have developed *in vitro* and *in vivo* approaches to CRISPR/Cas9-based genetic screening in primary T cells to interrogate metabolic pathways. In this method, we design small-scale targeted guide RNA libraries, which are transduced into primary T cells. For the *in vitro* assays, these cells can be cultured under selective pressures, such as with differentiation cytokines, and sorted for populations of interest. For the *in vivo* assays, the transduced cells are adoptively transferred into host mice of inflammatory or cancer models, such as airway inflammation or melanoma. We have constructed and tested a library targeting the solute carrier (SLC) family of transporters, which import and export essential nutrients required to fuel the metabolic programs. Preliminary results show the glutamine transporter, SLC38a2 (SNAT2), in addition to the previously identified transporter SLC7a5 (LAT1), to be critical for primary T cell proliferative capacity. This approach provides a powerful tool for identifying critical nodes within metabolic pathways of interest in primary T cells that can potentially serve as therapeutic targets.

Increased inflammatory CX3CR1+GPR56+CD57+ CD4+ T cells in fat from HIV+ diabetics

Celestine N. Wanjalla, Mona Mashayekhi, Samuel Bailin, Curtis L. Gabriel, Wyatt J. McDonnell, Ramesh Ram, Abha Chopra, Rama Gangula, Shay Leary, Beverly O. Woodward, Mark A. Pilkinton, Alyssa Hasty, Simon A. Mallal, Spyros A. Kalams, and John R. Koethe

Persons with HIV are at higher risk of diabetes mellitus compared to the general population, which may be due, in part, to immune-mediated alteration of lipid metabolism and storage. Compared to HIV^+ non-diabetics, adipose tissue from HIV^+ diabetics is enriched for CX_3CR1^+ $GPR56^+$ $CD57^+$ (i.e., 'C-G-C') $CD4^+$ T cells; however, the mechanism by which they alter adipose tissue inflammation and whether these cells are unique to diabetic persons with HIV is not known. To assess this, we performed subcutaneous abdominal liposuction and T cell isolation on 11 diabetic persons (6 HIV+ and 5 HIV-negative, all subjects cytomegalovirus (CMV)+), followed by flow cytometry phenotyping and single-cell sorting of memory T cells with RNA transcriptomic profiling. We found that adipose tissue from HIV+ diabetics had a larger proportion of C-G-C^+ $CD4^+$ T cells (23% versus 3% in HIV-negative, $p<0.05$), while $CD69^+$ $CD4^+$ T cells trended higher in the HIV-negative diabetics (54% versus 28% in HIV-positive, $p= 0.18$). Using tetramer staining, we showed that C-G-C^+ $CD4^+$ are largely cytomegalovirus-specific T cells. Regardless of HIV status, the RNA transcriptome of C-G-C^+ $CD4^+$ T cells had higher expression of inflammatory genes compared to $CD69^+$ $CD4^+$ T cells. Thus, adipose tissue of diabetic HIV+ is enriched for an inflammatory population of potentially anti-viral $CD4^+$ T cells expressing CX_3CR_1, GPR56 and CD57, which are present at far lower levels in HIV-negative diabetics. Adipose tissue serves as a reservoir for HIV, CMV, and other viruses, and further studies will determine if C-G-C^+ $CD4^+$ T cell responses target viral antigens and may impair adipocyte function.

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Broadly and potent neutralizing hantavirus monoclonal antibodies isolated from human survivors of natural Sin Nombre virus infection

Taylor B. Engdahl, Natalia Kuzmina, Nurgun Kose, Rachel E. Sutton, Apoorva Mehta, Thomas Ksiazek, Alexander Bukreyev, and James E. Crowe

Hantaviruses are high priority emerging pathogens predominantly carried by rodents and transmitted to humans by aerosolized feces. While sporadic in North and South America, a significant number of infections occur in Europe and Asia with mortality ranging from 10-40% depending on the hantavirus species. There are currently no FDA-approved vaccines or therapeutics for hantaviruses, and the only treatment for infection is supportive care for respiratory and kidney failure. It has been shown that hantavirus neutralizing antibodies are protective in animal models, and a high neutralizing antibody titer is correlated with a higher chance of survival in patients. However, the humoral immune response to hantavirus infection is incompletely understood, especially the location of major antigenic sites and conserved neutralizing epitopes. Thus, we used the human hybridoma method to probe the breadth and potency of the human antibody response to natural hantavirus infection. Through this approach and a mammalian cell surface display screening strategy, we have isolated a panel of 21 monoclonal antibodies (mAbs) from human survivors of Sin Nombre hantavirus infection that are reactive to hantavirus glycoproteins and show a diversity of binding patterns among 6 of the major pathogenic hantavirus species. We determined that broadly reactive and neutralizing mAbs generally target the fusion protein, glycoprotein C, and bind to at least 2 antigenic sites. These studies will further our understanding of natural humoral immune response to hantavirus infection, including critical broadly neutralizing epitopes on Gc. Isolation and characterization of these mAbs can be used for antibody guided vaccine design, and similar approaches can be widely applicable to vaccination strategies for other viral families.

Spatially-Targeted Proteomics for Analysis of Staphylococcus aureus Abscess Formation

Emma R. Guiberson, Andy Weiss, Daniel J. Ryan, Eric P. Skaar, Richard M. Caprioli, and Jeffrey M. Spraggins

Intact protein characterization *in situ* using MALDI mass spectrometry approaches is challenging owing to limited sequence coverage by traditional tandem mass spectrometry methods of low charge state ions. microLESA enables *in situ* protein identification using liquid extraction surface analysis (LESA) and spatially targeted enzymatic digestion. Here we apply microLESA for the investigation of proteins directly related to the abscess formation in *Staphylococcus aureus* infection. *S. aureus* forms abscess communities within infected tissues, but the makeup of these communities is poorly understood. We used microLESA to probe the proteome of the abscess, the leading edge surrounding the abscess, and the cortex, to determine proteomic differences between these three different regions during infection. The microLESA approach was performed on specific regions of *S. aureus* infected mouse kidney. Samples from 4- and 10- days post-infection were analyzed to highlight time-dependent proteomic differences. Data was searched in Protalizer against known Newman strain and mouse protein databases. From LC-MS/MS data, 2329 unique proteins were identified (minimum 2 peptides per protein and present in multiple technical and biological replicates), of which 50 were of bacterial origin. Of these, we have identified proteins exclusively observed in specific biological regions including 7 that were associated with the abscess, 11 with the abscess leading edge, and 27 within the normal cortex. By combining spatial and temporal proteomic data, this unique approach was able to elucidate localized pathway information for this infection model. More specifically, when considering only the human proteins observed in the analysis, 21 different pathways were detected. These include known infection and disease pathways as well as pathways associated with the biosynthesis of antibiotics. The microLESA identified proteins, and the ability to probe the proteomic differences across time points in a spatially-resolved manner, provide insight into the dynamic processes of infection and abscess heterogeneity.

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Iron deficiency promotes *Helicobacter pylori*-induced carcinogenesis via targeted disruption of inflammatory and metabolic pathways

Jennifer M. Noto, James D. Carmichael, M. Blanca Piazuelo, Judith Romero-Gallo, Ayushi Sharma, Margaret M. Allaman, Keith T. Wilson, M. Wade Calcutt, Kevin L. Schey, Charles R. Flynn, and Richard M. Peek, Jr.

Helicobacter pylori is the strongest known risk factor for gastric cancer. However, the majority of infected individuals fail to develop this malignancy and pathologic outcomes are mediated by complex interactions among bacterial, host and environmental determinants. We previously demonstrated that iron deficiency accelerates the development of *H. pylori*-induced gastric inflammation and cancer in Mongolian gerbils and is associated with increased assembly of the cag type 4 secretion system. To define the effects of *H. pylori* infection on specific host factors within the context of iron deficiency, C57BL/6 and INS-GAS mice were maintained on iron-replete or iron-depleted diets and challenged with Brucella broth or the wild-type *cag*$^{+}$ *H. pylori* strain PMSS1. Mice maintained on iron-depleted diets harbored significant reductions in parameters of iron deficiency, including hemoglobin, hematocrit, and mean corpuscular volume. *H. pylori* induced significantly higher levels of chronic inflammation in C57BL/6 and INS-GAS mice compared to uninfected mice, differences that were significantly augmented under conditions of iron deficiency. In addition, the incidence of gastric dysplasia was higher among *H. pylori*-infected iron-deficient INS-GAS mice (50%), compared to infected INS-GAS mice maintained on iron-replete diets (20%). Compared to previous data in gerbils, the iron-deficient phenotype in mice was not mediated by enhanced function of the *cag* T4SS. Thus, to investigate host effectors that may mediate this phenotype, Luminex was utilized to assess expression levels of 25 different chemokines/cytokines within murine gastric tissue. Levels of KC, IP-10, MIP-1α, MIP-1β, MIP-2, and RANTES were significantly upregulated following *H. pylori* infection in an iron-dependent manner in C57BL/6 mice, while levels of KC, IP-10, MIP-1α, IL-1α, and IL-17 were significantly upregulated in an iron-dependent manner in INS-GAS mice. To define mechanisms and potential effectors on a broader level, discovery-based and targeted metabolomics approaches were employed to identify novel pathways induced by *H. pylori* and iron deficiency. Bile acid biosynthesis pathways were significantly upregulated by *H. pylori* in an iron-dependent manner in both mouse models. Among 15 upregulated bile acids, isoforms of muricholic acid were the most abundantly expressed followed by deoxycholic acid and chenodeoxycholic acid, hydrophobic bile acids that have previously been shown to promote carcinogenesis. Collectively, these data demonstrate that iron deficiency augments *H. pylori*-induced injury in two independent mouse models. This phenotype is tightly linked to enhanced proinflammatory chemokine and cytokine host responses as well as heightened levels of bile acids, which may directly promote gastric carcinogenesis.

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The human B cell response to Sosuga virus infection

Helen M. Parrington, Nurgun Kose, Rachel Sutton, Erica Armstrong, Robert Carnahan, and James E. Crowe, Jr.

Sosuga virus (SOSV) is a recently discovered, zoonotic paramyxovirus in the Rubulavirinae subfamily. SOSV was discovered in 2012 through deep cDNA nucleotide sequence analysis of blood RNA from a wildlife researcher who had been hospitalized with a severe, acute febrile illness after returning to the United States from a field-study collecting bat samples in Uganda. While the researcher survived the infection, the severity of the disease and the high mortality rates of other bat-borne paramyxoviruses suggest that SOSV may have the potential to become an epidemic threat to public health. Also, this case offered the unique opportunity to study how the human immune system adapts in response to novel, zoonotic paramyxoviruses in the very first instance of human exposure and infection. In this study, we generated the first human monoclonal antibodies (mAbs) against SOSV using peripheral blood mononuclear cells isolated from the only known previously infected donor. We used cell-surface expression of the two SOSV glycoproteins and High-Throughput Flow Cytometry (HTFC) to select virus-specific memory B cells. In total, we isolated 24 SOSV-reactive mAbs, of which 6 are specific to the attachment (HN) glycoprotein and 18 to the fusion (F) glycoprotein. Further characterization of these mAbs could provide insight into the specificity and function of the human B cell response to SOSV and could contribute to the development of therapeutic, diagnostic, or laboratory agents against this zoonotic virus that was highly pathogenic in its index case.

The role of Toll-like receptors 2 and 9 in host responses to *Staphylococcus aureus* osteomyelitis

Jenna R. Petronglo, Nicole E. Putnam, Caleb A. Ford, Jacob Curry, and Jim E. Cassat

Staphylococcus aureus is the most common etiologic agent of bone inflammation, or, osteomyelitis (OM). During *S. aureus* OM, difficulty of treatment is compounded by inflammatory processes that dysregulate skeletal homeostasis, altering the differentiation and function of bone-building osteoblasts and bone-resorbing osteoclasts (OCs) to favor bone loss. In this study, we sought to determine the role of Toll-like receptors (TLRs) 2 and 9 in immune responses and bone loss during *S. aureus* OM. Using *in vitro* assays, we discovered that *S. aureus*-induced OC differentiation proceeds in a TLR2-and TLR9-dependent manner. To evaluate this finding *in vivo*, we surgically induced *S. aureus* OM in $Tlr2^{-/-}$ and $Tlr9^{-/-}$ mice. Despite increased mortality in $Myd88^{-/-}$ mice, we found that TLR2 and TLR9 were individually dispensable for host survival and bacterial control. Moreover, in contrast to our *in vitro* findings, there were no differences in OC number or in trabecular bone loss compared to wildtype (WT). We posited that compensation between TLR2 and TLR9 masked the role of TLR2/9 in OM. To test this, we induced OM in $Tlr2/Tlr9^{-/-}$, but found no defects in bacterial burden control over an infection time course. However, we did find a significant decrease in reactive, cortical bone formation in $Tlr2/Tlr9^{-/-}$ mice compared to WT, suggesting TLRs2/9 are involved in the osteoblast response to infection. We have begun *in vitro* experiments to further understand how TLR2/9 functions in skeletal cells responding to *S. aureus*. Overall, our data suggest that redundancy in innate immune responses to *S. aureus* in bone renders TLR2 and 9 individually expendable, but TLR signaling in the skeletal niche is a regulator of homeostasis in infection.

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Defining the battle for manganese between *Staphylococcus aureus* and the host

Valeria M. Reyes Ruiz, Jessica R. Sheldon, Lillian J. Juttukonda, Caroline Grunenwald, and Eric P. Skaar

Nutrient metals are essential for the function of approximately 40% of all proteins. Therefore, the battle between the host and pathogens over essential metals is critical to the outcome of disease. *Staphylococcus aureus* is the leading cause of bacterial endocarditis and a frequent agent of bloodstream infections. Manganese (Mn) homeostasis is important for *S. aureus* disease outcome. However, the mechanism by which Mn affects *S. aureus* pathogenesis is poorly understood. We sought to investigate the impact of dietary Mn on *S. aureus* pathogenesis. Mice fed a high Mn diet show elevated levels of Mn in different tissues and enhanced staphylococcal infection of the heart. However, different mouse strains exhibit key differences in the impact of high Mn on susceptibility to S. aureus infections, suggesting that there are host factors essential for control of *S. aureus* during altered Mn abundance. We are currently identifying the bacterial and host factors that are important for colonization of the heart and disease outcome when Mn homeostasis is altered. To uncover host genes that are associated with increased susceptibility to staphylococcal heart colonization, we are utilizing the Collaborative Cross, a collection of genotyped murine strains that enable the mapping of alleles associated with traits of interest. In addition, *in vitro* studies show that *S. aureus* are metal starved by murine macrophages. We are currently identifying the macrophage factors that are responsible for staphylococcal metal starvation. These results have the potential to uncover novel factors required for vertebrate Mn homeostasis and *S. aureus* pathogenesis in the heart.

Development of a Human Monoclonal Antibody that Protects Against Polio-like Paralysis Caused by Enterovirus D68

Matthew R. Vogt, Nurgun Kose, Lauren E. Williamson, Yury A. Bochkov, James E. Gern, and James E. Crowe, Jr.

Enterovirus D68 (EV-D68) generally causes a mostly harmless respiratory illness, but in some children viral infection may result in a polio-like paralyzing condition—acute flaccid myelitis (AFM). Outbreaks of EV-D68 infection and AFM regularly recur every two years, with the next expected worldwide outbreak beginning this summer. Perplexingly, all adults and most children have neutralizing antibodies to EV-D68 in their serum, yet these AFM epidemics persist. We hypothesized that AFM likely only occurs in patients who lack these antibodies. To better understand the role of human antibody in modifying disease, we generated hybridomas that produce fully human monoclonal antibodies (mAbs) from the peripheral blood mononuclear cells (PBMCs) of donors with known EV-D68 infection. We isolated > 60 naturally occurring anti-EV-D68 human mAbs. These mAbs bind to many different clades of recent EV-D68 isolates. Many mAbs neutralize EV-D68 quite potently *in vitro*, with [ng/mL] half maximal effective concentrations. We identified at least three, but likely more, major antigenic sites on the virus at which mAb binding leads to neutralization. When given as either prophylaxis before or treatment after EV-D68 inoculation, one specific mAb, EV68-228, protects mice from both respiratory disease and AFM-like paralytic disease, whereas equivalent doses of polyclonal human immunoglobulin protect only partially. MAb EV68-228 is a potent candidate therapeutic mAb that not only prevents paralysis but does so across viral clades, which will prevent treatment failure if clades shift, which happened in 2016. Through government and industry partnerships, we have produced material suitable for use in humans and are working to gain FDA approval for its use. As no licensed vaccines or treatments exist for EV-D68, our mAbs that have therapeutic benefit *in vivo* show promise for the prevention and/or treatment of EV-D68 related diseases in humans including paralysis.

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Polyunsaturated fatty acids: host-derived molecules that kill *Staphylococcus aureus*

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

Staphylococcus aureus is a pathogen capable of infecting nearly every organ in the vertebrate host, causing over 900,000 severe infections annually in the United States. The pervasiveness of this pathogen and its impact on human health presents the desperate need to identify and validate targets for the development of new therapies. Arachidonic acid (AA) is a polyunsaturated fatty acid (PUFA) produced by humans, but not by bacteria. We discovered that AA is bactericidal to *S. aureus* through a lipid peroxidation mechanism, where AA is oxidized to isolevuglandin (IsoLG). IsoLGs elicit toxicity in eukaryotic cells by reacting irreversibly with the ε-amine of lysine, often compromising protein function; however, the mechanism of toxicity has not been defined in bacteria. AA toxicity is alleviated in *S. aureus* with the administration of antioxidants as well as IsoLG specific scavengers. Further, this toxicity is exacerbated through the increased generation of reactive oxygen species. Taken together, these results reveal that AA is bactericidal through a lipid peroxidation mechanism, and that *S. aureus* can modulate AA toxicity by reducing endogenous levels of reactive oxygen species. The antimicrobial mechanism of lipid peroxidation is not unique to AA. We discovered that all PUFA kill S. aureus through a lipid peroxidation mechanism, and the bactericidal ability is directly related to the rate constant of lipid peroxidation for each PUFA. Currently, we are using ω-alkynyl arachidonic acid, click chemistry, affinity purification, and quantitative proteomics to identify lipid electrophile protein targets in *S. aureus*. These targets are potential therapeutic weakness that can be targeted by future *S. aureus* treatments. Also, the use of molecules that undergo lipid peroxidation are being explored in vivo as potential future therapeutic interventions due to their low toxicity to the host and the inability of *S. aureus* to simultaneously resist the toxicity and maintain pathogenicity.

SERINC5 Inhibits HIV-1 Infectivity by Altering the Conformation of gp120 on HIV-1 Particles

Austin Featherstone and Christopher Aiken

Human T cells express a 10-12 transmembrane domain protein called SERINC5 that is incorporated into budding HIV-1 particles and reduces HIV-1 infectivity and inhibits virus-cell fusion. HIV-1 susceptibility to SERINC5 is determined by sequences in the viral Env protein. However, the mechanism of how SERINC5 inhibits HIV-1 infectivity is still unclear. Previous studies suggested that SERINC5 affects Env conformation. To further test whether the antiviral mechanism of SERINC5 involves altering the conformation of HIV-1 Env, we assayed the effects of SERINC5 on binding of HIV-1 particles bearing the SERINC5-sensitive Env protein from HIV-1(HXB2) to a panel of epitope-specific monoclonal antibodies immobilized on plates. We observed that SERINC5 reduced the binding of HIV-1 particles to gp120-specific antibodies that recognize the V3-loop, a sCD4-induced epitope, and an N-linked glycan. By contrast, SERINC5 did not affect antibody binding to the MPER epitope in gp41 nor to the CD4 binding site or the V3 loop on gp120. We also observed that binding of HIV-1 particles to recombinant CD4 was unaffected by SERINC5. To determine whether these effects are related to the antiviral action of SERINC5, we performed assays using HIV-1 particles bearing the Env from the HIV-1(JRFL) strain, which is less sensitive to SERINC5. Antibody binding to the JRFL Env was unaffected by SERINC5. Combined with our results from the quantification of SERINC5 on HIV-1 particles (see accompanying abstract), our findings suggest that SERINC5's antiviral activity results from its ability to bind to gp120 and perturb its conformation. These results further elucidate the antiviral action of SERINC5 and provide evidence of a novel antiviral mechanism targeting HIV-1 Env.

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

Jessica R. Sheldon and Eric P. Skaar

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

Pathogenic allodiploid hybrids of *Aspergillus fungi*

Jacob L. Steenwyk, Abigail L. Lind, Laure N. A. Ries, Thaila F. dos Reis, Lilian P. Silva, Fausto Almeida, Rafael W. Bastos, Thais Fernanda de Campos Fraga da Silva, Vania L. D. Bonato, André Moreira Pessoni, Fernando Rodrigues, Huzefa A. Raja, Sonja L. Knowles, Nicholas H. Oberlies, Katrien Lagrou, Gustavo H. Goldman, and Antonis Rokas

Interspecific hybridization substantially alters genotypes and phenotypes and can give rise to new lineages. Hybrid isolates that differ from their parental species in infection-relevant traits have been observed in several human-pathogenic yeasts and plant-pathogenic filamentous fungi, but have yet to be found in human-pathogenic filamentous fungi. We discovered 6 clinical isolates from patients with aspergillosis originally identified as *Aspergillus nidulans* (section *Nidulantes*) that are actually allodiploid hybrids formed by the fusion of *Aspergillus spinulosporus* with an unknown close relative of *Aspergillus quadrilineatus*, both in section *Nidulantes*. Evolutionary genomic analyses revealed that these isolates belong to *Aspergillus latus*, an allodiploid hybrid species. Characterization of diverse infection-relevant traits further showed that *A. latus* hybrid isolates are genomically and phenotypically heterogeneous but also differ from *A. nidulans, A. spinulosporus,* and *A. quadrilineatus*. These results suggest that allodiploid hybridization contributes to the genomic and phenotypic diversity of filamentous fungal pathogens of humans.

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A putative lytic transglycosylase and transcriptional regulator confer susceptibility to aminoglycoside-inducible host defenses in *Acinetobacter baumannii*

Christiaan D.M. Wijers and Michael Noto

Acinetobacter baumannii is a Gram-negative, opportunistic pathogen that is a common cause of nosocomial infections such as pneumonia, wound infections, and sepsis. Of growing concern is the emergence of multi-drug resistant (MDR) strains of *A. baumannii*. As such, furthering our understanding of *A. baumannii* infection biology and identifying novel therapeutic targets are imperative. Prior work from our lab has demonstrated that *A. baumannii*, after exposure to aminoglycoside antibiotics, interacts with the innate immune system in the murine lung to enhance the clearance of multiple Gram-negative pathogens. Here, we demonstrate that aminoglycosides induce these antibacterial interactions between exposed bacteria and the host innate immune system in a dose-dependent manner. Further, we show that binding of aminoglycosides to the bacterial outer membrane, but not internalization into the bacterial cytosol, is required for these interactions. In addition, using an *A. baumannii* transposon mutant library, we have identified two genetic determinants of the bacterial response to the aminoglycoside-inducible host defenses in the murine lung: a putative lytic transglycosylase (*A1S_3027*) and a putative transcriptional regulator (*A1S_3086*). Here, we show that both genes confer susceptibility to the host defenses induced by aminoglycoside-exposed bacteria. Further, our data indicate that the putative lytic transglycosylase is required for *A. baumannii* surface motility, optimal growth kinetics, and cell envelope stress resistance. Finally, we demonstrate that the putative transcriptional regulator contributes to oxidative stress resistance in *A. baumannii*. Collectively, our data provide preliminary mechanistic insights into the antibacterial interactions between aminoglycoside-exposed *A. baumannii* and the innate immune system in the murine lung. Furthermore, our data implicate roles for two previously uncharacterized genes in the infection biology of this pathogen. Further investigations into the mechanism by which aminoglycoside-exposed bacteria interact with the innate immune system to enhance bacterial clearance may lead to novel therapeutic strategies for treating infections with MDR, Gram-negative pathogens.

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Microbiota-Derived Aspartate Drives *Salmonella* Typhimurium Expansion During Colitis

Woongjae Yoo, Jacob K. Zieba, Teresa P. Torres, Julia D. Thomas, Catherine D. Shelton, Nora J. Foegeding, Erin E. Olsan, and Mariana X. Byndloss

Salmonella enterica serotype Typhimurium (*S.* Typhimurium)-induced intestinal inflammation boosts the availability of electron acceptors creating a favorable niche for this pathogen to outcompete the commensal microbiota in the gut. However, the mechanisms linking inflammation-mediated changes in luminal metabolites and *S.* Typhimurium intestinal bloom are not completely understood. In this study we show that mucosal inflammation induced by *S.* Typhimurium infection results in increased intestinal levels of the amino acid aspartate. Interestingly, *S.* Typhimurium genome encodes an aspartate ammonia-lyase (*aspA*) whose function is to convert aspartate into fumarate. We hypothesized that aspartate-derived fumarate is used as an alternative electron acceptor promoting anaerobic respiration-mediated *S.* Typhimurium expansion during colitis. Indeed, *S.* Typhimurium used *tar* (encoding methyl-accepting chemotaxis protein to aspartate), *aspA* and *dcuA* (succinate:L-aspartate antiporter, C4-dicarboxylate transporter) to bloom in the murine large intestine during inflammation, in a fumarate respiration-dependent manner. Interestingly, aspartate utilization did not confer a growth advantage for *S.* Typhimurium in the gut of germ-free mice. However, mono-association of gnotobiotic mice with *Bacteroides caecimuris* (phyla Bacteroidetes), a major aspartate producer, restored aspartate utilization in *S.* Typhimurium, suggesting that the gut microbiota is the main source of aspartate during intestinal inflammation. Taken together, our data provide a novel role for microbiota-derived aspartate in driving respiration-dependent pathogen expansion during colitis.

Aspartate Respiration by Enteropathogenic Colibactin-Producing Escherichia Coli in the Gut

Jacob K. Zieba, Woongjae Yoo, Julia Thomas, Teresa P. Torres, Mandy Truelock, Jennifer Battle, Jessica Mo, Nora Foegeding, and Mariana X. Byndloss

Colonization of colibactin producing species of E. Coli has been shown to increase the risk for colon cancer. These facultative anaerobic microbes scavenge for metabolites in the nutrient deficient environment of the gut and aspartate is one such metabolite that microbes utilize for growth. This study shows that aspartate, derived from other members of the microbiome rather than the host, is utilized by these problematic facultative anaerobes and increases the chances for the host developing cancer. In-vitro experiments using aspartate ammonia lyase (AspA) knockouts show the microbiota have a growth disadvantage compared to the wild-type. Further characterization shows these E. Coli convert aspartate into fumarate for fumarate respiration. AspA gene disruption showed a competitive advantage of the wild-type over the mutant in murine models. In a germ-free background aspartate levels only increase in the presence of Bacteroides species which are often susceptible to the effects of inflammation. We conclude that aspartate is an important metabolite for enteric pathogens during intestinal inflammation.

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Hyperglycemia alters *Staphylococcus aureus* physiology and dissemination during osteomyelitis

Casey E. Butrico, Aimee D. Potter, Caleb A. Ford, Jacob M. Curry, Christopher Good, Elizabeth Neumann, Jeffrey M. Spraggins and Jim E. Cassat

Staphylococcus aureus is a leading cause of antibiotic-resistant bacterial infections and is the most common causative agent of osteomyelitis, or inflammation of the bone. Osteomyelitis is considered one of the most difficult to treat infections and often necessitates long-term antibiotic treatment and surgical intervention. To better understand how *S. aureus* survives during osteomyelitis, we sought to identify genes essential for staphylococcal growth *in vivo* using transposon sequencing (TnSeq). Several central metabolism genes were identified, and mono-infections with *S. aureus* mutants inactivated for various central metabolic pathways revealed that *S. aureus* relies heavily on glycolysis during osteomyelitis. Because hyperglycemia is a known risk factor for osteomyelitis, we decided to elucidate the effects of this comorbid condition on *S. aureus* metabolic regulation and physiology *in vivo*. We induced hyperglycemia with a beta cell toxic agent, streptozotocin (STZ), and then surgically induced osteomyelitis. Abscess architecture was altered, and bacterial burdens increased in hyperglycemic animals. Increased bacterial dissemination to distant organs further supported previous studies that suggest hyperglycemia leads to a defect in innate immune response. Moving forward, we plan to utilize novel imaging approaches and fluorescent transcriptional reporters for central metabolism genes to spatially resolve bacteria physiology *in vivo*. Additionally, a neutrophil reporter mouse model will be paired with IHC to identify innate immune cells present near bacterial microcolonies in bone. Together, our data suggest that bacteria require glucose to perform glycolysis during osteomyelitis and increased systemic glucose influences *S. aureus* physiology *in vivo*.

Functional cooperativity by human antibodies targeting the henipavirus attachment glycoprotein

Michael P. Doyle, Nurgun Kose, Viktoriya Borisevich, Marcus Nagel, W. Hayes McDonald, Edward Annand, Robert W. Cross, Thomas W. Geisbert, Kevin L. Schey, and James E. Crowe, Jr.

Hendra (HeV) and Nipah (NiV) are zoonotic viruses in the *Henipavirus* (HNV) genus known to cause severe disease across six mammalian orders, including humans. Because these viral agents can result in case fatality rates as high as 90%, the WHO has classified henipaviruses as priority pathogens with an urgent need for accelerated research efforts. Despite these efforts, no licensed pharmaceuticals are available for human use, and significant gaps in knowledge about the immune response to these viruses exist. To address these gaps, we used a highly efficient human B cell hybridoma technique to isolate a large panel of monoclonal antibodies (mAbs) from a subject inadvertently exposed to the Hendra equine vaccine. Competition binding and HDX-MS studies identified at least seven distinct antigenic sites on HNV-G. Antibodies corresponding to multiple antigenic sites potently neutralize Nipah and/or Hendra virus isolates *in vitro*. The most potent class of cross-reactive antibodies achieves neutralization by blocking viral attachment to host cell receptors ephrin-B2 and ephrin-B3. Antibodies from this class mimic receptor binding by inducing conformational changes to HNV-G, exposing an epitope that putatively lies at the interface between protomers within the HNV-G tetramer. Antibodies that recognize this interface epitope also potently neutralize both HeV and NiV. Flow cytometric studies using cell surface displayed HeV-G show that cross-reactive, neutralizing mAbs from each of these classes cooperate for binding. Antibodies from these classes also synergistically neutralize pseudotyped vesicular stomatitis virus bearing HeV glycoproteins. Our studies identified multiple candidate mAbs that could be used in a therapeutic cocktail approach to achieve synergistic potency and reduced risk of virus escape.

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Impact of High-Fat Diet on Colonocyte Function Leading to the Bloom of Tumorigenic *E. coli*

Nora J. Foegeding, Jacob K. Zieba, Catherine D. Shelton, Woongjae Yoo, Teresa P. Torres, Jeffrey C. Rathmell, and Mariana X. Byndloss

The human colon is home to a complex microbial ecosystem which contributes to host nutrition, immune education, and niche protection. A severe disruption of the balanced gut microbial community is linked to a range of gastrointestinal diseases, including colorectal cancer. In particular, increasing evidence suggests that an intestinal bloom of strains of *Escherichia* coli capable of producing the genotoxin, colibactin, may promote colorectal tumorigenesis. However, factors driving the intestinal expansion of colibactin-producing *E. coli* remain incompletely understood. We have found using mouse models of diet-induced obesity that consumption of high-fat diet shifts the metabolism of colonocytes away from mitochondrial β-oxidation, permitting oxygen levels in the colonic epithelium to become elevated. This in turn drives the growth of colibactin-producing *E. coli*, which use the newly available oxygen to perform aerobic respiration. Using *in vitro* metabolism assays, we show that exposure of Caco-2 cells to palmitate, a saturated fatty acid present in high-fat diet, reduces the capacity of mitochondria to perform oxidative metabolism. We also show that exposure of cells to palmitate results in the downregulation of complex I mitochondrial genes and an increase in the level of reactive oxygen species. These results suggest that high-fat diets rich in saturated fatty acids perturb colonocyte mitochondrial function and metabolism, causing the expansion of *E. coli* linked to the development of colorectal cancer.

Connecting TCA cycle flux and epigenetic regulation of hematopoiesis

Dalton L. Greenwood, and Jeffrey C. Rathmell

Hematopoietic stem cells (HSCs) undergo self-renewal or differentiation to generate a variety of myeloid, erythroid, and lymphoid cells through hematopoiesis. Hematopoiesis precisely balances many stimuli to maintain HSC self-renewal and differentiation. Among these signals, metabolic molecules can directly influence epigenetic state, chromatin accessibility, and cell lineage maturation via regulation of epigenetic modifying enzymes. However, understanding remains limited of how HSCs balance metabolic and other microenvironmental stimuli to regulate self-renewal and differentiation.
Glutamine and citrate metabolism regulate methylation and acetylation, respectively, and we investigated how these pathways may impact hematopoiesis. The Rathmell lab has previously shown that Glutaminase (GLS)-deficiency promotes Th1 effector differentiation and we have now shown that ATP-Citrate Lyase (ACLY) inhibition can drive myeloid differentiation. Here we hypothesize that glutamine metabolism and citrate metabolism play an integral role to balance the fate decision of HSCs and that targeting GLS and ACLY will impact epigenetic modifications and gene expression to affect cell fate. We treated methylcellulose-culture HSCs with small-molecule inhibitors of ACLY and GLS to determine if inhibition of these enzymes alters lineage commitment of HSCs to myeloid vs. erythroid fate decisions. We observed that inhibition of ACLY using SB-204990 drives HSC differentiation into myeloid fates, indicated by increased CD11b expression. Further, treatment with SB-204990 decreased population of the conventional stem compartment Sca-1+ c-Kit+ Lin-. Single-cell RNA-sequencing and chromatin accessibility assays further entice out specific differentiation pathways for Lin- HSPCs treated with ACLYi and GLSi. Unraveling the link between glutaminolysis, citrate metabolism, and stem cell function presents an opportunity to develop a more nuanced picture of metabolism's increasingly discussed role as a regulator of cellular development. Ultimately, elucidating possible mechanisms to promote hematopoietic lineage differentiation presents a novel therapeutic opportunity for various hematologic malignancies and disorders.

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Diversion or double-edged sword? Antibody cross-reactivity in HIV/HCV co-infection

Kelsey Pilewski, Nagarajan Raju, Rohit Venkat, Ian Setliff, Andrea Shiakolas, Kevin Kramer, Amyn Murji, Rutendo E Mapengo, Jordan Salas, Steven Wall, Juliana Qin, Emilee Friedman Fechter, Spyros A. Kalams, Justin Bailey, Lynn Morris, and Ivelin S. Georgiev

Investigating the human antibody response to HIV and HCV has led to significant advances towards preventative therapeutics and vaccines against these highly mutable pathogens. Yet, despite the high prevalence of HIV/HCV co-infection, little is known about antibody responses in this context. To address this deficit, we investigated the effect of chronic co-infection with HIV and HCV on the development of virus-specific humoral responses. We hypothesized that chronic HIV/HCV co-infection leads to the development of HIV and HCV cross-reactive antibodies. To investigate this, we used LIBRA-seq (Linking B cell Receptor to Antigen specificity by Sequencing), a technology recently developed in our laboratory that uses DNA-barcoded antigens to map B cell specificity through single-cell next-generation sequencing. We investigated class-switched B cells that recognize viral envelope glycoproteins (HIV Env gp160, HCV E1E2) from a chronic HIV/HCV co-infected donor ~3.59 years post HIV infection (ypi) and identified multiple unique HIV/HCV cross-reactive B cells. We mapped their specificity to a non-neutralizing epitope on HIV gp41 and a neutralizing epitope on HCV E2 corresponding to antigenic region 5 (AR5) by PDB structural alignment, competition ELISA, overlapping peptide ELISA, and *in vitro* neutralization assay. Importantly, isolated cross-reactive antibodies show extraordinary HCV neutralization breadth, neutralizing 19/19 viruses tested (surpassing the broadest antibody previously tested in this panel, HEPC74, which neutralized 17/19 viruses). Deep sequencing from the same donor (0.79ypi) revealed multiple relatives of our cross-reactive antibodies, and their characterization by recombinant expression revealed that these HIV/HCV cross-reactive antibodies develop from a low-polyreactive, HCV-specific lineage that is hijacked after HIV infection, and subsequent somatic hypermutation leads to increased affinity for HIV. This study is the first to directly demonstrates that antibody lineages raised against one pathogen can enter the immune response against a different, genetically unrelated pathogen. Finally, the extraordinary anti-HCV capacity of identified antibodies suggest they may impact clinical outcome or be developed as potent therapeutics.

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Role of intestinal epithelial function in antibiotic-associated obesity

Catherine Shelton, Julia Thomas, Teresa Torres, Nora Foegeding, Woongjae Yoo, Jacob Zieba, and Mariana Byndloss

Emerging evidence indicates environmental factors, such as early-life antibiotic use and a high-fat (HF) diet, increase the risk for childhood obesity. However, the mechanism connecting early-life antibiotic use, a HF diet, and obesity remains unknown. Both early-life antibiotic use and a HF diet disrupt the intestinal microbial community. The gut microbiota secretes metabolites which regulate the function of intestinal epithelial cells (enterocytes). Specifically, peroxisome proliferator activator gamma (PPAR-γ) is activated by compounds produced by the gut microbiota. PPAR-γ plays a key role in metabolism by regulating expression of angiopoietin-like protein 4 (ANGPTL4), a lipoprotein lipase inhibitor that reduces fat storage. The importance of intestinal PPAR-γ activity is evident in preliminary findings that young intestinal epithelial-specific PPAR-γ knockout mice, fed a HF diet, gained more fat more than wildtype littermate controls. Our *hypothesis* is that the use of antibiotics during early-life depletes microbiota responsible for PPAR-γ activation in intestinal epithelial cells, which results in decreased production of ANGPTL4 and increased fat storage. Our data indicate that mice exposed to low doses of penicillin (LDP) and a HF diet develop greater adiposity compared to mice exposed to a HF diet alone. In addition, mice treated with LDP and fed a HF diet have decreased expression of PPAR-γ and ANGPTL4 in their enterocytes compared to mice given only a HF diet. Interestingly, in mice exposed to LDP and HF diet, we also observed a significant decrease in intestinal *Lactobacillus*, highlighting changes in the microbiota that occur during early-life perturbations. The role of *Lactobacillus* was investigated by exposing Caco-2 cells to *Lactobacillus* isolates and measuring PPAR-γ and ANGPTL4 expression. Both PPAR-γ and ANGPTL4 expression increased, indicating that the loss of *Lactobacillus* during early-life may impair PPAR-γ signaling. This work provides new insight into the mechanism by which early-life antibiotic treatment promotes obesity.

Effects of extracellular vesicle-mediated transmission on *Reoviridae* infection

Sydni Smith, Ariana von Lersner, Andries Zijlstra, and Kristen Ogden

The *Reoviridae* family contains important viruses that infect humans and other animals. Reovirus is in advanced clinical trials as an oncolytic therapeutic and has been linked to onset of celiac disease. Rotavirus is a leading cause of diarrheal gastroenteric mortality in children aged 5 years or younger in the developing world. Traditionally, viruses have been thought to transit between cells as independent particles. This viewpoint is being challenged with the recent discoveries that many virus families utilize extracellular vesicles (EVs) for non-lytic transport. EV-mediated transmission potentially enables viral evasion of immune responses and collective transmission to drive enhanced productive infection. Recently, rotavirus was shown to egress host cells in large EVs, and EV-mediated transmission enhances rotavirus virulence *in vivo*. Mechanisms of reovirus egress are incompletely defined, and effects of multiparticle transmission on rotavirus and reovirus genetic complementation are unknown. Using cultured cells, microflow cytometry, and genetically barcoded viruses, we provide evidence that i) reovirus particles egress in large EVs in a virus strain- and cell type-dependent manner, ii) rotavirus upregulates EV release from infected cells, and iii) EV-mediated transmission increases the frequency of reovirus genotype mixing. Taken together, these data suggest that multiple particles of reovirus and rotavirus egress in large, virus-modulated EVs, and that transmission in EVs increases segment complementation compared to transmission as free particles. These discoveries may be broadly applicable to viruses that travel in EVs and will contribute to general principles of virus transmission and diversification. Continued studies will illuminate the specific cellular pathways reovirus and rotavirus utilize for successful egress. These pathways may prove to be critical targets for the improvement of vaccines and oncolytic therapy.

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Interleukin-23 receptor signaling impairs Treg cell function to promote intestinal inflammation

Justin Jacobse, Jing Li, Sarah P. Short, Rajeev Tyagi, Lori A. Coburn, M. Kay Washington, Edmond H.H.M. Rings, Janneke N. Samsom, and Jeremy A. Goettel

Background

Genome-wide association studies identified variants in the interleukin-23 receptor (*IL23R*) with altered risk for inflammatory bowel disease (IBD). Likewise, the cytokine IL-23 is involved in IBD pathogenesis as antibody-mediated IL-23 blockade is efficacious. *IL23R* is enriched on a subset of highly suppressive colonic regulatory T cells (Tregs) expressing forkhead box P3 (FOXP3), but the function of this Th17 type receptor on Tregs is unknown. We hypothesize that IL-23R signaling in Tregs modifies their transcriptional landscape, diminishing suppressive function, and thereby promotes chronic intestinal inflammation.

Methods

Published data was examined for tissue-wide, and Treg-specific, expression of *IL23R* during intestinal inflammation and carcinogenesis. The role of IL23R in Tregs was determined via conditional targeting of *Il23r* in FOXP3^{+} cells using *Il23r*$^{fl/fl}$*Foxp3*$^{YFP\text{-}Cre}$ mice. YFP^{+} Treg cell numbers were quantified at baseline for various compartments. The Treg specific expression of IL-23R was investigated during chronic DSS mediated inflammation, after mice received three cycles of 3.5% DSS for 5 days, with 10 days rest in between. Finally, *in vivo* suppressive capacity was investigated by co-injecting IL-23R-sufficient or IL-23R-deficient Tregs with CD45RB^{high} T cells into Rag1$^{-/-}$ recipient mice at a 1:20 Treg:Tnaïve ratio.

Results

Compared to control areas, *IL23R* expression was increased in colorectal cancer, and on Tregs specifically in ileal Crohn's disease. *In vivo*, in *Il23r*$^{fl/+}$*Foxp3*$^{YFP\text{-}Cre}$ mice, YFP^{+} Treg frequency in the colonic lamina propria was reduced compared to *Il23r*$^{fl/fl}$*Foxp3*$^{YFP\text{-}Cre}$ mice. During chronic DSS mediated inflammation, expression of IL-23R on colonic Tregs increased. Adoptive transfer experiments indicate that IL-23R-sufficient Tregs may be inferior to IL-23R-deficient Tregs in their ability to suppress effector T cell response *in vivo*.

Conclusions

IL-23R signalling may alter Treg function by destabilizing FOXP3 directly or by modulating stability/survival of FOXP3^{+} cells. These findings are especially relevant within an inflammatory microenvironment. The underlying mechanisms are being explored.

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B Lymphocytes in Human Autoimmune Disease

Eunice Kim, Austin B. Santhin, Bryan A. Joosse, James H. Jackson, Mason V. Forchetti, Daniel J. Moore, and Rachel H. Bonami

Type I diabetes (T1D) is caused by the killing of insulin-producing beta cells in the pancreas through the immunocompromised B cell and T cell interface. Previous research has shown that the presence of insulin autoantibody (IAA) predicts T1D risk in humans, emphasizing the importance of anti-insulin B cells (AIBCs) in T1D. Mouse models have also shown that AIBCs and their insulin-binding B cell receptors (BCR) play significant roles in disease progression. To identify the extent to which AIBC expansion and BCR modification impacts glucose tolerance deterioration that marks T1D, B cells in pre-symptomatic stage 1 and stage 2 T1D participants were compared. Hybridoma technology allowed for AIBC capture and BCR characterization in these stages. We have found a trend towards an enriched frequency of AIBCs in stage 2 pre-symptomatic T1D donor PBMCs than in stage 1. Not only that, AIBCs were present in both IAA+ and IAA- T1D donors. These findings suggest that AIBC populations are expanding with disease progression, influencing glucose tolerance impairment, and this can occur regardless of IAA secretion. Subsequent studies in cloning V(D)J genes from BCR antibodies of these AIBCs will provide insight into BCR insulin affinity regulation and in turn, the mechanisms of AIBC expansion. Recognizing AIBC function especially in the early stages of T1D is critical in disease research, as understanding its expansion can lead to understanding its prevention.

Human colon cancer-associated biofilms alter host immune populations and promote tumorigenesis

Nicholas O. Markham, Julia L. Drewes, Jada Domingue, Alan J. Simmons, Cody N. Heiser, Mary C. Macedonia, Marisol A. Ramirez, Erin N. Laubacher, Won Jae Huh, Martha J. Shrubsole, Qi Liu, Robert J. Coffey, Cynthia L. Sears, and Ken S. Lau

Recent advances in microbiome biology suggest invasive biofilm formation and the spatial organization of the gut microbiota may initiate and/or accelerate colorectal cancer (CRC). The mechanisms and host-microbe interactions responsible for the earliest tumorigenic changes are not understood. To investigate the critical bacterial species and host immunity alterations involved in biofilm-associated CRC development, we colonized germ-free *Apc*$^{Min/+}$ mice with a single gastric gavage of mucosal slurries derived from biofilm-positive human CRCs. Using this model, we have shown previously that a mixture of five human CRC-associated biofilms colonized the distal mouse colon and were tumorigenic. Here, biofilms isolated from individual patient human CRCs were compared. Interestingly, induction of colon tumorigenesis in germ-free *Apc*$^{Min/+}$ mice varied widely with the different human mucosal biofilm inocula at 14 weeks post-inoculation. We performed in-depth analyses on two inocula: 3728T, which was associated with strong colon tumorigenesis (median = 6 macroadenomas, n = 8) and 3979T, which was weakly tumorigenic (median = 0, n = 7). At 2 weeks and 10 weeks post-inoculation, colonic tissue was harvested for assessment of tumor burden, histopathological dysplasia/neoplasia, and single-cell transcriptomics. Fecal samples were collected throughout the experiment. After two weeks, 3728T-inoculated mice had an average 2-fold increase in crypt depth in the distal colon compared to 3979T-inoculated mice. At this time point, 3728T-inoculated mice also had multiple microadenomas with elevated β-catenin expression, whereas no microadenomas were observed in the 3979T-inoculated mice. Our preliminary scRNA-seq analysis revealed altered host immune populations, including increased naïve T cells at 2 weeks and increased M2 macrophage activation at 10 weeks, in the tumorigenic 3728T inoculum. Multiplex immunofluorescent validation of these data is underway, and 16S rRNA amplicon sequencing of the human CRC-associated biofilms and recipient mouse fecal microbiota is in progress. In summary, we present data defining the immune correlates of human mucosal biofilm-associated colon tumorigenesis in mice.

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T-B lymphocyte interactions mediated by SLAM-associated protein (SAP) are essential for diabetes in anti-insulin, VH125Tg NOD mice

Dudley H. McNitt, Rachel H. Bonami, Chrys Hulbert, Jamie L. Felton, and James W. Thomas

Type 1 diabetes (T1D) is an autoimmune disease characterized by T cell-mediated destruction of insulin-producing beta cells. Beta cell attack is preceded by the development of high-affinity islet autoantibodies that predict disease, but do not directly mediate pathological damage. This paradox makes T follicular helper cells (Tfh) a prime suspect in T1D pathogenesis. This novel subset of T cells interacts with B lymphocytes to generate germinal centers (GCs) that produce class switched high-affinity antibodies. A key facilitator of T-B interactions is signaling lymphocytic activation molecular-associated protein (SAP), that governs Tfh differentiation in GCs (GC Tfh). To understand the role of GC Tfhs in T1D, we created SAP deficienct transgenic VH125 (VH125Tg) non-obese diabetic mice, in which accelerated disease is driven by an anti-insulin B lymphocyte receptor. We found that SAP deficiency significantly impacts GC B lymphocyte formation, while the number of GC Tfh was similar between SAP-sufficient and deficient mice. SAP is also essential for T1D development, as SAP deficient VH125Tg mice did not develop T1D. Strikingly, SAP has only a limited role in the development of T1D in conventional mice. Thus, we find GC Tfh develop without SAP regardless of the genetic background and without strong GC formation, suggesting that GC Tfh can form extrafollicularly. In addition, the data suggests that the composition of the B lymphocyte receptor repertoire dictates the genetic program of GC Tfh. Future studies aim to compare the differential programing of diabetogenic GC Tfh in SAP sufficient versus deficient non-obese diabetic mouse models.

Leukotriene-B4 in diabetes: when systemic "metaflammation" status dictates macrophage function

Nayara Pereira, Theresa Ramalho, Niels O. Saraiva Câmara, Carlos Henrique Serezani, and Sonia Jancar

In type 1 diabetes (T1D), leukotriene-B4 contributes to the systemic inflammation and pro-inflammatory macrophages phenotype. Leukotriene-B4 was also reported to cause lipolysis-dependent hyperlipidemia. In macrophages, fatty acid uptake and uncoupling mitochondrial respiration is LTB4-dependent. Here we investigated if hyperlipidemia is the cause for systemic inflammation and if macrophage metabolism affects its activation. T1D was induced in 5-lipoxygenase *knock out* ($5LO^{-/-}$) mice, unable to produce leukotrienes, and in correspondent Wild Type (WT) by inoculation of streptozotocin. Peritoneal macrophages from diabetic-5-lipoxygenase knock out mice ($5LO^{-/-}$) produce fewer IL-12, IL-1β, and IL-10 when compared to diabetic-WT. Also, peritoneal macrophages from diabetic $5LO^{-/-}$ have less basal respiration, ATP production, and proton leak compared to diabetic WT mice. Considering the macrophage inflammatory phenotype, higher fatty acids uptake and mitochondrial activity in macrophages are leukotriene-dependent, we then assayed the effect of metabolic inhibitors on macrophage phenotype. In peritoneal macrophages, leukotriene-dependent production of IL-12, IL-1β and *Ucp1* gene expression (mitochondrial uncoupling protein) decreased dramatically with etomoxir (fatty oxidation inhibitor) treatment. The same was observed in the treatment with 2DG (glycolysis inhibitor) but Ucp1 increased expression in a leukotriene-dependent manner. Moreover, lipolysis in diabetic-$5LO^{-/-}$ is less pronounced, and their epididymal fat produces fewer IL-12, IL-6, MCP-1, and TNF-α compared to diabetic-WT. Our results suggest that inflammatory status LTB4-dependent is associated with adiposity loss, and macrophage function LTB4-dependent occurs in a dependent manner to metabolic reprogramming towards the fatty acid oxidation pathway.

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Increased YAP/TAZ Signaling in COPD Airway Secretory Cells Is Associated with Impaired Mucosal Immunobarrier Function

Jessica B. Blackburn, Rui-Hong Du, Jacob Schaff, Arun C. Habermann, Austin J. Gutierrez, Carla L. Calvi, Matthew K. Xin, Chase J. Taylor, Ciara M. Shaver, Lorraine B. Ware, Matthew J. Bacchetta, Nicholas E. Banovich, Jonathon A. Kropski, Timothy S. Blackwell, and Bradley W. Richmond

Chronic obstructive pulmonary disease (COPD) is a debilitating lung disease caused primarily by long-term exposure to cigarette smoke. Loss of the secretory IgA (SIgA) immunobarrier in small airways is common in COPD and is sufficient to induce a COPD-like phenotype in mice. We hypothesized that loss of the SIgA immunobarrier in patients with COPD results from reduced expression of the polymeric immunoglobulin receptor (pIgR) which is required for SIgA transport across the airway epithelium. We obtained human lung tissue from the explanted lungs of patients undergoing lung transplantation for severe COPD and organ donors without COPD whose lungs were rejected for transplantation. *PIGR* expression was determined by single-cell RNA sequencing (scRNA-seq), RNA in situ hybridization (RNAscope), and immunostaining. HBEC3-KT cells were used to perform mechanistic investigations into regulation of *PIGR* expression in vitro. scRNA-seq data indicated that *PIGR* expression was restricted to epithelial cells and was highest in basal and secretory cells. RNAscope and immunostaining confirmed that secretory cells are the dominant producer of *PIGR*/ pIgR in human airways. *PIGR* expression was reduced in secretory cells from COPD patients but was increased in other cell types, particularly basal and multiciliated cells. RNAscope showed COPD airways had reduced numbers of *PIGR*$^{+}$*SCGB3A2*$^{+}$ secretory cells, suggesting reduced *PIGR* expression in COPD may also result from a loss of these cells. Further analysis of scRNA-seq data indicated increased expression of *CTGF* and *CYR61* in secretory cells from COPD patients, suggesting increased YAP/TAZ activity. In HBEC3-KT cells, *PIGR* expression was suppressed by XMU-MP-1, a pharmacologic activator of YAP/TAZ signaling. These results suggest that in COPD loss of the SIgA immunobarrier results from reduced expression of *PIGR* in secretory cells and reduced numbers of *PIGR*$^{+}$*SCGB3A2*$^{+}$ cells. Increased YAP/TAZ activity within secretory cells suppresses *PIGR* expression in vitro and may reduce pIgR expression in COPD airways.

Phosphogluconate dehydrogenase antagonism by regucalcin impairs pancreatic tumorigenesis

Rana V. Smalling, Matthew E. Bechard, and Oliver G. McDonald

Pancreatic ductal adenocarcinoma (PDAC) is a highly metastatic, chemoresistant and extremely lethal cancer. The glucose metabolizing enzyme phosphogluconate dehydrogenase (PGD) is a pro-metastatic driver constitutively activated in PDAC metastases (PGD^{high}). PGD^{high} status is achieved by routing glucose into unconventional metabolic pathways that produce the PGD substrate. We show that blocking such pathways by overexpressing regucalcin (RGN), a gluconolactonase enzyme which siphons substrates away from PGD, can impair metastatic tumorigenesis. RGN expression is extremely low in patient derived PDAC metastasis subclones. Enzymatic assays on extracts from PGD^{high} cells expressing exogenous RGN indicate that RGN significantly lowers PGD catalysis by reducing the PGD substrate 6-phosphogluconate. This is associated with significant decreases in 3D tumor organoid size and number, reduction in glucose uptake and reversal of histone acetylation marks associated with pro-metastatic changes. This study highlights PGD as a therapeutic target for PDAC.

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Desiccation tolerance increases the pathogenicity of *Acinetobacter baumannii*

Erin R. Green and Eric P. Skaar

Acinetobacter baumannii is an important opportunistic pathogen that infects critically ill patients in hospital settings. Because of its rapid acquisition of antibiotic resistance, infections caused by *A. baumannii* have become extremely difficult to treat, underlying the importance of identifying new antimicrobial targets for this pathogen. *A baumannii* is frequently found contaminating hospital surfaces, which serve as a major reservoir for hospital-acquired infections with this pathogen. The ability of *A. baumannii* to survive extended periods of desiccation is thus thought to be a critical factor contributing to hospital outbreaks; however, the mechanisms by which *A. baumannii* survives desiccation and the impact these processes have on its pathogenicity have not been well defined. We established an *in vitro* system to experimentally model desiccation tolerance and found that *A. baumannii* remained viable after up to 7 months of desiccation, and that desiccated *A. baumannii* exhibited heightened resistance to oxidative stress and enhanced virulence in a murine pneumonia model of infection relative to a liquid-grown control. To determine the mechanisms by which *A. baumannii* survives desiccation, a Tn-Seq screen was performed. We identified a number of gene products promoting desiccation tolerance, including the transcriptional regulator *bfmR*. A *ΔbfmR* mutant was found exhibited enhanced sensitivity to oxidative stress and attenuated virulence in a pneumonia model of infection. Additionally, a mutant lacking the negative regulator of this system, *ΔbfmS*, displayed heightened desiccation tolerance relative to a WT strain, but was attenuated for virulence. Together, these data suggest that the BfmRS two-component system serves as an important environmental sensor in *A. baumannii* and may play a regulatory role in mediating the link between desiccation survival and pathogenicity in this organism, but must be carefully regulated during infection. Future directions will be aimed at characterizing genes regulated by BfmR that promote desiccation survival and virulence in *A. baumannii*.

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Hop Outer Membrane Proteins as Determinants of *Helicobacter pylori* Fitness *in vitro* and *in vivo*

M. Lorena Harvey, Aung S. Lin, John T. Loh, Matthew Scholtz, Jennifer H. Shuman, Mark S. McClain, and Timothy L. Cover

Introduction: The *H. pylori* genome encodes more than 60 predicted outer membrane proteins (OMPs). Several are known to function as adhesins, but the functions of most remain unknown. Here, we tested the hypothesis that specific OMPs provide *H. pylori* strains with a selective advantage in intraspecies competition experiments and facilitate gastric colonization. Methods: We generated two libraries of *H. pylori* strains containing unique nucleotide barcodes at target loci. The control library consists of strains with unique nucleotide barcodes inserted in an intergenic region predicted to be a neutral locus (unrelated to bacterial fitness). We also generated an "OMP library" of strains in which OMP-encoding genes are disrupted by insertion of an antibiotic cassette and unique nucleotide barcodes. We passaged each of the libraries *in vitro* on various types of media (TSA-blood agar plates or Brucella agar plates containing varying concentrations of sodium chloride) and orogastrically infected mice with each of the libraries. We then studied compositional changes in these populations via high-throughput sequencing at multiple timepoints. Results: The control library proliferated as a stable community *in vitro*, without any substantial changes in the proportional abundance of individual strains. In contrast, there were substantial changes in composition of the OMP library after passage *in vitro*. Specific OMP mutants exhibited fitness advantages or disadvantages compared to a control strain. The altered fitness characteristics of several OMP mutants were observed in experiments with multiple types of culture media, whereas the fitness alterations of other OMP mutants were specific for only one type of culture medium. We also observed changes in the composition of the OMP library during colonization of the mouse stomach. Analysis of *H. pylori* strains cultured from mice revealed that one OMP mutant was frequently overrepresented (>50% of sequence reads in half of the mice) while other OMP mutants had fitness defects compared to the control mutant. Conclusions: These data indicate that specific outer membrane proteins are determinants of *H. pylori* fitness in different environments.

Mechanisms of zinc homeostasis in *Acinetobacter baumannii*

Laura E. Hesse, Zachery R. Lonergan, William N. Beavers, and Eric P. Skaar

Acinetobacter baumannii is an opportunistic bacterial pathogen capable of causing a variety of infections including pneumonia, sepsis, and wound and burn infections. *A. baumannii* is an increasing threat to public health due to the prevalence of multidrug-resistant strains, leading the World Health Organization to declare *A. baumannii* a Priority 1: Critical Pathogen, for which the development of novel antimicrobials is desperately needed. Zinc (Zn) is an essential nutrient which pathogenic bacteria, including *A. baumannii*, must acquire from their hosts in order to survive. However, at high concentrations, Zn is toxic to *A. baumannii*, potentially through mismetallation of non-cognate proteins. Vertebrate hosts have defense mechanisms that exploit the dual nature of this ion by sequestering Zn from invading bacteria in certain niches, but intoxicating pathogens with Zn in other niches. To identify mechanisms used by *A. baumannii* to maintain Zn homeostasis, we screened a transposon mutant library for insertions in genes that altered sensitivity to low or high levels of Zn. This Zn-deplete screen revealed genes important for *A. baumannii* Zn acquisition, including genes encoding an ABC transporter and outer membrane TonB-dependent receptor. In conditions of excess Zn, insertions in genes involved in phosphate metabolism protected *A. baumannii* from experiencing toxicity. Future studies will determine the contribution of phosphate accumulation to Zn homeostasis.

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Structural and Functional Analysis of Large Clostridial Toxins and Their Interactions with Host Epithelial Cell Receptors

Jaime L. Jensen, Michael J. Sheedlo, John A. Shupe, Melissa A. Farrow, and D. Borden Lacy

Clostridioides difficile infections (CDIs) are the leading cause of nosocomial diarrhea in the United States. Symptoms may range from mild diarrhea to pseudomembranous colitis, sepsis, and death. The primary virulence factors of C. difficile are the homologous glucosyltransferase exotoxins TcdA and TcdB. Intoxication of host cells is initiated by toxin binding to host cell receptors. Several receptors have been identified for TcdB, including Frizzleds-½/7, poliovirus receptor-like 3 (PVRL3 or Nectin-3), and chondroitin sulfate proteoglycan 4 (CSPG4). Direct receptor binding has previously not been observed for TcdA. TpeL, an orthologous toxin from *C. perfringens*, has been shown to bind LDL receptor-related protein 1 (LRP1). The goal of this work is to characterize the binding mechanisms of TcdA, TcdB, and TpeL with their respective receptors through structural and functional analysis. We demonstrate that Fzd2 is capable of binding TcdA within the C-terminus of the CROPs domain, distinct from TcdB's Fzd2 binding site. Two novel receptors have been identified for TcdB, and while TcdB is capable of forming multi-receptor complexes, co-receptor interactions appear to preclude receptor association with TcdB. Lastly, we show that a TpeL point mutation, L1098K, mitigates cytotoxic "cell-rounding" effects observed with the wild-type toxin.

Structural and Functional Characterization of the Clostridioides difficile Transferase Toxin

Michael J. Sheedlo, David M. Anderson, Jaime L. Jensen, Audrey K. Thomas, and D. Borden Lacy

Clostridioides difficile is a gram-positive, pathogenic bacterium and is currently the leading cause of hospital acquired diarrhea in the United States. The pathology associated with *C. difficile* infection (CDI) has largely been attributed to the activity of two homologous toxins known as toxin A (TcdA) and toxin B (TcdB). Some of the most common clinical strains also produce a third toxin, however, known as the *C. difficile* transferase toxin or CDT. Though it is suspected that CDT enhances the severity of CDI, the underlying mechanisms associated with CDT pathology have not been well defined. CDT is a member of the binary toxin family and consists of two different polypeptide chains; an enzymatic component (known as CDTa) and a cell-binding, pore-forming component (known as CDTb). To establish a framework to better understand the effects of CDT during intoxication we sought to define the structure of CDT by cryo-EM. This analysis led the discovery of a previously unidentified glycan binding domain within the pore forming component, CDTb, as well as several distinct structures which depict CDTb as it exists at different points along the pore formation pathway. From this analysis we present a potential mechanism that describes the transition of CDTb from the soluble state to a fully-formed, membrane-inserted pore. We have also solved a structure of the complete toxin, CDTa in complex with CDTb. This study demonstrated a mode of toxin assembly that is distinct from prototypical binary toxins and raises new questions about how the toxin is delivered into the host. Taken together, these studies represent the first complete structural analysis of CDT and provide a means to better understand this clinically important toxin.

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Timing of coinfection influences reovirus reassortment frequency

Timothy W. Thoner Jr., Madeline M. Meloy, Julia R. Diller, Jacob M. Long, and Kristen M. Ogden

Reassortment, the process through which viruses exchange segments of their multipartite genomes, can increase viral pathogenesis by promoting generation of virus strains with enhanced host immune evasion, zoonotic transmission, and virulence properties. Within dense, cytoplasmic inclusions, mammalian orthoreovirus (reovirus) packages ten segments of positive sense (+) RNA, which it replicates to form genomic double-stranded RNA. Compartmentalizing replication, and the induction of antiviral innate immune responses, may limit reovirus reassortment by disrupting coordination of genome packaging by coinfecting viruses. To understand how reovirus replication influences reassortment, we eliminated the confounding variable of parent virus incompatibilities by generating a genetically-barcoded reovirus strain with identical replication kinetics to wild type. The inserted genetic barcodes alter the melt temperature of each gene segment, enabling determination of the parental origin of gene segments, and quantification of reassortment frequency, by post-PCR genotyping methods. With this genetic barcoding system, we have determined that while reovirus reassorts frequently during coinfection, reassortment frequency is diminished with increasing time separating primary and secondary infection. To assess whether antiviral innate immune responses restrict secondary infection, thereby limiting reassortment, using antibodies specific for a peptide-tagged reovirus and indirect immunofluorescence, we quantified infectivity of a secondary virus after primary infection with reovirus strains that either initiate strong (T3D) or weak (T1L) interferon responses. Our data indicate that primary infection with T3D reovirus restricts secondary infection while T1L primary infection has no effect on infectivity, suggesting that antiviral cellular responses may preclude reovirus secondary infection. To explore whether the compartmentalized replication processes of reovirus may influence reassortment, we have also established methods to visualize viral +RNA localization within inclusions during coinfection by branched DNA fluorescence in situ hybridization. Future studies will further explore the mechanism of reovirus secondary infection restriction and will determine if viral +RNA localization and virus-synthesized inclusion bodies influence reassortment.

Discovering How *Clostridioides difficile* Usurps Host Metal-binding Factors to Persist in the Gut

Aaron G. Wexler, Emma Guiberson, William N. Beavers, Richard Caprioli, Jeffrey Spraggins, and Eric P. Skaar

Clostridioides difficile is the leading cause of nosocomial and antibiotic-associated intestinal infections, predominantly affecting patients with a disrupted intestinal microbiota. Compounding this problem, antibiotics are the primary treatment option for *C. difficile* infections (CDI), yet they further disrupt the microbiota and lead to multiple infection relapses in up to 20% of patients. Enteric pathogens like *C. difficile* largely acquire their nutrients from the diet of their host, and we have previously reported a positive correlation between dietary zinc abundance and CDI susceptibility in mice. This suggests zinc is a limiting resource over which *C. difficile* competes with the host and commensal microbes during colonization. Indeed, we find that a defined mixture of nutrient metals, including zinc, is sufficient to determine the outcome of paired competitions between *C. difficile* and a diverse panel of human-derived commensal bacteria. Bile acids are both host-derived metal binding factors and potent germination factors for *C. difficile*. In our mouse model of CDI, we observe a substantial increase in the abundance of the bile acid taurocholate throughout the intestinal lumen during CDI using next-generation imaging mass spectrometry. We hypothesize that elevated taurocholate levels benefit *C. difficile* by facilitating spore germination, and by increasing dietary metal availability in the large intestine, where *C. difficile* primarily resides. Indeed, mice provided the bile acid sequestering drug cholestyramine are delayed for *C. difficile* colonization, suggesting high taurocholate levels promote *C. difficile* colonization and persistence. Our ongoing work is directed at understanding the contribution of taurocholate to *C. difficile* nutrient metal access and pathogenesis. These studies may reveal new strategies to prevent or mitigate CDI in patients.

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Comparative Multiplexed Interactomics of SARS-CoV-2 and Homologous Coronavirus Non-Structural Proteins Identifies Unique and Shared Host-Cell Dependencies

Jonathan P. Davies, Katherine M. Almasy, and Lars Plate

Human coronaviruses (hCoV) have become an increasing threat to global health and society, as evident from the SARS outbreak in 2002 to the most recent COVID-19 pandemic caused by SARS-CoV-2. Despite the high sequence similarity between SARS-CoV-1 and -2, each strain has distinctive virulence. A better understanding of the basic molecular mechanisms mediating changes in virulence is needed. Here, we profile the virus-host protein-protein interactions of several CoV non-structural proteins (nsp) that are critical for virus replication. We use tandem mass tag-multiplexed quantitative proteomics to sensitively compare and contrast the interactome of nsp2 and nsp4 from three betacoronavirus strains: SARS-CoV-1 from the 2002 outbreak, SARS-CoV-2 from the current pandemic, as well as CoV-OC43 – an endemic strain associated with the common cold. This approach enabled us to identify both unique and shared host cell protein binding partners and further compare the enrichment of common interactions across homologs from the related strains. We identified common nsp2 interactors involved in endoplasmic reticulum (ER) homeostasis, including the ERLIN½ complex enriched for SARS-CoV-1, as well as unique interactors like FOXK1 for SARS-CoV-2 and STOML2 for SARS-CoV-1. We also identified nsp4 interactors unique to each strain, such as the CTLH E3 ligase complex for SARS-CoV-1, ER homeostasis factors for SARS-CoV-2, and an oxidative-stress pathway component for CoV-OC43. In addition, common nsp4 interactors include N-linked glycosylation machinery, unfolded protein response (UPR) associated factors, and anti-viral innate immune signaling factors. This work improves our understanding of the role these coronavirus proteins play in the infection cycle, as well as host factors they may mediate the divergent pathogenesis of OC43 from SARS strains. Our mass spectrometry workflow enables rapid, robust comparisons of multiple bait proteins, which can be applied to additional viral proteins. Furthermore, the identified, common host-dependencies may present new targets for exploration by host-directed anti-viral therapeutics.

Diflunisal-loaded Poly(propylene sulfide) nanoparticles ameliorate *Staphylococcus aureus*-mediated bone destruction during osteomyelitis

Caleb A. Ford, Thomas J. Spoonmore, Mukesh K. Gupta, Craig L. Duvall, James E. Cassat, and Scott A. Guelcher

Osteomyelitis is a debilitating infection of bone and carries substantial morbidity and mortality due to difficulty in eradicating infection from bone. The recalcitrance of these infections to pharmacologic therapies necessitates the development of improved drug delivery platforms. Staphylococcus aureus is the most commonly isolated pathogen causing bone infections and boasts an arsenal of virulence factors such as phenol soluble modulins (PSMs) that contribute to bone destruction. Previous studies have reported that diflunisal, a non-steroidal anti-inflammatory drug (NSAID), ameliorates infection-mediated bone destruction during osteomyelitis through suppression of agr-mediated toxin production (e.g. PSMs). However, due to the limited aqueous solubility of diflunisal, drug efficacy required administration via a local device. Despite standard-of-care antibiotic therapy during diflunisal delivery, bacterial colonization of the local delivery device was observed. Therefore, diflunisal delivery using parenterally delivered poly(propylene sulfide) nanoparticles (PPS NPs) was assessed to circumnavigate infectious complications with local delivery. We first characterized the size and encapsulation of diflunisal-loaded PPS NPs. Next, we demonstrated the ability of PPS NPs to selectively accumulate at the infectious focus in a murine model of osteomyelitis. Finally, we determine that diflunisal-loaded PPS NPs ameliorate S. aureus-mediated bone destruction during osteomyelitis. Thus, PPS NPs may provide a parenteral delivery platform for hydrophobic small molecule therapeutics in the context of osteomyelitis without the complications associated with local delivery depot methods.

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Phagocyte-derived SOCS1 disrupts *Staphylococcus aureus* skin host defense

Nathan Klopfenstein, Stephanie L. Brandt, and C. Henrique Serezani

Methicillin-resistant *Staphylococcus aureus* (MRSA) skin infection is controlled by the actions of skin resident macrophages and the formation of a neutrophilic abscess that prevents bacterial spread and tissue damage. We and others have shown that myeloid differentiation factor 88 (MyD88) is required for the clearance of MRSA skin infection in mice. MyD88 expression is controlled by the balance between STAT1 and the suppressor of cytokine signaling 1 (SOCS1). Here, we hypothesized that SOCS1 inhibits antimicrobial effector functions and the inflammatory response, leading to poor abscess formation and tissue injury during MRSA skin infection. Our data show that MRSA skin infection enhances SOCS1 expression. Infection in myeloid-specific SOCS1 deficient mice displays decreased lesion size, lower bacterial loads, and increased abscess thickness when compared to WT mice. When we treated infected mice with a peptide that specifically inhibits the kinase inhibitory region (KIR) of SOCS1, it also improved infection outcome. Examining the mechanisms by which SOCS1 enhanced skin host defense, we observed increased phagocytosis and bacterial killing in SOCS1 deficient macrophages and KIR peptide-treated cells. Increased antimicrobial effector function correlated with enhanced STAT1 activation and increased production of IFNγ *in vivo* and *in vitro*. Next we tested whether IFNγ is crucial to improved host defense in KIR-treated mice. Our data show that the beneficial effect of SOCS1 KIR peptide in skin host defense was abrogated in mice treated with an IFNR blocking antibody and in the IFNR deficient mice. Overall, these data show that preventing SOCS1 actions enhances microbial clearance and host defense during MRSA skin infection.

Defining the effects of dietary trace metals on commensal microbiota community structure and dynamics

Caitlin C. Murdoch and Eric P. Skaar

The intestines of animals are colonized by dense communities of microorganisms, referred to as the gut microbiota. Animals must tightly regulate the growth of bacteria within the intestine in order to maintain homeostasis and preserve organismal health. This is achieved in part through sequestration of trace metals, nutrients essential for life, via a process termed nutritional immunity. It is well established that elevated metal concentrations in the intestine can predispose hosts to enteric microbial infections. However, the effects of metal bioavailability on inter-microbial interactions, as well as microbiota community structure and function, remain largely unknown. We are using gnotobiotic zebrafish as a model to study metal metabolic circuits and impacts of metal exposures on microbiota colonization by combining high resolution in vivo imaging with genetic analyses of both microorganisms and the host. We are engineering bacterial reporters of metal bioavailability in natural commensal bacteria isolated from the zebrafish intestine. We will monitor bacterial responses to metal supplementation and sequestration in mono- or poly-microbial communities in vivo. Moreover, genetic loss- and gain-of-function approaches (through mutation or overexpression) of host metal transporters and chaperones will define how host factors influence intestinal microbiota composition and metal homeostasis in bacterial communities. This work will help identify how micronutrients modulate bacterial physiologies in the microbiota during homeostasis and how dysregulation of bacterial and host metal metabolism may lead to differential outcomes during infectious challenge. Ultimately, we aim to identify novel targets for therapeutic intervention in order to mitigate enteric pathogenesis in humans and animals.

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Dietary zinc deficiency compromises immunity to *Acinetobacter baumannii* pneumonia

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

Zinc deficiency affects approximately one third of the global population and is estimated to contribute to 1 in 6 cases of pneumonia. In the United States, intensive care unit patients are at increased risk for both zinc deficiency and infection by *Acinetobacter baumannii*. *A. baumannii* is a leading cause of ventilator associated pneumonia and a critical public health threat due to increasing rates of multi-drug resistance. Determining the role of host and *A. baumannii* metabolism during infection has the potential to identify new therapeutic targets to support host immunity and inhibit essential bacterial processes. To test whether dietary zinc deficiency contributes to *A. baumannii* pathogenesis, we established a murine model of dietary zinc deficiency and acute *A. baumannii* pneumonia. In this model, zinc deficient mice have increased *A. baumannii* burdens and suffer significantly higher mortality. During infection, zinc deficient mice produce more pro-inflammatory cytokines, including the type 2 cytokine IL-13 that is typically associated with asthma and parasite infection. Importantly, neutralizing IL-13 protects zinc deficient mice from *A. baumannii* induced mortality. This discovery could lead to a new immunotherapy for zinc deficient pneumonia patients, such as anti-IL-13 antibody therapies that are well-tolerated in humans. Furthermore, this work identifies a previously unrecognized link between zinc deficiency and type 2 immunity, consistent with an emerging paradigm that certain nutrient deficiencies promote type 2 immunity to prevent parasite infection at the expense of immune protection from bacterial and viral pathogens.

Identification and characterization of coronavirus cross-reactive antibodies using LIBRA-seq

Andrea Shiakolas, Kevin Kramer, Daniel Wrapp, Nianshuang Wang, Steven Wall, Rohit Venkat, Emilee Friedman Fechter, Rita Chen, David Martinez, Kelsey Pilewski, Michael Diamond, Ralph Baric, Robert Carnahan, James Crowe, Jason McLellan, and Ivelin Georgiev

As of May 18, 2020, SARS-CoV-2 has infected approximately 4.7 million people, and over 316,000 people have died from health complications associated with COVID-19. The emergence of the novel coronavirus (CoV) SARS-CoV-2, the causative agent of COVID-19, has produced a worldwide pandemic, devastating healthcare systems and national economies. Currently, there are no vaccines or therapeutics available, so the pursuit of treatments and the study of human immune responses to CoVs is incredibly important. The spike protein on the CoV virion engages with host cell receptors to mediate viral entry and is the main antigenic target of neutralizing antibodies. Here, we focus our efforts toward the discovery of antibodies that can cross-react with spike proteins from multiple CoVs. To identify these antibodies, we performed an assay recently developed in our lab called LIBRA-seq (Linking B cell receptor to antigen specificity through sequencing), which enables the recovery of paired heavy/light chain antibody sequences along with antigen reactivity information for thousands of single B cells simultaneously. In preliminary studies, we used LIBRA-seq to screen B cells from a SARS-CoV-1 convalescent donor using an antigen screening library composed of stabilized prefusion spike proteins from pandemic strains (SARS-CoV-2, SARS-CoV-1, MERS-CoV) and endemic strains (HKU1, OC43), resulting in paired antibody sequence-antigen specificity information for 2526 B cells. A number of antibodies that were cross-reactive against SARS-CoV-2, SARS-CoV-1, and MERS-CoV, and in some cases additionally against HKU1 and OC43, were identified and validated in ELISA binding assays. Antibody candidates are being evaluated in various functional assays, with planned testing of additional antibodies from the current set, as well as additional LIBRA-seq experiments on human samples. Elucidation of cross-reactive CoV epitopes will help inform rational vaccine design strategies, both for the current and potential future CoV pandemics.

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Local delivery of diflunisal limits bone destruction but fails to improve systemic vancomycin efficacy during *Staphylococcus aureus* osteomyelitis

Thomas J. Spoonmore, Caleb A. Ford, Jacob M. Curry, Scott A. Guelcher, and James E. Cassat

Diflunisal is a non-steroidal anti-inflammatory drug that has previously been identified as an osteoprotective adjunctive therapy for osteomyelitis. Diflunisal inhibits *S. aureus* quorum sensing and subsequent quorum-dependent toxin production (e.g. phenol soluble modulins). When delivered locally during experimental osteomyelitis, diflunisal significantly limits bone destruction without affecting bacterial burdens. However, because diflunisal may have "quorum-quenching" activity, antibiotic recalcitrance could theoretically be altered and it is critically important to evaluate this adjunctive therapy in the context of standard of care antibiotics. The objective of this study is to evaluate the efficacy of vancomycin to treat osteomyelitis during local diflunisal treatment. We first determined that systemic vancomycin effectively reduces bacterial burdens in a murine model of osteomyelitis, and identified a dosing regimen that decreases bacterial burdens without eradicating infection. Using this dosing scheme, we found that vancomycin activity is unaffected by the presence of diflunisal *in vitro* and *in vivo*. Similarly, locally-delivered diflunisal still potently inhibits osteoblast cytotoxicity *in vitro* and bone destruction *in vivo* in the presence of sub-therapeutic vancomycin. However, we also found that the resorbable polyurethane foams used to deliver diflunisal serve as a nidus for infection. Taken together, these data demonstrate that diflunisal does not significantly impact standard of care antibiotic therapy for *S. aureus* osteomyelitis, but also highlight potential pitfalls encountered with local drug delivery.

Molecular characterization of the staphylococcal abscess microenvironment through spatially targeted proteomic analysis

Andy Weiss, Emma R. Guiberson, Daniel J. Ryan, Andrew J. Monteith, William J. Perry, Richard M. Caprioli, Jeffrey M. Spraggins, and Eric P. Skaar

Staphylococcus aureus is one of the leading causes of healthcare-associated infections, and this organism is able to withstand numerous frequently used antibiotics. In order to develop novel treatment approaches, a deeper understanding of the complex interaction between host and pathogen is required. This is particularly true for the processes during development of tissue abscesses, which are important contributors to acute and persistent staphylococcal infections. Nºtably, the formation of staphylococcal abscesses takes place in distinct stages. Consequently, studies aiming to elucidate the underlying mechanisms of abscess development have to take temporal and spatial components into account.

State-of-the-art proteomic technologies offer insight into complex biological systems. However, the pairing of spatial information and deep proteomic analysis is challenging, complicating the targeted analysis of relatively small-scale and heterogeneous structures like organ abscesses. To overcome existing technical limitations, we have previously introduced the use of a spatially targeted liquid extraction surface analysis (LESA) workflow termed "microLESA". In our current study, we perform microLESA to investigate the processes at the host-pathogen interface during development staphylococcal kidney abscesses in a murine model of systemic infection. For this investigation, regions from the abscess community, the interface surrounding the abscess, and the cortex of infected kidneys were extracted and analyzed at both 4- and 10-days post infection. By defining the proteome of different abscess regions across the course of infection, we followed the immune response and bacterial contribution to abscess development through spatial and temporal proteomic changes.

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Single-cell systems neuroimmunology reveals a highly immunosuppressive microenvironment in human glioblastomas contacting the lateral ventricle stem cell niche

Todd Bartkowiak, Sierra Barone, Madeline Hayes, Nalin Leelatian, Justine Sinnaeve, Allison R. Greenplate, Caroline E. Roe, Akshitkumar M. Mistry, Bret C. Mobley, Lola B. Chambless, Reid C. Thompson, Kyle D. Weaver, Rebecca A. Ihrie, and Jonathan M. Irish

Glioblastomas make up more than 60% of adult primary brain tumors and carry a 15-month overall survival despite aggressive standard-of-care therapy. Recent advances in immunotherapy offer an appealing alternative that may improve outcomes for patients with glioblastoma; however, predictive features have yet to be identified identified that may inform therapeutic responsiveness and clinical stratification. Patients whose tumors demonstrate radiographic contact with the lateral ventricle have reduced survival compared to patients whose tumors do not contact the lateral ventricle. Further, we have demonstrated an association between immune infiltration and tumor phenotypes that are prognostic of poor outcome. We therefore hypothesized that the lateral ventricle acts as a previously unappreciated immunosuppressive microenvironment within the brain that promotes tumor growth by suppressing anti-tumor immunity. Primary human glioblastomas were disaggregated into single-cell suspensions and mass cytometry (CyTOF) measured >30 parameters assessing immune phenotype, checkpoint receptor expression and phosphor-protein readouts in response to cytokine stimulation in thirteen immune populations infiltrating human glioblastomas. Machine-learning tools identified key differences in the abundance and phenotypes of T cells, B cells, NK cells, microglia, and peripheral macrophages infiltrating ventricle-contacting gliomas. Further, enriched expression of immune checkpoint receptors (PD-1, TIGIT, LAG-3, TIM3) and phosphor stimulation (p-STAT3, p-STAT5) correlated with ventricle contact and patient outcome. These results provide key insights into the immune microenvironment of glioblastomas and elucidate several clinically actionable immunotherapeutic targets that may be used to optimize treatment strategies for glioblastoma patients based on V-SVZ contact status.

Tissue-specific NK cell diversity in solid tumors

Zerick Dunbar and Anil Shanker

Cytotoxic innate lymphoid cells, conventionally known as natural killer (NK) cells, play major roles in cancer immunity largely due to their natural cytolytic and immune regulatory functions. However, due to the extensive heterogeneity and plasticity seen among NK cells, their functions have yet to be fully harnessed in immunotherapy. The objective of this study is to elucidate NK cell diversity via maturation, recruitment and effector function profiling in solid tumor microenvironments. We hypothesize that NK cells from different tissue locations display genetic and functional profiles that can predict NK effectiveness in solid tumor microenvironments. Here, we show the biological diversity that exists among NK cells from different locations pre- and post-interaction with solid breast and lung cancer tumors, including heterogeneity of NK cell specific cluster of differentiation markers and gene expression perspectives based on flow cytometry, qPCR, and bioinformatics analyses. Understanding NK cells in the tumor microenvironment could help answer critical NK cell research questions and lead to advancements in cancer immunotherapy applications, ultimately helping to mitigate a growing cancer resources-to-results discrepancy.

This work was supported by the NIH RISE grant R25 GM059994.

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The Role of TDP-43 in Activation of RIG-I Like Receptors

William Dunker and John Karijolich

The activation and regulation of double-stranded RNA (dsRNA) sensors is paramount for initiating a robust anti-microbial gene expression response to an infection and for preventing an aberrant immune response to endogenous ligands. As two of the primary sensors, RIG-I Like Receptor (RLR) family members retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5) are responsible for recognizing distinct classes of RNAs via specific structural and chemical moieties and signal through the adapter protein mitochondrial antiviral-signaling protein (MAVS) to promote an immune response. Recent findings have identified RNA processing proteins that function at essential junctions of RNA metabolism to prevent the accumulation of immunostimulatory intermediate dsRNAs. The ubiquitous RNA binding protein TAR DNA-binding protein 43 (TDP-43) has been associated with a wide range of RNA processes including alternative splicing, miRNA biogenesis, and paraspeckle formation, although the function of TDP-43 has not been fully elucidated. Here, we demonstrate that TDP-43 functions at a pivotal stage of RNA metabolism where its depletion induces in innate immune response. Loss of TDP-43 leads to the accumulation of immunostimulatory dsRNA and induces an antiviral Type I and III IFN response in neuroblastoma and clear-cell renal cell carcinoma cell lines during basal conditions. The antiviral IFN state is completely dependent upon the RLR signaling pathway as loss of TDP-43 in a MAVS, IRF3, or RIG-I knockout, but not MDA5, does not promote an immune state. Furthermore, TDP-43 depletion results in cell death that is rescued by a MAVS knockout. Importantly, we determined that a TDP-43 knockout is viable if constructed in a MAVS knockout setting, suggesting both a novel epistatic interaction between TDP-43 and MAVS; and that TDP-43's regulation of gene expression is not essential for life. Collectively, these data determine how loss of TDP-43 activates a lethal immune response and how it is rescued.

Mechanisms of inflammasome-mediated pro-tumorigenesis in Head and Neck Squamous Cell Carcinoma

Cara Lang, Juan Fu, Qi Zeng, and Young J. Kim

Tumor associated myeloid cells, including Myeloid Derived Suppressor Cells (MDSCs), are important mediators of immune evasion in cancer. While most of the immunosuppressive mechanisms occur through the inhibition of T cell effector function which facilitates tumor growth and metastasis, we showed that tumor infiltrating myeloid cells utilize the inflammasome signaling pathway as an alternate mechanism to promote T-cell independent tumor growth. The inflammasome is a multi-protein signaling pathway in myeloid and epithelial cells that releases IL-1β and IL-18 to induce pyroptosis. *In vivo*, growth rates of B16 and MOC2 were significantly blunted in chimeric mice adoptively transferred with caspase 1 null bone marrow cells but not gasdermin D null bone marrow, suggesting that gasdermin D independent, non-pyroptotic mechanisms of IL-1β release can drive the pro-tumor phenotype.. We also show that caspase 1 and gasdermin D knockout tumor infiltrating myeloid cells can suppress T cell proliferation *in vitro*. When we examined the TME, we noted no significant changes in T or NK cell infiltration, but a significant reduction in myeloid cell infiltration suggesting that IL-1β release is required for myeloid cell trafficking into the tumor. Together, our findings suggest that non-pyroptotic IL-1β secretion, independent of gasdermin D, is a key mediator of tumor growth and can be targeted in combination with checkpoint blockade to improve tumor response.

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Immunologic Effects of GLP-1 Activation in Obese Adipose Tissue

Mona Mashayekhi, Celestine Wanjalla, Mark Pilkinton, Christian Warren, Samuel Bailin, Curtis Gabriel, Spyros A. Kalams, Nancy J. Brown, and John R. Koethe

Objectives: Obesity is associated with systemic inflammation that stems, in part, from adipose tissue (AT). Obese AT is characterized by pro-inflammatory T cells that promote macrophage activation. The incretin GLP-1 has been shown to have anti-inflammatory effects in AT of animals, and we hypothesized that increased GLP-1 would reduce inflammation in association with an increase in anti-inflammatory invariant natural killer T cells (iNKTs), group 2 innate lymphoid cell (ILC2s) and regulatory T cells (Treg) in blood and AT in humans.

Methods: Obese adults with pre-diabetes were randomized to pharmacologic treatment resulting in increased GLP-1 signaling (liraglutide or sitagliptin), or hypocaloric diet. This ongoing study is blinded, so the effects of liraglutide and sitagliptin are combined in analyses and referred to as "drug". Subcutaneous AT and blood were collected at baseline ("pre") and after 12 weeks ("post").

Results: Using Nanostring's inflammation panel, we found a number of pro-inflammatory genes were significantly downregulated in whole AT after treatment with drug, including CD163, CD86, MCP-2, and MCP-4 (N=22). Blood and AT ILC2s were significantly decreased with drug (blood: pre 3.95%±3.05, post 1.71%±1.65, p=0.01 N=8; AT: pre 2.04%±1.67, post 1.32%±1.58, p=0.07 N=4), but not diet (blood: pre 2.17%±1.91, post 1.46%±1.68, p=0.18 N=3). We did not detect a change in Treg or iNKT numbers after treatment with either diet or drug.

Conclusions: In a small pilot study of obese patients treated with drugs that activate GLP-1 signaling (liraglutide or sitagliptin) or hypocaloric diet, transcriptional analysis of whole AT suggested decreased inflammation with drug therapy. However, we found decreased percentages of ILC2 cells (considered anti-inflammatory in adipose) in both blood and AT after drug treatment. Future experiments will further characterize the function of these cell types and evaluate other immune subsets in blood and AT that may be responsible for decreasing inflammation.

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Mechanisms of bone loss during inflammatory bowel disease

Christopher T. Peek, Caleb A. Ford, Nicole Putnam, Blanca Piazuelo, Margaret Allaman, Justin Jacobse, Jeremy Goettel, Keith Wilson, and Jim Cassat

Inflammatory bowel disease (IBD) is characterized by severe gastrointestinal inflammation and changes in the intestinal microbiota. Patients with IBD often experience extra-intestinal disease manifestations. Skeletal abnormalities are a frequent extra-intestinal manifestation of IBD, and patients exhibit a 40% increased fracture risk compared to that of the general population. Many factors, including malabsorptive malnutrition and glucocorticoid use, contribute to IBD-associated bone loss. Yet, nutritionally replete and glucocorticoid naive patients remain susceptible to bone loss. We used complementary mouse models of colitis-driven bone loss to test the hypothesis that IBD-associated bone loss is triggered in part by changes in innate and adaptive immunity. We observed significant reductions in trabecular bone volume over total volume BV/TV in mice subjected to both a chemically induced colitis with dextran sodium sulfate (DSS) and an adoptive T-cell transfer model of colitis (ACT). We found that with DSS-colitis, there was an increase in osteoclasts and monocytic osteoclast precursors *in vivo* prior to the onset of bone loss. When sorted, these monocytic osteoclast precursors formed more osteoclasts *ex vivo* compared to controls. Bone loss with DSS was not dependent on the adaptive immune system, as $Rag1^{-/-}$ were still susceptible to bone loss. However, because T-cells play a central role in the pathogenesis and treatment of IBD and are capable of altering bone homeostasis, we sought to understand the polarization and function of T-cells in bone during colitis. Relative to control mice without colitis, we observed a significant shift towards $T\text{-}bet^{+}$, IFNg producing T-cells, a profile classically associated with Th1 polarization. We also noted a concurrent decrease in IL-17 producing $Rorgt^{+}$ T-cells, classically associated with Th17 polarization, in mice suffering from colitis. Collectively, these data suggest that both changes in innate and adaptive immunity alters skeletal homeostasis during gastrointestinal inflammation.

Gastric inflammation drives positive selection of specific *Helicobacter pylori* mutations in a Mongolian gerbil model

Jennifer H. B. Shuman, John T. Loh, Aung Soe Lin, Mark S. McClain, Natalie Favret, Abha Chopra, and Timothy L. Cover

Helicobacter pylori persistently colonizes the human stomach, resulting in varying severity of gastric inflammation. We hypothesized that *H. pylori* strains harboring specific mutations are positively selected in response to severe gastric inflammation. To test this hypothesis, we experimentally infected Mongolian gerbils with an *H. pylori* strain in which expression of the *cagUT* operon (required for Cag type IV secretion system functionality) is controlled by a TetR/*tetO* system. At three months post-infection, *H. pylori*-infected gerbils consuming chow containing doxycycline (to de-repress Cag type IV secretion system activity) had significantly higher levels of gastric inflammation than those consuming drug-free chow. Output strains from animals in each group were analyzed by whole genome sequencing, allowing identification of polymorphisms that were undetectable in the input strain but present in >90% of sequence reads from individual output strains (likely representing positively selected polymorphisms). The output strains from 15 animals on doxycycline-containing chow and output strains from 17 animals on drug-free chow did not differ significantly in the number of single nucleotide polymorphisms (SNPs) (2.9 + 2.3 and 3.2 + 4.3, respectively), deletions or insertions meeting these criteria. Mutations within *fabZ* (encoding β-hydroxyacyl-acyl carrier protein (ACP) dehydratase, which has a role in fatty acid biosynthesis) were detected in multiple output strains from animals in both groups. Mutations upstream of *katA* (encoding catalase, an enzyme that helps protect against damage from oxidative stress) were detected (100% of sequence reads) in 6 of 15 output strains from gerbils that received doxycycline and none of 17 output strains from gerbils that received drug-free chow ($p = 0.0055$). These data suggest that high levels of inflammation are associated with positive selection of specific *H. pylori* mutations that may confer survival advantages in an inflammatory gastric environment.

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