Vanderbilt Institute

for

Infection, Immunology & Inflammation

virtually presents

4th Annual VI4 Research Symposium

Thursday, April 15th, 2021

VANDERBILT UNIVERSITY

MEDICAL CENTER

Welcome to the 4th Annual

VI4 Research Symposium

virtually presented by

Vanderbilt Institute

for

Infection, Immunology & Inflammation

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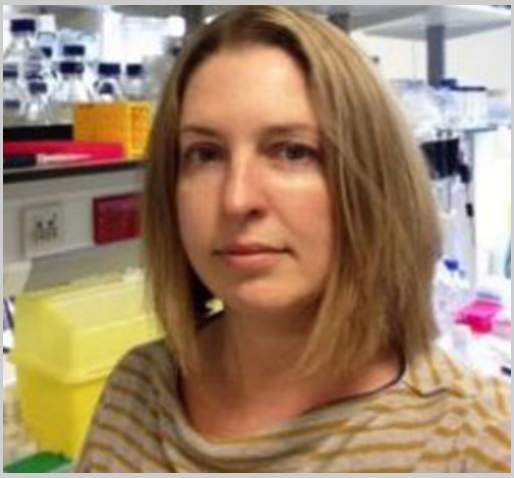
Agenda

9 am - 4 pm Central Daylight Time

9:00 - 9:05 am	Welcome: **Eric Skaar, PhD, MPH**
9:05 - 9:50 am	**Sian Henson, PhD** **Senior Lecturer - Queen Mary College of London** *"Mitochondrial Dysfunction: GATA3 keeps your T cells fit"* @DrSianH
	Break
10:00 - 11:00 am	Virtual Poster Session #1
	Break
11:15 - 12:00 pm	**Nassos Typas, PhD** **Group Leader & Senior Scientist - European Molecular Biology Lab** *"Probing the interface of drugs and microbes"*
12:00 - 1:00 pm	Virtual Poster Session #2
	Break
1:15 - 2:00 pm	**Liza Konnikova, MD, PhD, FAAP** **Assistant Professor - Yale University School of Medicine** *"In utero Intestinal Metabolome"* @LizaKonnikova
2:00 - 3:00 pm	Virtual Poster Session #3
	Break
3:15 - 4:00 pm	Award Announcements: **Eric Skaar, PhD, MPH**
	Graduate Student Presentation: **Connor Beebout**
	Postdoctoral Fellow Presentation: **Erin Green, PhD**
	Closing Remarks: **Eric Skaar, PhD, MPH**

Guest Speakers



Sian Henson, PhD

Queen Mary College of London

Dr. Sian Henson has spent the past 20 years researching immune senescence and its impact in age-related diseases. More recently she has become interested in the metabolic requirements of senescent T cells and her group at QMUL investigate the deregulation of T cell metabolism during human ageing and how it maintains an inflammatory deleterious state.

Dr Henson serves as a committee member for the BBSRC and the Dunhill Medical Trust. She is the Chair of the British Society of Immunology affinity group for Immune senescence. Sian is also an Associate Editor for Frontiers of Immunology. Current academic roles include module lead for Immunology for the Biomedical Sciences BSc.

Liza Konnikova, MD, PhD, FAAP

Yale University School of Medicine

Dr. Liza Konnikova is an attending Neonatologist, and an Assistant Professor in Pediatrics and Obstetrics, Gynecology and Reproductive Sciences as well as a member of the Human and Translational Immunology Program at the Yale School of Medicine. Dr. Konnikova received a BA from Brandeis University and an MD PhD from Tufts School of Medicine. She then went on to train in pediatrics and neonatology at Boston Childrens' Hospital where she stayed to complete her post-doc training under the supervision of Dr. Scott Snapper. She started her own group in 2017 at the University of Pittsburgh and has recently been recruited to Yale.

Dr. Konnikova research focuses on the development of neonatal immunity at barrier sites such as the GI tract and the maternal-fetal interface and its role in the pathogenesis of diverse diseases such as sepsis, preterm labor, necrotizing enterocolitis (NEC), very early onset (VEO) and pediatric IBD.

Nassos Typas, PhD

European Molecular Biology Lab

Dr. Nassos Typas is a Senior Scientist in the Genome Biology Unit in Heidelberg, where he has been running his group since 2011. His group combines automated platforms and genome-wide approaches with molecular mechanism to study bacterial cellular networks, and how bacteria interact with the environment, the host and with each other. Key focal areas of the lab are on antibiotics, bacterial pathogenesis, and the human gut microbiome.

Nassos is a trained biochemist, geneticist, and systems biologist. He did his undergraduate studies at the Aristotle University of Thessaloniki, his PhD at the Free University of Berlin with Regine Hengge and his postdoctoral research at University of California, San Francisco with Carol Gross. He has received a number of awards (NIH K99/R00, Sofja Kovalevskaja Award from the Humboldt Foundation, ERC Consolidator Grant, VAAM 2021 Award) and is a member of the European Academy of Microbiologists.

Poster Abstracts

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

Session 1
10:00 - 11:00 AM

Room A

Join Here

Alexandra Abu-Shmais
Katherine Almasy
Benjamin Fowler
Caitlin Murdoch
Nicholas Markham
Amyn Murji
Jenna Petronglo
Catherine Shelton
Jennifer Shuman

Room B

Join Here

Connor Beebout
John Brannon
Erin Green
Jennifer Gribble
Emma Guiberson
Lorena Harvey
Shannon Kordus
Lauren Walker
Christiaan Wijers

Session 2
12:00 - 1:00 PM

Join Here

Casey Butrico
Kara Eichelberger
Samantha Grimes
Catherine Leasure
Grace Morales
Matthew Munneke
Nicolas Shealy
Sirena Tran
Michelle Wiebe

Join Here

Kaitlyn Bunn
Nora Foegeding
Kevin Kramer
Cara Lang
Matthew Madden
Louise Rollins-Smith
Andrea Shiakolas
Wenqiang Song
Britton Strickland
Matt Vukovich

Session 3
2:00 - 3:00 PM

Join Here

Jonathan Davies
Taylor Engdahl
Dillon Kunkle
Jeanette Miller
Andrew Monteith
Rebecca Moore
Haley Overby
Hualiang Pi
Valeria M Reyes Ruiz
Jessica Sheldon

Join Here

Wei-Yao Chin
Carlos Detres-Roman
Megan Erwin
Natalie Favret
Justin Jacobse
Kathleen McClanahan
Luke Postoak
Michael Rudloff
Steven Scaglione
Guan Yang

Poster Abstracts

Session 1 - Room A

Generation of a Human Antibody-Antigen Atlas

Alexandra A. Abu-Shmais, Grant S. Hansman, M. Gordon Joyce, Masaru Kanekiyo, and Ivelin S. Georgiev

The B cell compartment of the adaptive immune system plays a critical role in various disease settings, particularly in the generation of potent antigen-specific antibodies against invading pathogens. Despite antibody discovery research efforts, there is limited data linking human antibody sequence to antigen specificity. Recently our laboratory has developed a technology termed Linked B cell Receptor to Antigen Specificity by Sequencing (LIBRA-seq) that enables the rapid identification of antigen-specific B cells by turning B cell receptor (BCR):antigen interactions into sequence-able events. Application involves physically mixing human B cells with a pool of oligonucleotide conjugated antigens, sorting for antigen positive B cells by fluorescence-activated cell sorting, and co-encapsulation of single B cells with bead-delivered oligos using droplet microfluidics. Here, I am proposing to leverage LIBRA-seq to search for BCRs capable of recognizing several biomedically relevant antigens including those associated with influenza, parainfluenza, norovirus, hepatitis B, hepatitis C, dengue, zika, human immunodeficiency virus, cytomegalovirus, and endemic as well as zoonotic coronavirus within pathogenically naïve donor repertoires. Implementation of a diverse antigenic library, representing multiple strains across 8 distinct viral families, will lead to a detailed analysis of the antibody response towards common infections and vaccinations and may yield the discovery of novel antibody therapeutics and antibody-based vaccine templates. LIBRA-seq will be of immense utility for high-throughput mapping of antibody sequence to antigen specificity, providing the basis for building a human antibody-antigen atlas.

Disrupting host-pathogen protein-protein interactions using host-targeted protein folding regulators

Katherine Almasy, Jonathan Davies, Samantha Lisy, Reyhaneh Tirgar, Sirena Tran, and Lars Plate

The COVID-19 pandemic has highlighted the importance of rapidly responding to emerging pathogens. The use of Remdesivir, initially investigated as a broad-acting RNA virus therapeutic, as a treatment for SARS-CoV-2 shows the advantage of having existing antiviral drugs which may potentially be used against future outbreaks. However, several classes of viruses have no current therapeutic options available; among these are the flaviviruses, which include such members as dengue, Zika, and yellow fever. Though several direct-acting antiviral compounds against these pathogens have been examined, many of these ultimately fail due to the development of resistance by the viral genome. We and others propose that instead of directly targeting viral proteins, another option is to target host processes required for the viral life cycle and disrupt macromolecular interactions to block viral replication. To this end, we have shown the ability of compounds **147** and **263**, initially designed to assist in host protein folding pathways, to act as antivirals against different strains of dengue and Zika virus. Mechanistic investigations of **147** have shown that the molecule likely causes perturbation of the structure of progeny virions after initial infection, thus affecting their ability to further infect cells. This occurs via covalent modification of cysteine residues on host proteins, and quantitative mass spectrometry approaches for target ID combined with RNAi screening of potential targets indicate that the combined modification of several (largely) endoplasmic reticulum resident proteins is likely responsible for the effect. Viral titers are lowered up to 99% across all strains tested, excitingly showing the potential of these host-targeted molecules to a) be used against several existing viruses and b) be starting points for future drug development against similar pathogens yet to come.

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Poster Abstracts

Session 1 - Room A

Human monoclonal antibodies to *Escherichia coli* bind proteins conserved across multiple pathotypes

Benjamin D. Fowler, Eric P. Skaar, and James E. Crowe Jr.

Escherichia coli typically is a harmless commensal organism in the human intestine. However, some *E. coli* strains are pathogenic, infecting many organ systems and causing distinct symptom profiles associated with specific *E. coli* pathotypes. This bacterium is concerning because of the spread of carbapenem-resistant or extended spectrum beta lactamase (ESBL)-producing strains, which the Centers for Disease Control and Prevention have respectively classified as Urgent or Serious threats. In the United States, these antimicrobial resistant strains annually infect over 200,000 people, killing over 10,000, causing unmeasured morbidity, and creating billions of dollars in healthcare costs. The pace of emergence of antimicrobial resistance exceeds the pace of antimicrobial development and requires urgent solutions. One class of antibacterial agents that has not yet been studied in detail is monoclonal antibodies (mAbs). MAbs have shown efficacy against viral and autoimmune diseases, most recently evidenced during the SARS-CoV-2 pandemic during which at least 24 mAbs began clinical trials. Their role as antibacterial agents has received less attention, but notable early clinical successes have been reported against *Staphylococcus aureus* (MEDI4983, 514G3, AR301) and *Pseudomonas aeruginosa* (MEDI3902). We screened human PBMCs for B cells encoding antibodies against proteins from *E. coli* outer membranes. We isolated and assessed five human mAbs that bind *E. coli*. Using a panel of isolates representing important pathotypes (UPEC, EPEC, & ETEC), we found that four mAbs reacted broadly and potently to all tested wild-type isolates, while one mAb bound five of seven WT isolates less potently. Using available knockout strains and recombinant proteins, we determined that four mAbs specifically targeted OmpA, the main outer membrane protein of *E. coli* and a virulence factor associated with numerous activities. We hypothesize that these mAbs will inhibit *E. coli* virulence, and are continuing to assess functional characteristics, epitopes, and therapeutic potential of these mAbs.

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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Session 1 - Room A

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

Nicholas O. Markham, Julia L. Drewes, Jada C. Domingue, Bob Chen, Cody N. Heiser, Molly A. Bingham, J. Alan Simmons, Austin N. Southard-Smith, Mary Catherine Macedonia, Marisol A. Ramirez, Erin N. Laubacher, Won Jae Huh, Martha J. Shrubsole, Qi Li, Robert J. Coffey, Cynthia L. Sears, and Ken S. Lau

Recent advances in microbiome biology suggest that, in addition to specific pro-carcinogenic driver bacteria, changes in the spatial organization of the gut microbiota known as invasive biofilm formation 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 cell alterations involved in biofilm-associated CRC development, we colonized germ-free $Apc^{Min/+}$ mice with a single gastric gavage of mucosal bacterial slurries derived from biofilm-positive human CRCs. Using this model, we have shown previously that mucosal slurries from a mixture of five human CRC-associated biofilms colonized the distal mouse colon mucosa 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 further in-depth analyses on two inocula: 3728T, which was associated with strong colon tumorigenesis (median = 6 macroadenomas, range 0-14, n = 8 mice) and 3979T, which was weakly tumorigenic (median = 0, range 0-2, n = 7 mice). 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 twofold 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 single-cell RNA sequencing (scRNA-seq) analysis revealed altered host immune populations, including increased tumor-associated neutrophils with the tumorigenic 3728T inoculum. At 2 weeks, 3728T induced consistent upregulation of MHC II and ROS-generating genes indicative of a mounting innate and adaptive immune response across colonocytes, goblet cells and stem cells. Also in corroboration of our increased β-catenin immunofluorescence, we saw evidence of Wnt pathway activation. 3979T induced upregulation of genes *suppressing* the Wnt pathway and some known to be downregulated in CRC. 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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Cross-clade Response to Multimeric HIV-1 Nanoparticle Immunogens

Amyn Murji, Juliana Qin, Tandile Hermanus, Lynn Morris, and Ivelin 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. BG505 nanoparticle and CZA97 nanoparticle cocktails as well as multivalent nanoparticles bearing both Env trimers elicited high titers in mice and guinea pigs. Guinea pigs, but not mice, immunized with nanoparticle immunogens neutralized vaccine-matched viruses. Furthermore, guinea pig sera neutralized heterologous viruses and recognized Envs from multiple, different clades. These results provide promise for multimerizing antigens on a nanoparticle-based platform. As such, our efforts are generalizable to vaccine design for other viruses that exhibit high levels of sequence diversity.

Determining the involvement of inflammasome activation in the osteoclast response to *Staphylococcus aureus*

Jenna R. Petronglo and Jim Cassat

Osteomyelitis, or infection in bone, is a debilitating inflammatory disease most commonly caused by the gram-positive pathogen *Staphylococcus aureus*. When *S. aureus* becomes entrenched in bone, the differentiation and function of the resident bone resorbing cell, the osteoclast, becomes altered. Increased formation of osteoclasts (osteoclastogenesis) and altered resorption by mature cells contribute to the collapse of bone homeostasis to favor bone loss. In published work, we demonstrated the important role the pro-inflammatory cytokine Interleukin-1 β (IL-1β) plays in promoting osteoclast-mediated bone loss during osteomyelitis. Macrophages release IL-1β in response to *S. aureus* through inflammasome activation, resulting in cytokine release and pyroptotic cell death. Whether osteoclasts, which share a common myeloid progenitor with macrophages, also engage an inflammasome when confronted by *S. aureus* remains largely unexplored. Using *in vitro* assays, we have found that osteoclasts are able to mount a caspase-1-dependent IL-1β response when stimulated with canonical inflammasome stimuli such as LPS and Nigericin. We show that osteoclasts have a reduced capacity to release of IL-1β in response to *S. aureus* supernatants, compared to macrophages. Moreover, because osteoclasts are specialized bone cells meant to polarize to a bone surface, we also investigated whether cell sensing of bone substrates influences release of IL-1β. We found a significant reduction of IL-1β release in osteoclasts and, to a lesser extent, macrophages responding to positive control and *S. aureus* stimuli while cultured with a bone substrate. These findings indicate that osteoclasts are capable of releasing IL-1β in response to a variety of stimuli, but inflammasome activation in response to *S. aureus* is likely environment-specific.

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Session 1 - Room A

Early-life disruption of gut microbiota and epithelial interactions promotes obesity

Catherine Shelton, Jessica Mo, Julia Thomas, Gillian Fitz, Nora Foegeding, Teresa Torres, Jacob Zieba, Matthew Tyska, and Mariana Byndloss

Emerging evidence indicates environmental factors, such as early-life antibiotic use and an obesogenic high-fat (HF) diet, increase the risk for childhood obesity. However, the mechanism by which antibiotic use, either alone or combined with a HF diet, increases adiposity remains unknown. The gut microbiota secretes metabolites that affect fat storage, partially via regulation of lipid absorption and secretion by the intestinal epithelium through largely unknown mechanisms. A potential regulator of intestinal lipid metabolism is peroxisome proliferator activator gamma (PPAR-γ), a nuclear receptor activated by compounds produced by the gut microbiota. We hypothesize that the use of antibiotics during early life depletes microbiota members responsible for PPAR-γ activation in the small intestine, which disrupts lipid metabolism in the intestinal epithelium and promotes obesity. To model early-life environmental perturbations, I exposed 3-week-old mice to low doses of penicillin (LDP) and an obesogenic HF diet. These mice developed greater adiposity compared to mice exposed to a HF diet alone. Mice treated with LDP and fed a HF diet displayed decreased expression of *Pparg* in their enterocytes as well as changes in several genes associated with lipid absorption and secretion. The downregulation of PPAR-γ signaling in mice exposed to antibiotics was associated with a significant decrease in intestinal *Lactobacillus* and *Lactobacillus*-derived metabolites. Importantly, a *Lactobacillus* species activated PPAR-γ signaling *in vitro* in intestinal epithelial cells. To identify the role of intestinal PPAR-γ activity in regulating body fat, we used epithelial-specific PPAR-γ knockout (iPPAR-γ^{KO}) mice. We determined that young iPPAR-γ^{KO} mice fed a HF diet developed increased adiposity compared to wildtype littermate controls. Together, our data reveal a novel mechanism by which early-life antibiotic treatment combined with a HF diet depletes *Lactobacillus* from the small intestine microbiota, resulting in reduced PPAR-γ signaling and promoting excess adiposity.

Gastric lipid alterations in response to *Helicobacter pylori* infection

Aung Soe Lin, Jennifer H. B. Shuman, Ankita Kotnala, Jeff A. Shaw, Amber C. Beckett, Jennifer L. Harvey, Michael Tuck, Beverly R. E. A. Dixon, Michelle L. Reyzer, Holly M. Scott Algood, Kevin L. Schey, M. Blanca Piazuelo, Timothy L. Cover

Helicobacter pylori colonization of the stomach results in chronic gastric inflammation and is a strong risk factor for development of peptic ulcer disease and, stomach cancer. In this study, we tested the hypothesis that *H. pylori* infection triggers alterations in gastric lipid composition. Mongolian gerbils were experimentally infected with *H. pylori* for 3 months. Conventional histologic staining revealed gastric inflammation in the infected animals but not in uninfected control animals. Atrophic gastritis, gastric mucosal hyperplasia, dysplasia and/or gastric cancer were detected in stomachs from several infected animals. We then used imaging mass spectrometry to analyze the relative abundance and spatial distribution of gastric lipids. We detected numerous lipids that differed in abundance when comparing gastric tissue from infected and uninfected animals. Analysis of gastric tissue from an independent cohort of *H. pylori*-infected gerbils and uninfected control animals confirmed many of these changes. Three phosphatidylcholine species (PC O-32:0, PC P-38:6, and PC 34:4), phosphatidylserine 38:5, and phosphatidylethanolamine 40:7 were more abundant in gastric tissues from infected gerbils than gastric tissues from uninfected gerbils. Lyso-phosphatidylethanolamine 18:1 and triglyceride 48:8 were detected in the corpus of uninfected stomachs and were reduced in abundance in *H. pylori*-infected stomachs that exhibited atrophic gastritis and gastric mucosal hyperplasia, but not in the stomachs of infected animals with less severe gastritis or in uninfected stomachs. Liquid chromatography tandem mass spectrometry analysis of lipid extracts from homogenized gastric tissues provided additional supportive evidence for identification of several of the differentially abundant lipids. These findings indicate that *H. pylori* infection can lead to alterations in gastric lipid composition and provide a new approach for identifying biomarkers of gastric atrophy and premalignant changes.

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Cytochrome bd promotes Escherichia coli biofilm antibiotic tolerance by regulating accumulation of noxious chemicals

Connor Beebout, Levy Sominsky, Allison Eberly, Gerald Van Horn, and Maria Hadjifrangiskou

Biofilms are multicellular bacterial communities commonly encountered in the environment and during infection. Within biofilms, bacteria consume or alter chemicals as they diffuse through the community, thereby generating a variety of nutrient gradients that ensure individual bacteria are exposed to highly variable local environmental conditions. This environmental heterogeneity induces bacteria to differentiate into metabolically distinct, and oftentimes cooperative, subpopulations which enhance the overall resilience and versatility of the community. We previously determined that oxygen availability spatially organizes respiration in uropathogenic *Escherichia coli* biofilms, and that the high affinity respiratory quinol oxidase cytochrome *bd* is necessary for extracellular matrix production and biofilm development. In this study we investigate the physiologic consequences of cytochrome *bd* deficiency in biofilms and determine that loss of cytochrome *bd* induces a biofilm-specific increase in expression of general diffusion porins, leading to elevated outer membrane permeability. In addition, loss of cytochrome *bd* impedes the proton mediated efflux of noxious chemicals by diminishing respiratory flux. As a result, loss of cytochrome *bd* enhances cellular accumulation of noxious chemicals and increases biofilm susceptibility to antibiotics. These results identify an undescribed link between *E. coli* biofilm respiration and stress tolerance, while suggesting the possibility of inhibiting cytochrome *bd* as an anti-biofilm therapeutic approach.

Mapping the Network of Two-component Systems in Uropathogenic *Escherichia coli*

J. R. Brannon, S. Reasoner, S. C. Wall, T.L. Dunigan, M.A. Wiebe, C. J. Beebout, T. Ross, A. Bamidele, D. Aronoff, and M. Hadjifrangiskou

Sensory systems allow pathogens to differentiate between different niches and respond to the stimuli within them. A major mechanism through which bacteria sense and respond to stimuli in their surroundings is two-component systems (TCSs). TCSs serve as bacterial logic gates that process sensory input with the net output result often being a change in gene expression. The primary pathogen that causes urinary tract infections (UTIs) is uropathogenic *Escherichia coli* (UPEC). Upon exiting the intestinal tract, UPEC's ascension begins with vaginal colonization, followed by transurethral ascension to the bladder. In the bladder, adherence to the urothelium triggers *E. coli* invasion of bladder cells and an intracellular pathogenic cascade. Some invading UPEC form metabolically quiescent intracellular reservoirs from which they may re-emerge and seed recurrent UTIs. Intracellular *E. coli* are safely hidden from host neutrophils, competition from the microbiota, and antibiotic that kill extracellular *E. coli*. Surviving all these diverse niches requires *E. coli* to sense their local environment and coordinate metabolic and virulence systems to balance survival requirements with limited nutrients. We hypothesize that specific TCSs allow UPEC to sense theses diverse environments encountered during infection. We utilize a targeted library of isogenic TCS deletion mutants to begin mapping the signaling networks contribution to phenotypes relevant to infection. We assessed the contribution of the TCSs for their contribution to planktonic and biofilm growth, aberrant metabolic activity, adherence and invasion on bladder epithelial cells, and in an acute UTI mouse model. We found that while some TCS have no apparent impact on *E. coli* in these growth conditions the deletion of other TCSs had remarkable changes in biofilm formation and cell adherence. The results indicate that while some systems are expendable, potentially a result of network redundancies, other TCSs are indispensable.

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A bacterial dehydrin promotes pathogen desiccation tolerance

Erin R. Green, Joseph Fakhoury, Andrew J. Monteith, David P. Giedroc, and Eric P. Skaar

The emerging bacterial pathogen *Acinetobacter baumannii* is a leading cause of hospital-acquired infections, and is found ubiquitously on hospital surfaces, which are considered a major reservoir for its transmission. Moreover, the COVID-19 pandemic has caused disruptions to standard infection control practices within hospitals, leading to recent surges in *A. baumannii* cases and underscoring the potential for future outbreaks with this pathogen. *A. baumannii* tolerates an onslaught of stresses encountered during persistence on environmental surfaces, including water loss due to extended periods of dryness, a process termed desiccation. However, the mechanisms underlying the extreme desiccation tolerance of *A. baumannii* and the impact of desiccation on pathogen transmission and infection severity remain largely unexplored. Here, we show that *A. baumannii* is hypervirulent following rehydration from desiccation and we uncover a central mechanism dictating desiccation tolerance in this pathogen. We report the discovery of the first bacterial dehydrin, the intrinsically disordered protein (IDP), DtpA, which is required for desiccation tolerance in *A. baumannii*. Recombinant DtpA is sufficient to protect purified enzymes from desiccation and heat inactivation, and heterologous expression extends the desiccation tolerance of a probiotic bacterium, indicating a broadly applicable function for DtpA in the preservation of proteins during denaturing conditions. Additionally, we show that DtpA expression is controlled by a conserved protease, revealing a key regulatory mechanism utilized by *A. baumannii* to tolerate environmental stress. In summary, our results uncover a previously unknown connection between environmental persistence and pathogenicity in *A. baumannii* and provide a mechanism for the extreme desiccation tolerance of this organism. Furthermore, these findings highlight a previously unexplored role for IDPs in bacterial stress tolerance and infection, and reveal potential applications for bacterial dehydrins to preserve activity of protein- and live bacteria-based pharmaceuticals that require desiccation for long term storage and transport.

The Coronavirus nsp14 Exoribonuclease is Required for High-Level RNA Recombination and Virus Fitness

Jennifer Gribble, Laura Stevens, Jordan Anderson-Daniels, Jim Chappell, Xiaotao Lu, Andrea J. Pruijssers, Andrew L. Routh, and Mark Denison

Coronaviruses (CoVs) are a genetically diverse group of positive-sense single-stranded RNA viruses containing the largest known genome of any RNA virus, >30 kb, and responsible for the current COVID-19 pandemic (SARS-CoV-2). CoV RNA-RNA recombination is essential for CoV sub-genomic mRNA synthesis and defective viral genome generation (DVG), and is proposed to be critical for novel CoV genome generation, evolution, and repair of mistakes during RNA synthesis. Here, we show that multiple betacoronaviruses including murine hepatitis virus (MHV), MERS-CoV, and SARS-CoV-2, generate extensive and diverse recombination products during replication in vitro. We further show that the nsp14 3′-to-5′ exoribonuclease (nsp14-ExoN) is required for normal CoV recombination and determines recombination junction site selection during replication and transcription. We further show that MHV can adapt to replication defects resulting from genetic inactivation of nsp14-ExoN activity, and that adaptation is associated with increased recombination that equals or exceeds WT levels. These results define the extent and patterns of CoV recombination and its association and requirement for virus fitness. The results also identify a novel recombination function for nsp14-ExoN in addition to demonstrated roles in fidelity, resistance to nucleoside analogs, antagonism of innate immunity, and in vivo virulence. The results increasingly emphasize the importance of nsp14 as a conserved and vulnerable target for inhibition and attenuation.

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Session 1 - Room B

Increased bile acid abundances in the small intestine during *Clostridioides difficile* infection

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

Clostridioides difficile is a spore-forming pathogen that impacts half a million people annually in the U.S. alone. *C. difficile* infections (CDI) are caused by the introduction of metabolically inactive spores to the gastrointestinal tract, which begin germinating in the presence of certain biological markers. One such germination factor is taurocholate (TCA), part of the biological class of molecules known as bile acids. Preliminary data using matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS) showed highly elevated TCA in the intestine of infected mice as compared to their mock-infected counterparts. LC-MS/MS data showed this elevation in a variety of other bile acids (TbMCA, CA, bMCA), with differences existing as early as 24 hours post infection with *C. difficile* spores. To determine if these changes are due to a secondary effect of CDI, samples from mice treated with dextran sulfate sodium to mimic inflammation from CDI were collected and analyzed for bile acid concentrations. DSS-treated and mock-infected mice showed no significant differences in concentrations, indicating that the influx of bile acids is specific to *C. difficile*. To see the potential impact on germination with limited access to bile acids, mice were then treated with cholestyramine, a bile acid binding resin, and infected with *C. difficile*. While bile acid concentrations showed minimal impact, mice treated with cholestyramine had hindered germination as compared to their mock-infected counterparts. This indicates that bile acids, and their increased abundance early in infection, are crucial for the success of *C. difficile*, giving insight into potential treatment and diagnostics for this increasingly prevalent infection.

Hop Outer Membrane Proteins as Determinants of *Helicobacter pylori* Fitness

M. Lorena Harvey, Aung Soe Lin, Lili Sun, Tatsuki Koyama, Jennifer H. B. Shuman, John T. Loh, Holly M. Scott Algood, Matthew B. Scholz, Mark S. McClain, and Timothy L. Cover

Helicobacter pylori genomes encode >60 predicted outer membrane proteins (OMPs). Several OMPs in the Hop family act as adhesins, but the functions of most Hop proteins are unknown. Here, we investigated the contributions of Hop OMPs to *H. pylori* fitness. We generated a library of *hop* mutant strains, each containing a unique nucleotide barcode, as well as a library of control strains, each containing a nucleotide barcode in an intergenic region predicted to be a neutral locus unrelated to bacterial fitness. We passaged each of the libraries *in vitro* and then analyzed changes in the populations over time. The control library proliferated as a relatively stable community *in vitro*, without substantial changes in the proportional abundance of individual strains. In contrast, there were marked changes in composition of the OMP mutant library after passage *in vitro*. Fitness defects of *hopE* and *oipA* mutants were consistently observed in experiments with multiple types of culture media. After orogastric infection of mice with the control library, there was a reduction in the population diversity and marked variation in the dominant strains recovered from individual animals, consistent with the existence of a non-selective bottleneck *in vivo*. Analysis of *H. pylori* cultured from mice infected with the OMP mutant library revealed a fitness advantage of the *babA* mutant. These data provide evidence that specific outer membrane proteins are determinants of *H. pylori* fitness, both *in vitro* and *in vivo*.

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The mechanistic basis for toxin secretion in *Clostridioides difficile*

Shannon Lynn Kordus, Heather Kroh, Audrey Thomas, Ben Spiller, and D. Borden Lacy

Understanding how large macromolecules are transported across a cell wall is a complex and poorly understood biological process. The nosocomial pathogen *Clostridioides difficile* produces and secretes two large toxins that are responsible for causing disease. Although much is known about the effector functions of the toxins, very little is known about how the toxins are secreted, as the toxins lack a secretion tag and are released independent of cell lysis. The toxins are encoded on a pathogenicity locus which also encodes TcdE, a holin-like protein and TcdL, the N-terminal remnant of an endolysin. While bacteriophages use holin/endolysin systems to trigger bacterial cell lysis and escape, multiple reports now suggest that TcdE is used for the secretion of the toxins. We will address the outstanding questions of how TcdE and TcdL interact with *C. difficile* toxins to create a pore and secrete toxins without causing cell lysis. TcdL has recently been discovered and its role in toxin secretion has not been fully explored. Our work showed that TcdL is important in maintaining cell wall homeostasis during toxins secretion. Specifically, deletion of *tcdL*, but not *tcdE*, led reduced of motility, increased biofilm formation, and resistance to cell wall stressors. Interestingly, TcdL phenotype is dependent on the presence of TcdE. This work will shed new on our understanding of toxin secretion in *C. difficile*.

The impact of gut commensal bacteria on HIV-1 vaccine Immunogenicity

Lauren Walker, Nagarajan Raju, Catie Shelton, Mariana Byndloss and Ivelin Georgiev

At present, there are ~40 million people worldwide infected with Human Immunodeficiency virus (HIV-1). It is estimated that between 0.5 million – 1.1 million people die of HIV-1-related illness each year. A key element in the fight against HIV-1 infection is the development of an effective prophylactic vaccine. Yet, after numerous vaccine efficacy trails, no candidate vaccine has been approved for clinical use. Failure of vaccine candidates can in part be attributed to heterogenous immune responses elicited by trial participants. Several host factors are thought to contribute to these variations in vaccine response. Recent evidence implicates the gut microbiome as a mediator of both viral and bacterial vaccine responses. With respect to HIV-1, particular HIV-1 monoclonal antibodies (mAbs) are cross-reactive with gut commensal antigens, suggesting the gut microbiome may be shaping the HIV-1 antibody response. To identify microbial antigens targeted by HIV-1 mAbs a screen was performed in which lysate from the bacterium Limosilactobacillus reuteri (*L. reuteri*) was probed for reactivity to known HIV-1 mAbs by western blotting. *L. reuteri* is a well-studied probiotic bacterium and can be found in the gastrointestinal tract of many mammals, including humans and mice. This uncovered mAb CH58, an antibody specific to HIV-1's envelope (Env) protein, binding to *L. reuteri* lysate. CH58 targets the V1V2 region on HIV-1 Env protein and antibodies targeting this region have been correlated with protection from infection in both human and non-human primate studies. Immunoprecipitation and mass spectrometry analysis have identified candidate proteins being targeted by CH58. Prior antibody response to commensal antigens, such as *L. reuteri*, prime the humoral immune response to HIV-1 vaccination and a better understanding of this relationship will have the ability to directly impact vaccine design and prevention strategies.

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Increased cardiolipin synthase gene content promotes cell envelope stress resistance and modulates innate immune cell interactions in *Acinetobacter baumannii*

Christiaan D.M. Wijers and Michael J. Noto

Pneumonia is a leading cause of morbidity and mortality worldwide. In bacterial pneumonia, both pathogen-specific factors and the host inflammatory response contribute to pathogenesis. Cardiolipin is a lipid found in both mitochondrial and bacterial membranes. Mitochondrial cardiolipin is a direct agonist of the NLRP3 inflammasome, leading to the production of pro-inflammatory cytokines such as IL-1β. Release of mitochondrial cardiolipin into extracellular fluid contributes directly to lung injury in pneumonia. In contrast, the contribution of bacterial cell envelope cardiolipin content to pneumonia pathogenesis is incompletely understood. *Acinetobacter baumannii* is a nosocomial pathogen and a causative agent of bacterial pneumonia. Here, the discovery that two otherwise isogenic variants of *A. baumannii* strain ATCC 17978 differ based on the presence of a 44-kb accessory genetic locus is described. This accessory locus encompasses several putative pathogenesis genes, including a gene predicted to encode a cardiolipin synthase (ClsC), an enzyme that catalyzes the synthesis of cardiolipin. Deletion of the accessory *clsC* gene significantly decreased *A. baumannii* virulence in a mouse model of pneumonia. Additionally, the accessory *clsC* gene contributed to *A. baumannii* surface associated motility and cell envelope stress resistance, and promoted bacterial uptake by macrophages, implicating a dichotomous role for this gene in *A. baumannii* pathogenicity. Overexpression of the accessory *clsC* gene increased the production of pro-inflammatory IL-1β, anti-inflammatory IL-10, as well as the murine neutrophil chemokine KC by infected macrophages. Finally, pneumonic infection of mice with the variant of *A. baumannii* naturally harboring the accessory *clsC* gene promoted a shift toward neutrophilic, and away from lymphocytic, lung inflammation. Further investigations into the effects of variable cell envelope cardiolipin content on inflammation and lung injury may uncover novel pathways at the host-microbe interface relevant to pneumonia pathogenesis.

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Staphylococcus aureus central metabolism regulation during osteomyelitis infection

Casey Butrico, Christopher Good, Brittney Gimza, and Jim Cassat

Staphylococcus aureus is a leading cause of antibiotic-resistant bacterial infections and can infect nearly every organ of the human body. One common manifestation of *S. aureus* disease is invasive bone infection, known as osteomyelitis. Osteomyelitis is considered one of the most difficult to treat infections, often necessitating long-term antibiotic treatment and surgical intervention. Hyperglycemia, characterized by increased blood glucose concentration, increases the risk for developing osteomyelitis. In mice treated with streptozotocin (STZ) to induce hyperglycemia and infected with our osteomyelitis model, we discovered increased bacterial burdens within infected femurs as well as other organs. Hyperglycemia is widely associated with increased inflammation and decreased directed immune cell functionality. Dissemination and reduced immune cell function may lead to *S. aureus* metabolic adaptations to survive in the altered nutrients, metabolites, and inflammatory state. To determine temporal and spatial *S. aureus* adaptations to stressors associated with hyperglycemia *in vitro*, we developed fluorescent reporters responsive to bacterial central metabolites and neutrophil inflammatory molecules. Confocal microscopy validated that *pckA*, a glucose-responsive gluconeogenesis gene, was repressed in the presence of glucose in in vitro models of bacterial abscess communities. Furthermore, RNAIII, a transcript responsible for repressing a number of *S. aureus* virulence factors, was expressed in the absence of glucose, suggesting the ability of *S. aureus* to initiate toxin production in the presence of abundant nutrients during infection. To obtain a comprehensive understanding of *S. aureus* metabolic and virulence changes during hyperglycemic osteomyelitis, we are conducting a transposon sequencing experiment to identify genes essential for staphylococcal growth *in vivo* in STZ treated mice. Finally, we are harnessing the power of Imaging Mass Spectrometry to understand the heterogeneity of lipids and metabolites *S. aureus* is exposed to *in vivo* during osteomyelitis, and we plan to apply these tools to hyperglycemic infected bone.

Characterizing *Candida albicans-Staphylococcus aureus* interactions during osteomyelitis

Kara R. Eichelberger and James E. Cassat

Polymicrobial infections pose a significant clinical problem, as the presence of multiple infecting organisms can drastically alter disease outcome and treatment. Due to many shared niches in the human body, the fungal pathogen *Candida albicans* and Gram-positive bacterium *Staphylococcus aureus* are commonly co-isolated from polymicrobial infections. Direct and indirect interactions between *C. albicans* and *S. aureus* contribute to lethal synergism during invasive co-infection, but the mechanisms underlying these interactions remain to be fully elucidated. Osteomyelitis is a devastating invasive infection of bone that can result from contiguous infectious spread. Treatment of osteomyelitis requires long-term antibiotic delivery and often surgical debridement of necrotic bone. Osteomyelitis is most frequently caused by *S. aureus*, but approximately 30% of cases that develop from a contiguous infection are polymicrobial. Due to the difficulty in treatment and the frequency of polymicrobial disease, osteomyelitis serves as a model infectious niche to study mechanisms of invasive polymicrobial infections. To explore interactions of *C. albicans* and *S. aureus* during osteomyelitis, I developed an in vitro workflow to examine the effects of *C. albicans-S. aureus* co-culture on cytotoxicity towards various skeletal cell populations. I determined that *C. albicans* enhanced *S. aureus* cytotoxicity towards bone cells in vitro, including osteoblasts and bone-marrow macrophages (osteoclast precursor). Interestingly, *C. albicans* also enhanced the cytotoxicity of *S. aureus* mutants that are non-toxic when grown in monoculture. This indicates that the presence of *C. albicans* can alter the *S. aureus* secreted toxin repertoire with varying impacts on bone cells. Additionally, *C. albicans* can impact *S. aureus*-induced differentiation of bone-marrow macrophages to osteoclasts. Pre-exposure of bone-marrow macrophages to *C. albicans* reduced osteoclast differentiation upon subsequent *S. aureus* stimulation. In summary, the presence of *C. albicans* can dramatically change *S. aureus* cytotoxicity and induced osteoclast differentiation, which may have profound impacts on osteomyelitis outcome.

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Coronavirus Helicase Mutation Contributes to Remdesivir Resistance

Samantha L. Grimes, Alexandra Abu-Shmais, Maria L. Agostini, Jennifer Gribble Bowser, Xiaotao Lu, Andrea J. Pruijssers, and Mark R. Denison

Coronaviruses (CoVs) have caused three outbreaks of severe disease in humans in the past 20 years, including the current global pandemic caused by Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2). This pandemic highlights the importance of developing countermeasures against emerging viruses and understanding current treatments for SARS-CoV-2. Currently, remdesivir (RDV), a nucleoside analogue with broad-spectrum activity against human and zoonotic CoVs, is the only FDA-approved antiviral in use to treat COVID-19, the disease caused by SARS-CoV-2. Widespread use of RDV increases the risk for potential resistance pathways to the drug. We previously reported that serial passaging of murine hepatitis virus (MHV) in GS-441524, the parent nucleoside of the prodrug remdesivir, yielded two resistance mutations located in the RNA-dependent RNA polymerase (RdRp). Reverse engineering of these two mutations together in a wild-type background resulted in a partial recapitulation of the resistance phenotype observed in the parental lineage. Four additional nonsynonymous mutations were identified in this resistant lineage, including a substitution mutation in the helicase, a key enzyme in the CoV replication-transcription complex (RTC). Modeling of this mutation locates the substitution near the predicted RNA-binding channel of the helicase. To determine if this mutation contributed to the resistance phenotype observed in passage, we engineered and characterized this helicase mutation. Our data suggest the helicase mutation by itself confers partial resistance to RDV, independent of the previously characterized RdRp mutations. Further, an engineered virus with the helicase mutation plus the RdRp mutations fully recapitulated the resistance phenotype of the passaged virus. This mutation did not confer alterations to replication kinetics. Importantly, the triple mutant was still sensitive to EIDD-2801, another nucleoside analogue currently in phase 2/3 clinical trials for the treatment of COVID-19. Together, these data suggest an important role for the helicase in RDV antiviral activity and expand an understanding of the molecular mechanisms underlying RDV resistance.

Heme binding to glutamyl tRNA reductase in *Staphylococcus aureus* does not impact GtrR post-translational regulation

Catherine S. Leasure, Jacob E. Choby, and Eric P. Skaar

Staphylococcus aureus is an important human pathogen responsible for a variety of disease states. The combination of increasing antibiotic resistance in this pathogen and the lack of an efficacious vaccine underscores the importance of understanding how *S. aureus* maintains homeostasis to succeed as a pathogen. Within the human host, *S. aureus* must regulate cellular levels of the molecule heme to support enzymatic activities without encountering heme toxicity. Previous work has identified the initial heme biosynthetic enzyme glutamyl tRNA reductase (GtrR) as an important node of regulation within the heme synthesis pathway in bacteria, archaea, and plants. In many of these organisms, heme status negatively regulates GtrR abundance and activity to control flux through the heme synthesis pathway. Using UV/vis spectrometry, we determined that *S. aureus* GtrR is capable of binding heme *in vitro*. Systematic mutagenesis of GtrR identified R214 as an important residue for GtrR binding to heme but strains expressing $GtrR^{R214}$ showed no difference in GtrR abundance and did not show signs of heme stress seen in strains with dysregulated GtrR. Taken together, these results suggest that a direct interactions between GtrR and heme does not impact heme synthesis in *S. aureus*.

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Characterizing the Mobilome of Urinary and Fecal *Escherichia coli* Isolates

Grace Morales, Benjamin Abelson, Maria Hadjifrangiskou, and Jonathan Schmitz

Escherichia coli is a diverse organism responsible for approximately 75% of urinary tract infections. It also colonizes the gut as a commensal. With a genome that ranges in size between 4.5-5.5Mb, *E. coli* is genomically diverse with what has been described as "open pangenome." Much of this genomic diversity is attributed to horizontal gene transfer mechanisms. The goal of this study was to evaluate how prophages and other mobile genetic elements contribute to *E. coli* diversity and carriage of fitness factors. Using 138 urinary *E. coli* isolates from the *micro*VU collection at Vanderbilt University Medical Center (VUMC) and the genomes of 50 fecal isolates from the NCBI, we preformed genomic analysis using predictive software to identify mobile genetic elements in each sample. The *micro*VU isolates underwent genomic DNA isolation, sequencing, and assembly, followed by annotation using Prokka. To characterize the mobilome, all *E. coli* isolates were queried for prophage sequences using two independent methods, PHASTER and Prophage Hunter. Integrative and conjugative elements (ICE) were identified using ICEfinder. We identified that fecal isolates carry a higher proportion of prophage sequences compared to the urinary isolates. These prophage sequences additionally do not significantly contribute to fitness factor or antibiotic resistance gene carriage in the urinary isolates. We conclude that the diversity seen in urinary isolates is likely attributed to other mechanisms of horizontal gene transfer.

Excess zinc alters metabolic homeostasis in *Clostridioides difficile*

Matthew J. Munneke, Reece J. Knippel, Hualiang Pi, Andy Weiss, and Eric P. Skaar

Clostridioides difficile is a problematic human pathogen that is the leading cause of antibiotic-associated nosocomial infections and is responsible for 500,000 infections and 20,000 deaths annually. *C. difficile* infection (CDI) typically proceeds following disruption of the gut microbiota by antibiotics or other perturbations. The incidence and recurrence of CDI has increased in the last decade due to the limited efficacy of current therapeutic strategies. This highlights the need to develop an understanding of the factors that allow *C. difficile* to establish and maintain colonization of the mammalian gastrointestinal tract to identify new therapeutic targets. Transition metals are an essential nutrient for cellular physiology, and colonization of a host requires mechanisms to maintain metabolic homeostasis in dynamic metal environments. High dietary zinc (Zn) increases Zn concentrations in the murine gut, alters the community structure of the gut microbiota, and decreases the amount of antibiotics necessary to confer susceptibility to CDI. We have discovered that in response to excess Zn, *C. difficile* responds transcriptionally to excess Zn by increasing the abundance of transcripts involved in carbohydrate transport and metabolism and decreasing the abundance of transcripts involved in translation and transition metal import. The transcript abundance for *CD196_RS15350* (*15350*), a putative energy coupled factor nucleoside transporter is increased following treatment with a bolus of Zn. In addition, the transcript abundance for *CD196_RS15345* (*15345*) is increased in response to excess Zn, and 15345 shares an intergenic region with *15350* that is also conserved across several anaerobic bacteria. Three-dimensional modeling of 15345 revealed the conservation of key catalytic residues for 2-thiouracil desulfuration. We hypothesize that 15345 and 15350 function together in thiouracil salvage and conversion in response to excess Zn. Future studies will investigate the fate of the product from 15345 and 15350 activity during Zn toxicity.

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Microbiota-derived Amino Acids Enable *Salmonella* Typhimurium to Overcome Enterobacteriaceae Mediated Colonization Resistance

Nicolas G. Shealy, Teresa P. Torres, Woongjae Yoo, Jacob Zieba, Catherine D. Shelton, Nora Foegeding, and Mariana X. Byndloss

The gut microbiota protects the host against pathogens (colonization resistance), via nutrient competition. To cause disease, *Salmonella enterica* serovar Typhimurium (*S.* Tm) must overcome colonization resistance. However, the mechanisms used by *S.* Tm to outcompete the microbiota remain unclear. *S.* Tm infection elicits gut inflammation, causing release of host-derived antimicrobial molecules (e.g., reactive oxygen species, ROS). This inflammatory response alters the gut's metabolic landscape. It promotes pathogen expansion via respiration-dependent oxidative metabolism. However, the nutrient sources in the inflamed gut that fuel *S.* Tm growth are largely unknown. I have shown that *S.* Tm-dependent intestinal inflammation causes a significant increase in aspartate (Asp) bioavailability in the gut in a microbiota-dependent manner. *I hypothesize that ROS produced during intestinal inflammation disturb gut commensal homeostasis, allowing S. Tm to take advantage of increased Asp availability to outcompete the microbiota.* To test this hypothesis, I have conducted *in vitro* and *in vivo* experiments using conventional and germ-free mice to determine the mechanism by which the microbiota releases Asp and how *S.* Tm uses Asp to overcome colonization resistance. Using a drug-inducible suicide model, I observed that microbiota members' lysis promotes *S.* Tm expansion in an Asp-dependent manner. Commensal *E. coli* strains play a key role in resistance against *S.* Tm. Interestingly, *S.* Tm significantly outcompetes *E. coli in vitro* when cultured in the presence of Asp, nitrate, and glycerol. Lastly, deletion of the enzymes necessary for Asp-dependent fumarate respiration abrogates the advantage of *S.* Tm over *E. coli* under *in vitro* conditions that resemble those of the inflamed gut. My results suggest that *S.* Tm uses newly available resources (i.e., Asp) more effectively than the resident Enterobacteriaceae, likely due to differential gene regulation. This study provides a novel mechanism used by *S.* Tm to thrive in the inflamed gut via metabolic adaptation.

Essential Role of the CagY Antenna Projection in *Helicobacter pylori* Type IV Secretion System Activity

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

Helicobacter pylori is a gram-negative bacterium that colonizes the stomach in about 50% of the world's population. Most individuals colonized with *H. pylori* remain asymptomatic, but the presence of these bacteria is a risk factor for peptic ulcer disease and gastric adenocarcinoma. *H. pylori* strains harboring a chromosomal genetic element known as the *cag* pathogenicity island are more likely to cause disease than are strains lacking this genetic element. The *cag* pathogenicity island encodes a secreted protein (CagA) that contributes to cancer pathogenesis and components of a type IV secretion system (T4SS) that delivers CagA into host cells. Recent cryo-EM and cryo-ET analyses provided insight into the molecular architecture of the *H. pylori* Cag T4SS. "Antenna projections" [AP]) of CagY, a component of the T4SS that extends from the inner membrane to the outer membrane, are hypothesized to form a channel through the outer membrane and contact host cells. The goals of this project are to elucidate the functional properties of the CagY AP and define its location in relation to the outer membrane. I have successfully generated multiple *H. pylori* strains that produce mutant forms of CagY. An in-frame deletion of the *cagY* region encoding the CagY AP abolished the capacity of *H. pylori* to stimulate NF-kB activation in gastric epithelial cells (a phenotype dependent on T4SS activity). Insertion of an HA epitope tag into an unstructured portion of the AP did not alter T4SS activity and allowed immunopurification of CagY. Conversely, anti-HA antibodies did not block the capacity of this strain to cause T4SS-mediated alterations in gastric epithelial cells. These results indicate that the CagY AP is required for T4SS activity and provide further insight into structural features of CagY.

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BtsS-YpdB two-component system cross regulation constitutes a new acid-sensing system in uropathogenic *E. coli*

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

Uropathogenic *E. coli* (UPEC), the principal cause of urinary tract infections (UTIs) worldwide, has an arsenal of acid-stress response mechanisms to deal with severe acid stress. Yet the mechanisms by which UPEC respond to milder acidic conditions, such as those encountered in the vaginal space – where it forms a reservoir - remain undefined. In this work we describe an acid-sensing signal transduction mechanism that facilitates UPEC resistance to mild acid stress via the de-amination of L-serine. We show that in response to a pH range of 4-5, the BtsS histidine kinase and the non-cognate YpdB transcription factor control expression of the *yhjX* gene that codes for a putative Major Facilitator Superfamily (MFS) transporter. Induction of yhjX in a BtsS-YpdB dependent manner occurs in response to several acids, or L-serine and is abrogated in mutants lacking *btsS* and *ypdB* or in mutants defective for L-serine deamination or import. L-serine is imported in the cell by thee SdaC transporter and deaminated to generate ammonia and pyruvate by the L-serine deaminases SdaA and SdaB. Deletion of *yhjX, btsSypdB* or *sdaA* diminish the ability of UPEC to resist acid. Deletion of *yhjX* alters intracellular and extracellular pyruvate levels. Based on these observations, our current model supports that BtsS-YpdB constitute a previously uncharacterized acid-sensing system in *E. coli* that controls intracellular pH via L-serine de-amination. Future studies will focus on determining the role of YhjX as a pyruvate exporter.

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T cell-derived extracellular vesicles in the allergic airway

Kaitlyn E. Bunn and Heather H. Pua

T cells, immune cells that drive allergic airway inflammation, are known to secrete extracellular vesicles (EVs) following stimulation. *In vitro*, it has been shown that T cell-derived EVs can induce pro-inflammatory behaviors in target cells. *In vivo*, the presence and relevance of T cell-derived EVs to allergic airway pathology is not known. We previously found that immune cell-derived EVs are increased in the airways of mice with induced allergic airway inflammation compared to control mice. The goal of this study was to determine if T cells contribute to these immune cell-derived EVs. We induced allergic airway inflammation in mice by ovalbumin sensitization and challenge and collected bronchoalveolar lavage fluid (BALF) from mice challenged with either vehicle or allergen in the airways. We used T cell specific labeling of EV membranes *in vivo* coupled with high sensitivity flow cytometry to detect T cell-derived EVs in the BALF. We additionally used fluorophore-coupled antibody labeling to assess the presence of surface cargoes carried by T cell EVs. We found that T cell-derived EVs were present in the BALF of mice challenged with allergen in the airways but absent from the BALF of vehicle-challenged mice. We identified that 3% of vesicles in the BALF of mice with induced allergic airway inflammation were of T cell origin and recovered over 6 million T cell-derived EVs per airway. We additionally found that T cell EVs carry CD9, a well-known surface marker of EVs. These results provide evidence that T cells recruited to the airways during allergic airway inflammation contribute to the population of immune cell-derived EVs in the airway and may also promote intercellular communication during the immune response and allergic asthma pathogenesis.

High-Fat Diet Induced Mitochondrial Dysfunction Links Intestinal Dysbiosis and Colorectal Cancer

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

Colorectal cancer (CRC) rates among young adults are increasing at an alarming rate. While the underlying causes remain unclear, one of the drivers of early-onset CRC may be a Western-style high-fat diet (HFD) and associated diet-induced obesity (DIO). Emerging evidence suggests alterations in gut microbiota composition (dysbiosis) may be an important mechanistic link between HFD consumption and CRC. Specifically, expansion of facultative anaerobic Enterobacteriaceae is associated with both HFD consumption and CRC, and tumorigenic strains of *Escherichia coli* (a member of the Enterobacteriaceae family) that produce the genotoxin colibactin are frequently overrepresented in the microbiota of CRC patients. Altogether, this suggests a key role of colibactin-producing *E. coli* expansion in CRC. Yet, factors that permit the intestinal bloom of tumorigenic *E. coli* are incompletely understood. Gut microbiota composition is largely shaped by the metabolism of colonocytes, which consume oxygen via mitochondrial β-oxidation, limiting oxygen diffusion into the colonic lumen. In this way, the anaerobiosis of the colon helps maintain the dominance of obligate anaerobes. Using mouse models of DIO, we have found that consumption of HFD alters colonocyte metabolism and colonic anaerobiosis to support the sustained bloom of colibactin-producing *E. coli*. Competitive infection studies reveal that expansion of colibactin-producing *E. coli* in the HFD gut is driven by the ability of *E. coli* to utilize oxygen, as well as reactive oxygen and nitrogen species, for aerobic and anaerobic respiration. Using *in vitro* metabolism assays, we show that exposure of an epithelial cell line to elevated levels of fatty acids, consumed at a high level on HFD, impairs mitochondrial respiration and causes an increase in reactive oxygen species. Collectively, our results suggest that obesogenic HFDs perturb colonocyte mitochondrial function and metabolism, causing the expansion of *E. coli* linked to CRC development.

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Cross-reactive coronavirus antibodies with diverse epitope specificities and Fc effector functions

Kevin J. Kramer, Andrea R. Shiakolas, Daniel Wrapp, Simone I. Richardson, Alexandra Schäfer, Steven Wall, Nianshuang Wang, Katarzyna Janowska, Kelsey A. Pilewski, Rohit Venkat, Robert Parks, Nelia P. Manamela, Nagarajan Raju, Emilee Friedman Fechter, Clinton M. Holt, Naveenchandra Suryadevara, Rita E. Chen, David R. Martinez, Rachel S. Nargi, Rachel E. Sutton, Julie E. Ledgerwood, Barney S. Graham, Michael S. Diamond, Barton F. Haynes, Priyamvada Acharya, Robert H. Carnahan, James E. Crowe Jr, Ralph S. Baric, Lynn Morris, Jason S. McLellan, and Ivelin S. Georgiev

The continual emergence of novel coronavirus (CoV) strains, like SARS-CoV-2, highlights the critical need for broadly reactive therapeutics and vaccines against this family of viruses. Coronavirus spike (S) proteins share common structural motifs that could be vulnerable to cross-reactive antibody responses. To study this phenomenon in human coronavirus infection, we applied a high-throughput sequencing method called LIBRA-seq (Linking B cell receptor to antigen specificity through sequencing) to a recovered SARS-CoV donor sample from more than ten years after infection. We identified and characterized a panel of six monoclonal antibodies that cross-reacted with S proteins from the highly pathogenic SARS-CoV and SARS-CoV-2, and demonstrated a spectrum of reactivity against other coronaviruses. Epitope mapping revealed that these antibodies recognized multiple epitopes on SARS-CoV-2 S, including the receptor binding domain (RBD), N-terminal domain (NTD), and S2 subunit. Functional characterization demonstrated that the antibodies mediated phagocytosis - and in some cases trogocytosis - but not neutralization *in vitro*. When tested *in vivo* in murine models, two of the antibodies demonstrated a reduction in hemorrhagic pathology in the lungs. The identification of cross-reactive epitopes recognized by functional antibodies expands the repertoire of targets for pan-coronavirus vaccine design strategies that may be useful for preventing future coronavirus outbreaks.

Efferocytosis of apoptotic cancer cells in the TME drives myeloid inflammasome signaling and Gasdermin D independent secretion of IL-1β to promote tumor growth

Cara Lang, Sohini Roy, Yu Wang, Michael Korrer, and Young Kim

Myeloid intrinsic caspase-1 signaling can promote T-cell independent tumor growth *in vivo*, however the mechanism driving myeloid inflammasome signaling remains unclear. To characterize inflammasome signaling in the human tumor microenvironment (TME), we performed both bulk and single cell RNA-sequencing of untreated human HNSCC and found that inflammasome transcriptomic activity was more prominent in the tumor infiltrating myeloid cells rather than the epithelial cancer cells. In order to characterize the pro-tumorigenic effects of myeloid inflammasome signaling, we examined the TME of tumors grown in caspase-1 knockout mice, and we noted a reduction in myeloid cell infiltration while no differences in T cell infiltration were found, suggesting that myeloid cell turnover is inversely regulated by inflammasome activation in the TME. Tumor infiltrating myeloid cells from caspase-1 null mice demonstrated a higher apoptosis rate *ex vivo* but when cultured with tumor cell conditioned media, we saw an increase in overall myeloid cell viability. *In vivo*, we noted significant tumor growth suppression in NLRP3 null and caspase-1 null mice and not in Gasdermin D null mice. Blocking IL-1β *in vivo* also resulted in a blunted tumor growth rate indicating the utilization of Gasdermin D independent methods of cytokine secretion in the TME. We present analysis of single cell transcriptomic profiles of human HNSCC which demonstrate elevated inflammasome transcriptomic activity in the tumor infiltrating myeloid cells including the inflammasome sensor NLRP3, but not AIM2. Next we determined NLRP3 dependent inflammasome signaling and IL-1β production within the tumor infiltrating myeloid cells was directly linked to efferocytosis of dying tumor cells. Cumulatively we demonstrate that efferocytosis drives myeloid inflammasome dependent tumor growth.

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Glucose and glutamine partitioning in the tumor microenvironment

Matthew Z Madden, Bradley I Reinfeld, Melissa M Wolf, Anna Chytil, Jackie E Bader, Andrew R Patterson, Allison S Cohen, Rachel A Hongo, Kirsten L Young, Rachel E Brown, Vera M Todd, Tessa Huffstater, Abin Abraham, Fuxue Xin, Noor M Tantawy, W David Merryman, Rachelle W Johnson, Christopher S Williams, Frank E Mason, Emily F Mason, Katy E Beckermann, H Charles Manning, Jeffrey C Rathmell, and W Kimryn Rathmell

The tumor microenvironment (TME) includes transformed cancer and infiltrating immune cells. Tumor cells can consume large quantities of glucose through Warburg metabolism that can be visualized with positron emission tomography (PET). Activated immune cells also can rely on glucose, but how immune cell metabolism is programmed or restrained by competition for nutrients with cancer cells remains uncertain. Here we used PET tracers to measure the accessibility of glucose and glutamine to cell subsets in the TME. Myeloid cells were the greatest consumers of intra-tumoral glucose, followed by T cells and cancer cells. Cancer cells, in contrast, had the highest glutamine uptake. This distinct nutrient partitioning was programmed through selective mTORC1 signaling and glucose or glutamine-related gene expression. Treatment with mTORC1 inhibitor rapamycin *in vivo* decreased glucose and glutamine uptake across tumor cell types and reduced the expression of key metabolic proteins. Inhibition of glutamine uptake enhanced glucose uptake across tumor cell types demonstrating that glucose is not limiting in the TME. Thus, cancer cells are not the only cells in tumors which exhibit high glucose uptake *in vivo* and instead preferentially utilize glutamine over other cell types. Intrinsic cellular programs can play a major role in the use of some nutrients. Together, these data argue that cell selective partitioning of glucose and glutamine can be exploited to develop therapies and imaging strategies to alter the metabolic programs of specific cell populations in the TME.

Amphibian Phagocyte Functions are Inhibited by the Deadly Chytrid Fungus, *Batrachochytrium dendrobatidis*

Louise A. Rollins-Smith, William G. Payne, and Laura K. Reinert

Previously, we showed that *Batrachochytrium dendrobatidis (Bd)* sporangia and supernatant factors inhibit amphibian and human lymphocytes and induce apoptosis. Here, we present evidence that the inhibition extends to phagocytic cells. Peritoneal phagocytes (enriched for MΦs and neutrophils) were induced by injection of killed bacteria into *Xenopus laevis* adult frogs and exposed to *Bd* sporangia or cell-free supernatant factors (*Bd* Sup) for 24 hr. Following the cell co-culture, fungal cells were removed by treatment with amphotericin B and a wash step to remove the antifungal drug. Treated phagocytes or untreated control cells were then exposed to pHrodo Green™ Zymosan Bioparticles™, and fluorescence of beads in the phagolysosomal compartments was measured after 2 hr. Phagocytes co-cultured with *Bd* Sup engulfed about 58.5 ± 9.9% fewer zymosan particles than untreated control cells (N=7 replicate experiments) while those directly exposed to *Bd* sporangia engulfed 61.4 ± 9.5% fewer zymosan beads that control cells (N = 3 replicate experiments) demonstrating that *Bd* cells can also inhibit this important cell function. Overall, these finding suggest another mechanism by which the fungal cells can evade immune clearance in the skin (Support NSF IOS-1557634 and IOS-2011291).

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Rapid and efficient discovery of potently neutralizing SARS-CoV-2 antibodies using LIBRA-seq with ligand blocking

Andrea Shiakolas, Kevin Kramer, Daniel Wrapp, Nicole Johnson, Naveenchandra Suryadevara, Kelsey Pilewski, Nagarajan Raju, Natalia Kuzmina, Alexander Bukreyev, Jason McLellan, James Crowe Jr, and Ivelin Georgiev

The emergence of a novel coronavirus (CoV), SARS-CoV-2, has resulted in a worldwide pandemic, threatening the lives of millions and imposing an immense burden on healthcare systems and the global economy. The devastating effects of the COVID-19 pandemic have highlighted the critical need for rapid, high-throughput screening tools for antibody discovery against viral pathogens. Antibodies can be utilized as therapeutic molecules, and studying antibody-antigen interactions can be exploited in vaccine design strategies during both pandemic emergencies and for other health concerns as well. With typical antibody screening tools, hundreds to thousands of antibodies must be screened, expressed, and tested to identify neutralizing antibody candidates for further characterization. In particular, though therapeutic antibody discovery efforts against SARS-CoV-2 have been generally successful, they have been associated with the production of large numbers of antibodies with low hit rates for the identification of lead candidates. Here, we incorporated antibody-ligand blocking as part of LIBRA-seq, the high throughput sequencing platform for antibody discovery. By using SARS-CoV-2 spike (S) and its receptor ACE2, we applied the LIBRA-seq with ligand blocking technology to convalescent SARS-CoV-2 samples and demonstrated high rates of neutralizing antibody identification (90% of predictions confirmed), including the discovery of several ultra-potent SARS-CoV-2 antibodies. The antibodies identified targeted diverse epitopes across the S protein and bound to several major circulating S variants. A better understanding of the sequence features, epitopes, and functional characteristics of potent, SARS-CoV-2 neutralizing antibodies may translate into strategies for current vaccine design efforts and additional measures to counteract potential future pandemic variants. Overall, leveraging LIBRA-seq with ligand blocking will enable general antibody discovery targeting the disruption of antibody-ligand interactions and can ultimately facilitate the creation of better vaccines and therapies in a variety of disease settings.

Adipocyte-specific PIK3C3/VPS34 controls adipogenesis, metabolically-triggered inflammation, insulin resistance, and adaptive thermogenesis in mice

Wenqiang Song, Guan Yang, J. Luke Postoak, Jianhua Zhang, Lan Wu, and Luc Van Kaer

Interactions between cells of the immune and metabolic systems play critical roles in controlling immune responsiveness and metabolic health. When these interactions are dysregulated, metabolic diseases such as obesity or lipodystrophy may develop. We have focused on cellular processes related to autophagy, a cellular self-degradation process, in controlling interactions between the immune and metabolic systems. In this study, we analyzed mice with an adipocyte-selective deficiency in PIK3C3/VPS34, a key early player in autophagy. These animals showed reduced white and brown adipose tissue (WAT and BAT) with enhanced macrophage infiltration, systemic immune activation, ¬and spontaneous insulin-resistance. The phenotype of these animals bears a striking resemblance with the disease manifestations seen in individuals with lipodystrophy. Mechanistically, we found that conditional *Pik3c3*-deficiency inhibits adipogenesis in both WAT and BAT by downregulating glucose uptake and fatty acid metabolism, and impairs the thermogenic function of BAT by downregulating the expression of key regulators (*Prdm16, Ucp1,* and *Cidea*). Upon acute cold exposure, *Pik3c3*-deficient mice became cold-sensitive under fasting conditions due to insufficient thermogenesis in BAT. In contrast, these animals exhibited blunted beige fat thermogenesis in subcutaneous WAT in response to administration of a b3-adrenergic receptor agonist. Collectively, our data demonstrate that PIK3C3 is a vital regulator of adipose tissue development, metabolically-triggered inflammation, insulin resistance, and adaptive thermogenesis. Hence, we identify PIK3C3 as a potential therapeutic target for metabolic diseases such as type 2 diabetes.

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Novel host-microbiome interactions reduce severe disease outcomes caused by Respiratory Syncytial Virus (RSV) infection

Britton A. Strickland, Meghan H. Shilts, Helen Boone, Hunter Brown, Mira C. Patel, Arash Kamali, Wei Zhang, Daniel Stylos, Marina S. Boukhvalova, Seesandra V. Rajagopala, Fang Yan, Jorge C. G. Blanco, and Suman R. Das

RSV acute respiratory infection during infancy strongly correlates with subsequent childhood recurrent wheezing and asthma. We and others have shown that airway microbial communities, specifically members of the genus *Lactobacillus*, protect from RSV disease severity and wheezing outcomes in infants; however, limited data are available to establish the mechanisms of *Lactobacillus*-mediated protection of early-life RSV disease outcomes. The cotton rat (genus *Sigmodon*) is the gold-standard animal model for respiratory viral diseases, especially RSV immuno-pathology. Recently we have comprehensively characterized the gut microbiome (16S and metagenomics) of two inbred cotton rat species, *S. hispidus* and *S. fulviventer*, to show distinct species-level differences in gut microbiome communities: *S. hispidus* has a strikingly higher abundance of *Lactobacillus* (~2.5 log2fold higher) that correlated with reduced disease severity. Isolation of *Lactobacillus* from *S. hispidus* and gavage into *Lactobacillus*-deficient *S. fulviventer* revealed reduced lung histopathology upon RSV infection. Based on these findings, we hypothesize that presence and abundance of *Lactobacillus* in gut is protective for severe RSV disease in cotton rats through systemic immunomodulation (gut-lung axis). To determine the more specific mechanisms of protection, we conducted *Lactobacillus* metabolite conditioning of RSV-infected epithelial cell lines using a previously described secreted protein *p40*. Pre-treatment of epithelia with *p40* revealed increased activation of cell proliferation pathways, interference with viral pathogenicity pathways, reduction of viral load, and protection against viral-mediated apoptosis. Further experimentation includes RNA-seq of infected epithelia for transcriptomic analysis and *in vivo* testing of intranasal *p40* on RSV infectivity. This project will greatly improve our understanding of host-microbiome-mediated protection against severe RSV outcomes.

Multivalent Immunogens for the Elicitation of HIV-1 Env Broadly Neutralizing Antibodies

Matthew J. Vukovich, Nagarajan Raju, and Ivelin S. Georgiev

The incredible diversity of the HIV-1 envelope glycoprotein (Env) between strains makes creation of a protective vaccine especially challenging. Attempts at incorporating Env diversity in vaccination strategies have had limited success, likely due to relatively arbitrary strain selection. Our lab developed a multi-objective optimization algorithm that simultaneously optimized Env strain selection based on factors that favor the elicitation of broadly neutralizing antibodies (bNAbs) including glycan shield coverage, availability of bNAb epitopes, and the level of sequence diversity between strains. We expect that immunizations of guinea pigs with this optimized strain set will elicit antibodies with a wider breadth of Env neutralization compared to previous immunization studies. Furthermore, we are comparing different immunization strategies with our strain set including sequential, cocktail, and multivalent display on nanoparticles. Nanoparticles that display multiple different Env strains on one particle can more strongly engage low affinity bNAb precursor B cell receptors due to increased avidity. We are using a modified form of insect ferritin that has been truncated and fused to Env to construct nanoparticles that each display two different Env strains from our optimized set. To increase nanoparticle stability, we added a mutation that introduces a disulfide bond between monomers. Additionally, we are producing DNA nanoparticles that have the potential for precise attachment of greater than two strains of Env per nanoparticle. We found that crosslinking DNA with AMT increased the stability of DNA in serum, thereby offering a method to increase the stability of our DNA nanoparticles in vivo. Thus, we are approaching a leading idea in the field both through innovative strategies for immunogen design and through a systematic evaluation of critical variables, with the goal of developing a vaccine candidate that can elicit bNAb responses.

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Comparative multiplexed interactomics of SARS-CoV-2 and homologous coronavirus nonstructural proteins

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

Human coronaviruses (hCoVs) are a threat to global health and society, as evident from the SARS outbreak in 2002, the MERS outbreaks in 2012 and 2014, and the most recent COVID-19 pandemic. Despite the sequence similarity between these severe disease-causing hCoVs, 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 three CoV nonstructural proteins (nsps) that are critical for virus replication. We use tandem mass tag-multiplexed quantitative proteomics to sensitively compare and contrast the interactome of nsp2, nsp3, nsp4 from three betacoronavirus strains: SARS-CoV-1, SARS-CoV-2, and hCoV-OC43 – an endemic strain associated with the common cold. In addition, we include nsp3 homologs from MERS-CoV and a common cold alphacoronavirus, hCoV-229E. 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. Both nsp2 and nsp4 common interactors are strongly enriched for proteins localized at mitochondria-associated ER membranes suggesting a new functional role for modulating host processes, such as calcium homeostasis, at these organelle contact sites. The nsp3 constructs showed more variation from strain to strain, including interactions with nuclear import machinery for hCoV-229E and with rRNA processing for MERS-CoV. Our results shed light on the role these CoV proteins play in the infection cycle, as well as host factors that may mediate the divergent pathogenesis of common cold CoVs from SARS/MERS 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.

Broad and potently neutralizing monoclonal antibodies isolated from human survivors of New World hantavirus infection

Taylor B. Engdahl, Natalia A. Kuzmina, Adam J. Ronk, Chad E. Mire, Matthew A. Hyde, Nurgun Kose, Rachel E. Sutton, Apoorva Mehta, Rachael M. Wolters, Nicole M. Lloyd, , Francisca R. Valdivieso, Thomas G. Ksiazek, Alexander Bukreyev, and James. E. Crowe, Jr.

Hantaviruses are high-priority, emerging pathogens that are carried predominantly by rodents and transmitted to humans following inhalation of aerosolized feces. New World hantaviruses (NWHs) are endemic in Nºrth and South America, and cause hantavirus cardiopulmonary syndrome (HCPS), with a case fatality rate of up to 40%. There are currently no FDA-approved vaccines or therapeutics for NWHs, and the only treatment for patients is supportive care to prevent respiratory failure. Hantavirus-specific antibodies protect against a severe disease in animal models, and a high serum neutralizing antibody titer correlates with a higher chance of survival in human patients. However, there is limited understanding of the natural humoral immune response to NWH infection. Here, we describe a panel of human monoclonal antibodies (mAbs) isolated from individuals previously infected with Sin Nºmbre virus (SNV) or Andes virus (ANDV). Most of the SNV-reactive mAbs showed broad recognition across NWH and OWH species, weak neutralizing activity, and primarily target the fusion protein Gc. In contrast, many of the ANDV-reactive mAbs primarily target Gn and showed specificity and potently neutralizing activity for ANDV only. MAb ANDV-44, isolated from an ANDV survivor, and mAb SNV-53, isolated from a SNV survivor, compete for binding to a distinct site on the ANDV surface glycoprotein, and show potently neutralizing activity for both New and Old World species. Four mAbs also showed therapeutic efficacy at clinically relevant doses in post-exposure treatment of the golden Syrian hamster model. These findings indicate that NWH infections elicit a convergent and potently neutralizing antibody response and suggest the therapeutic potential for human mAbs against HCPS.

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Modulation of *Acinetobacter baumannii* membrane carbohydrates facilitate metal acquisition

Dillon E. Kunkle, Will N. Beavers, Matt J. Munneke, and Eric P. Skaar

Acinetobacter baumannii is a multidrug-resistant nosocomial bacterial pathogen that causes a range of diseases including respiratory and wound infections. *A. baumannii* is the leading cause of hospital-acquired pneumonia and has been identified as a major pathogen coinfecting COVID19 patients. The WHO has categorized *A. baumannii* as the bacterial pathogen that poses the greatest threat to human health. Nutrient transition metals are essential to nearly all life forms, including pathogenic bacteria. Vertebrates exploit this requirement by sequestering metals from invading pathogens in a process known as nutritional immunity. We have recently shown that the struggle for nutrient metals at the host-pathogen interface is a critical determinate of *A. baumannii* infection outcome. However, the mechanisms that *A. baumannii* employs to respond to, and overcome, nutritional immunity remain poorly understood. We have identified a gene, *A1S_3410*, which encodes for an extracytoplasmic carbohydrate-targeting acyltransferase that is induced during nutrient metal limitation. Preliminary evidence suggests that A1S_3410 targets LOS core carbohydrates for acetylation and thereby alters LOS metal binding properties. Inactivation of *A1S_3410* results in a reduced capacity to obtain environmental metals, loss of membrane barrier function, and hypersusceptibility to the LOS-targeting antimicrobial polymyxin B. Collectively, these results implicate structural alteration of membrane carbohydrates as a previously unappreciated adaptive response to impact nutrient acquisition and the maintenance of membrane integrity.

A Lytic Transglycosylase Intersects with a Metallochaperone to Ensure Zinc Homeostasis in *A. baumannii*

Jeanette M. Miller, Erin R. Green, Matthew J. Munneke, and Eric P. Skaar

Acinetobacter baumannii is an opportunistic human pathogen and a leading cause of nosocomial pneumonia. Multi-drug resistant *A. baumannii* infections have few therapeutic options available, thus emphasizing the need for novel drug development. During infection, *A. baumannii* must acquire nutrient metals to colonize and survive in the host. In zinc (Zn) deplete environments, *A. baumannii* upregulates the expression of a metallochaperone of the COG0523 subfamily of GTPases named ZigA. To better understand Zn stress in *A. baumannii*, a transposon sequencing (Tn-seq) screen was performed to profile the fitness of wildtype (WT) 17978 and Δ*zigA* transposon libraries in Zn deplete media. Using genetic interaction mapping a mutation in *A1S_3027* (Δ*3027*) was selected against in low Zn in the WT transposon library. In the Δ*zigA* library, the fitness of Δ*3027* (Δ*3027*Δ*zigA*) was not reduced in low Zn, indicating that A1S_3027 could be a suppressor of the physiologic impact of ZigA function. A1S_3027 is a predicted soluble lytic transglycosylase, a cell-wall interacting enzyme family important for cell wall recycling, signaling, and insertion of proteins into the cell membrane. The mechanism by which transglycosylases influence Zn homeostasis, however, is unclear. Here, through employing marked deletion strains (Δ*3027* and Δ*3027*Δ*zigA*), we demonstrate sensitivity to Zn deficiency as predicted by the Tn-seq results. Incubation of Δ*3027* and Δ*3027*Δ*zigA* in TPEN, a Zn chelator, with equimolar ZnCl2 resulted in complete restoration of WT growth indicating that this sensitivity is Zn specific. To define the role of A1S_3027 in Zn homeostasis, we quantified Zn^{70} uptake using ICP-MS with these strains and determined that Δ*3027*, Δ*3027*Δ*zigA*, and Δ*zigA* have decreased Zn^{70} uptake indicating that A1S_3027 and ZigA contribute to Zn homeostasis. Ongoing work focuses on determining how A1S_3027 and ZigA interact to allay Zn dysregulation. Defining mechanisms by which *A. baumannii* maintains Zn homeostasis may identify viable antimicrobial targets.

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S100A9 modulates mitochondrial metabolism thereby altering neutrophil function in response to *Staphylococcus aureus*

Andrew J. Monteith, Jeanette M. Miller, William N. Beavers, and Eric P. Skaar

Professional phagocytes form the backbone of the innate immune response and are equipped with an arsenal of antimicrobial processes. Neutrophil activation plays a critical role in protecting the host from *S. aureus* infections; however, a delicate balance is required to ensure pathogen clearance while limiting damage to host tissue. The proteins forming calprotectin, S100A8 and S100A9, are the most abundant cytosolic proteins in neutrophils and play a critical role in regulating neutrophil function. Specifically, S100A9-deficeint ($A9^{-/-}$) neutrophils produce higher levels of mitochondrial superoxide, which results in lower suicidal neutrophil extracellular trap (NET) formation in response to *S. aureus* compared to wild-type neutrophils. Herein, we demonstrate that intracellular S100A9 regulates ion flux through the mitochondrial calcium uniporter whereby $A9^{-/-}$ neutrophils accumulate higher levels of calcium and iron in the mitochondria, thereby disrupting mitochondrial metabolism. Pharmacologically and genetically modifying the mitochondrial calcium uniporter in neutrophils significantly skews disease outcome during systemic *S. aureus* infections suggesting that targeting mitochondrial homeostasis may provide an efficacious approach in treating *S. aureus* infections. Based on these results, intracellular S100A9 plays a critical role in regulating mitochondrial immunometabolism in response to *S. aureus* by modulating intracellular distribution of metal.

Antimicrobial properties of human milk oligosaccharides in Group B Streptococcus

Rebecca E. Moore, Jennifer A. Gaddy, and Steven D. Townsend

One of the most challenging problems facing the United States healthcare establishment is combatting hospital-acquired infections caused by bacteria that have developed antibiotic resistance. Several species of bacteria are responsible for this serious threat, including the Gram-positive, opportunistic bacterium *Streptococcus agalactiae* (Group B Streptococcus, GBS). Many strategies are currently employed in the clinic to prevent and treat GBS infections, one of which is combination therapy in which an adjuvant is administered along with the drug as this not only helps increase its efficacy, but also helps with the antibiotic resistance problem by lowering the dosages of the drugs required. In the Townsend Lab we are interested in exploring the putative antimicrobial activity conferred by human milk oligosaccharides (HMOs) and its ability to act as an adjuvant. In recent studies, a heterogeneous mixture of HMOs was found to possess antimicrobial and antibiofilm activity against GBS, as well as increase the efficacy of intracellular-targeting antibiotics through increased cell membrane permeability. Following up on the combination studies of HMOs with antibiotics in GBS, we expanded our studies to examine how HMOs will react when in coculture with commensal bacteria. We hypothesize that due to observed GBS growth inhibition in the presence of HMOs, cocultures with will allow HMOs to replace antibiotics in treatment of GBS and other multidrug resistant infections.

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Co-culture of Human Fecal Microbiota with Human Intestinal Cells Reveals Significant Participant-Specific Effects on Cellular Respiration

Haley B. Overby and Jane F. Ferguson

The gut microbiome has emerged as both a driving factor and a therapeutic target for a variety of diseases, including diabetes, obesity, and cardiometabolic diseases. However, many microbiome studies remain descriptive, with limited translation from observations of disease-related compositional differences towards mechanistic insight. New models for efficient translation from phenotypic association to functional characterization are urgently needed. We aimed to develop an *in vitro* system for rapid bedside-to-bench translation of clinical microbiome research into mechanistic knowledge. Furthermore, we aimed to investigate interindividual differences in evoked phenotypes, to determine whether differences in participant demographics (sex, race, BMI) could be recapitulated *in vitro* and whether this could provide mechanistic insight into the host:microbe relationship. Fecal microbiota were extracted from fresh human stool samples obtained from an ongoing clinical trial in our lab (healthy individuals, N=80, 4 samples/person, NCT04417218). Microbial extracts were co-cultured with human colonocytes (Caco-2), and cellular respiration was assessed using Seahorse XFe96 Analyzer. Data were analyzed using Wave 2.6.1 and GraphPad Prism 9. Differences between individuals were assessed by ANOVA and Tukey's post hoc tests. In preliminary analyses (n=4 individuals, 2 samples/person), we observed significant differences by sample (overall $p<0.0001$) in basal and maximal respiration, as well as measures of non-mitochondrial oxygen consumption and ATP production. Post-hoc analyses revealed significant inter-individual differences when compared to the cells-only control ($p<0.005$) and to other individuals ($p<0.001$), whereas there were no significant differences between samples taken from the same individual one week apart. Our data suggest that differences in microbiome function between and within individuals can be re-capitulated *in vitro*, and that our model of *ex vivo* co-culture of microbial extracts may reveal novel mechanisms underlying disease risk and etiology. Future work will investigate differences by participant characteristics, determine effects on cytotoxicity and membrane formation, and further probe mechanisms via gene expression analysis.

Dissecting a posttranscriptional regulatory mechanism of two component signaling in *Bacillus anthracis*

Hualiang Pi and Eric P Skaar

The molecular mechanisms by which bacterial two component systems (TCSs) drive signal transduction are well known, however, important questions remain regarding regulation of TCSs. HitRS detects and responds to cell envelope damage in the vertebrate pathogen *Bacillus anthracis*. Here, our data revealed that KreA (ComK repressor in *B. anthracis*) functions as an RNA binding protein that plays a critical regulatory role in HitRS activation through modulating mRNA stability. We discovered that KreA binds to hitRS transcripts *in vivo*, exhibits no detectable nuclease activity *in vitro*, and conceivably functions as an RNA chaperone that interacts with RNA degradation machinery to facilitate mRNA turnover. Additionally, transcriptomics experiments uncovered the effects of KreA-mediated RNA regulation on its direct targets and captured the broad influences in gene expression and coregulatory gene networks modulated by KreA function. This study elucidates a new regulatory mechanism of TCS signaling and provides insights into bacterial posttranscriptional gene regulation.

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Defining *S. aureus* regulatory systems that respond to environmental stresses at the host-pathogen interface

Valeria M. Reyes Ruiz, Anderson Miller, Sydney Drury, and Eric P. Skaar

Bacterial pathogens cause considerable morbidity and mortality worldwide. Of particular importance is *Staphylococcus aureus*, a leading cause of skin and soft tissue infection, endocarditis, and a frequent agent of bloodstream infections. While *S. aureus* is mainly considered an extracellular pathogen, recent studies describe an intracellular reservoir of *S. aureus*, which is poorly accessed by antibiotics. Moreover, *S. aureus* that grow and persist inside macrophages can disseminate and cause disease in mouse models of infection. Studies examining the interaction of intracellular *S. aureus* with macrophages would provide insight into the development of therapeutics to treat this bacterial reservoir, which circumvents antibiotic efficacy. To survive and adapt to the host environment, *S. aureus* has evolved intricate regulatory networks that allow the bacterium to produce a diverse array of virulence factors and defense mechanisms. These include two-component signal transduction systems (TCSs), in which a histidine kinase (HK) responds to extracellular stimuli and transfers the signal to a response regulator (RR) that mediates regulation of gene expression and an output response. Additionally, metalloregulators can sense levels of nutrient metals, which are essential for both the host and pathogen to carry out physiologic processes. *S. aureus* contains a diverse array of environmental sensing systems, including 16 TCSs and at least 3 major metalloregulators. We have engineered reporter constructs for each of these sensing systems in *S. aureus*. Through the application of advanced imaging modalities, we will determine the *S. aureus* regulatory systems that are activated and are essential to interact with immune cells. Additionally, we aim to discover host factors responsible for the activation of these regulatory systems with the use of an arrayed CRISPR screen. These studies will elucidate novel innate immune factors required for the control of bacterial infections, as well as revealing the bacterial signaling pathways essential for overcoming host-imposed stress.

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, baumannoferrins 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*.

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Adenylate kinase 4 Promotes Inflammatory Gene Expression via Hif1α and AMPK in Macrophages

Wei-Yao Chin, Chi-Ying He, Tsun Wai Chow, Qi-You Yu, Liang-Chuan Lai and Shi-Chuen Miaw

Macrophages comprise the front line of defense against various pathogens. Classically activated macrophages (M1), induced by IFN-γ and LPS, highly express inflammatory cytokines and contribute to inflammatory processes. By contrast, alternatively activated macrophages (M2) are induced by IL-4 and IL-13, produce IL-10, and display anti-inflammatory activity. Adenylate kinase 4 (Ak4), an enzyme that transfers phosphate group among ATP/GTP, AMP and ADP, is a key modulator of ATP and maintains the homeostasis of cellular nucleotides which is essential for cell functions. However, its role in regulating the function of macrophages is not fully understood. Here we report that Ak4 expression is induced in M1 but not M2 macrophages. Suppressing the expression of Ak4 in M1 macrophages with shRNA or siRNA enhances ATP production and decreases ROS production, bactericidal ability and glycolysis in M1 cells. Moreover, Ak4 regulates the expression of inflammation genes, including *Il1b, Il6, Tnfa, Nos2, Nox2* and *Hif1a*, in M1 macrophages. We further demonstrate that Ak4 inhibits the activation of AMPK and forms a positive feedback loop with Hif1α to promote the expression of inflammation-related genes in M1 cells. Furthermore, RNA-seq analysis demonstrates that Ak4 also regulates other biological processes in addition to the expression of inflammation-related genes in M1 cells. Interestingly, Ak4 does not regulate M1/M2 polarization. Taken together, our study uncovers a potential mechanism linking energy consumption and inflammation in macrophages.

Targeting Tumor Stiffness to Activate Anti-Myeloma CD8 T Cells

Carlos R. Detres-Roman, Minna K. Apostolova, Logan Northcutt, Marjan Rafat, and Mary Philip

Multiple myeloma (MM) is an incurable plasma cell malignancy. Although it commonly arises in the bone marrow, after treatment patients can relapse in extramedullary sites, leading to considerable morbidity and mortality. Extramedullary MM is often more resistant to therapy compared to medullary MM. Stiffness is a biophysical property reflecting a tissue's resistance to deformation, and stiffness varies widely in different tissues and pathologic states, due to changes in the composition and structure of the extracellular matrix (ECM). Solid organs and tumors have higher stiffness than the bone marrow. While increased stiffness increases cancer invasiveness and metastasis, we know little about how increased ECM stiffness impacts T cell function. We hypothesize that increased ECM stiffness in extramedullary MM inhibits T cell proliferation and effective cytolytic function. To study this, we have developed an *in vivo* mouse model to study CD8 T cell responses against myeloma. We transduced DP42 cells, a mouse myeloma cell line, to express an immunogenic CD8 T cell recognized peptide epitope ($OVA_{257-264}$) fused to EGFP (DP42OVA). By implanting DP42OVA cells via the intravenous (iv) or subcutaneous (sc) route in immunocompetent mice, we established medullary and extramedullary MM tumors, respectively. We found that tumor-specific CD8 T cells infiltrating extramedullary MM upregulated inhibitory receptors such as PD1 and CD39. In addition, we have developed a tunable stiffness 3D culture system and shown that increased stiffnesses alters the morphology of DP42OVA MM cells. We will use this system to study how ECM stiffness alters CD8 T cell function and proliferation. By gaining insights into how biophysical properties of tumors such as stiffness impact anti-tumor T cell responses, we can design better immunotherapies for solid tumors.

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Dissecting persistence mechanisms in late dysfunctional tumor-specific CD8 T cells

Megan Erwin and Mary Philip

Tumor specific CD8 T cells (TST) specifically recognize cancer cells and have the potential to kill malignant cells. However, TST found in patient tumors fail to produce effector cytokines, allowing tumors to progress. Previous work demonstrated that TST cells differentiate through two epigenetically encoded dysfunctional states; an early, plastic state from which TST effector function can be rescued after removal from chronic antigen stimulation and proliferation, and a late, fixed state from which TST cannot be rescued. Despite being dysfunctional, TST persist long-term in tumors; however, the mechanism of TST persistence is not known. Naïve and memory CD8 T cell persistence relies on signaling from homeostatic cytokines IL7 and IL15 and/or self-peptide-MHC. We analyzed the proliferative capacity of early and late dysfunctional TAG epitope-specific CD8 T cells (TCR_{TAG}) from tumors in AST;Cre-ERT2 mice, a mouse model of TAG-driven liver cancer. Early and late dysfunctional TCR_{TAG} were adoptively transferred into lymphopenic hosts to evaluate their responsiveness to homeostatic cytokines in the presence and absence of cognate antigen. After 21 days, all hosts were challenged with *L. monocytogenesTAG* (LM_{TAG}) infection to assess TCR_{TAG} proliferation in response to TCR stimulation. Circulating TST and TST harvested from livers and spleens of host mice were analyzed using flow cytometry to quantify proliferation, expansion, and absolute numbers of early and late TST. While both early and late dysfunctional TCR_{TAG} proliferated in response to homeostatic cytokine signaling, late dysfunctional TCR_{TAG} failed to proliferate in response to TCR stimulation, suggesting homeostatic cytokine signaling capability is preserved while TCR-dependent proliferative capacity is lost during differentiation to the fixed dysfunctional state. Elucidating altered signaling in dysfunctional TST can inform the development of novel strategies to reprogram dysfunctional TST for cancer immunotherapy.

Kill or divide: balancing CD8 T cell cytotoxicity and cycling

Natalie R. Favret and Mary Philip

CD8 T cells play a critical role in pathogen clearance and anti-tumor defense. Following activation during acute infection, CD8 T cells destined to become effectors undergo multiple rapid cycles of cell division while differentiating to gain cytotoxic function to generate a sufficiently large population to clear infected cells. Interestingly, CD8 T cells activated in tumors proliferate robustly but fail to gain cytotoxic function, allowing cancers to progress. Early effector differentiation has been understudied, and little is known about how proliferation and cytotoxicity is balanced. Comparing cell cycle kinetics and cytotoxic function in CD8 T cells activated during infection and in tumors could elucidate the mechanisms regulating the balance between proliferation and differentiation. To study this, we adoptively transferred naïve TAG epitope-specific CD8 T cells (TCR_{TAG}) into *L.monocytogenes*TAG (LM_{TAG})-infected B6 mice and AST;Alb-Cre mice (mouse model of TAG-driven liver cancer). 2.5 days after transfer, we harvested spleens and livers from all mice and used flow cytometry to determine cytotoxic capacity (IFNg, granzyme B) and DNA content/cell cycle phase distribution (DAPI). Surprisingly, TCR_{TAG} from infected mice and TCR_{TAG} from liver tumor bearing-mice cycled at comparable rates, with a similar distribution of cells in each cycle phase. However, while TCR_{TAG} from infected mice expressed granzyme B, TCR_{TAG} from tumor-bearing mice failed to express IFNg or granzyme B. Interestingly, we found that expression of the transcription factor TCF1, generally associated with quiescent stem/progenitor programs, correlated with TCR_{TAG} cycling speed but inversely correlated with cytotoxic capacity. By dissecting the mechanism by which TCF1 regulates cell cycle and cytotoxic differentiation, we may ultimately be able to shift this balance in dysfunctional CD8 T cells in cancer to improve immunotherapy.

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Selenoprotein-deficient T cells exhibit impaired thymopoiesis

Justin Jacobse, Zaryab Aziz, Jeremy A. Goettel, and Yash Choksi

Background

Eosinophilic esophagitis (EoE) is an allergy-mediated inflammatory disorder, characterized by a T helper 2 (Th2) immune response. As a consequence of chronic inflammation in EoE, lamina propria fibrosis occurs, leading to esophageal stricture. The inflammatory cytokines in the microenvironment of EoE have been suggested to promote reactive oxygen species (ROS), and ROS production is hypothesized to promote fibrosis. Oxidative stress is regulated in part by selenoproteins, which have antioxidant and anti-inflammatory properties. Selenoproteins have been implicated in T cell function; however, the role of selenoproteins in EoE and its defining T cell subsets is unknown. Previously, we showed that Th2 cells in patients with active EoE have increased expression of selenoproteins compared to normal controls. Here, we examined the functional role of selenoproteins in T cells using a conditional murine model.

Methods

Trsp$^{fl/fl}$ mice (*Kumaraswamy et al.*) have LoxP sites flanking the *Trsp* gene, which encodes a transfer RNA required for selenoproteins (tRNA[Ser]^{Sec}). To generate mice that are deficient for selenoproteins in all T and B cells, *Cd2*^{Cre} mice were crossed with *Trsp*$^{fl/fl}$ mice, resulting in *Trsp*$^{-/-}$*Cd2*^{Cre} mice. Immunophenotyping was performed using Fluorescent Activated Cell Sorting (FACS).

Results

The thymus of *Trsp*$^{-/-}$*Cd2*^{Cre} in 6-8-week old mice was largely deconvoluted compared to TRSP-sufficient littermates (*Cd2*^{Cre}). In conditional *Trsp*$^{fl/fl}$ mice, the apoptosis marker Annexin-V was increased in double negative (DN) thymocytes, the frequency of DN cells was increased, and within the DN subset the frequency of thymocytes in stage 3 (CD44^{-}CD25^{+}) was increased with a concomitant decrease in stage 4 (CD44^{-}CD25^{-}). In the spleen and mesenteric lymph nodes, the proportion of CD4^{+} and CD8^{+} T cells was decreased and within the CD4^{+} T cell subset, the frequency of naïve T cells was decreased whereas the frequency of T effector memory cells was increased.

Conclusions

Our results suggest that selenoproteins are essential for physiological T cell development.

Milk-derived osteopontin shapes the development of the intestinal microbiota

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Breast-feeding is important for the growth and development of infants, delivering nutritional support as well as a wealth of immunological factors that provide protection while the infant immune system matures. Formula-fed infants have an increased risk of negative health outcomes, including obesity, autoimmunity, and infections, and display dysbiosis of the intestinal microbiota compared to breast-fed infants. Of the many components of milk, bioactive proteins provide a potential mechanism by which breast milk can impact the infant microbiota. One protein of interest is osteopontin (OPN), a highly glycosylated phosphoprotein involved in a range of physiological processes including bone mineralization, inflammation, wound healing, and homeostasis of lymphoid cells in the intestinal epithelium. Osteopontin constitutes about 2% of the protein component of milk; however, the impacts of milk-derived osteopontin on the development of the infant microbiota are not yet well understood. We propose that milk-derived osteopontin plays a critical role in the development of the neonatal gut microbiota, and that lack of osteopontin intake during the post-natal period leads to gut dysbiosis.

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Thymic epithelial cells require autophagy-related protein Vps34 for homeostasis and CD4 T cell selection

J.L. Postoak, G. Yang, W. Song, S. Xiao, L. Wu, N.R. Manley, and L. Van Kaer

The generation of a functional, self-tolerant T cell repertoire depends on interactions between developing thymocytes and antigen-presenting thymic epithelial cells (TECs). The presentation of self-antigens to developing thymocytes involves a variety of cellular processes, including proteolysis, endocytosis, vesicle trafficking, and autophagy. In our current study, we focus on the class III PI3K, Vps34, which has been implicated in autophagy and vesicle trafficking. To address the contribution of Vps34 to TEC function, we have generated mice with a TEC-specific Vps34 deficiency. Conditional mutants display progressively hypoplastic thymi with reduced TEC cellularity and altered TEC subset ratio. Additionally, when TCR transgenic lines were bred to these animals we found profound defects in the positive selection of the CD4 T cell lineage but not the CD8 T cell lineage. Interestingly, cortical TECs from mutant mice display an increased abundance of unprocessed antigen bound to surface MHCII molecules indicating altered antigen processing. To test the role of autophagy, we generated mice with a TEC-specific loss of Atg5, a molecule essential for autophagy. Interestingly, MHCII-restricted TCR transgenic T cells were positively selected in the Atg5-conditional mutants. Collectively, these studies identify an autophagy-independent role for the PI3K Vps34 in maintaining TEC homeostasis and contributing to the repertoire of selecting ligands processed and presented by TECs.

CD8 T cell fate determination occurs during priming prior to cell division

Michael Rudloff, Natalie Favret, Paul Zumbo, Friederike Dundar, Doron Betel, and Mary Philip

Tumor specific CD8 T cells (TST) can eliminate malignant cells; however, TST often become dysfunctional resulting in cancer progression. Dysfunctional TST lack effector function, including cytokine (TNFα, IFNγ) and cytolytic molecule (granzyme B, perforin) production, and express high levels of inhibitory receptors (PD1, LAG3). The current paradigm is that TST dysfunction is driven by chronic antigen stimulation over the course of days to weeks. However, our previous studies showed that TST are completely dysfunctional just 4-5 days after entering tumors. It is currently unknown how early TST become dysfunctional and what transcriptional and epigenetic programs mediate effector function loss. Using a murine model of hepatocellular carcinoma (HCC) in conjunction with adoptively-transferred, HCC specific CD8 T cells (TCR_{TAG}) labeled with proliferation dye, we now show that TST primed in tumors completely lose effector cytokine production capacity and induce high-level inhibitory receptor expression within hours, even prior to the first cell division. Strikingly, even functional effector and memory TST rapidly lost effector cytotoxic function hours after entering tumors, indicating that activation in tumors overrides pre-existing functional epigenetic and transcriptional programs. To identify the earliest transcriptional and epigenetic drivers of dysfunction, we sorted TCR_{TAG} T cells 6, 12, and 24 hours after activation in liver tumors or from mice infected with Listeria monocytogenes expressing the TAG epitope and interrogated them by ATAC- and RNA-SEQ. Large-scale chromatin and transcriptional changes distinguished functional from dysfunctional TCR_{TAG} and interestingly, chromatin accessibility patterns characteristic of late-stage (day 7-60) dysfunctional T cells were observed within 6 hours of activation in tumors. Our studies demonstrate that TST dysfunction is induced immediately upon activation in tumors, mediated by epigenetic and transcriptional remodeling that does not require cell division, challenging the previous paradigm. By targeting the earliest drivers of TST dysfunctional differentiation, we can potentially rescue TST and boost anti-tumor immunotherapies.

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Anti-insulin B cell receptors display skewed immunoglobulin gene usage and limited mutation in pre-symptomatic type 1 diabetes donors

Steven R Scaglione, Bryan A Joosse, Camille Westlake, Eunice Kim, Austin B Santhin, Mason V Forchetti, Alberto Cisneros, Daniel J Moore, Scott A Smith, and Rachel H Bonami

Antibodies targeting insulin and other islet antigens predict type 1 diabetes (T1D) onset. Insulin autoantibody (IAA)-positive individuals progress to diabetes more rapidly, yet little is known about how IAA-precursor anti-insulin B cells (AIBCs) recognize insulin. Complementarity-determining regions (CDRs) of the B cell receptor (BCR) are structurally diverse loops that constitute the antigen-binding site, and amino acid variability in BCRs increases the breadth of repertoire antigen-specificities. To investigate anti-insulin BCRs during the earliest detectable stages of T1D, we enrolled eight insulin therapy-naïve Type 1 Diabetes TrialNet Pathway to Prevention participants who were positive for ≥2 islet autoantibodies and thus at high risk for diabetes. We stimulated donor peripheral blood mononuclear cells to drive BCR secretion as antibody, screened for AIBC-containing wells by ELISA, and immortalized these cells as hybridomas. We validated 25 monoclonal anti-insulin hybridoma lines and sequenced 16 heavy chain (IgH) and 14 light chain (IgL) immunoglobulin genes. 25% of V_H and 50% of V_L sequences contained at least one mutation within a CDR compared to germline, with 13% of V_H and 29% of V_L sequences having ≥6 mutations within the V-region. We surveyed previously and currently reported AIBC V_H (n=25 BCRs), which revealed skewed V_H and J_H gene use compared to the total polyclonal IgH repertoire (n= 8790 BCRs, $p < 0.001$ and $p < 0.05$, respectively, chi-squared test). These data expose unique features of insulin recognition among AIBCs isolated from pre-symptomatic T1D donors, including V and J gene usage bias and potential for germline IgH insulin recognition, providing insight into AIBC origins.

Dendritic cell PIK3C3/VPS34 controls the pathogenicity of CNS autoimmunity independently of LC3-associated phagocytosis

Guan Yang, J. Luke Postoak, Wenqiang Song, Jennifer Martinez, Jianhua Zhang, Lan Wu, and Luc Van Kaer

The PIK3C3/VPS34 subunit of the class III phosphatidylinositol 3-kinase (PtdIns3K) complex is a key player in macroautophagy/autophagy and MAP1LC3/LC3-associated phagocytosis (LAP), both of which play critical roles in mediating dendritic cell (DC) function. In this study, we assessed the contribution of PIK3C3 to DC function in the context of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS). We found that *Pik3c3*-deficient DCs exhibited attenuated ability in reactivating encephalitogenic T cells in the central nervous system, leading to reduced incidence and severity of EAE in DC-specific *Pik3c3*-deficient mice. Additionally, animals with DC-specific deficiency of *Rb1cc1* (essential for autophagosome nucleation but not LAP) but not *Rubcn* (required for LAP but not autophagy) were protected against EAE, suggesting that the EAE phenotype of DC-specific *Pik3c3*-deficient mice is associated with the lack of canonical autophagy rather than LAP. Collectively, our studies have revealed a critical role of PIK3C3 in DC function and in the pathogenicity of these cells during EAE. Our findings also have important implications for the development of immunotherapies to treat autoimmune diseases such as MS by targeting PIK3C3-containing complexes.

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