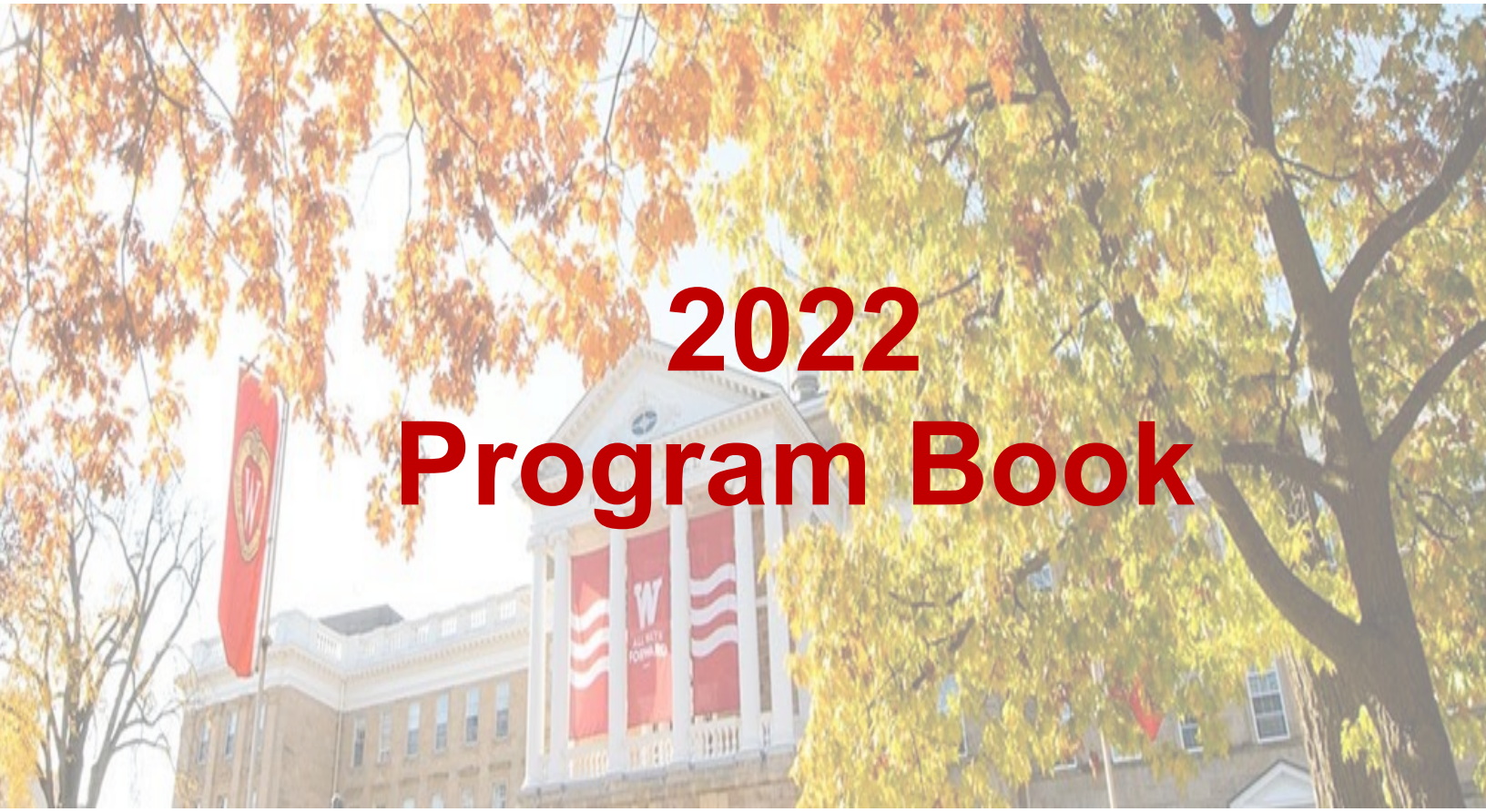
28th Midwest Microbial Pathogenesis Conference
University of Wisconsin-Madison



2022 Program Book

September 30-October 1, 2022
Madison, WI

2022 MIDWEST MICROBIAL PATHOGENESIS CONFERENCE

September 30-October 1, 2022
Madison, Wisconsin

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CONFERENCE SCHEDULE

	Friday, September 30	
12:00p – 6:30p	**Registration**	**Annex Room**
4:00p	**Welcome Reception**	**Tripp Commons**
6:30p	**Opening Session**	**Shannon Hall**
	Island Exploration in *Vibrio cholerae* Dr. Chris Waters, Michigan State University	
	Staphylococcus aureus Utilizes a Multifaceted Strategy to Satisfy the Requirement for Nutrient Sulfur Dr. Neal Hammer, Michigan State University	
7:30p	**Session 1**	**Shannon Hall**
	Characterization of *Streptococcus pneumoniae* Secreted PPIase Chaperones Required for Virulence Dr. Laty Cahoon, University of Pittsburgh	
	CagC Gates Effector Passage Through the Helicobacter pylori cag T4SS Translocation Channel Mackenzie Ryan, University of Kentucky	
	Regulation of Klebsiella pneumoniae Mucoidy by the Bacterial Tyrosine Kinase Wzc Dr. Laura Mike, University of Toledo	
	Amino Acid Catabolism in Acinetobacter baumannii Colonization Dr. Lauren Palmer, University of Illinois at Chicago	

	Saturday, October 1	
7:15a – 3:00p	**Registration**	**Annex Room**
7:15a	**Parfait Breakfast**	**Shannon Hall Lobby**
8:00a	**Session 2**	**Shannon Hall**
	Emergent Metabolic Dynamics in Microbial Communities Dr. Arthur Prindle, Northwestern University	
	Towards Elucidating the Structure of the Pseudomonas aeruginosa Biofilm Matrix Protein Called CdrA Dr. Courtney Reichhardt, Washington University - St. Louis	
	Sensory Regulation of Bacterial Biofilm Development Dr. Sampriti Mukherjee, University of Chicago	
	Urinary Catheterization-induced Fibrin Accumulation Promotes M2 Macrophage Polarization to Enhancing Bacterial Colonization Armando Marrufo, University of Notre Dame	
9:15a	**Break**	**Shannon Hall Lobby**
9:30a	**Session 3**	**Shannon Hall**
	A Short chain Fatty Acid-centric View of *Clostridioides difficile* Pathogenesis Dr. Andrew Hryckowian, University of Wisconsin-Madison	
	The Sweet Science – How Enteric Pathogens Take Advantage of a Sugar-Rich Diet Dr. James Collins, University of Louisville	
	Staphylococcal* Secreted Cytotoxins are "Competition Sensing" Signals for *Pseudomonas aeruginosa Dr. Dominique Limoli, University of Iowa	
	Coxiella burnetii Effector Protein with FFAT Motif (EPF1) Mediates Membrane Contact Sites Between Host Lipid Droplets and Endoplasmic Reticulum Rajendra Angara, University of Nebraska Medical Center	
10:45a	**Break**	**Shannon Hall Lobby**
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	Decoding Key Players of Plasmodium Nuclear Biology using Expansion Microscopy Dr. Sabrina Absalon, Indiana University	
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	Structural and Mechanistic Insights into the Clostridioides difficile Transferase Dr. Michael Sheedlo, University of Minnesota	
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	Unraveling the Adaptation of Staphylococcus aureus During Bloodstream Infection Dr. Victor Torres, New York University	
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GENERAL INFORMATION

Registration
The registration desk is located in the Memorial Union, Second Floor, Annex Room.

Hours
Friday, September 30 –12:00p-6:30p
Saturday, October 1 – 7:15a – 3:00p

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Please load your presentation before your scheduled session in Memorial Union's Shannon Hall. Bring your presentation on a flash drive; you will not be able to use your personal computer. We will provide both a Mac and a PC. Please follow the speaker entrance sign and go backstage to download your presentation. There will be an AV tech there to assist you.

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Poster board dimensions are 4' high x 4' wide. Push pins and poster numbers will be provided. Your poster number can be found in the program book. All posters must be removed by the designated time.

Poster setup starts on Saturday at 1:00p. All posters must be removed immediately after the conference ends. Poster room assignments can be found below:

Poster #s 25-80	Main Lounge, 2nd Floor
Poster #s 81-248	Great Hall, 4th Floor
Poster #s 249-276	Multicultural Greek Council Room, 4th Floor

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Additional transportation can be found on the conference website.

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Masks are not mandated in an indoor setting. However, we strongly encourage participants to wear masks throughout the event. Masks are available at registration, as well as all Langdon & Park Street entrances of the Wisconsin Union.

We recommend that all participants screen themselves daily for symptoms. Antigen tests may be available upon request at registration.

We encourage attendees who wish to physically distance themselves to do so. All breaks and meals have easy access to outdoor space. Attendees may choose to eat their meals outside or throughout the public spaces in the building.

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The Midwest Microbial Pathogenesis Conference is committed to providing an environment a friendly, safe, and welcoming environment for all, regardless of gender, sexual orientation, disability, race, ethnicity, religion, national origin, or other protected characteristics. We expect all attendees, media, speakers, volunteers, organizers, venue staff, guests, and exhibitors to help us ensure a safe and positive conference experience in which participants can discover, examine, critically, and transmit knowledge that will improve the quality of life for all.

If you witness or experience conduct that violates the code of conduct, the conference staff will be happy to help participants contact hotel/venue security or local law enforcement, and otherwise assist those experiencing harassment, to enable them to feel safe for the duration of the conference. We value your attendance and want to make your experience as productive and professionally stimulating as possible.

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2. Please respect others with social media postings. Refrain from photographing, recording, or videotaping during oral presentations or during poster sessions. Violators may be asked to leave the session. Please do not post images with data or people without permission.

3. Abide by University of Wisconsin and venue rules, including policies for alcohol consumption.

4. Participants should report any concerns, including experiencing or witnessing harassment, to Conference staff (at registration or by email at conferences@union.wisc.edu) and/or Conference Organizers (Mark Mandel (mailto:mmandel@wisc.edu), JD Sauer (mailto:sauer3@wisc.edu). Additional reporting mechanisms can be found at http://go.wisc.edu/report.

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ORAL PRESENTATIONS & POSTERS

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Chris Waters

2 **Staphylococcus aureus Utilizes a Multifaceted Strategy to Satisfy the Requirement for Nutrient Sulfur**
Neal Hammer

3 **Characterization of *Streptococcus pneumoniae* Secreted PPIase Chaperones Required for Virulence**
Laty Cahoon

4 **CagC Gates Effector Passage Through the Helicobacter pylori cag T4SS Translocation Channel**
Mackenzie Ryan, Prashant P. Damke, Carrie L. Shaffer

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Arthur Prindle

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Courtney Reichhardt

9 **Sensory Regulation of Bacterial Biofilm Development**
Sampriti Mukherjee

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ORAL PRESENTATIONS

Oral Presentation #1

Island Exploration in *Vibrio cholerae*

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The central challenge that all bacteria face is sensing and adapting to environmental change and stress. *Vibrio cholerae*, a Gram-negative proteobacterium that is the causative agent of cholera, resides in many different environments from aquatic environmental reservoirs to infection of the human host. To survive and multiply, *V. cholerae* has evolved multiple systems that integrate environmental information into the appropriate phenotypic responses. In this talk, I will discuss my laboratories progress in understanding the role of chemical signaling pathways and molecular mechanisms of biological defense in the evolution and ecology of *V. cholerae*.

Oral Presentation #2

Staphylococcus aureus Utilizes a Multifaceted Strategy to Satisfy the Requirement for Nutrient Sulfur

Neal Hammer[1]
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Bacterial pathogens must acquire sulfur to proliferate during infection. Prior in vitro studies demonstrated that, Staphylococcus aureus utilizes glutathione (GSH), an abundant sulfur containing metabolite in host tissues, as a source of nutrient sulfur. However, mechanisms of GSH acquisition are unresolved. Using a genetic approach, we reveal that a putative ABC-transporter and γ–glutamyl transpeptidase (ggt) promote S. aureus proliferation in medium supplemented with either reduced or oxidized GSH (GSSG) as the sole source of nutrient sulfur. Consequently, we name this transporter the Glutathione import system (GisABCD). Bioinformatic analyses establish that only Staphylococcus species closely related to S. aureus encode GisABCD-Ggt homologues. Homologues are not detected in Staphylococcus epidermidis. Accordingly, we demonstrate that GisABCD-Ggt provides a competitive advantage for S. aureus over S. epidermidis in a GSH- and GSSG-dependent manner. These results describe a nutrient sulfur acquisition system in S. aureus that targets GSH and promotes competition against other staphylococci commonly associated with the human microbiota. From the host perspective, we monitored spatial distribution of GSH and GSSG in infected and healthy tissues using matrix assisted laser desorption/ionization imaging mass spectrometry. This analysis revealed considerable sulfur metabolite redistribution in infected kidneys and uncovered a new source of nutrient sulfur. Finally, a mutant strain harboring a transposon within a gene encoding a predicted sulfur transporter exhibits decreased heart colonization, indicating that yet another host-derived metabolite satisfies the sulfur requirement in the heart. These findings underscore the multifaceted approach S. aureus utilizes to acquire a critical element during host colonization.

Oral Presentation #3

Characterization of *Streptococcus pneumoniae* Secreted PPIase Chaperones Required for Virulence

Laty Cahoon[1]
[1]*Department of Biological Sciences, University of Pittsburgh*

For the Gram-positive bacterium *Streptococcus pneumoniae* to colonize the human nasopharynx and transition into an invasive infection, the pathogen relies on several proteins that are translocated across the bacterial membrane and destined for surface exposure, cell-wall attachment, or release into the host. The majority of these proteins are secreted unfolded into the space between the membrane and cell wall. This space is solvent exposed, contains high concentrations of cations, and a high density of negative charge which presents a challenging environment for protein folding and function. Therefore, *S. pneumoniae* has two peptidyl-prolyl isomerase (PPIase) secretion chaperones: SlrA and PrsA that work between the membrane and cell-wall. SlrA is a cyclophilin PPIase that catalyzes the *cis* to *trans* conversion of peptide bonds N-terminal to proline residues while PrsA shares homology to the parvulin family of PPIases but no longer retains PPIase activity. We hypothesize that SlrA and PrsA regulate folding and activity of several secreted virulence factors. We determined that *slrA* and *prsA* are required for host cell adhesion, hemolysis, and mouse colonization and invasive disease. To identify proteins that interact with PrsA and SlrA, we used a Stable Isotope Labeling by Amino acids in Cell culture affinity pull-down approach which suggests that several virulence factors interact with both chaperones including the pore forming toxin, pneumolysin. We determined using microscale thermophoresis and affinity isothermal titration calorimetry that the interaction of SlrA and PrsA with Ply is direct. We anticipate that several additional virulence factors have direct interactions with SlrA and PrsA.

Oral Presentation #4

CagC Gates Effector Passage Through the Helicobacter pylori cag T4SS Translocation Channel

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Helicobacter pylori exploits *cag* type IV secretion system (*cag* T4SS) activity to alter the mucosal microenvironment by delivering diverse immunostimulatory cargo into gastric cells. However, the mechanism by which the *cag* T4SS transports substrates across the bacterial envelope is undefined. Using techniques to monitor uncontrolled effector release, we show that the pilin ortholog CagC plays a critical role in regulating cargo delivery to target cells. Inactivation of *cagC* resulted in mislocalization of protein and DNA effectors to the bacterial cell surface, suggesting that CagC governs substrate passage across the outer membrane. Using live cell assays to detect cell surface-exposed DNA, we demonstrate that significantly more *H. pylori cagC* were immunocaptured by dsDNA monoclonal antibodies compared to WT and the complemented strain. Treatment with DNase prior to incubation with dsDNA antibody restored immunocaptured *cagC* bacteria to WT levels. Using reporter cell lines to analyze *cag* T4SS-dependent TLR9 activation, we show that *cagC* is required for DNA translocation to host cells and demonstrate that TLR9 stimulation is significantly decreased in the presence of DNase I or dsDNase, but not RNase or exonuclease I, suggesting that dsDNA cargo is exposed to the extracellular milieu prior to entering host cells. TLR9 activation was also significantly impaired in the presence of dsDNA antibodies, supporting a two-step DNA secretion model. Collectively, these data demonstrate that CagC orchestrates the passage of effector molecules through the secretion apparatus and support a model whereby *cag* T4SS cargo is transported to an extracellular site before delivery to the gastric epithelium.

Oral Presentation #5

Regulation of Klebsiella pneumoniae Mucoidy by the Bacterial Tyrosine Kinase Wzc

Laura Mike[1], Saroj Khadka[1], Brooke Ring[1], Keila Acevedo Villanueva[1], Lindsey Krzeminski[1], Matthew Hathaway[1], Ryan Walker[2], Harry Mobley[2]
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Klebsiella pneumoniae is a nosocomial pathogen associated with urinary tract infections (UTI), pneumonia, and septicemia. Two challenging lineages include the hypervirulent strains, causing invasive community-acquired infections, and the carbapenem-resistant isolates, most frequently isolated from UTI. Clinical isolates from UTI are typically non-mucoid, while invasive hypervirulent isolates are often characterized by a hypermucoid (adherent) colony phenotype. We hypothesized that environmental conditions may drive K. pneumoniae adaptation to the urinary tract and regulate mucoidy. We found that K. pneumoniae suppressed mucoidy when cultured in urine without altering capsule abundance. This indicated that mucoidy is a phenotype distinct from capsule abundance, contrary to the historical perspective that hypermucoidy is due to over-production of capsule. We performed a transposon screen to identify genes that regulate mucoidy in response to urine. We found that within single transposon insertion mutants, some colonies gained the ability to be mucoid on urine, while others did not. Whole genome sequencing identified that colonies with a constitutive mucoid phenotype encoded secondary mutations in the wzc tyrosine kinase, which regulates capsule biosynthesis. Wzc mutations alter autophosphorylation and are sufficient to increase mucoidy in a clinical UTI isolate without affecting capsule abundance. Combined, these data implicate mutation-driven modulation of Wzc activity as a global mechanism boosting K. pneumoniae mucoidy, which may provide site-specific fitness. Wzc activity may represent the lynchpin that coordinates capsule biosynthesis and mucoidy, explaining why these two phenotypes have been historically intertwined.

Oral Presentation #6

Amino Acid Catabolism in Acinetobacter baumannii Colonization

Lauren Palmer[1], Xiaomei Ren[1], Dziedzom Bansah[1], John Geary[1], R. Mason Clark[1], Bradford Winkelman[2], Jonathan Winkelman[2]
[1]*University of Illinois Chicago,* [2]*Trestle, LLC*

Acinetobacter baumannii is an important cause of healthcare-associated infections including ventilator-associated pneumonia and a major public health threat due to multidrug resistance. Previous work identified arginine metabolism as important for lung infection in a murine model. Here, we found that *A. baumannii* utilizes the arginine succinyl transferase (AST) pathway to catabolize arginine. Using ortholog and phylogenetic analyses, we uncovered a second, partial duplication of the *ast* operon in pathogenic *Acinetobacter* and a related clade including *Acinetobacter colistiniresistens* and species that are occasional pathogens and commonly vertebrate commensals. Clades consisting primarily of environmental *Acinetobacter* do not encode the *ast2* locus. We determined that AstA2 and the associated regulator are required for ornithine catabolism but dispensable for arginine catabolism. The double mutant strain *astA1sup*^{H229A} Δ*astA2* lose the ability to replicate in media with arginine or ornithine as the only carbon source but do not have a major defect in a murine model of lung infection. Because the *ast2* locus in maintained in *Acinetobacter* that are non-pathogenic commensals, we hypothesized that *ast* duplication is important for colonization. We developed a mouse model of *A. baumannii* gut carriage and determined that the *astA* double mutant had a colonization defect compared to the WT strain. Thus, arginine and ornithine catabolism are critical for *A. baumannii* gut colonization. The gastrointestinal tract has been proposed as a reservoir for *A. baumannii* to develop multidrug resistance. Therefore, understanding the mechanisms governing *Acinetobacter* gut colonization has the potential to limit the acquisition and dissemination of multidrug resistant infections.

Oral Presentation #7

Emergent Metabolic Dynamics in Microbial Communities

Arthur Prindle[1]
[1]*Northwestern University*

Emerging research of the human microbiome has generated new insights into the role of human-associated microbes in health and disease. In particular, microbes that colonize the gastrointestinal tract play a central role in host metabolism, immunity, and homeostasis, and can change in response to external perturbations such as dietary alterations, chemical exposures, and physiological or psychological stressors. However, the microbiome field currently lacks essential knowledge for how microbial cell-to-cell interactions give rise to higher-order community behavior, which is a key roadblock in the path towards next-generation microbiome-based products and therapies. In this talk, I will describe our various efforts to address these challenges, including how our multi-scale microfluidic platform for microbial community analysis is contributing to the new field of bacterial electrophysiology. My lab is currently deciphering the underlying mechanisms of bacterial biofilm electrophysiology with the goal of better understanding and engineering natural microbial community behavior. These next-generation microbial community analysis and engineering tools provide a foundational platform for bridging synthetic biology technologies to the microbiome field.

Oral Presentation #8

Towards Elucidating the Structure of the Pseudomonas aeruginosa Biofilm Matrix Protein Called CdrA

Courtney Reichhardt[1]
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A hallmark feature of chronic infections is the formation of microbial communities called biofilms. Within biofilms, microbes are encased in a mesh-like, biopolymer-rich extracellular matrix. The matrix promotes microbial cell-cell interactions, adherence to host tissues, and protection from antimicrobials. The Pseudomonas aeruginosa biofilm matrix can include the exopolysaccharides (EPS) Psl and Pel, extracellular DNA (eDNA), and proteins. CdrA was the first P. aeruginosa biofilm matrix protein to be discovered and is required for robust biofilm formation. CdrA promotes aggregation via CdrA-EPS interactions. Homology modeling predicted that CdrA has several binding motifs including sites for binding to exopolysaccharides. However, the high molecular weight and repetitive structure of CdrA has made determining its structure, including its binding motifs, challenging. As such, we still have limited structural information about CdrA despite that it has been known to be an important matrix protein for over a decade. Our early results provide evidence of CdrA structure and interactions, and how these properties relate to its function as a biofilm adhesin. Since CdrA is similar to other structural biofilm matrix proteins including that it has a repetitive primary structure, binds to EPS, and has both cell-associated and secreted forms, we believe that our findings may provide general insight into biofilm assembly, and the approaches that we are using to study CdrA should be translatable to similar adhesins.

Oral Presentation #9

Sensory Regulation of Bacterial Biofilm Development

Sampriti Mukherjee[1]
[1]*University of Chicago*

Bacterial responses to self-generated and environmental stimuli influence their survival, persistence in particular niches, and lifestyle transitions between individual behaviors such as swimming and collective behaviors such as formation of structured communities encased in extracellular matrix called biofilms. Biofilms represent an abundant form of bacterial life on Earth, exhibit superior resilience to antimicrobials and host immune responses in medical settings, while they clog devices and foul surfaces in industrial settings. How the information encoded in multiple sensory inputs is integrated to control collective behaviors is largely mysterious. Our primary research goal is to combine bacterial genetics, molecular biology, biochemistry, fluorescence microscopy, and genome-scale studies to address fundamental questions about bacterial sensory signal detection, relay, integration, and the consequences to collective behaviors. Our model system is the human pathogen *Pseudomonas aeruginosa* because it is a notorious biofilm former, and biofilms are associated with virulence in this pathogen. We are currently investigating how quorum-sensing, photo-sensing and nutrient-sensing pathways separately and combinatorically control different stages of biofilm development in *P. aeruginosa*. Our data suggest that individually the processes of population density sensing via quorum-sensing receptor RhlR, light sensing via photoreceptor BphP, and carbon: nitrogen balance sensing via the nutrient-sensing two-component system CbrA/CbrB repress the formation of biofilms by controlling the expression of different biofilm matrix components. Ultimately, understanding how information encoded in diverse sensory inputs is extracted and integrated to drive collective behaviors will be foundational for designing successful synthetic strategies to enhance or to inhibit biofilms and for developing novel therapeutic interventions.

Oral Presentation #10

Urinary Catheterization-induced Fibrin Accumulation Promotes M2 Macrophage Polarization to Enhancing Bacterial Colonization

Armando Marrufo[1], Christopher Gager[1], Marissa J. Andersen[1], Peter V. Stuckey[1], Kurt Kohler[1], Jonathan J. Molina[1], Matthew J. Flick[2], Francis J. Castellino[1], Felipe H. Santiago-Tirado[1], Ana L. Flores-Mireles[1]
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While urinary catheterization is utilized for impaired bladders, catheterization leads to the development of catheter-associated urinary tract infections (CAUTIs). Upon catheterization, fibrinogen (Fg) released into the bladder is polymerized into fibrin by thrombin for tissue healing. However, deposition of Fg & fibrin on catheters provided a scaffold for uropathogenic *Escherichia coli* (UPEC) and *Enterococcus faecalis* to establish infection. Using a CAUTI mouse model, we found that significant recruitment of macrophages (Mϕs) was ineffective at controlling infection. We unveiled that Fg or fibrin polarize Mϕs to either proinflammatory M1 or anti-inflammatory M2 by upregulating inducible nitric oxide synthase or Arginase-1, respectively. Therefore, we hypothesized that Fg & fibrin differentially polarizes Mϕs: where Fg favors M1 polarization with enhanced antimicrobial activity, while fibrin induces M2 polarization, suppressing pathogen control. To test our hypothesis, wildtype and coagulation-transgenic mice, such as AEK-mutated Fg mice (AEK-Fg) and plasminogen (Pg)-deficient mice (fibrin accumulation), were catheterized & infected with UPEC or *E. faecalis* for 24 hours. We found that bacterial burden decreased in bladders of AEK-Fg mice, where Fg does not polymerize to fibrin, while burden increased in Pg-deficient mice. Furthermore, we observed higher presence of M1s than M2s in AEK-Fg catheterized bladders; in contrast, there was elevated presence of M2s in Pg-deficient bladders for both pathogens. This suggests that Fg shifts Mϕs towards M1 where it controls infection, while fibrin polarize to M2, where pathogens persist during catheterization. Understanding how dysfunction in Mϕ polarization contributes to CAUTI pathogenesis will enhance our understanding of impaired immune response.

Oral Presentation #11

A Short chain Fatty Acid-centric View of *Clostridioides difficile* Pathogenesis

Andrew Hryckowian[1]
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Clostridioides difficile is a diarrheal pathogen that causes significant human morbidity and mortality worldwide. The primary risk factor for *C. difficile* infection (CDI) is a disrupted microbiome, which is often engendered by antibiotic use. Therefore, strategies that support a healthy microbiome are likely to be important for mitigating *C. difficile* infection in at-risk individuals. Given that diet is one of the most powerful and easily manipulatable factors that affects the gut microbiome, we hypothesized that diet may play important roles in determining CDI outcomes. Using a mouse model of CDI, we showed that mice fed diets deficient in dietary fiber exhibit persistent infection. Conversely, mice fed fiber-rich diets clear *C. difficile* from their gastrointestinal tracts within days. Interestingly, these effects are not generalizable to all fiber types and effective/ineffective fiber types are differentiated based on whether they lead to elevated production of the short chain fatty acid (SCFA) butyrate by the gut microbiome. Our ongoing efforts, aimed at understanding the specific molecular mechanisms underlying SCFA-driven impacts on CDI will lead to a better understanding of *C. difficile*, the gut microbiome, and the host immune system. This understanding will be exploited to develop rationally designed diet-based strategies for combatting *C. difficile* and perhaps other bacterial pathogens.

Oral Presentation #12

The Sweet Science – How Enteric Pathogens Take Advantage of a Sugar-Rich Diet

James Collins[1]
[1]*University of Louisville*

In the last 50 years, the human diet has changed dramatically. Ultra-processed foods now account for 60% of all calories consumed. One of the most significant changes has been the increase in the consumption of sugars, with Americans consuming more than 150 g of caloric sweeteners per day. Not only has the quantity of sugar increased, the variety of sugars and non-calorific sweeteners has also increased significantly. High sugar consumption increases the bioavailability to both indigenous gut microbiota and opportunistic enteric pathogens. Furthermore, a high-sugar diet promotes systemic inflammation and dysregulation of the gut epithelial barrier. High-sugar diets can therefore benefit opportunistic pathogens while also worsening outcomes in several ways: i) inflammation and increased gut permeability may exacerbate disease, and ii) pathogens that have adapted to utilize these nutrient sources can take advantage of the increased bioavailability of simple sugars. We show how two distantly related pathogens, vancomycin-resistant *Enterococcus faecium* and *Clostridioides difficile*, have adapted to this new nutritional environment, potentially increasing their ability to spread and cause disease.

Oral Presentation #13

Staphylococcal Secreted Cytotoxins are "Competition Sensing" Signals for *Pseudomonas aeruginosa*

Dominique Limoli[2], Grace Z. Wang[1], Elizabeth Warren[2], Allison Welp[3], Brett Lomenick[4], Megan Kiedrowski[5], Jennifer Bomberger[3]
[1]California Institute of Technology, [2]University of Iowa, [3]University of Pittsburgh, [4]Beckman Institute, [5]University of Alabama at Birmingham

Interspecies interactions between two notorious opportunistic pathogens that dominate chronic infection in the cystic fibrosis (CF) airway, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, critically affect CF disease progression and treatment outcome. Here, we used a combination of microscopy, genetics and chemo-selective proteomics approaches to uncover a previously unknown "competition sensing" model where *Staphylococcal* secreted cytotoxic phenol-soluble modulin (PSM) peptides are key interspecies signals that mediate *P. aeruginosa* antagonistic response.

First, we show that *P. aeruginosa* is attracted to *S. aureus* and travels up a gradient of *Staphylococcal* secreted factors using type IV pilus mediated motility. PSM-deficient *S. aureus* also exhibit increased survival in co-culture compared to wild-type. Time-resolved proteomic analysis of *P. aeruginosa* immediate global response to PSM peptides and *S. aureus* cells further revealed systematic induction of the pyoverdine biosynthesis cluster and the type VI secretion system (T6SS)—an interspecies weaponry that loads and fires effectors targeting both mammalian and prokaryotic cells. Single-cell microscopy of a fluorescent reporter of T6SS activity confirmed that PSMs are sufficient to induce *P. aeruginosa* deployment of T6SS.

S. aureus PSMs possess lytic activity towards mammalian cell through disruption of membrane integrity; however, they did not affect the viability of *P. aeruginosa* or permeabilize the outer or inner membranes. Yet, a subset of proteins necessary for cell envelope stress were induced, suggesting that transient, non-lethal membrane perturbations may activate T6SS. These observations provide a paradigm-shifting model for T6SS-mediated antagonism by a Gram-positive bacterium and its secreted signal.

Oral Presentation #14

Coxiella burnetii Effector Protein with FFAT Motif (EPF1) Mediates Membrane Contact Sites Between Host Lipid Droplets and Endoplasmic Reticulum

Rajendra Angara[1], Stacey D. Gilk[1]
[1]*University of Nebraska Medical Center*

Membrane contact sites (MCS) are areas of close apposition between two membranes and are involved in inter- or intra-organelle communication, primarily exchange of small metabolites, ions, and lipids. FFAT motif-containing proteins bind the endoplasmic reticulum (ER) VAP proteins to mediate MCS between the ER and different cell organelles such as mitochondria, golgi, and peroxisomes. Several bacterial pathogens, including *Coxiella burnetii*, are known to hijack VAP in order to establish MCS between the host ER and the bacteria-containing vacuole. *Coxiella* is the causative agent of Q fever endocarditis, a difficult to treat disease with a high fatality rate. *Coxiella* is found in lipid droplet (LD)-rich macrophages in the cardiac valves of Q fever endocarditis patients, and *Coxiella*-infected macrophages accumulate LDs in vitro. We recently discovered that *Coxiella* Type 4B Secretion System (T4BSS) effector proteins manipulate LD metabolism in macrophages; however, the specific *Coxiella* T4BSS effector proteins and their function in modulating host LD homeostasis is unknown. Here, we show that *Coxiella* secretes a T4BSS effector protein with FFAT motif (EPF1) that localizes to the host endoplasmic reticulum (ER) at LD biogenesis sites and translocates to the surface of mature LDs. EPF1 binds VAPB, a VAP protein on the ER, to form MCS between the host ER and LDs. This is the first report of bacteria-induced membrane contact sites outside of the bacteria-containing vacuole. EPF1-mediated membrane contact sites could be a novel strategy adopted by *Coxiella* to interfere in host inter-organelle communication and regulate host LD metabolism to support *Coxiella* infection.

Oral Presentation #15

Decoding Key Players of Plasmodium Nuclear Biology using Expansion Microscopy

Sabrina Absalon[1], Benjamin Liffner[1]
[1]*Indiana University School of Medicine*

Infection by the protozoan parasite Plasmodium falciparum causes the most severe form of human malaria. Clinical manifestation of malaria begins with the parasite invasion and proliferation in human red blood cells (RBC). Following the invasion of an RBC, a single-celled parasite replicates into ~ 20 new daughter parasites through an atypical cell division process named schizogony. Unlike its human host, P. falciparum undergoes multiple rounds of asynchronous DNA replication and nuclear division without cytokinesis to form a multinucleated parasite, followed by a single round of specialized cytokinesis where individual nuclei and subcellular organelles are partitioned to produce daughter parasites. This distinctive replication mode relies on timely DNA replication, segregation, and nuclear division within an intact nuclear envelope in a shared cytoplasm. To study the molecular processes occurring within an ~1 µm Plasmodium nucleus, we turned to Ultrastructure expansion microscopy (U-ExM), a recently developed sample preparation method for microscopy. U-ExM allowed us first to achieve the resolution needed to visualize intranuclear microtubule dynamics during nuclear multiplication. Moreover, we developed the first Plasmodium nuclear envelope staining enabled by expansion microscopy. We are now successfully applying our microscopy advances to determine the role of assembly/disassembly of microtubules, kinase, and nuclear condensate in DNA segregation and nuclear division. Our laboratory aims to uncover critical processes for the atypical cell division of malaria parasites that remain poorly understood and will likely reveal druggable targets for developing novel antimalarials urgently needed due to the emergence of resistance against all current treatments.

Oral Presentation #16

Bacteria, Fungus and Malaria: The Multifaceted Role of Mosquito Leucine-rich Repeat Immune Factor, APL1

Michelle Riehle[1], Tullu Bukhari[2], Christian Mitri[3], Kenneth Vernick[3]
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Mosquitoes within the genus Anopheles are the insect vectors of malaria infection and rely solely on innate immunity in their defense against pathogens. As the work to identify additional malaria control tool intensifies, understanding the role of mosquito immune factors against an array of pathogens in a variety of mosquito species remains essential. The role of mosquito APL1 in response to malaria infection in *Anopheles coluzzii* is well known. However, the role of APL1 in response to other pathogens is less characterized. Further, the APL1 gene family has evolved within the Anopheles genus expanding from a single gene in a majority of species to a 3 gene family in a small number of species. When the unique APL1 gene was knocked down within *A. stephensi*, a vector of malaria in SE Asia, mosquitoes experienced marked mortality which was rescued by antibiotic treatment suggesting a role for APL1 in conferring protection from or tolerance to bacterial pathology. Upon closer examination knockdown of APL1 was shown to lead to altered midgut abundance of two main taxa within the Enterobacteriaceae and not a generalized midgut dysbiosis. Following depletion of APL1C in *A. coluzzii*, a major vector of malaria in Sub Saharan African, challenged with entomopathogenic fungus, *Metarhizium anisopliae*, showed increase mortality. Both the genes involve and their regulatory pathways suggest a similarity in Anopheles responses to fungal and parasitic pathogens.

Oral Presentation #17

Cross-Kingdom Interactions: C. albicans Enhances Salmonella Typhimurium Pathogenesis

Olivia Todd[1], Kanchan Jaswal[1], William Santus[1], Judith Behnsen[1]
[1]*University of Illinois Chicago*

Salmonella enterica serovar Typhimurium (STm) is a food-borne bacterium that causes over 1 million infections in the United States alone. *Candida albicans* is a ubiquitous fungal opportunistic pathogen that colonizes the skin and gut of ~40-60% of the human population. As a major component of the human gut mycobiome, *C. albicans* presumably interacts with STm during infection, however this cross-kingdom interaction is currently almost entirely uncharacterized. We therefore stably colonized CBA/J mice with *C. albicans* and subsequently orally infected mice with STm. Mice pre-colonized with *C. albicans* showed higher STm dissemination to the spleen than mice infected with STm alone. Presence of *C. albicans* during STm infection also resulted in a 13% mortality rate whereas STm infection alone was non-lethal. In vitro, *C. albicans* induced expression of several STm virulence genes that enhanced STm invasion of epithelial cells. We determined that a number of *C. albicans* virulence factors are necessary for this enhancement, as mutants deficient in a major adhesin (Δ/Δ *als3*), quorum sensing molecule production (Δ/Δ *dpp3*), candidalysin toxin production (Δ/Δ *ece1*), and hyphal morphogenesis (Δ/Δ*efg1*/Δ/Δ *cph1*) did not augment STm invasion. Overall, we have found that *C. albicans* and STm interact synergistically to enhance pathogenesis and have begun to detangle the complex mechanism of this common cross-kingdom interaction.

Oral Presentation #18

Mechanisms of Anti-phage Immunity in Pathogenic Staphylococci

Asma Hatoum-Aslan[1], Lucy Chou-Zheng[1], Nayeemul Bari[1]
[1]*University of Illinois Urbana-Champaign*

Staphylococci are major constituents of the skin microbiome and leading causes of antibiotic-resistant infections. Of the ~40 skin-associated species, *S. aureus* and *S. epidermidis* have the greatest pathogenic potential: *S. aureus* is the leading cause of skin and soft tissue infections, and *S. epidermidis* is the most common cause of infections associated with indwelling medical devices. Thus, understanding the major factors that impact the survival and pathogenesis of these organisms is absolutely critical. Among such factors, staphylococcal phages and the immune systems targeted against them have profound and opposing effects. For instance, lysogenic phages can boost the pathogenic potential of staphylococci by mobilizing pathogenicity islands and carrying virulence factors that integrate along with the phage into the host. In contrast, the strictly lytic variety can kill the host within minutes of infection and are being exploited for therapeutic applications. Anti-phage defenses target lytic and lysogenic phages alike and therefore counter these opposing effects; However, little is known about the full battery of defenses that staphylococci employ and the mechanisms by which they operate. This presentation will discuss new insights into anti-phage immunity in staphylococci with a focus on our most recent findings using *S. epidermidis* and its phages as a model host-virus system. These efforts are expected to inform the development of more robust anti-staphylococcal therapeutics.

Oral Presentation #19

Career Panel

Aileen Rubio[1], Greg Richards[2], Victor Torres[3], Caitlin Brennan[4], Jonathan Jones[5], Jonathan Lenz[6],
[1]ClearB Therapeutics, Inc. [2]Univeristy of Wisconsin-Parkside, [3]New York University, [4]National Institute of Allergy and Infectious Diseases, [5]Genetech/Roche, [6]Catalent Pharma Solutions

Oral Presentation #20

The Genetics of Antibiotic Function in Acinetobacter baumannii

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Antibiotics kill bacteria by inhibiting essential cellular processes, but our knowledge of antibiotic-essential gene interactions in Gram-negative pathogens is severely limited. Here, we use CRISPRi knockdowns to comprehensively phenotype essential genes in Acinetobacter baumannii—a Gram-negative pathogen that is considered an "urgent threat" to escape antibiotic killing. We identified essential genes in A. baumannii that are most vulnerable to depletion; thus, providing an enticing list of potential antibiotic targets. To characterize interactions between essential genes and antibiotics of last resort, we developed a computational framework that combines machine learning prediction of CRISPRi knockdown efficacy with classic dose response curves. In doing so, we found a surprising new mechanism for killing by polymyxin antibiotics and uncovered the genetic basis for synergy between polymyxins and rifamycins—two antibiotic groups with unrelated cellular targets. Our results elucidate the mechanisms of antibiotic function in A. baumannii and point to new weaknesses that may be exploited in future therapies.

Oral Presentation #21

A siRNA Screen to Uncover the Mechanisms Underlying Plasma Membrane Repair of Mammalian Cells Damaged by Bacterial Pore-forming Toxins

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Plasma membrane disruption by pore-forming toxins is a most common and ancient strategy used by bacterial pathogens to infect their host and evade the host immune responses. *L. monocytogenes* is a facultative intracellular pathogen that infects a large variety of host cells including epithelial cells and macrophages. *L. monocytogenes* major virulence factor is a 57 kDa pore-forming toxin called listeriolysin O (LLO) that binds cholesterol-rich membranes to assemble a large transmembrane pore complex allowing for bacterial proliferation in the host cell cytosol. Despite LLO attack, the infected cells repair their plasma membrane to maintain homeostasis. To uncover the plasma membrane repair machineries of cells exposed to LLO, we developed a fluorescence-based siRNA screen. The selected library targets 287 host genes. HeLa cells stably expressing Histone-2B-GFP were reverse transfected with siRNAs and were exposed to 0.5 nM LLO and 1 µM TO-PRO-3 (a small dye that only labeled damaged cells) in Ca2+-containing (repair permissive conditions) and Ca2+-free (repair restrictive conditions). The fluorescence intensities of TO-PRO-3 and GFP were then recorded at regular time intervals in a multimode plate reader. TO-PRO-3 fluorescence intensity was used to assess cell integrity and GFP fluorescence to enumerate cells via image cytometry. Statical analysis confirmed that this assay has the robustness necessary to identify potential “hits”. In conclusion, this screen identified 70/287 targets, that when knocked down, altered membrane resealing efficiency. We identified genes encoding proteins controlling lysosomal biogenesis, membrane fusion, intracellular trafficking, exocytosis, and autophagy.

Oral Presentation #22

Enrichment and Validation of the Small Proteome from Mycobacteria

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Small proteins 'Sproteins' are polypeptides typically <50 amino acids in length. They are an abundant class of protein encoded by all forms of life and likely represent at least ~20% of the 'Dark Proteome.' However, identification of Sproteins is hampered by their biochemical properties; non-standard amino-acids, low mass, and dominance of the nominal proteome. This makes Sproteins challenging to annotate and detect. They are typically missed by traditional gene annotation, and standard mass-spectrometry preparations are poorly suited for their direct identification. As a consequence, we still know very few small proteins even in well studied prokaryotes and pathogens. Systematic evaluation of techniques to identify Sproteins are also lacking. Here, we have implemented a novel combination of strategies to detect Sproteins in Mycobacteria referred to as 'Anti-proteomes.' We prepared samples for proteomics on fractions from lysates and supernatants of M. smegmatis and M. tuberculosis and then performed MS analysis on the fractions nominally discarded from these sample workups. Analysis of these samples was performed by combining FASTA sequences of the canonical genome with custom protein sequences generated using ribosomal profiling approaches to enhance protein representation. Confirmation of candidate sProtein genes is enhanced by overlapping orthogonal techniques and generation of synthetic sProteins for empirical validation. We have identified more than 300 candidate and confirmed sProtein gene products in pathogenic/nonpathogenic Mycobacteria, which represents the largest single data collection to-date. Our data suggests that sProteins are pervasive and concatenated molecular and analytical strategies provide the best source for their census and ultimately evaluating function.

Oral Presentation #23

Structural and Mechanistic Insights into the Clostridioides difficile Transferase

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Clostridioides difficile is currently the leading cause of hospital-acquired infectious diarrhea in the United States. The pathology associated with *C. difficile* infection is the result of the activity of up to three toxins that are secreted by the bacterium at the site of infection known as Toxin A, Toxin B, and the *C. difficile* transferase (CDT). Although Toxin B alone has long been thought to drive the pathology associated with the disease state, recent studies have emerged that link the presence of CDT to increased disease severity and poorer patient outcomes. We are interested in understanding how CDT delivers its toxic cargo into host cells. As a part of our work, we have used cryo-electron microscopy to generate several structural snapshots of the pore-forming component of CDT which paint a picture of how the toxin transitions from the soluble state to a membrane embedded pore. From this work, we have determined the structure of the receptor binding domain and, using mutational analysis, we have proposed a receptor interacting surface. In conjunction with these studies, we determined the structure of the assembled toxin which revealed an unexpected mode of assembly and provided insight into how the translocation of cargo is accomplished. Together, these studies highlight marked differences in the architecture and functionality of CDT when compared to distantly related toxins.

Oral Presentation #24

Unraveling the Adaptation of Staphylococcus aureus During Bloodstream Infection

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In this presentation I will discuss our recent studies that take advantage of epidemiology, clinical specimens, microbial comparative genomics, and pathogenesis to understand microbial adaptation in humans. Specifically, I will discuss our findings on how the epidemic community-acquired methicillin-resistant Staphylococcus aureus (CA-MRSA) USA300 lineage, which is responsible for most skin and soft tissue infections in healthy individuals, has now become a leading cause of hospital-associated bloodstream infections (BSI). We have leveraged this recent introduction into hospitals and the limited genetic variation across USA300 strains to identify and study adaptive mutations. We found that bloodstream USA300 isolates exhibit altered virulence. Using comparative genomics, we discovered independent single-nucleotide variants in transcriptional regulators that are associated with altered virulence in these bloodstream isolates. Transcriptional analyses revealed that these mutations increased expression of a surface adhesin, ClfB, and decreased expression of proteases. Notably, the identified mutations increased virulence in a murine model of BSI, a phenotype dependent on ClfB. Altogether, these findings highlight ongoing adaptation of a major contemporary MRSA lineage to the hospital environment and suggest that USA300 strains can optimize their fitness in hospitals through evolution of altered regulation of virulence.

Poster Presentation #25

Regulation of Neisseria gonorrhoeae Peptidoglycan Dynamics in Different Infection Niches

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The inflammatory response to *Neisseria gonorrhoeae* (GC) infection of the Fallopian tubes drives the loss of ciliated cells from the epithelium. Gonococcal peptidoglycan (PG) fragments induce this inflammation. While infections of the Fallopian tubes are pro-inflammatory and damaging, infections of the cervix are more frequently asymptomatic. The underlying mechanism of these differences has yet to be determined; however, we hypothesize that gonococcal regulation of peptidoglycan breakdown and recycling dynamics plays an important role. In support of this idea, we have observed that purified GC tripeptide monomer, a PG fragment agonist of NOD pattern-recognition receptors, induces ciliated cell sloughing in human Fallopian tube in organ culture (FTOC). Further, RNAseq experiments show that treatment of FTOC with NOD agonist replete gonococcal supernatant suppresses transcripts involved in cell junction and extracellular matrix maintenance, while NOD agonist deplete supernatant does not. Investigation of PG recycling and fragment producing proteins demonstrated that they are regulated by infection niche. Levels of the major gonococcal lytic transglycosylase for producing PG fragments (LtgA) were higher in FTOC infection compared to cervix infection. By contrast, levels of the PG recycling enzyme AmpG are increased in cervix. These data suggest that GC may decrease toxic PG fragment production and enhance recycling when in the cervix to reduce the inflammatory response. Proteomics analysis of GC recycling mutants identified NGO1982 as a transcriptional regulator of PG-related proteins and ChIPSeq using NGO1982 identified binding to six PG-related genes. Its role in the coordinated, niche-dependent regulation of PG dynamics is being investigated further.

Poster Presentation #26

Stenotrophomonas maltophilia Pili and Their Role in Causing Chronic Infection

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Stenotrophomonas maltophilia, a Gram-negative, multidrug-resistant, opportunistic pathogen, is increasingly identified as a cause of numerous human infections, including respiratory tract, urinary tract, skin, blood, brain, and heart infections. It causes also chronic infections through biofilm formation on biotic and abiotic surfaces. However, *S. maltophilia* biofilm and virulence have been severely understudied. The SMF-1 pilus is suggested to play a major role in biofilm development, but the evidence supporting SMF-1's role in biofilm is mainly correlative. To address this issue, we isogenically deleted the *smf-1* pilin gene. The *Δsmf-1* strain was significantly defective for attachment and biofilm on biotic and abiotic surfaces, compared to wild type. Through bioinformatics, we found two other putative pilus systems, CBL and CUP, and isogenic deletion of their respective pilin genes (*cblA*, *cupA*) also inhibited bacterial attachment and biofilm growth. Analysis of clinical and environmental S. maltophilia strains indicates that the three pilin genes are nearly universally distributed and display high sequence conservation across strains. Importantly, clinical strains lacking *smf-1*, *cblA*, or *cupA* showed biofilm phenotypes like our isogenic mutants in type strain K279a, indicating vital importance for pathogenesis. We also found that mutation of one pilus fails to compensate for loss of another, and double and triple mutant strains demonstrate unexpected biofilm phenotypes, suggesting a unique regulatory crosstalk between the pilus systems. Intriguingly, pilus mutants were more virulent in a *Galleria melonella* infection model. Understanding how these 3 pili impact biofilm and pathogenesis could lead to new therapies to treat this emerging multidrug-resistant pathogen.

Poster Presentation #27

The Sodium Proton Exchanger NHE9 Regulates Phagosome Maturation and Bactericidal Activity in Macrophages

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Acidification of phagosomes is essential for the bactericidal activity of macrophages. Targeting machinery that regulates pH within the phagosomes is a prominent strategy employed by various pathogens that have emerged as major threats to public health. Nascent phagosomes acquire the machinery for pH regulation through a graded maturation process involving fusion with endolysosomes. Meticulous coordination between proton pumping and leakage mechanisms is crucial for maintaining optimal pH within the phagosome. However, relative to mechanisms involved in acidifying the phagosome lumen, little is known about proton leakage pathways in this organelle. Sodium proton transporter NHE9 is a known proton leakage pathway located on the endosomes. As phagosomes acquire proteins through fusions with endosomes during maturation, NHE9 seemed a promising candidate for regulating proton fluxes on the phagosome. Here, using genetic and biophysical approaches, we show NHE9 is an important proton leakage pathway associated with the maturing phagosome. NHE9 is highly expressed in immune cells, specifically macrophages; however, NHE9 expression is strongly downregulated upon bacterial infection. We show that compensatory ectopic NHE9 expression hinders the directed motion of phagosomes along microtubules and promotes early detachment from the microtubule tracks. As a result, these phagosomes have shorter run lengths and are not successful in reaching the lysosome. In accordance with this observation, we demonstrate that NHE9 expression levels negatively correlate with bacterial survival. Together, our findings show that NHE9 regulates lumenal pH to affect phagosome maturation, and consequently, microbicidal activity in macrophages.

Poster Presentation #28

CteG Mediated Control of Centrin-2: Getting to the Center of How Chlamydia trachomatis Infection Causes Centrosome Amplification

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Chlamydia trachomatis (C.t.) is the most prevalent bacterial sexually transmitted infection and the leading cause of non-congenital blindness worldwide. Infections are often asymptomatic, leading to severe consequences like pelvic inflammatory disease, ectopic pregnancy, and sterility. Moreover, individuals with current or prior infections are at an increased risk of ovarian or cervical cancers. C.t. infection has been correlated with blocked cytokinesis, supernumerary centrosomes, and multipolar spindles. However, the mechanisms behind how C.t. induces these cellular abnormalities are unknown. From the confines of its inclusion, C.t. must engage numerous host organelles and signaling pathways to generate a niche that is permissive for replication. To accomplish these feats, C.t. releases an array of proteins into the host cell via a type III secretion system (T3SS). Using affinity purification-mass spectrometry, we show that T3SS effector protein, CteG, binds to centrin-2 (CETN2), a key structural component of centrosomes and regulator of centriole duplication. Using sequential truncations, we have determined that the C-terminus of CteG binds to the C-terminus of CETN2, a region containing a key calcium binding domain. Significantly, deletion of CteG leads to normal centrosome number, indicating that C.t. infection-induced centrosome amplification occurs in a CteG-dependent manner. Uniquely we have identified the first effector to target centrins and have begun to address how C.t. induces gross cellular abnormalities. Understanding the mechanistic underpinnings of the effect of CteG-CETN2 interaction, its impact on the centrosome amplification, and the long-term consequences this has on host cells could explain why chlamydial infection leads to an increased cancer risk.

Poster Presentation #29

A Transposon in Acinetobacter baumannii 17978 Encodes a Microcin System with Antimicrobial Properties to Engage in Contact-independent Competition

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Acinetobacter baumannii is a multidrug-resistant opportunistic pathogen that persists in the hospital environment and produces a wide variety of clinical infections, primarily affecting immunocompromised patients. Here, we report that *A. baumannii* strain 17978 (AB17978) encodes the subclass II microcin 17978 (Mcc17978) with antimicrobial activity through a contact-independent mechanism, and that the Mcc17978 system is upregulated under low-iron conditions commonly found in the host environment. Using classical bacterial genetic approaches, we determined that the molecular receptor of Mcc17978 in *Escherichia coli* is the iron-catecholate transporter Fiu. We also identified the genetic locus encoding the Mcc17978 system in AB17978. In bacteria, the Ferric uptake regulator (Fur) positively regulates siderophore systems and microcin systems under iron-deprived environments. We identified a putative Fur binding site upstream of the *mcc17978* gene and we observed that low iron levels increased the antimicrobial activity of Mcc17978. Lastly, we found that AB17978 can exhibit antimicrobial activity against different *E. coli* K12 and *Acinetobacter* strains.

IMPORTANCE Despite the cumulative knowledge on microcins, little is known about the microcins produced by AB17978 and their relevance in bacterial competition. Here, we described the subclass II microcin Mcc17978 utilized by AB17978 to compete with other bacteria and demonstrated that this system is upregulated under iron deficiency typically found in the host environment, suggesting that *A. baumannii* may utilize microcins to compete for resources during infection.

Poster Presentation #30

A New Toxin Class in Pseudomonas aeruginosa Mediates Interbacterial Competition and Impacts Virulence

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Microbes compete for limited resources within their environment and have evolved numerous strategies to antagonize competitors. One competitive strategy involves producing effector proteins that directly interfere with the growth of neighboring cells. These effectors are often modular proteins where the N-terminus guides the secretion and delivery of a C-terminal "toxin" domain into a neighboring bacterium. The action of this toxin domain restricts replication of the intoxicated cell. Siblings of toxin-producing strains are protected because they encode a cognate immunity factor, which binds and neutralizes the toxin. These proteins are often polymorphic, meaning the same protein in a different bacterium may contain a different C-terminal toxin domain.

We uncovered a new class of polymorphic effectors in Pseudomonas aeruginosa. Predictive modeling suggests this new class forms a heterodimeric shell that encapsulates a variable C-terminal toxin domain. We demonstrated that heterologous expression of the toxin domain attenuates bacterial replication and that including a cognate immunity factor rescues growth. Further, we observed that the holotoxin could inhibit the replication of susceptible target cells in mixed culture. This inhibition also occurred when only filtered culture supernatant from a toxin-producing strain was used, suggesting this is a secreted toxin. Sequence variation in the N-terminus of the protein may indicate that this domain steers the holotoxin toward different target cell receptors to facilitate toxin delivery. We also observed that toxin mutants are attenuated in a bloodstream infection model, suggesting a potential role for this protein in competition and virulence.

Poster Presentation #31

Not So 'Secret'ome: An Analysis of The Secretion Profiles of Streptococcus Pneumoniae Secretion Chaperone Mutants

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To be released from the bacteria, proteins must first translocate across the bacterial cell membrane, fold into their final state, and become active in the space between the cell membrane and the cell wall. The membrane-wall space is a challenging environment that inhibits many proteins from folding themselves and necessitates secretion chaperones to perform this function. Investigations into the mechanism underlying bacterial secretion have historically been performed in Gram-negative bacteria which have a thin cell wall sandwiched between inner and outer cell membranes. However, due to structural differences, much remains unclear about the processes responsible for protein secretion in Gram-positive bacteria which have a single cell membrane followed by a thick cell wall. Streptococcus pneumoniae is a Gram-positive, extracellular bacterium that is a common inhabitant of the human upper respiratory tract. In S. pneumoniae, three secretion chaperones work in the membrane-cell wall space: PrsA, SlrA and HtrA. Preliminary research has determined that each of these chaperones is required for host cell adhesion. This led us to hypothesize that these chaperones are required for the folding and activity of proteins involved in processes such as host cell adhesion. To investigate the varied roles of secretion chaperones, we used proteomic techniques to determine the secretion profiles of *ΔprsA, ΔslrA* and ΔhtrA mutants. The secretion profiles of these mutants were analyzed to identify proteins that are altered secretion levels in the absence of each secretion chaperone. The "client proteins" identified in these secretion profiles will be investigated for roles in host cell adhesion.

Poster Presentation #32

The Yersinia pestis Type 3 Secretion System Triggers LTB4 Synthesis by Leukocytes in an Inflammasome-independent Manner

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Yersinia pestis causes the human disease known as plague. A key manifestation of plague is a delay in neutrophil infiltration into Y. pestis infected tissues. Because this delay in neutrophil recruitment is required for virulence, we are interested in defining the molecular mechanisms used by Y. pestis to evade immune recognition. Eicosanoids are lipids produced early during infection and necessary to initiate a rapid inflammatory response. Despite the importance of these lipids in mediating inflammation, the role of the eicosanoids' during plague has not been previously investigated. Using the mouse model, we determined the kinetics of eicosanoid synthesis during pneumonic plague. While the cyclooxygenase pathway was rapidly induced, the lipoxygenase pathway was significantly delayed, including leukotriene B4 (LTB4), which is a potent chemoattractant and essential in initiating the inflammatory cascade. Using primary leukocytes, we demonstrated that neutrophils and macrophages do not synthesize LTB4 in response to Y. pestis unless the bacteria lack the type 3 secreted Yop effector proteins. Surprisingly, the synthesis of LTB4 by leukocytes in response to the Yop effector mutant was dependent on the expression of the bacterial type 3 secretion system (T3SS) but independent of host caspase 1/11 and inflammasome activation. Together, these data represent the first characterization of the eicosanoid response during pneumonic plague and suggest that Y. pestis inhibition of LTB4 synthesis is important for the delayed inflammatory response associated with plague. Furthermore, it suggests a previously undescribed inflammasome-independent mechanism used by leukocytes to sense and respond to the bacterial T3SS.

Poster Presentation #33

Unraveling Candida albicans Dispersion by Screening Transcription Factor Mutants

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Candida albicans is an opportunistic pathogenic fungus that develops biofilms on medical devices, such as catheters. Biofilms release "dispersed" cells into the bloodstream, causing systemic infections with mortality rates approaching 50%. Candida strains deficient for dispersion are less virulent suggesting that dispersion plays an important role in disease progression. To identify genetic factors regulating dispersion, we screened a library of 165 transcription factor mutants for dispersion phenotypes. The library was screened using both an improved 96 well plate assay and an underoil droplet assay. The 96 well plate assay uses both microscopy and a metabolic assay to determine growth during biofilm initiation, mature biofilm metabolic output, dispersed cell morphology and quantity, and dispersed cell metabolic output. The underoil droplets use double-exclusive liquid repellency to create droplets that allow visualization of all stages of biofilm growth. Using these methods, we discovered multiple mutants that showed increased dispersion when compared to wildtype; most notably the Rob1 transcription factor mutant which is known to regulate biofilm development. We will use candidate's gene expression to potentially link specific genes to the dispersion phenotype.

Poster Presentation #34

Multiple Domains within DciA Contribute to its Roles During Mycobacterial DNA Replication

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The loading of the replicative helicase is a key step during DNA replication in bacteria. In Eschericha coli and Bacillus subtilis, the replicative helicase is loaded by a AAA+ ATPase helicase loader. However, most bacteria do not encode homologs to these ATPases. Instead, most bacteria encode a domain of unknown function (DUF)721-containing protein, DciA, which lacks an ATPase domain. DciA homologs interact with the replicative helicase, are essential for bacterial viability, and depletion of DciA in mycobacteria leads to a block in DNA replication. These data are evidence that DciA performs an analogous function to helicase loaders and likely constitute the predominant mechanism of helicase operation during DNA replication in bacteria. However, the mechanism of DciA helicase operation is unknown. DciA homologs are defined by the presence of the DUF721 (DciA) domain. However, we have found that DciA proteins from diverse bacterial phyla vary in the position of the DciA domain within the DciA protein. The DciA homolog in mycobacteria, a Group 3 DciA protein, contains amino acid extensions both N and C-terminal to the DciA domain. The DciA domain of mycobacterial DciA is not sufficient to support viability, highlighting the importance of the sequence extensions, which are not conserved in all DciA proteins. We have identified residues both within the N-terminal extension and the DciA domain that are critical for mycobacterial growth and cell cycle progression, demonstrating that both regions of DciA are critical for its function. Therefore, DciA proteins in different groups may have different mechanisms of helicase operation.

Poster Presentation #35

ISG15 Modification of the Arp2/3 Complex Restricts Pathogen Spread

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During infection the ubiquitin-like protein, ISG15, can act as a cytokine or can covalently modify host and pathogenic proteins. While progress has been made in identifying sites of modification on target proteins, the molecular consequences of ISGylation on individual protein substrates are still unknown. Here by using a model of enhanced ISGylation, we identify that ISG15 modifies the ARP2/3 complex following both bacterial and viral infection. ISGylation significantly slows *Listeria*-actin comet tail speed leading to morphologically shorter and denser comet tails. For Vaccinia virus, this results in reduced spread, whereas while *Listeria* is initially restricted, over time the bacteria divide while remaining tightly attached to actin filaments resulting in multi-headed comet tails that move as a group. These structures contribute to spread in both human and murine cells and in vivo in mice with unchecked ISGylation, which leads to increased mortality. ISG15 modification of the Arp2/3 complex also affects cortical actin density, cell motility, and adhesion. Furthermore, ISG15-deficient neonates have aberrant epidermal epithelia, which correlates with observed defects in wound healing in a subset of human patients who lack ISG15. Our discovery identifies a conserved molecular mechanism of ISG15 modification of the Arp2/3 complex which directly restricts pathogen spread.

Poster Presentation #36

Antimicrobial Efficacy Against Antibiotic-Tolerant Staphylococcus aureus Depends on the Mechanism of Antibiotic Tolerance

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Background
Bacteria can adopt alternate metabolic states during infection. In Staphylococcus aureus this is induced by nutritional limitation, host responses and bacterial competition. Isogenic "tolerant" subpopulations have variable responses to antibiotics and can remain viable. Survivors resume growth upon antibiotic cessation and cause relapse infection. In this study we compare the capability of antibiotics to reduce viability of S. aureus made tolerant by different mechanisms.
Methods

Overnight S. aureus SH1000 was induced with mupirocin (nutritional), HQNO (competitive), peroxynitrite (oxidative) or serum (humoral). Tolerant cultures were exposed to ceftaroline, daptomycin, gentamicin, levofloxacin, oritavancin or vancomycin at physiological concentrations and viability assessed (dilution plating). Time to bactericidal activity (TBA) and 24h viability reduction were calculated independently for three biological replicates. Significance ($P < 0.05$) was determined using Student's t-test.

Results
TBA was prolonged for most antibiotics tested regardless of mechanism of tolerance. In contrast to other agents, oritavancin's TBA was only modestly prolonged with nutritional tolerance and not prolonged with other forms of tolerance. Reduction in 24h viability was mitigated for nutritionally- (5/6), competitively- (2/6), oxidatively- (3/6) and humorally- (5/6) tolerant staphylococci. Again, oritavancin uniquely demonstrated sustained viability reduction at 24h against uninduced and tolerant cultures.

Conclusion
Tolerance can alter both time to bactericidal effect and the extent of killing. Both the antibiotic and the mechanism of tolerance impact TBA and 24h viability. Oritavancin was the only antibiotic that maintained the same extent of killing regardless of tolerance mechanism. Further studies to evaluate additional antistaphylococcal antibiotics and different tolerance inducers are warranted.

Poster Presentation #37

Staphylococcus aureus Promotes Melanoma Growth and Invasion

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The gut microbiome can impact melanoma tumor growth and anti-cancer therapy response; however, little is known about the impact of the skin microbiota. *Staphylococcus* species are found on both healthy skin and in melanoma. Thus, I aim to determine how the skin microbe *S. aureus* can affect cutaneous melanoma growth and progression. To evaluate cancer progression in vivo, zebrafish melanoma cells (ZMel) were cultured with *S. aureus* bacterial supernatant before injection into the larval zebrafish hindbrain. In vivo imaging revealed increased melanoma growth and metastasis after incubation with *S. aureus* supernatants, compared to control. To determine the mechanism driving melanoma invasion, I utilized an in vitro cell cluster assay. Melanoma cells showed increased clustering with bacterial supernatant, correlating to a more invasive phenotype. Furthermore, only supernatants from the gram-positive *S. aureus* bacteria caused melanoma clustering, while *S. epidermidis* or gram-negative bacteria did not. Charcoal stripping of *S. aureus* supernatant prevented cluster formation indicating that the molecule promoting metastasis is likely a lipid. Accordingly, melanoma culture with *S. aureus* lipase mutant *gehB::tn* resulted in smaller clusters. Finally, *S. aureus* supernatants were shown to decrease TFAP2 expression in melanoma, a transcription factor known to inhibit cancer cell invasion. Thus, *S. aureus*-derived lipids may activate pro-metastatic signaling pathways in melanoma. Understanding the interface between the skin microbiome and the melanoma tumor microenvironment will be informative for identification of novel therapies to inhibit cancer cell metastasis.

Poster Presentation #38

InvL, an Invasin-Like Adhesin, is a Type II Secretion System Substrate Required for Acinetobacter baumannii Uropathogenesis

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Acinetobacter baumannii is an opportunistic pathogen of growing concern, as isolates are commonly multidrug resistant. While A. baumannii is most frequently associated with pulmonary infections, a significant proportion of clinical isolates come from urinary sources, highlighting its uropathogenic potential. The type II secretion system (T2SS) of commonly used model Acinetobacter strains is important for virulence in various animal models, but the potential role of the T2SS in urinary tract infection (UTI) remains unknown. Here, we used a catheter-associated UTI (CAUTI) model to demonstrate that a modern urinary isolate, UPAB1, requires the T2SS for full virulence. A proteomic screen to identify putative UPAB1 T2SS effectors revealed an uncharacterized lipoprotein with structural similarity to the intimin-invasin family, which serve as type V secretion system (T5SS) adhesins required for the pathogenesis of several bacteria. This protein, designated InvL, lacked the β-barrel domain associated with T5SSs but was confirmed to require the T2SS for both surface localization and secretion. This makes InvL the first identified T2SS effector belonging to the intimin-invasin family. InvL was confirmed to be an adhesin, as the protein bound to extracellular matrix components and mediated adhesion to urinary tract cell lines in vitro. Additionally, the invL mutant was attenuated in the CAUTI model, indicating a role in Acinetobacter uropathogenesis. Finally, bioinformatic analyses revealed that InvL is present in nearly all clinical isolates belonging to international clone 2, a lineage of significant clinical importance. In all, we conclude that the T2SS substrate InvL is an adhesin required for A. baumannii uropathogenesis.

Poster Presentation #39

Genetic Dissection Uncovers a Relationship Between Undecaprenyl-PP Biosynthesis and Maintenance of Lipid Asymmetry in A. baumannii

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Acinetobacter baumannii is a Gram-negative opportunistic pathogen responsible for causing nosocomial infections and is a major health concern due to increasing multidrug resistance. The *A. baumannii* envelope includes an inner (IM) and outer membrane (OM). The OM has an asymmetric composition that is important for structural integrity and barrier to the environment. Due to this, bacteria have mechanisms to uphold this asymmetry. The maintenance of lipid asymmetry system (Mla) removes glycerophospholipids from the OM outer leaflet and transports them to the IM. Loss of this system results in attenuated virulence and increased susceptibility to membrane stressors and some antibiotics. We recently reported two variants of the type strain ATCC 17978, 17978VU and 17978UN. In these two strain backgrounds, we observed different phenotypes of Δ*mlaF* strains for colony morphology and membrane stress resistance. Therefore, we used comparative genetics to identify interactions between ATCC 17978 variant alleles and *mlaF*. We identified a SNP in the essential gene encoding undecaprenyl pyrophosphate (Und-PP) synthase, *uppS*, that confers the opposite phenotype when alleles are exchanged. Und-PP is a lipid glycan carrier implicated in the biosynthesis of multiple bacterial envelope components. Additionally, we identified multiple suppressor mutations within the isoprenoid biosynthetic pathway that are predicted to modulate flux to UppS. Our data suggests the cellular level of Und-PP is critical for tolerance to membrane stressors and some antibiotics in the absence of the Mla system. These findings uncover synergy between Und-PP synthesis and the Mla system in maintaining the *A. baumannii* outer membrane and stress resistance.

Poster Presentation #40

Klebsiella pneumoniae Transposon Sequencing Reveals Multisite and Tissue-specific Fitness Mechanisms During Bacteremia

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Gram-negative bacteremia is a major cause of global morbidity involving three pathogenesis phases: initial site invasion, dissemination, and survival in the blood and filtering organs. Klebsiella pneumoniae (Kp) is a leading bacteremia pathogen and the lung is a common initial site. Defining shared and distinct Kp fitness strategies across bacteremia phases may identify therapeutic targets to prevent infection or subsequent sepsis. Previously, we found that capsule production is critical for initial site fitness in the lung, as is branched chain amino acid and purine biosynthesis pathways. To identify genes required for Kp dissemination and bloodstream survival, we performed transposon insertion sequencing (TnSeq) in a bacteremic pneumonia model and intravascular (tail-vein injection) model, respectively. Bacteremic pneumonia Tn-Seq identified the LPS inner core biosynthesis gene, gmhB, as important for dissemination and fitness in the spleen, but dispensable for lung fitness. Intravascular TnSeq data revealed 57 Kp genes enhancing bloodstream fitness. These include pdxA, contributing to a vitamin B6 biosynthesis pathway unique to Enterbacterales, which enhances spleen fitness. A pdxA mutant had a moderate splenic defect in the intravascular model that was exacerbated after a slight lung fitness defect in the bacteremic pneumonia model, which may reflect multiple rounds of dissemination from the lung. In contrast, purine biosynthesis genes were important across all three phases of bacteremia. Combined, these data from forward genetic screens in complementary animal models define both tissue-specific fitness strategies and mechanisms required across all phases of Gram-negative bacteremia.

Poster Presentation #41

The Role of TamAB in Salmonella Pathogenesis

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Salmonella can survive within macrophages during systemic infection by activating various virulence factors, including the PhoPQ two-component regulon. We showed that PhoP directly induces the transcription of *tamAB* operon both in vitro and in macrophages.

The TamA protein is structurally similar to BamA. We hypothesized that the Bam machinery struggles in the macrophage due to the harsh phagocytic environment; the TamAB system gets induced to compensate. Deletion of the *tamAB* operon does not result in significant virulence defect in mice. However, the *tamAB bamB* mutant has a synthetic effect, exacerbating the bamB virulence defect by 14-fold. This virulence defect can be recapitulated in tissue culture macrophages. Additionally, a similar synthetic phenotype is observed in vancomycin and SDS sensitivity assays in vitro and was complemented by a plasmid encoding *tamAB*.

We isolated suppressors of vancomycin sensitivity in *tamAB bamB* mutant. Sequencing of these suppressors showed multiple loss of function mutations in the LPS synthesis pathway such as *galE* and was confirmed by clean deletion of gal operon. Furthermore, *mlaA* deletion, presumably causing a phospholipid increase in the outer leaflet of the outer membrane, rescues the vancomycin sensitivity. These results suggest the role of *tamAB* in outer membrane lipid homeostasis.

Finally, we performed an LC/MS analysis of the *tamAB bam* double mutant outer membranes and compared them to the outer membranes of single mutants and wild-type *Salmonella*. We compared these results with RNA sequencing results under the same conditions. The results show various proteins present in less abundance than in single mutants.

Poster Presentation #42

An Atypical ABC Transporter is Involved in Antifungal Resistance and Host Interactions in the Pathogenic Fungus Cryptococcus neoformans

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Fungal infections kill close to 1.6 million people yearly, with the biggest burden in resource-limited regions. One of the leading causes of fungal infections is the environmental, opportunistic pathogen, *Cryptococcus neoformans*, which is responsible for over 180,000 deaths annually. Moreover, the population at risk to develop disease from this fungus is increasing, hence *C. neoformans* represents a serious health concern. Previous studies have correlated fungal uptake by macrophages with mortality, hence we performed a screen of 1,200 *C. neoformans* single-gene deletions for fungal regulators of phagocytosis. One of the mutants identified with increased uptake was missing the gene CNAG_06909. We have called this uncharacterized gene *PDR6* due to similarities to Pleiotropic Drug Resistance genes in other fungi and hypothesized that it may play a role in cryptococcal virulence. Sequence analysis showed that *PDR6* encodes a unique half-size ABC transporter protein. We found that in antifungal susceptibility assays the *pdr6Δ* strain was hypersensitive to azoles, the most common class of antifungal drug. Additionally, we found that the *pdr6Δ* strain has significantly less ergosterol in its plasma membrane when compared to the WT, which could explain defects in biofilm formation, capsule shedding, and host recognition exhibited by this mutant. Collectively, these results are consistent with a model where Pdr6 regulates ergosterol transport into the plasma membrane, affecting both azole resistance as well as host-fungal interactions. Determination of its biological functions will open new avenues of investigation in the cryptococcal field and more broadly, advance ABC transporter research given their evolutionarily conserved nature.

Poster Presentation #43

Regulation of Arginine and Ornithine Catabolism in Acinetobacter baumannii

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Acinetobacter baumannii is a gram-negative bacterium that is a common cause of hospital-acquired infections. *A. baumannii* poses an urgent public health threat worldwide due to its widespread multidrug resistance. During infection, *A. baumannii* must acquire all of its nutrients from the host. Arginine is an amino acid that can serve as a sole source of carbon or nitrogen for *A. baumannii*. Prior studies have suggested that *A. baumannii* use host-acquired arginine as a nutrient source during infection. *A. baumannii* catabolizes arginine via the arginine-succinyltransferase (AST) pathway, producing glutamate. Within the *A. baumannii* genome, we identified two partially duplicated operons that encode for enzymes predicted to play a central role in this pathway. The partially duplicated operon (*ast2*) is only encoded in pathogenic *Acinetobacter* and consists of two enzyme-encoding genes preceded by a gene encoding for a predicted transcriptional regulatory protein. We show that the *ast2* operon and the transcriptional regulator are essential for growth on ornithine, suggesting that the operon functions in ornithine utilization as well as arginine catabolism. We, therefore, named it the ornithine utilization regulator, OutR. Luciferase reporter gene assays suggest OutR activates the *ast2* operon during growth with arginine/ornithine and TCA cycle carbon sources. Future work will determine DNA binding activity of OutR through EMSA and define its regulon by ChIP-Seq. These results reveal a critical mechanism of nutrient acquisition for pathogenic *Acinetobacter* that may be a potential therapeutic target to overcome the multidrug resistance of pathogenic strains.

Poster Presentation #44

Disarming Bad Bugs: Targeting the Pneumolysin Toxin in *Streptococcus pneumoniae*

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Streptococcus pneumoniae is a deadly pathogen that can cause pneumonia, blood poisoning, and inflammation of the protective layers of the brain. To colonize and invade the host, *S. pneumoniae* secretes a wide repertoire of virulence factors that enables it to establish a replication niche within the host. The secreted proteins and virulence factors must be folded within the challenging cell wall-cell membrane compartment. *S. pneumoniae* encodes three post-translocation secretion chaperones SlrA, HtrA and PrsA. All three chaperones have been implicated in the pathogenicity of *S. pneumoniae* where they are thought to function in the folding of secreted proteins and virulence factors at the bacterial membrane interface. In this study, we utilized SILAC-affinity pulldown to identify putative virulence factors that interacted with SlrA. Out of the total client proteins identified, approximately 30% are known virulence factors including the pneumolysin toxin, Ply. We determined that Ply binds to SlrA with a nanomolar affinity. Site directed mutations in the putative S1 and S2 pockets of SlrA significantly reduces the peptidyl prolyl isomerase activity of SlrA and binding to Ply. We showed that the addition of cyclosporine A, an immunosuppressive drug significantly reduces the binding of SlrA to Ply. Taken together, our results suggest that the pneumolysin toxin is bound by SlrA, and this interaction can be inhibited as a means of a targeted therapeutics.

Poster Presentation #45

Identification and Preliminary Functional Characterization of Chlamydia trachomatis Type III Secreted Effector Proteins

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Chlamydia trachomatis (C.t.) is an obligate intracellular pathogen that replicates in a parasitophorphorus vacuole termed the inclusion. Central to pathogenesis is a type III secretion system that translocates effector proteins into the host cell, which are predicted to play major roles in host cell invasion, nutrient acquisition, and immune evasion. However, until recently the genetic intractability of C.t. hindered identification and characterization of these potentially important virulence factors. Here we sought to expand the repertoire of identified effector proteins and confirm they are secreted during infection. Using a CyaA, BlaM, and GSK assay we confirmed secretion of most effectors that had previously been defined as secretion candidates using a surrogate system. Significantly, we identified several novel effector proteins that had not been previously reported. Additionally, we preliminarily characterized any effector proteins that were secreted. Here we show that a subset of these effectors traffic to distinct host organelles when ectopically expressed and perturb important eukaryotic cell pathways using Saccharomyces cervisiae as a model organism. By elucidating which effector proteins are secreted into the host cell and what their functions are, we will be able to know which ones are important or necessary for C. t. survival and pathogenesis.

Poster Presentation #46

PII-like Signal Transduction Protein PstA Modulates c-di-AMP-mediated Antibiotic Resistance in Listeria monocytogenes

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C-di-AMP is an essential second messenger in bacteria, and directly regulates many molecular targets within the bacterial cell. PstA is a conserved c-di-AMP-binding protein and is broadly present among c-di-AMP-producing bacteria. However, the biological functions of PstA remain elusive. In the intracellular pathogen Listeria monocytogenes, we found that PstA is important for β-lactam resistance, but its function is dependent on cytoplasmic c-di-AMP levels. In the wild-type strain and a low c-di-AMP mutant, over-expression of PstA increased β-lactam susceptibility, whereas its deletion increased resistance. Conversely, at high c-di-AMP levels, PstA is beneficial to β-lactam resistance. L. monocytogenes is a facultative anaerobe that ferments under anaerobiosis and respires with oxygen under aerobic growth. We further found that PstA most likely regulates aerobic metabolism under β-lactam stress. Manipulation of PstA only conferred β-lactam susceptibility phenotypes in aerobic cultures, but not under anaerobic growth. We next examined mutants abolished for components of the electron transport chain and the tricarboxylic acid cycle, which together enable aerobic metabolism in L. monocytogenes. Of these components, the absence of cytochrome oxidases completely reversed the b-lactam resistance phenotypes of delta-pstA mutants. Taken together, our data suggest that PstA regulates the aerobic electron transport chain in L. monocytogenes.

Poster Presentation #47

Investigating the Role of the Methylcitrate Cycle and Remodeling of Lipid Metabolism During ATP Depletion in Mycobacterium tuberculosis.

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Mycobacterium tuberculosis *(Mtb)* adopts a non-replicating state in response to host-derived stresses. Non-replicating bacilli are tolerant to several frontline antibiotics but remain vulnerable to drugs targeting energy homeostasis, such as the ATP synthase inhibitor bedaquiline. However, our understanding of how *Mtb* responds to perturbation of energy metabolism remains incomplete. We previously identified C10 as a compound that decreases oxygen consumption and ATP levels, making C10 a promising tool for dissecting the link between ATP homeostasis and drug tolerance. C10 induces methylcitrate cycle (MCC) enzymes PrpD, PrpC, and their transcriptional activator PrpR, and also increases levels of MCC metabolites propionyl-CoA, 2-methylcitrate, and methylmalonyl-CoA, which can be derived from odd-chain fatty acid catabolism. C10 treatment also altered *Mtb* lipid composition and inhibited the lipid-dependent processes of biofilm formation and cording. Supplementation with exogenous propionate rescued *Mtb* susceptibility to C10, suggesting increased propionate levels support growth during C10 treatment. The MCC metabolizes propionate into succinate and pyruvate but can also run in reverse to make propionyl-CoA, an important precursor cell envelope lipids. We hypothesize that MCC upregulation improves *Mtb* growth during C10 treatment and propose two possible models where 1) C10 blocks lipid biosynthetic pathways, inducing reverse MCC flux to generate propionyl-CoA for lipid synthesis or 2) C10 promotes forward MCC flux to regenerate succinate to replenish tricarboxylic acid cycle carbon units and compensate for ATP depletion. These experiments elucidate unexplored connections between *Mtb* energy homeostasis and lipid and central carbon metabolism, revealing novel mechanisms by which *Mtb* compensates for ATP depletion.

Poster Presentation #48

Spike IgG, but Not IgA, is Elevated in Rheumatology Patients following COVID-19 mRNA Vaccine Boost

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The objective of this study was to assess the impact of different DMARDs on antibody response to the 3rd/booster dose of mRNA COVID-19 vaccines in rheumatic patients and compare overall antibody titers to those of healthy controls. Samples were collected from fully vaccinated adults being treated for rheumatic diseases at the Medical College of Wisconsin, 30 age and sex matched controls, and 15 pre-pandemic controls. Titers of Spike S1 (IgG, IgA), Spike RBD (IgG, IgA), and Nucleocapsid (IgG) were quantified using the LegendPlex assay (Biolegend). Pre-boost titers of Spike and RBD IgG were significantly lower in rheumatic subjects than matched controls and all IgA levels were low. However, both IgG and IgA titers were rectified to similar of those in matched controls following the boost. Vaccine non-responders were using DMARD treatments of B cell depleting therapy or JAK inhibitors, suggesting a variable response to the vaccine in rheumatic patients based on the type of therapeutic intervention in use. Results of this study are consistent with previously published work reporting decreased vaccine response in immunocompromised individuals, variable vaccine response in patients using DMARD treatments, and transient IgA response to mRNA vaccines. Importantly, this reinforces the importance of COVID-19 vaccine boosts, especially in immunocompromised populations. This data also suggests poor IgA response may contribute to the high rate of COVID-19 reinfections and breakthrough infections.

Poster Presentation #49

High Levels of Cyclic di-guanylate Interfere with Initiation of a Beneficial Symbiosis

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The binary symbiosis between *Vibrio fischeri* and the Hawaiian bobtail squid (*Euprymna scolopes*) is a valuable model system to study host-bacterial signal transduction *in vivo*. Using this system, we examined how global c-di-GMP levels impact the ability of bacteria to form a productive host association and identified emergent behaviors in the host that were not evident in culture. C-di-GMP inhibits motility and promotes biofilm formation in numerous bacteria. *V. fischeri* encodes 50 proteins predicted to synthesize and/or degrade c-di-GMP. Deletion of multiple genes encoding enzymes that synthesize or degrade the molecule yielded high and low c-di-GMP strains, respectively. In the host as early as 18 hours post inoculation, the high c-di-GMP strain was defective in colonization, while the low c-di-GMP strain had no defect. Colonization by the high c-di-GMP strain was rescued by expression of a *V. cholerae* phosphodiesterase, arguing that c-di-GMP levels, not signaling specificity, caused the colonization defect. Elevated c-di-GMP levels resulted in altered biofilm aggregates in the host mucus compared to those made by a low c-di-GMP strain. Gene expression in the aggregates revealed a novel regulatory interplay between two distinct polysaccharides: Syp expression, which is required for host colonization, is repressed by c-di-GMP-induced cellulose. These interactions do not occur in culture-grown cells, emphasizing the importance of studying signal transduction *in vivo*. Together, our data show that proper retention of a low c-di-GMP state is critical for establishment of the vibrio-squid symbiosis and that even modest increases impact biofilm composition, aggregation, and motility in the host.

Poster Presentation #50

Discovery of Natural Antimicrobials from the Cheese Ripening Microbiota

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Listeria monocytogenes is a leading cause of mortality among foodborne illnesses. L. monocytogenes is abundantly present in the natural and agricultural production environments, and frequently contaminates the human food production chain. Furthermore, L. monocytogenes is notoriously adaptable under various stress conditions, and its ability to form biofilm on many materials adds further challenge to controlling this pathogen. Fermented foods harbor diverse and complex microbial communities that have been shown to exhibit antimicrobial activities. Among various fermented foods, cheese is exceptionally well studied for microbiota composition and diversity. Furthermore, on the surface of wooden boards used in cheese ripening, we found that L. monocytogenes was greatly inhibited. Thus, we hypothesize that the wooden cheese board microbiota is a source of natural antimicrobials against L. monocytogenes. We systematically isolated bacterial species from wooden boards used for different cheese types and have thus far identified three species that inhibits L. monocytogenes: Serratia sp., Bacillus sp., and Lactococcus sp. Inhibition by Serratia sp. and Bacillus sp. occurs via secreted antimicrobial factors, and is specific towards L. monocytogenes. Work is in progress to identify those antimicrobials and their mechanisms of action.

Poster Presentation #51

Fibroblast-like Synoviocytes Activate Natural Killer Cells and Shape Inflammation and Wound Healing in Lyme Arthritis

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Lyme arthritis (LA) is caused by infection with *Borrelia burgdorferi* (Bb) and can persist for months or years after antibiotic therapy. Fibroblast-like synoviocytes (FLS) are the most abundant cell type found in the synovial lesion and are critical in immune responses to Bb. However, cellular mechanisms of FLS-NK cell interactions remain incompletely understood. FLS were cultured from human synovial tissue or joints of uninfected and Bb-infected C57BL/6 (B6) mice and stimulated with IFNɣ, Bb, and/or peptidoglycan (PG). Flow cytometry and cytokine profiling were used to characterize immune activation. FLS were co-cultured with NK cells, and NK cell activation was assessed by proliferation assay and cytokine secretion. FLS wound healing responses were determined by scratch assays. When treated with IFNɣ, FLS were hyper-responsive to Bb and produced high levels of inflammatory mediators, including IL-15, which induces NK cell proliferation. FLS pre-treated with PG + IFNɣ or PG alone induced marked NK cell proliferation and secretion of high levels of inflammatory cytokines, effects that were not seen in Bb and/or IFNɣ stimulation. Moreover, naïve B6 FLS treated with IFNɣ and/or Bb displayed significantly reduced wound healing compared to unstimulated FLS, and FLS from 4-week infected mice were more responsive to Bb than naïve FLS. In human FLS, inflammatory and wound healing phenotypes varied depending on patient diagnosis. Our data show that FLS are directly activated by IFNɣ produced by NK cells, which downregulates FLS wound healing responses; FLS in turn activate NK cells via IL-15, causing further cellular and tissue damage.

Poster Presentation #52

Bacterial Hydrophilins Promote Pathogen Desiccation Tolerance

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Acinetobacter baumannii is a leading cause of hospital-acquired infections, where outbreaks are driven by its ability to persist on surfaces in a desiccated state. Here, we show that *A. baumannii* causes more virulent pneumonia following desiccation and profile the genetic requirements for desiccation in this organism. We find that desiccation tolerance is enhanced upon disruption of Lon protease, which targets unfolded and aggregated proteins for degradation. Notably, two bacterial hydrophilins, DtpA and DtpB, are transcriptionally upregulated in Δ*lon* via the two-component regulator, BfmR. These proteins, both hydrophilic and intrinsically disordered, promote desiccation tolerance in *A. baumannii*. Additionally, recombinant DtpA protects purified enzymes from inactivation and improves the desiccation tolerance of a probiotic bacterium when heterologously expressed. Together, these results demonstrate a connection between environmental persistence and pathogenicity in *A. baumannii*, provide insight into mechanisms of extreme desiccation tolerance, and reveal potential applications for bacterial hydrophilins in preservation of protein- and live bacteria-based pharmaceuticals.

Poster Presentation #53

Peptidoglycan Acts as an Immune Adjuvant to Enhance CD4+ T Cell Activation by MHC-II+ fibroblast-like Synoviocytes

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Post-antibiotic Lyme arthritis (LA) is characterized by proliferative synovitis, autoimmune Th1/ IFNɣ responses and MHC-II+ fibroblast-like synoviocytes (FLS), a predominant cell type in inflamed joints. High levels of B. burgdorferi cell wall peptidoglycan (PG) have been identified in LA patient synovia. However, the effects of PG on host immune responses are not well characterized. We hypothesize that during LA, PG acts as an adjuvant to enhance antigen presentation and CD4+ T cell activation in the joint. To test this, murine macrophages and FLS, both abundant cell types in LA synovial lesions, were stimulated in vitro with IFNɣ, PG, or both. Macrophage stimulation led to upregulation of MHC-II (I-ab in mice) in an IFNɣ-dependent manner, as expected. Stimulation of FLS with IFNɣ led to upregulation of MHC-II and this response was enhanced by PG. FLS stimulated with the muramyl-dipeptide portion of PG, a known NOD2 ligand, upregulated production of pro-inflammatory cytokines IL-1β, IL-6, IL-12, and TNFα. To examine T cell responses, OT-II mouse T cells (with identical antigen-specific T cell receptors) were co-cultured with FLS and supplemented with the cognate OVA323-339 peptide. IFNɣ-primed MHC-II+ B6 FLS induced naïve OT-II mouse CD4+ T cell activation and proliferation, which was enhanced by PG stimulation. T cell supernatants showed upregulated IFNɣ and IL-12, indicating a Th1 response. Negative controls using irrelevant OVA257-264 peptide or NOD2-deficient FLS failed to induce T cell proliferation. These data show that PG acts as an immune adjuvant enhancing MHC-II peptide-specific CD4+ T cell activation by MHC-II+ FLS.

Poster Presentation #54

C-di-AMP Accumulation Diminishes PrfA Activity in Listeria monocytogenes

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C-di-AMP is an essential second messenger produced by thousands of bacterial species. Although c-di-AMP is required by many bacteria for growth and infection, its accumulation also greatly diminishes growth, stress response, and pathogenesis. Listeria monocytogenes is a foodborne pathogen that invades many mammalian cell types and replicates in the host cytosol. The intracellular lifecycle is dependent on a suite of virulence genes that are transcriptionally activated by PrfA. A L. monocytogenes mutant lacking c-di-AMP phosphodiesterases (hereafter denoted as the pde mutant) accumulates c-di-AMP and is greatly attenuated for virulence in the mouse infection model. We found that the pde mutant was defective for activation of the PrfA regulon, both at the basal level and under virulence-inducing conditions. A constitutively active PrfA variant failed to restore the expression of PrfA-regulated genes, suggesting that PrfA transcription contributes to the defect in PrfA activity. Furthermore, the pde strain was also deficient for glutathione, which allosterically activates PrfA during infection. Finally, the general stress response transcription factor, σB, has been shown to contribute to PrfA activation, but hyper-activation of σB did not rescue the defect in pde strain. Taken together, these data indicate that c-di-AMP accumulation diminishes PrfA activity both at the transcriptional and post-translational levels. Work is in progress to determine whether defective PrfA function is the major cause for virulence attenuation in the pde mutant.

Poster Presentation #55

Characterization of a Novel Regulator of the Mating Cycle in the Fungal Pathogen Cryptococcus neoformans

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Cryptococcus neoformans is an environmental pathogen responsible for ~200,000 cases globally of lethal meningoencephalitis yearly. With a mortality as high as 81%, there is a critical need for better, more effective antifungals, by characterizing genes of unknown function with a connection to virulence we may be able to open new lines of investigation to better understand and treat this disease.

We previously described a cryptococcal palmitoyl transferase important for virulence by acting on diverse substrates. One of the main substrates is the uncharacterized protein CNAG_02129. N. crassa contains the only partially characterized homolog named HAM13 found to be necessary for cell-to-cell communication via filament fusion. We hypothesize that cryptococcal HAM13 (CnHAM13) is also involved in cellular communication by playing a role in mating. During mating, MATα yeast cells respond to secreted pheromone from MATa cells through morphological transition into a filamentous form. Therefore, we investigated if CnHAM13 plays a role during this process. We found that ham13Δ mutants' filament faster and robustly but lack directionality in a confrontation scenario. Undergo higher cell-cell fusion but have reduced competitive fitness against a Wildtype counterpart. Exhibit altered induction of key genes in the mating cycle and cell surface defects. The mechanisms of mating regulation in this fungus are not completely known, however our results suggest that Ham13 is a negative regulator of the mating cycle with potential links to virulence capacity. Understanding how HAM13 dysfunction affect C. neoformans's ability to cause disease may uncover new ways to target and treat this disease.

Poster Presentation #56

Chlamydia Modulates the Expression of JAK-STAT Signaling Components to Attenuate the Type II Interferon Response in Epithelial Cells

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Chlamydia trachomatis is an obligate intracellular pathogen that causes genital tract infections in humans. Infections are often asymptomatic but can lead to reproductive tract inflammation with irreversible complications if left untreated. While the pathologic consequences of infection are well-established, its pathogenicity is not fully understood. *Chlamydia* has developed strategies to subvert signaling functions of its host to ensure its successful intracellular development. Following infection, lymphocyte-derived interferon-γ (IFNγ) induce epithelial cells to produce indoleamine-2,3-dioxygenase (IDO1) that starve *Chlamydia* of tryptophan. However, relative to mock-infected cells, IFNγ-induced expression of IDO1 is down-modulated and is concomitant with lower nuclear localization of its transcription factor Signal Transducer and Activator of Transcription 1 (STAT1) in infected cells, suggesting an altered interferon response. Thus, we hypothesize that *Chlamydia* targets the components of the IFNγ-JAK/STAT pathway to attenuate signaling. To test this, we infected Hep-2 cells with *C. trachomatis* serovar L2 for 24 hours before exposing to IFNγ. We observed a reduced phospho-activation of both STAT1 and its kinase Janus Kinase 2 (JAK2) in infected cells. This lower activation correlated with lower expression of both total STAT1 and JAK2 during infection. This decrease was rescued when we inhibit de novo chlamydial protein synthesis, restoring IFNγ-induced activation and expression of STAT1 target genes. Taken together, our findings suggest a mechanism for *Chlamydia* to dampen the interferon response by modulating its host cell to respond poorly to the cytokine. These findings provide insight into how *Chlamydia* can circumvent host immune responses by lowering the threshold of cytokine signaling.

Poster Presentation #57

The Murine Lyme Arthritis Immunopeptidome: Linking Infection, Arthritis, and Autoimmunity

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Introduction
Lyme arthritis (LA), which is caused by infection with *Borrelia burgdorferi* (*Bb*), is frequently accompanied by autoimmune B and T cell responses in humans, resulting in chronic inflammation. In this study, we used immunopeptidomics and histology to gain further insight into mechanisms of immune evasion and infection-induced autoimmunity by using two mouse models: C57BL/6 (B6) mice, which develop mild inflammatory, self-resolving LA, and B6 *Il10-/-* (IL-10 KO) mice, which develop severe, autoimmune-like LA.

Methods
B6 and IL-10 KO mice were inoculated with 2x103 *Bb*. Joint-draining inguinal and popliteal lymph nodes (LN) and tibiotarsal joints were harvested at 4- and 16-weeks post-inoculation. MHCII molecules were isolated from LN by immunoaffinity and peptides were identified by LC-MS/MS. Joints were stained with H&E, Masson's trichrome, and anti-CD31.

Results
Peptides derived from extracellular matrix (ECM) components including collagens, laminins, and fibronectin were abundant. Interestingly, the number of Apolipoprotein B-100 (ApoB-100) peptides, a human Lyme autoantigen, was greatly increased in lymph nodes of infected mice. Histological analysis of joints showed increased inflammatory infiltrate, fibrosis and neovascularization in infected mice.

Conclusion
This proteomics study provides a profile of immune-relevant proteins associated with LA development. Expansion of peptides derived from ECM proteins and proteins associated with vascular damage, such as ApoB-100, in draining lymph nodes corresponded with histopathological evidence of ECM remodeling and vascular involvement within inflamed joint tissue. Using LA as a model, these data provide a critical link between *B. burgdorferi* infection and development of infection-induced autoimmunity.

Poster Presentation #58

Proteus mirabilis Employs a Contact-dependent Killing System Against Competing Enterobacteriaceae

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Many bacterial species employ systems for interference competition with other microorganisms. Some systems are effective without contact (e.g., through secretion of toxins), while others (e.g., type VI secretion system *T6SS*) require direct contact between cells. Here, we provide the initial characterization of a novel contact dependent competition system for Proteus mirabilis. In neonatal mice, a commensal P. mirabilis strain eliminated commensal Escherichia coli. We replicated the phenotype in vitro and showed that P. mirabilis efficiently reduced the viability of several Enterobacteriaceae species. Importantly, multiple P. mirabilis strains isolated from humans also killed E. coli. A reduction of viability occurred from early stationary phase to 24 h of culture and was observed in shaking liquid media as well as on solid media. Killing required contact but was independent of T6SS, which is the only contact-dependent killing system described for P. mirabilis. Expression of the killing system was regulated by osmolarity and components secreted into the supernatant. E. coli increased expression of genes involved in stress response and DNA damage repair machinery during co-culture with P. mirabilis. In support of a potential DNA-targeting mechanism, E. coli maintained cellular integrity during co-culture with P. mirabilis and did not take up propidium iodide but showed decreased DNA staining. In a transposon screen, we identified P. mirabilis mutants unable to kill E. coli and are currently determining the genetic components of this novel P. mirabilis killing system. In summary, we provide the initial characterization of a potentially novel interbacterial competition system used by P. mirabilis.

Poster Presentation #59

A Novel Two-component Signaling System, PieRS and its Associated Regulon that Contribute to Listeria monocytogenes Infection and Stress Resistance

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Listeria monocytogenes (Lm) is an environmental Gram-positive non-spore-forming bacterium that is able to transition into a pathogen following ingestion by susceptible hosts. During this process, Lm encounters multiple physiological stress conditions that can navigate successfully by using two-component signaling systems (TCS). TCS systems represent one example of a signaling system designed to detect changes at the bacterial cell surface; these systems are often composed of a surface sensor histidine kinase (HK) and a response regulator (RR) that modifies patterns of gene expression in response to a stimulus. Here we identify a novel two-component signaling system, PieRS, that regulates the expression of the virulence chaperones PrsA1, PrsA2, and HtrA as well as several additional gene products of unknown function. Analysis of strains containing individual in-frame deletion mutations in target genes indicates varying contributions of gene products to stress resistance with non-overlapping functional roles. In addition, using human colon epithelial cell lines indicates a role for PieRS regulon members in host cell invasion. Taken together, our experimental evidence supports our hypothesis that PieRS and its regulon members contribute to bacterial survival and growth under various stress conditions present in the gastrointestinal tract and within the outside environment as well as in the GI tract to promote bacterial colonization. Understanding the role and contributions of PieRS regulon members will increase knowledge of how Lm adapts to a variety of environmental stressors in mechanistic contrast to its gram-negative relatives who boast the barrier of an outer membrane.

Poster Presentation #60

Structure-Function Analysis of the Dual-Functioning Mycobacterial Virulence Factors EspE and EspF

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Mycobacterium tuberculosis is the world's second leading infectious disease killer, with approximately 1.5 million people succumbing to the disease annually. Protein export by the ESX-1 secretion system is essential for the virulence of pathogenic mycobacteria, including *M. tuberculosis* and nontuberculous mycobacteria (NTMs) such as *Mycobacterium marinum*. ESX-1 mediates rupture of the phagosomal membrane, allowing mycobacteria to gain access to the cytoplasm of host macrophages. Phagosomal lysis is required for host cell death and mycobacterial cell-to-cell spread. Our previous work demonstrated that the ESX-1 substrates EspE and EspF are required for the membranolytic activity of M. marinum. Our lab recently showed that EspE and EspF are the last set of substrates secreted by the ESX-1 system and require each other for secretion. Moreover, EspE and EspF regulate gene expression in the mycobacterial cytoplasm. Using a targeted genetic approach, we are conducting a structure-function analysis to determine if protein interaction is required for the hemolytic and regulatory activities of EspE and EspF. We show that the disruption of a predicted salt bridge between EspE and EspF abrogates hemolytic activity and EspE secretion. In addition, the protein-protein interface between EspF and EsxA is necessary for hemolytic activity. Disruption of this region leads to a substantial decrease in EspE secretion. This, combined with EsxB secretion data, shows that EspE-EspF interaction is required for secretion, but may not be required for gene regulation. These data provide new insight into the unique ESX-1 substrates EspE and EspF in gene regulation, protein secretion, and virulence.

Poster Presentation #61

A Conserved N-terminal Acetyltransferase Acetylates the ESAT-6 in Pathogenic Mycobacteria

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Mycobacterium tuberculosis was responsible for approximately 1.3 million deaths in 2021. An additional approximate 9.9 million people fell ill with tuberculosis (TB) in that same year. N-terminal protein acetylation (NTA) is a ubiquitous post-translational modification. N-terminal acetylation and the enzymes that mediate it remain poorly understood in bacteria. In M. tuberculosis approximately 11% of the detectable proteome is N-terminally acetylated, including the Early Secreted Six Kilodalton Antigenic Target (ESAT-6), a major virulence factor. We used ESAT-6 to identify an enzyme responsible for N-terminal acetylation of mycobacterial proteins. Using an antibody that specifically recognizes the acetylated N-terminus of ESAT-6, but not the unacetylated variant of the same epitope, we screened a library of M. marinum NAT deletion strains. M. marinum is a pathogenic mycobacterial species that serves as a model for studying TB. Using this approach, we identified a putative conserved NAT that was required for the N-terminal acetylation of ESAT-6. We confirmed our genetic approach using both MALDI-MS and Label Free Quantitation Mass Spectrometry. We also identified additional potential substrates of MMAR_1839 which we plan to characterize. Our work reveals one of the first bacterial NATs identified, outside of those associated with ribosomal proteins, which provides a valuable starting point for the study of the role of NTA in bacterial systems and virulence. Additionally, the methodology used here will be used to identify additional mycobacterial NATs/Substrate pairs. This work will advance the field by beginning to determine the mechanism and function of NTA in bacteria.

Poster Presentation #62

Antibacterial Activity of a Host-derived Peptide Against Salmonella Typhimurium

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Non-typhoidal salmonellae are a leading cause of bacterial food-borne gastroenteritis worldwide. The increased incidence of antibiotic resistant non-typhoidal *Salmonella* infections presents a growing challenge for effective patient treatment, making it essential to develop new therapeutic strategies. Here, we evaluate the effect of a peptide derived from the mammalian protein, Myristoylated Alanine Rich C-Kinase Substrate (MARCKS), has on *Salmonella*. First, we identified that MARCKS is active at the site of *Salmonella* invasion in HCT116 epithelial cells *in vitro*. We also found that a peptide which inhibits the phosphorylation of the MARCKs effector domain, referred to as Anti-*Salmonella* Peptide (ASP), reduces the intracellular bacterial population in HCT116 epithelial cells. Next, we show that in the calf ligated ileal loop model, ASP treatment alters the course of infection by reducing *Salmonella* growth and increasing fluid accumulation. Next, we found that ASP decreases the *in vitro* survival of *Salmonella* in various media, regardless of bacterial growth phase or atmospheric conditions. We utilized fluorescent chemical uptake assays to demonstrate that ASP permeabilizes both outer and cytoplasmic membranes. We hypothesized the membrane damage caused by ASP might enhance the bactericidal effects of antibiotics. We found that combinatory treatment of ASP with sub-inhibitory concentrations of multiple different classes of antibiotics caused enhanced killing of antibiotic-sensitive *Salmonella*. Overall, these results highlight that ASP has the potential to enhance effectiveness of multiple classes of antibiotics against *Salmonella* which could be employed as a treatment strategy against the escalating problem of antibiotic resistant *Salmonella*.

Poster Presentation #63

Proliferation of Specific Bacterial Populations and Microbial Functions in a Vertically Transmitted Dysbiotic Gut are Associated with Host Physiology and Gene Expression

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The interactions of the microbiota with the host are crucial in examining complex autoimmune diseases such as Inflammatory Bowel Disease (IBD). We examined how re-introducing a microbiota to dysbiotic IL-10 knockout mice during development impacted outcomes of colitis, host response, and microbial functions. Vertically transmitted dysbiotic pups received fecal microbiota transplantation (FMT) from control mice, at 2, 3, and 8 weeks after birth. After 23 weeks, remaining mice were supplemented with 2.5% dextran sulfate sodium to induce colitis. FMT mice displayed less colon inflammation and showed similar histology scores as well as microbial makeup to control mice, suggesting successful FMT. We used shotgun metagenomics to investigate the microbial function and assembled 190 non-redundant Metagenome-Assembled Genomes (MAGs) from pup fecal content, revealing several community differences in FMT mice compared to dysbiotic mice. *Akkermansia muciniphila* was highly detected in all mice, while two *Enterococcus sp.* and two Enterobacteriaceae sp. were highly detected in dysbiotic mice only. These MAGs shared a large number of antimicrobial resistance genes, with *A. muciniphila* harboring over 30 resistance genes. The Enterobacteriaceae sp. displayed several virulence genes such as a T4SS, multidrug efflux pumps, protection from reactive oxygen species, and toxin-antitoxin systems, suggesting these MAGs fueled dysbiotic conditions in mice. In addition, host genes associated with IBD and chronic inflammatory conditions such as MTA2, ABCA2, FOXP2, and ACSS1 were upregulated in dysbiotic mice. Putting these all together, the increase in these bacterial populations coincided with increased colon inflammation, upregulation of IBD associated genes and perpetual dysbiosis.

Poster Presentation #64

Pathogen-driven Induction of a Host Transcriptome Facilitating Pro-fibrotic Signaling

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Chronic infection of the female reproductive tract by Chlamydia trachomatis can cause severe fibrotic sequelae, such as tubal factor infertility and ectopic pregnancy. While the prevailing model of chlamydial pathogenesis links this outcome to the immune response to infection, the scarring pathologies observed in subclinical or asymptomatic infections remain unexplained. Recent work demonstrates that infection induces the pro-fibrotic process of epithelial-to-mesenchymal transition (EMT), but it remains unclear how chlamydial EMT drives tissue-wide fibrotic pathology. Using an in vitro model of chlamydial infection, we observe that coculture of infected epithelial cells with uninfected fibroblasts stimulates the latter's production of collagen I, a critical biomarker of scar formation. Subsequent analysis of the host cell transcriptome revealed gene induction consistent with the activity of Yes-associated protein (YAP), a transcriptional coactivator associated with pro-fibrotic signaling. Consistent with this result, we observe increased YAP nuclear translocation during infection, as well as induction of fibroblast-activating signaling factors (CTGF, BMP2, INHBA) in a YAP-dependent fashion. We find that induction of YAP bypasses canonical phosphoinhibition by the Hippo kinase cascade at S127, instead enhancing phosphoactivation of YAP at Y357. Further, chlamydial YAP activation requires the activity of host Src-family kinases, as well as the myosin-modulating chlamydial effector CTL0480. Comparing the host response to ΔCTL0480-mutant and wild-type infection revealed attenuated expression of EMT-associated genes and pro-fibrotic signaling factors. Collectively, our data presents a novel mechanism for Chlamydia-directed modulation of host pro-fibrotic gene expression, providing insight into potential means by which infection promotes scarring in an immune-independent fashion.

Poster Presentation #65

T3SS-mediated Pathogenesis in V. campbellii is Co-regulated by Quorum Sensing and Other Environmental Signals

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Vibrio campbellii is a marine bacterial pathogen that utilizes a multi-component type III secretion system (T3SS) during host infection to directly inject exotoxins through a needle complex into the host cell cytoplasm, leading to cytotoxicity and cell lysis. We and others have shown that the ~40 genes in T3SS in V. campbellii are regulated by a complex network of transcription factors in response to changes in the cell's environment: cell density (quorum sensing), temperature, calcium, and host cell presence. The master T3SS transcription factor ExsA activates expression of the four structural T3SS operons required for needle formation. It has been proposed that environmental signals are combinatorially integrated via ExsA, thus indirectly controlling the entire T3SS program. To test this model, we examined regulation of exsA and each of the four T3SS structural operons under varying conditions. Our data show that the master quorum sensing transcription factor LuxR separately represses both exsA and expression of the effector vopN. In addition, RNA-seq analysis shows that ExsA and LuxR co-regulate several operons outside the four known structural operons. These genes include a YopT homolog and genes of unknown function located next to putative T3SS chaperones. We identified two of these proteins in supernatant extracts by mass spectrometry, suggesting that they are secreted through the T3SS. We hypothesize that these genes encode novel T3SS effectors. Our future experiments will examine the function of these putative effectors and the regulatory network that controls them in vitro and in vivo in a brine shrimp infection model.

Poster Presentation #66

Ex Vivo Human and Porcine Skin Effectively Model Candida auris Colonization, Differentiating Robust and Poor Fungal Colonizers

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Background
Candida auris is a recently emerged fungal pathogen that has become a major source of nosocomial infection. *C. auris* colonizes and persists on the skin of patients, often leading to health care-associated infections with high mortality. This capacity for persistent skin colonization surpasses that of other *Candida* species which more typically colonize the gastrointestinal tract. It is important to develop reliable skin models to better understand *C. auris* skin colonization. Methods: Here, we describe two clinically relevant skin models for *C. auris* colonization. We compare the growth of *C. auris* on human skin to that of porcine skin, testing numerous *C. auris* isolates. Additionally, we examine the growth of several other *Candida* species both in vitro and on skin in comparison to *C. auris*.

Results
We show that *C. auris* grows comparably on human and porcine skin and confirm that *C. auris* isolates from distinct geographic clades all grow similarly on skin. Further, we demonstrate that other *Candida* spp., including those with phylogenetic similarity to *C. auris*, do not display high growth in the skin microenvironment.

Conclusions
These studies highlight the utility of 2 ex vivo models of *C. auris* colonization that allow reproducible differentiation among *Candida* spp., which should be useful for comparison of *C. auris* clinical isolates and genetically mutated strains. These findings mirror clinical observations that *C. auris* displays an enhanced capacity for skin growth and provide an important tool for developing strategies to reduce or eliminate *C. auris* colonization of patient skin.

Poster Presentation #67

Wastewater Analyses and RNA-sequencing as a Surveillance Tool for COVID-19 Spread

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COVID-19 has fundamentally altered daily life since its emergence. SARS-CoV-2, the virus that causes COVID-19, is transmitted rapidly partly due to its clinical presentation variability. Infected individuals can be asymptomatic and transmit disease through respiratory droplets and feces. We have employed a method to analyze the raw wastewater collected from wastewater treatment plants (WWTPs) in the Greater Toledo area. Our project aimed to determine if wastewater analysis is a helpful tool in predicting potential outbreaks in our communities. Raw sewage samples from Greater Toledo WWTPs were analyzed for RNA extraction by Ohio Coronavirus Wastewater Monitoring Network (OCWMN). The RNA isolated from the wastewater samples was analyzed by qRT-PCR for viral gene copies and was further analyzed by high throughput sequencing to identify SARS-CoV-2 variants. We measured SARS-CoV-2 gene copies in the samples collected from the Toledo area WWTPs. The 7-day average case numbers in the Toledo area were obtained from the Ohio Department of Health website. There was a moderate correlation with increased wastewater SARS-CoV-2 gene copies preceding case increases in the tested counties. We conclude that wastewater data can be used to help bolster our efforts against the COVID-19 pandemic. The data shows a predictive effect in locating viral wastewater spikes to an increased number of reported cases and new viral variants. Wastewater surveillance can be used as an early warning system to improve community health protocols.

Poster Presentation #68

Assessing Factor H-Fc Fusion Proteins for Controlling Burkholderia pseudomallei Infection

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Burkholderia pseudomallei (Bp) is a Gram-negative bacterium that causes melioidosis, a major septic disease. While melioidosis cases are rising globally, and Bp is classified as a Tier 1 select agent and potential bioweapon, no vaccines or effective treatments exist due to the bacteria's strong antibiotic resistance. Bp thrives in serum, indicating an innate resistance to the host complement immune system. Hosts protect themselves from complement using the 20-domain inhibitory protein Factor H (FH). Many pathogens can sequester this regulator via FH-binding proteins (FHbp) to evade clearance by complement, making these FHbp(s) a therapeutic target. After identifying a FHbp in Bp, we have used a series of chimeric proteins engineered to combine regions of FH with the Fc portion of IgG (FH-Fc) to both block the FHbp and promote complement activation via the classical pathway, clearing infection. In vitro binding assays using ELISA determined that chimeras containing the 18-20 FH domains bound strongly to Bp while those containing domains 6-7 possessed only minimal binding. ELISA analyses to assess complement C3-deposition on Bp determined that only the 18-20 chimeras possessing the IgG3 Fc region promoted deposition, whereas those containing the IgG1 Fc region did not support deposition. ELISA analyses also determined a similar trend for those chimeras that promote membrane attack complex (MAC) deposition on Bp. Direct killing assays are currently being performed to determine whether these findings correlate with the ability to promote complement-mediated killing of Bp, as well as to promote neutrophil-mediated phagocytosis and killing of Bp.

Poster Presentation #69

Mannosylation in Candida auris Cell Wall Plays a Role in Neutrophil Evasion with Pathways Distinct from Candida albicans and Candida glabrata

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Background
Candida auris is an emergent fungal pathogen causing invasive infections with mortality rates approaching 60% in healthcare facilities worldwide. Neutrophils are leukocytes important for responding to invasive fungal infections; however, when compared to other *Candida* spp., neutrophils fail to produce robust antifungal activities in response to *C. auris*. Currently, little is known regarding the mechanism of immune evasion observed for *C. auris*. We hypothesized that cell wall mannosylation of *C. auris* contributes to the evasion of neutrophil responses ex vivo and in a zebrafish infection model.

Methods
We disrupted predicted and putative mannosylation pathways of *C. auris* through gene disruption via homologous recombination. We quantified changes to the cell walls of these *C. auris* mutants via gas chromatography, NMR analysis, and transmission electron microscopy, and we measured exposure of inner cell wall layers via immunofluorescence. We isolated human neutrophils for examining neutrophil-*Candida* interaction and utilized the larval zebrafish hindbrain injection model to perform in vivo studies.

Results
Genetic disruption of mannosylation pathways (*PMR1* and *VAN1*) caused unmasking of immunostimulatory components, reduced outer cell wall mannan, and provoked neutrophil phagocytosis and killing. When disrupting these pathways in related *Candida* spp. (*C. albicans* and *C. glabrata*), we did not observe an effect on *Candida*-neutrophil interaction.

Conclusion
C. auris mannosylation contributes to evasion of antifungal neutrophil response using genetic pathways divergent from other common pathogenic *Candida* spp. These findings shed light on innate immune evasion for this emergent pathogen.

Poster Presentation #70

Intravital Microscopy Reveals Novel Motility Defects within Murine Tissues for a Borrelia burgdorferi cheY2 Chemotaxis Mutant

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Borrelia burgdorferi (Bb) is an extracellular spirochetal bacterium and the causative agent of Lyme disease. Response regulator genes (cheY) are important for changing direction within environments, and Bb possesses 3 forms of this gene (cheY1-3). Here, we use intravital confocal fluorescence microscopy (IVM) to visualize effects of the cheY2 gene on motility within murine skin tissues, and qPCR to determine bacteria levels in target tissues.

Injection into murine skin showed that cheY2-deletion mutant (ΔcheY2) reached lower levels in skin tissues than wild-type (WT), during early and late infection. While WT-Bb were able to achieve a persistent level, ΔcheY2 demonstrated much lower capacity to persist in tissues. Visual counting of Bb in ear skin demonstrated that ΔcheY2 numbers were similar to WT until day 4 post-infection and their peak numbers were >10-fold less than WT until days 10-21, where numbers were again similar to WT. While WT numbers remained constant after day 28, ΔcheY2 numbers gradually dropped to almost 100-fold less than WT and were eventually cleared. While WT-Bb needed 7-10 days to migrate from one ear to another, ΔcheY2-Bb required over 60 days to achieve this. Analysis of ΔcheY2 in ear tissues indicated that they reversed motility direction at higher rates than WT, starting at 6h and persisting throughout infection. Dissemination from injection site was slower than WT, and also demonstrated slower velocities. We demonstrate identification of a chemotaxis gene in Bb that is differentially active depending on environment, providing useful information on aspects of chemotaxis/motility important for murine persistence.

Poster Presentation #71

S. aureus Toxins Impair Aortic Endothelial Wound Healing and Angiogenesis

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Staphylococcus aureus infective endocarditis (IE) is an infection of the cardiac endothelium characterized by bacteria induced lesions and inflammatory tissue damage. While various pathogens can cause IE, *S. aureus* is the leading etiology and the most tissue destructive form causing endothelial dysfunction and life-threatening complications.

Of the many *S. aureus* virulence factors, secreted toxins directly promote the IE phenotype. Using a rabbit-derived aortic ring model, we determined that *S. aureus* toxins significantly inhibit angiogenic neovessel formation following injury ($p \leq 0.0001$). Sprout development varied between aortic rings originating from the thoracic region compared to those from the abdominal region in both toxin-treated and media control conditions, with abdominal samples exhibiting more robust sprouting over 14 days. Further, differing toxin types – β-toxin and the staphylococcal superantigens SEC and TSST1 – elicited varying phenotypic responses. While rings treated with TSST1 and SEC demonstrated matrix degradation, β-toxin treated rings did not, suggesting toxin-specific mechanisms contribute to differential repair phenotypes. To further elucidate toxin-mediated endothelial responses, we utilized immortalized human aortic endothelial cells (iHAECs) to assess biological effects to TSST1 and SEC. Significant decreases in monolayer barrier integrity in permeability assays and increased expression of ICAM1 and VCAM1 were detected following exposure to TSST1. Finally, deficits in gap closure were observed in iHAECs treated with TSST1 and SEC in wound healing assays, demonstrating toxin-associated physiological responses at the cell level. Collectively, this work contributes valuable knowledge for regenerative vascular biology with potential to influence new approaches for treatments and improve clinical outcomes.

Poster Presentation #72

Identification of Factors Contributing to Gastrointestinal Colonization by Klebsiella pneumoniae

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Gastrointestinal colonization by *Klebsiella pneumoniae* is a risk factor for subsequent infection as well as transmission to other patients. Colonization is achieved by many strain types which exhibit high diversity in genetic content. Thus, we aimed to study strain-specific requirements for *K. pneumoniae* GI colonization by developing and optimizing a mouse model. To disrupt the endogeneous GI flora, we administered intraperitoneal injections of vancomycin, one of the most highly utilized antibiotics in the United States. The vancomycin regimen was optimized by piloting various doses and durations of administration. Male and female mice were given 350 mg/kg (a mouse equivalent of a human dose of 1 g/day calculated through the FDA guidelines for estimating safe dosing) for 5 days prior to gavage with *K. pneumoniae*. Mice had stable fecal loads of around 10^7 CFU/g feces for at least 60 days post-gavage. We next used transposon insertion sequencing (INSeq) to identify *K. pneumoniae* genes important for colonization. Transposon mutant libraries were generated in three strains (a carbapenem-resistant strain, an extended spectrum beta-lactamase producing strain, and a non-epidemic antibiotic-susceptible strain), which were gavaged into individual mice. Three days after gavage, DNA was extracted from feces, processed, and sequenced. Several genes, such as those encoding efflux pumps and purine metabolism were required by all three strains for colonization, while other genes, such as hemolysin expression-modulating protein (*hha*), were uniquely required by a single strain. Generation of in-frame mutants is ongoing to verify the results of these screens.

Poster Presentation #73

Two Roles, One Pheromone: A Listeria monocytogenes Peptide Pheromone that Contributes to Both Vacuole Escape and Viability

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The bacterium, Listeria monocytogenes (Lm), transitions from an environmental bacterium to an intracellular pathogen following entry into mammalian cells, where infections can lead to serious invasive disease and even death. It has been previously shown that pPplA, a small peptide secreted by Lm, contributes to bacterial virulence by enhancing escape of Lm from the vacuoles of non-professional phagocytic cells. Peptide pheromones are generally associated with the coordination of multicellular activities including biofilm formation, however pPplA is required within the cell vacuole without bacterial neighbors. Evidence suggests that the secretion and re-uptake of the pPplA pheromone within the vacuole signals a confined space to Lm and leads to specific changes in bacterial gene expression that promote vacuole disruption. As genetic mutations were constructed to facilitate receptor identification, it became apparent that bacteria were negatively affected by the complete loss of pPplA. Transduction of a complete deletion of pplA into a WT Lm strain resulted in low recovery of transductants that only appeared after several days of incubation. Whole genome sequencing of independent transductants revealed mutations in genes encoding enzymes associated with RNA metabolism; subsequent RNA transcript analysis has identified several pheromone-regulated gene products that could potentially negatively impact Lm fitness. Overall, these results strongly suggest that second site suppressor mutations are required to restore full viability to Lm mutants lacking the pPplA pheromone. It is surprising and novel that a secreted pheromone contributes to bacterial viability in broth culture, suggesting that these peptide signals play a critical role in bacterial survival.

Poster Presentation #74

Ribosome Degradation in Staphylococcus aureus Lacking Hibernation Factor

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Hibernating 100S ribosomes are translationally silent dimer of the 70S complexes that are widespread among bacteria. The significance of the hibernating 100S ribosome has remained elusive until its critical roles in antibiotic resistance, host colonization and stress responses were revealed in recent years. Using the human opportunistic pathogen Staphylococcus aureus, we previously showed that a loss of hibernation-promoting factor (Hpf) attenuates S. aureus colonization that is associated with a massive degradation of ribosomes. Recently we reported that a 3'-5' exoribonuclease RNase R promotes ribosome degradation in the absence of hpf. Dimerization of 70S ribosome by Hpf sterically occludes RNase R binding. However, the deletion of rnr does not completely restore the ribosome population of hpf knockout to the wild-type level, suggesting an existence of unidentified RNase(s) in ribosome turnover. This presentation will focus on YhaM, a poorly characterized Firmicutes-specific exoRNase that has been implicated in mRNA decay but not ribosome degradation. Deletion of yhaM stabilizes ribosomes in a Δhpf null mutant, similarly to that of a ΔrnrΔhpf strain, whereas a combination of ΔrnrΔyhaMΔhpf achieves the highest ribosome stability. We found that S. aureus YhaM destabilizes ribosome indirectly by reducing the abundance of Hpf. Using structural and biochemical approaches, we are investigating how RNA structure and sequence context, as well as metal cations differentially influence YhaM specificity and catalysis. The knowledge gained from these studies will provide basic insights into a novel aspect of ribosome metabolism that is critical for bacterial pathogenesis and for enzyme engineering tailored to biotechnological application.

Poster Presentation #75

Basis of Narrow-spectrum Activity of Fidaxomicin on Clostridioides difficile

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Fidaxomicin (Fdx) is widely used to treat Clostridioides difficile (Cdiff) infections (CDIs), but the molecular basis of its narrow-spectrum activity in the human gut microbiome remains enigmatic. CDIs are a leading cause of nosocomial deaths. Fidaxomicin, which inhibits RNA polymerase (RNAP), targets Cdiff with minimal effects on gut commensals, reducing CDI recurrence. Here, we present the cryo-electron microscopy structure of Cdiff RNAP in complex with Fdx, allowing us to identify a crucial Fdx-binding determinant of Cdiff RNAP that is absent in most gut microbiota like Proteobacteria and Bacteroidetes. By combining structural, biochemical, genetic, and bioinformatic analyses, we establish that a single RNAP residue is a sensitizing element for Fdx narrow-spectrum activity. Our results provide a blueprint for targeted drug design against an important human pathogen.

Poster Presentation #76

Salmonella enterica Serovar Typhimurium chitinases Modulate the Intestinal Glycome and Promote Small Intestinal Invasion

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Salmonella enterica serovar Typhimurium (STM) is one of the leading causes of food-borne illnesses. To colonize the gastrointestinal tract, STM produces multiple virulence factors that facilitate cellular invasion. Chitinases have been recently emerging as virulence factors for various pathogenic bacteria, and the STM genome contains two annotated chitinases: *STM0018* (*chiA*) and *STM0233*. The role of these chitinases during infection is unknown. Chitinases typically hydrolyze chitin, which is absent in vertebrates. However, *chiA* was expressed in infection models and purified ChiA cleaved carbohydrate subunits (N-acetyllactosamine) present on mammalian surface glycoproteins. STM can bind underlying mannose residues to facilitate invasion, suggesting a role for chitinases in exposing mannose to promote invasion. Here, we demonstrate that both STM chitinases facilitate epithelial cell adhesion and invasion. Chitinase-deficient STM showed a 70% reduction in invasion of small intestinal epithelial cells in vitro. In a gastroenteritis mouse model, chitinase-deficient STM strains were significantly attenuated in the invasion of small intestinal tissue. This reduced invasion resulted in delayed STM dissemination to the spleen and the liver. STM chitinases are likely secreted since the invasion defect was rescued by the presence of wild-type STM. By analyzing N-linked glycans of small intestinal cells, we identified specific N-acetylglucosamine-containing glycans as potential targets of STM chitinases. This analysis also revealed changes in Lewis X/A-containing glycan abundance that is likely a result of host cell modulation due to the detection of STM chitinases. Overall, our results demonstrate that STM chitinases contribute to intestinal adhesion and invasion through modulation of the host glycome.

Poster Presentation #77

Interferon Regulatory Factor 3 Brakes Viral Inflammation Using RIKA

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Excessive inflammation during virus infections such as the cytokine storm in patients with severe COVID-19 is a primary culprit in viral pathogenesis associated with organ damage, morbidity, and mortality. Some viruses, such as human cytomegalovirus, could even thrive in inflammation. We uncovered a novel cellular anti-inflammatory mechanism, RIKA (Repression of IRF3-mediated NF-κB Activity), in which interferon (IFN) regulatory factor 3 (IRF3) prevents viral inflammation by inhibiting the activity of NF-κB. IRF3 is a critical transcription factor for IFN induction and anti-viral defense. In addition to being activated by phosphorylation for inducing IFNs and antiviral genes, we showed that IRF3 could be activated by polyubiquitylation to activate a pro-apoptotic pathway, which kills virus-infected cells. IRF3, however, does not require transcriptional or pro-apoptotic activities for RIKA. Mechanistically, IRF3 interacts with the NF-κB subunit p65 and prevents its nuclear translocation and subsequent inflammatory response. A mutant IRF3, defective in both transcriptional and apoptotic activity, can suppress NF-κB-induced inflammatory responses upon infections by respiratory viruses. RIKA, therefore, represents a novel defense mechanism against aberrant inflammation in virus infections, and it could also be potentially protective in other inflammatory diseases. Overall, the presentation will highlight how the IFN system utilizes IRF3 to function in multiple pathways for managing viral infection and pathogenesis.

Poster Presentation #78

Mechanisms of Borrelia Surface Lipoprotein Translocation Through the Outer Membrane

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Borrelia burgdorferi, the spirochetal agent of Lyme disease, is unique among diderm bacteria in its lack of lipopolysaccharide (LPS) in the outer membrane (OM) and its abundance of surface lipoproteins, which play major roles in bacterial transmission and virulence. Despite the importance of surface lipoproteins, little is known about how they are translocated to different cellular compartments. In this study, we characterized B. burgdorferi BB0838, an OM LPS assembly protein LptD homologue. Using a newly developed CRISPRi approach, we showed that BB0838 is essential for cell growth. Upon BB0838 knockdown, representative surface lipoproteins such as OspA and OspB were retained in the inner leaflet of OM, as determined by their inaccessibility to in situ proteolysis but presence in OM vesicles. The secretion, insertion and topology of the B. burgdorferi OM porin P66 remained unaffected. MudPIT mass spectrometry analysis of the B. burgdorferi membrane-associated proteome further confirmed the selective periplasmic retention of surface lipoproteins under BB0838 knockdown conditions. This indicates that BB0838 facilitates the essential terminal step in a distinctive spirochetal lipoprotein secretion pathway that evolved in parallel to the LPS secretion pathway in gram-negative bacteria. Hence, BB0838 and other essential lipoprotein secretion pathway components represent attractive novel targets for antimicrobials. Ongoing experiments are identifying and characterizing the periplasmic lipoprotein secretion pathway components that are feeding into the OM lipoprotein flippase and resolving lipoprotein secretion in time and space using fluorescent lipoprotein fusions.

Poster Presentation #79

Deciphering Uropathogenic Escherichia coli's Resistance to the Neutrophilic Antimicrobial Hypochlorous Acid

Sadia Sultana[1]
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Activated neutrophils generate reactive oxygen and chlorine species (RO/CS) to eliminate pathogens during phagocytosis. Hypochlorous acid (HOCl) is the most potent neutrophilic RO/CS that kills phagocytized pathogens through widespread oxidative macromolecular damage. We discovered that uropathogenic Escherichia coli (UPEC), the common pathogen of urinary tract infections, are more resistant to HOCl exposure and neutrophil-mediated killing compared to intestinal E. coli pathotypes. Our study identified an operon of three genes (i.e., rcrA, rcrR, rcrB) that is pivotal for UPEC's HOCl resistance.

We recently characterized RcrR as a HOCl-sensing transcriptional repressor that represses the operon during non-stress condition and becomes inactivated through cysteine oxidation during HOCl-stress, resulting in the expression of all three genes. However, one target gene of the RcrR regulon (i.e rcrB) is particularly crucial for UPEC's increased survival during HOCl-stress, as rcrB-deficient UPECs are similarly sensitive to HOCl exposure and phagocytosis as intestinal E. coli pathotypes. Alternatively, recombinant expression of rcrB in HOCl-sensitive intestinal E. coli renders the strains highly resistant to HOCl.

Our aim is now to decipher how RcrB contributes to bacterial HOCl resistance, which will help us to better understand pathogen survival strategies in HOCl-rich environments. We hypothesize that the putative membrane protein RcrB maintains intracellular redox balance during HOCl-stress. We examined the extent of intracellular oxidative damage as a proxy for intracellular HOCl level and found indeed substantially increased protein, lipid, and DNA damage in UPEC cells that lack rcrB. Now, further studies are underway to investigate the HOCl scavenging potential of RcrB.

Poster Presentation #80

Reducing Catheter Associated UTI by Decreasing Host Protein Deposition on Urinary Catheters

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Microbial adhesion to medical devices is common for hospital acquired infections, particularly, those associated with urinary catheters. If not properly treated these infections can lead to further patient complications and exacerbate antimicrobial resistance. Studies show catheter placement causes bladder inflammation by causing damage to the bladder epithelium. This prompts the release of host serum-proteins into the bladder, which are consequently deposited on the urinary catheter. Additionally, it's been shown that Enterococcus faecalis uses the serum protein fibrinogen as a scaffold to bind and persist in the bladder despite antibiotic treatments while inhibition of this interaction significantly reduces infection. Here, we show that deposited fibrinogen is advantageous for E. faecalis, E. coli, P. aeruginosa, K. pneumoniae, A. baumannii and C. albicans. Additionally, we show that a liquid-infused silicone catheter is able to decrease fibrinogen deposition on the catheter and reduce microbial burden in the bladders of mice with catheter associated urinary tract infection. Furthermore, this novel catheter reduces systemic dissemination for most microbes tested and proteomics revealed a significant decrease in deposition of most host-secreted proteins on catheter surfaces in vivo. Our findings suggest targeting microbial binding scaffolds would be an effective antibiotic-sparing prevention strategy for use against catheter-associated urinary tract infections and other infections associated with medical devices.

Poster Presentation #81

Modulating T-cell regulatory Molecules on Antigen Presenting Cells to Improve Immune Responses Against a Mycobacterium tuberculosis Infection

Olivia Beckman[1]
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Infections with Mycobacterium tuberculosis (Mtb) result in chronic disease due to immune evasion mechanisms. These mechanisms allow Mtb to prevent the robust activation of CD4+ T-cells, that are required for controlling infection. Mtb blinds T-cells from identifying infected cells by altering the expression of critical immune regulatory molecules on infected cells, suppressing immune activation. Yet how modulating these immune regulatory molecules on antigen presenting cells (APCs) alters Mtb-specific T-cell activation remains unclear. We hypothesize that an over-expression of T-cell regulatory molecules on Mtb-infected cells may overcome immune evasion mechanisms and drive protection. To test this, we are optimizing a novel ex vivo platform to probe interactions between Mtb-specific T-cells and macrophages engineered to express variable amounts of immune regulatory molecules. To begin, we examined activation of T-cells from a newly engineered transgenic mouse (p25) where all T-cells are specific for an Mtb antigen. We activated these cells ex vivo and are quantifying proliferation, activation, and cytokine production. In parallel, we are optimizing a gain-of-function approach using CRISPR-activation to drive the expression of T-cell regulatory molecules on APCs. Independent sgRNAs targeting these molecules along with catalytically dead Cas9 will be expressed in macrophages. Flow cytometry and RT-PCR will be used to quantify the expression of target genes. Future goals include combining p25 T-cells with CRISPR-activation macrophages. This will define how distinct immune regulatory molecules control T cell activation and identify new targets for immunotherapies. Thus, these experiments have major implications for treating Mtb alongside similar chronic infections.

Poster Presentation #82

Urine Supports Candida albicans' Biofilm and Hyphal Formation through Efg1 and Als1 during Catheter-associated Urinary Tract Infections

Alyssa La Bella[1], Ana Flores-Mireles[1], Felipe Santiago-Tirado[1]
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Biofilm formation is a key fungal virulence trait that poses a significant healthcare burden, especially when biofilms attach and develop on implanted medical devices. Urinary catheters, a frequently implanted medical device in healthcare settings, can act as a platform for these highly structured biofilms and allow fungal pathogens the opportunity to colonize, disseminate to the bloodstream, and lead to invasive systemic infections. Candida spp., specifically Candida albicans, are the second most common cause of catheter-associated urinary tract infections (CAUTIs). Yet, the cellular and molecular details of C. albicans CAUTI are poorly understood. With the steady increase in catheter usage, combined with fungal infection mismanagement and treatment challenges, this question is of utmost public health importance. Here, for the first time, we characterized fungal mechanisms during CAUTI, finding that the catheterized bladder environment triggers Candida virulent programing, inducing Efg1-dependent hyphal morphogenesis, required for CAUTI establishment. Moreover, Als1, an Efg1-downstream effector, is necessary for in vitro and in vivo fibrinogen-biofilm formation and Als1 expression levels were crucial for fungal persistence during CAUTI. These findings unveil the mechanisms for fungal CAUTIs and identify potential treatment targets.

Poster Presentation #83

Immunological Impact of Lipid Signaling in Aspergillus fumigatus Respiratory Infection

Breanne Steffan[1], Jun Yang[2], Bruce Hammock[2], Nancy Keller[1]
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Aspergillus fumigatus is an opportunistic respiratory pathogen with high mortality rates. The organism produces several oxygenated fatty acids, oxylipins, that are structurally similar to those found in mammals. We hypothesize that these fungal-specific oxylipins are produced in the lung during respiratory infection and can modulate host immune responses by interacting with the mammalian GPCRs. To address this hypothesis, we infected immunocompromised mice with *A. fumigatus* and ran lipidomic analysis on lung tissue. We were able to detect 5,8-diHODE, 8-HODE, and 10-HODE during infection, which are produced by *A. fumigatus*. Next, we used GPCR signaling assays to determine if the oxylipins have agonist or antagonistic activity against mammalian GPCRs. Initially, we identified 5,8-diHODE as an antagonist to the mammalian receptor, G2A, while 8-HODE and 10-HODE have agonist activity on this receptor. Using immunocompetent mice infected with *A. fumigatus*, we found that loss of G2A results in a survival advantage for the mice and is associated with increased neutrophilia. In vitro work with human neutrophils shows that 5,8-diHODE has some chemotactic activity on the cells especially when G2A is blocked. Future investigations will involve infection studies using fungi unable to produce the abovementioned oxylipins.

Poster Presentation #84

Understanding the Roles of TcdE and TcdL During Toxin Secretion in Clostridioides difficile

Shannon Kordus[1,2], Ruben Cano Rodriguez[1,2,3], Natalie Loveridge[1,2], D. Borden Lacy[1,2,3]
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Understanding how large macromolecules are transported across a cell wall is a complex and poorly understood biological process. The nosocomial pathogen *Clostridioides difficile* produces two large toxins, TcdA (308 kDa) and TcdB (270 kDa). Although toxin function in host cells has been extensively studied, little is known about how these toxins are secreted from the bacterium. TcdA and TcdB are encoded on a pathogenicity locus (PaLoc) which also encodes the holin-like protein TcdE and the remnants of a partial endolysin TcdL. While bacteriophages use holin/endolysin systems to trigger bacterial cell lysis and escape, multiple reports now suggest that TcdE is used for the secretion of the toxins by forming a channel within the cell membrane. While TcdL was only recently discovered, there are data to suggest that it can interact with TcdB to help facilitate toxin translocation. To further understand toxin secretion, we created strains with *tcdE*, *tcdL*, or *tcdE* and *tcdL* deleted. Our data indicate that both TcdE and TcdL are required for secretion. Furthermore, we found that TcdA and TcdB are secreted in a temporal manner where TcdA is secreted before TcdB. The research presented here will address the outstanding questions of how TcdE and TcdL interact with *C. difficile* toxins to create a pore and how the toxins can be released with mechanisms other than cell lysis.

Poster Presentation #85

Defining Novel Mechanisms Critical to Mycobacterium abscessus Uptake in Macrophages

Haleigh Gilliland[1], Andrew Olive[1]
[1]*Michigan State University*

Mycobacterium abscessus (MAB) is a highly antibiotic resistant, rapidly growing non-tuberculous mycobacterium that infects patients with chronic lung diseases, like cystic fibrosis. Phagocytosis by macrophages is critical to terminate invading pathogens. While interactions between MAB and macrophages are known to contribute to MAB pathogenesis, how macrophages bind and phagocytose MAB during early infection remains largely unknown. These interactions are further complicated by the ability of MAB to transition from a smooth to rough morphology. We hypothesize that understanding key MAB-macrophage interactions will identify critical host targets that can be leveraged to prevent infection. To test this hypothesis, we developed fluorescent reporters in smooth and rough MAB and optimized a range of macrophage assays to elucidate MAB-macrophage interactions both with and without antibiotic treatment. We used these tools to conduct a forward genetic screen using a genome-wide CRISPR-Cas9 knockout library in immortalized bone marrow derived macrophages (BMDM) to identify host pathways that contribute to MAB uptake four hours following infection. In addition to CD11b, M6pr and Rac1, our results show glycosaminoglycan (sGAG) synthesis in macrophages is required to efficiently take up both smooth and rough MAB during early infection. We are now dissecting how key regulators of sGAG biosynthesis, UGDH, B4GALT7 and B3GAT3, influence uptake of latex beads, heat-killed MAB, and paraformaldehyde fixed MAB in immortalized BMDMs and a novel alveolar macrophage model. Future work focuses on understanding how sGAGs modulate uptake of other pathogens, including Mycobacterium tuberculosis, with the goal of defining key mechanisms driving host-pathogen interactions in the lungs.

Poster Presentation #86

Investigating the Antibiotic Potentiating Effects of a Novel Reactive-oxygen Species Generating Antimicrobial

Greg Anderson[1], Gracious Donkor, Michael Stadler, Kevin-Pierre Hoffmann, Jan-Ulrik Dahl
[1]*Illinois State University*

The emerging antimicrobic resistance and decline in the discovery of new antibiotics has led to the development of alternative antimicrobials including silver-containing compounds which inhibit bacterial growth. Our work focuses on the antimicrobial effects of AGXX, a novel silver-containing antimicrobial surface coating, which produces highly reactive oxygen species (ROS causing extensive macromolecular damage. We investigated possible synergistic effects of AGXX on members of several antibiotic classes in the gram-negative opportunistic pathogen Pseudomonas aeruginosa, one of the major causes of acute and persistent infections. We discovered that combined treatment of sublethal concentrations of AGXX and aminoglycosides exponentially enhanced their bactericidal activity against P. aeruginosa and discovered that this synergy restored the sensitivity of a kanamycin-resistant P. aeruginosa strain. To explore the underlying mechanism, we utilized redox sensitive probes and found that the bactericidal effect of the combined treatment of AGXX and aminoglycosides is mediated through elevated ROS production. The increase in oxidative stress resulted in the disruption of iron homeostasis evidenced by a significant decrease in aconitase activity under combined AGXX and aminoglycoside treatment. Using fluorescent spectroscopy and microscopy, we further demonstrate that combined treatment of the two antimicrobials also caused significant membrane damage. Our goal is now to delineate the mechanisms that underpin this synergistic effect by exploring how the metabolic processes and cellular targets disrupted by AGXX contributes to the synergistic effects with aminoglycosides. Our findings will potentially provide an understanding of cellular targets that could be inhibited to increase the activity of conventional antimicrobials.

Poster Presentation #87

Uropathogenic Escherichia coli Employ a Highly Effective Defense System to Counter Neutrophil-mediated Killing

Jan-Ulrik Dahl[1], Sadia Sultana, Mary E Crompton, Kennadi Meurer, Greg Anderson
[1]*Illinois State University*

Activated neutrophils generate reactive oxygen and chlorine species (RO/CS) to eliminate pathogens in a process named phagocytosis. Hypochlorous acid (HOCl) is the most potent neutrophilic RO/CS that kills phagocytized pathogens through widespread oxidative macromolecular damage. We discovered that uropathogenic Escherichia coli (UPEC), the common pathogen of urinary tract infections, are more resistant to HOCl-stress and neutrophil-mediated killing compared to intestinal E. coli pathotypes. Our study identified an operon consisting of three genes (i.e. rcrA, rcrR, rcrB) that is responsible for UPEC's increased HOCl resistance. We identified RcrR as a HOCl-sensing transcriptional repressor that represses the operon during non-stress condition but becomes inactivated through cysteine oxidation during HOCl-stress, resulting in the expression of all three genes. One target gene of the RcrR regulon (i.e rcrB) is particularly crucial for UPEC's increased survival during HOCl-stress, as rcrB-deficient UPECs are similarly sensitive to HOCl exposure and phagocytosis as intestinal E. coli pathotypes. Alternatively, recombinant expression of rcrB in HOCl-sensitive intestinal E. coli renders the strains highly resistant to HOCl. Our aim is now to decipher how RcrB contributes to bacterial HOCl resistance, which will help us to better understand pathogen survival strategies in HOCl-rich environments. We hypothesize that the putative membrane protein RcrB maintains intracellular redox balance during HOCl-stress. We examined the extent of intracellular oxidative damage as a proxy for intracellular HOCl level and found indeed substantially increased protein, lipid, and DNA damage in UPEC cells that lack rcrB. Now, further studies are underway to examine the HOCl scavenging potential of RcrB.

Poster Presentation #88

Interrogating the Role of the Oral Microbiome on Head and Neck Squamous Cell Carcinoma

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The oral microbiome is an emerging field with high potential for uncovering new avenues for cancer therapy. Over 700 microbial species have been identified within the human aerodigestive tract, however the impact of many of these is still unknown. Head and neck squamous cell carcinoma (HNSCC) are the most frequently occurring malignancy of oral cavity cancers with an incidence of over 90%. Treatment options for HNSCC include surgery, chemotherapy, and radiotherapy; all of which still provide a low 5-year survival rate which indicates a need for additional treatment options. We hypothesized that the oral microbiome is distinct in patients from paired cancer-free controls. We have interrogated the profile of the oral microbiota by use of 16S rRNA amplicon sequencing of saliva samples from HNSCC patients and their significant others. The patients showed a higher relative abundance in *Prevotella, Neisseria, Fusobacterium*, and *Campylobacter* when compared to their significant others. *Fusobacterium nucleatum* is one of the most common cultivable microorganisms found within the oral cavity that has been shown to exhibit pathogenic properties. Here, we observed significantly higher relative abundance of *Fusobacterium nucleatum* when comparing patients to their significant others. In this research, we detailed the key properties involved with the anaerobic cultivation of *Fusobacterium nucleatum*, including optimized growth conditions and medium, as well as increased in-vivo proliferative properties in conjunction with metagenomic and metabolomic data. These studies provide a direct translational aspect with the potential for future studies to develop therapeutic techniques within a clinical setting.

Poster Presentation #89

Distinct Roles of Chlamydia trachomatis Effectors TarP and TmeA in the Regulation of Formin and Arp2/3 during Entry

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The obligate intracellular pathogen Chlamydia trachomatis manipulates the host actin cytoskeleton to assemble actin-rich structures that drive pathogen entry. The recent discovery of TmeA, which like TarP is an invasion-associated type III effector implicated in actin remodeling, raised questions regarding the nature of their functional interaction. Quantitative live-cell imaging of actin remodeling at invasion sites revealed differences in recruitment and turnover kinetics associated with TarP and TmeA pathways, with the former accounting for most of the robust actin dynamics at invasion sites. TarP-mediated recruitment of the actin nucleators formin and the Arp2/3 complex were crucial for rapid actin kinetics, generating a collaborative positive feedback loop that enhanced their respective actin-nucleating activities within invasion sites. In contrast, Fmn1 is neither recruited to invasion sites nor collaborates with Arp2/3 within the context of TmeA-associated actin recruitment. While the TarP-Fmn1-Arp2/3 signaling axis is responsible for the majority of actin dynamics, its inhibition had similar effects as deletion of TmeA on invasion efficiency, consistent with the proposed model that TarP and TmeA acting on different stages of the same invasion pathway.

Poster Presentation #90

Candida albicans Cell Wall Remodeling in Response to Commensal Gastroinestinal Bacteria

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The fungus *Candida albicans* commonly colonizes mucosal surfaces of healthy individuals; however, in cases of immunocompromise, microbial dysbiosis, or disrupted barrier integrity, *C. albicans* can cause superficial mucosal or life-threatening disseminated infections. The major interaction interface of *C. albicans* with the host is its multilayered cell wall, which is dynamic and highly responsive to the surrounding environment. *C. albicans* undergoes activate cell wall remodeling in response to changes in environmental pH, nutrient source, temperature, and oxygen levels. Alterations in the outer mannan layer have important downstream implications on the host response to *C. albicans*, as mannan can shield the immunodominant beta-glucan residue from recognition by host receptors. The ability of relevant bacteria to induce fungal cell wall remodeling has not been thoroughly characterized. I have demonstrated that the prominent gut bacterium, *Bacteroides thetaiotaomicron*, induces an increase in fungal cell wall mannan. Previous work demonstrated that *B. thetaiotaomicron* provides colonization resistance against *C. albicans* and these microbes co-localize in the outer colonic mucus layer, indicating the capacity for relevant interactions during gastrointestinal colonization. My preliminary results indicate that changes in the cell wall induced by *B. thetaiotaomicron* alter macrophage recognition to *C. albicans*, highlighting the importance of fungal-bacterial interactions on the potential host response to *C. albicans* colonization. Overall, my work seeks to determine how a commensal bacterium that has already been implicated in colonization resistance against *Candida* directly regulates fungal biology.

Poster Presentation #91

Yersinia pestis Actively Influences Extracellular Vesicle Production by Human Neutrophils

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Extracellular vehicles (EVs) are key mediators of intercellular communication during infection. These lipid-bound vesicles contain proteins, lipids, and nucleic acids that represent the immunologic state of a given cell. Upon release, EVs can fuse with other immune cells, establishing biochemical communication between cells. *Yersinia pestis* is the etiologic agent of the plague. A hallmark of plague is subversion of the host immune response by disrupting host signaling pathways required for inflammation. We and others have shown *Y. pestis* actively inhibits phagocytosis and degranulation by neutrophils. Manipulation of these vesicular trafficking pathways strongly suggests that *Y. pestis* also alters EV secretion, cargo selection, and/or trafficking in infected cells. However, the involvement of EVs during *Y. pestis* infection has not been elucidated. Our goals are to define the EVs produced by neutrophils in response to *Y. pestis* and how these vesicles influence inflammation. Toward these goals, EVs were isolated from human neutrophils infected with *Y. pestis* or a mutant in the T3SS. These EVs were subsequently analyzed via dynamic light scattering (DLS), nanoparticle tracking, and mass spectroscopy to determine size profile, concentration, and protein payloads, respectively. While our DLS data indicated similar EV size profiles from *Y. pestis* and T3SS mutant infected cells, mass spectrometry data revealed that cargoes packaged in EVs isolated from the mutant infected cells were enriched with antimicrobials and cytotoxic proteins and differed from uninfected and *Y. pestis* infected cells. Together, these data suggest that *Y. pestis* actively inhibits the production of antimicrobial EVs produced by neutrophils.

Poster Presentation #92

Genetic Determinants of Candida auris Colonization of Abiotic Surfaces

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Candida auris is an emergent fungal pathogen responsible for drug-resistant infections and healthcare-associated outbreaks worldwide. The ability of *C. auris* to colonize and persist on surfaces, medical instruments, and indwelling devices in healthcare environments drives transmission and outbreaks, requiring extensive infection prevention practices and exacerbating the burden of disease. The predicating step of colonization of abiotic surfaces is the irreversible attachment of cells. We have observed widespread variability in the capacity of diverse *C. auris* clinical isolates to adhere to inert surfaces. Our data suggests *C. auris* attachment is not substantially mediated by canonical fungal adhesin proteins or their associated regulators, despite their genetic conservation in *C. auris*. Instead, we have identified an uncharacterized, *C. auris*-specific adhesin belonging to the *FLO11* superfamily that substantially controls *C. auris* adhesion to abiotic surfaces. Transcriptional control of this adhesin is a major determinant of surface attachment in strains representing every *C. auris* clade. Furthermore, clinical *C. auris* isolates of diverse origins exhibit widespread variation in transcript abundance of this adhesin associated with variation in adhesion capacity. Several conserved transcriptional regulators that control attachment and adhesin expression in related fungal species do not control expression of this adhesin or *C. auris* attachment. Rather, transcriptional regulation of this adhesin is governed by the *SWI/SNF* chromatin remodeling complex and a novel, *C. auris*-specific Zinc-Finger protein. Together, our findings suggest *C. auris* has evolved novel mechanisms of surface colonization, which may in part explain the unique propensity of this fungal pathogen for transmission and healthcare-associated outbreaks.

Poster Presentation #93

Shikimate Biosynthesis Contributes to Citrobacter freundii Fitness During Bacteremia

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Bacteremia is a life-threatening bacterial bloodstream infection (BSI) for which an estimated 38-44% of cases are caused by Gram-negative bacteria (GNB). While *Escherichia coli* and *Klebsiella pneumoniae* are responsible for the majority of GNB bacteremia cases, other members of the family *Enterbacteriaceae*, including *Citrobacter freundii*, also cause BSI. *C.freundii* is a common constituent of the intestinal microbiota but can become an opportunistic pathogen in immunocompromised individuals with the capacity to infect diverse body sites. A recent Tn-seq study exploring the genetic requirements for *C. freundii* to cause bacteremia in a murine model (*Anderson et al. 2018*) suggested the shikimate biosynthesis pathway contributes to bacterial fitness. Importantly, the utility of the shikimate biosynthesis pathway during bacteremia has also been predicted in other GNB Tn-seq experiments (*E. coli* and *Serretia marcescens*). Using targeted mutants interrupting shikimate biosynthesis (*aroA, aroB, aroC, aroD, aroE,* and *aroK*) we validated the predicted contribution of this pathway during *C. freundii* bacteremia in the murine model. Shikimate biosynthesis is utilized by bacteria to produce chorismate; a biomolecule required to synthesize aromatic amino acids, enterobactin, folate, and quinones (ubiquinone and menaquinone). Further *in vivo* competitions using chorismite-dependent biosynthetic pathway mutants have, thus far, implicated ubiquinone (*ubiG* and *ubiH*) and folate biosynthesis (*pabC*) as the down-stream pathways primarily responsible for the *in vivo* fitness defect observed in shikimate biosynthesis mutants. Our future expansion of these findings in *C. freundii* to other GNB species may reveal shared strategies required to cause bacteremia, highlighting therapeutic targets to universally manage GNB bacteremia.

Poster Presentation #94

Pathogenicity of Multidrug-Resistant Klebsiella pneumoniae Clinical Isolates

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Klebsiella pneumoniae (Kp) strains have been divided into two categories: classical strains (cKp), which are frequently multidrug-resistant and infect compromised patients residing in hospitals and long-term care facilities, and hypervirulent strains (hvKP), which cause severe invasive and disseminated community-acquired infections. A major public health concern is that multidrug-resistant (MDR)-cKp isolates responsible for nosocomial infections will become highly virulent with acquisition of a hypervirulence plasmid, resulting in highly aggressive strains that are difficult to treat. Globally, there have been several reports of "convergence" of hvKP-MDR strains, but the prevalence and level of virulence expected for these isolates remains unknown. In this work, we performed whole genome sequencing and virulence studies on US "convergent" isolates. Based on our sequencing, we identified 11 MDR isolates containing a large virulence plasmid. These "convergent" isolates are found in 1.9% of U.S. genomes included in this study. Most convergent isolates are MDR high risk clones that have acquired a virulence plasmid. These isolates were all non-hypermucoviscous except for one isolate. In a chrome azurol S assay, convergent isolates produced 2-5-fold more siderophores than an enterobactin only control. In our mouse pneumonia model, only one convergent isolate had an LD50 consistent with hvKp while the others had LD50s, consistent with that of cKp. While siderophore production is necessary for virulence, it is not sufficient for hypervirulence. Convergent isolates are only hypervirulent if they contain the mucoid regulator operon (rmpADC) and are hypermucoviscous. Taken together, these data suggest that RmpADC mediated hypermucoviscosity is the most potent virulence factor.

Poster Presentation #95

PASTA Kinase-dependent Control of Peptidoglycan Synthesis is Required for Adaptation of Listeria monocytogenes to Cell Wall Stress in the Cytosol

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The mammalian cytosol is restrictive to microorganisms. Professional cytosolic pathogens must therefore possess adaptations that allow them to survive and replicate in this niche. Previously, we demonstrated that the penicillin-binding protein and serine/threonine-associated (PASTA) kinase in the intracellular pathogen Listeria monocytogenes, PrkA, is required for cytosolic survival and ultimately virulence. PASTA kinases sense cell wall stress and phosphorylate multiple targets to mediate global physiological changes in response. Using orthogonal phosphoproteomic and genetic approaches, we found that during β-lactam exposure PrkA phosphorylates 23 proteins with functions in cell wall homeostasis, central metabolism, stress responses, and more. We investigated the impact of PrkA phosphorylation of two substrates involved in cell wall homeostasis, ReoM and GpsB, and show that proper phosphoregulation is required for the resistance to cell wall stress. Using a click chemistry-based peptidoglycan (PG) labeling technique, we demonstrate that PrkA-mediated phosphorylation of ReoM results in an increase PG synthesis during exposure to the cephalosporin ceftriaxone. Using an in vitro kinase assay, we show that a non-phosphorylatable form of GpsB potentiates PrkA autophosphorylation and phosphorylation of a model kinase substrate, suggesting that appropriate phosphoregulation of GpsB is important for regulating PrkA activity. Ultimately, we find that PrkA-mediated regulation of ReoM and GpsB is required for survival in the cytosol and for virulence in a mouse model of systemic listeriosis. Cumulatively, this work sheds light on how a professional cytosolic pathogen uses cell wall stress cues sensed by a PASTA kinase to modulate its physiology and ability to cause disease.

Poster Presentation #96

Investigating Membrane Vesicle Biogenesis from Persistent Group B *Streptococcus* Isolates in the Context of Antibiotic Stress.

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Group B *Streptococcus* (GBS) colonizes ~30% of pregnant people recto-vaginally and when transferred to a fetus during pregnancy, causes severe neonatal disease. Over 60% of countries administer intrapartum antibiotic prophylaxis (IAP) to treat GBS-positive pregnant people. However, we have shown that certain GBS isolates withstand stress and persistently colonize the vaginal tract despite IAP. Those mechanisms that promote survival in antibiotic stress are poorly understood. One tolerance strategy employed by bacteria is the production of membrane vesicles (MVs), which have been demonstrated to aid in bacterial survival. GBS has been shown to produce MVs, but their role in the context of antibiotic stress remains elusive. Consequently, we selected clinical GBS isolates that persistently colonized pregnant people before and after IAP to determine how antibiotic stress impacts MV biogenesis. Cultures were treated with ampicillin, and prepped for microscopy and MV isolation, which were visualized with TEM, quantified via NanoSight, and analyzed for protein content via LC-MS/MS. Ampicillin-treated GBS significantly enhanced MV production relative to control ($p = 0.00086$, $n=6$). Thin-section TEM images showed areas of membrane weakening in the ampicillin-treated GBS, which could represent points of MV release. Upon completion of proteomics, we expect to uncover key proteins that are differentially abundant across treatment groups. These data suggest the hypothesis that excess quantities of MVs in the presence of antibiotics may enhance GBS survival in these conditions. Improving our understanding of GBS MVs and their role in persistent infections is important for directing the discovery of novel therapeutics in the future.

Poster Presentation #97

A CRISPRi-based Structure-function Analysis for Delineating Lipoprotein Localization in Borrelia burgdorferi

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Efficient lipoprotein localization is essential for cell survival in diderm bacteria, as lipoproteins mediate a wide variety of important cellular functions, including the assembly and function of essential outer membrane machinery like the Bam complex. In the Lyme disease spirochete Borrelia burgdorferi, differentially expressed surface lipoproteins also facilitate each individual stage of the bacterium's pathogenic lifecycle from tick vector colonization to dissemination and persistence in mammalian hosts. Similar to alpha and deltaproteobacteria, B. burgdorferi has all of the canonical localization of lipoprotein (Lol) pathway components found in E. coli with the exception of an identified LolB outer membrane lipoprotein acceptor. Since the soluble LolA periplasmic chaperone is the furthest identifiable downstream component of these systems, we solved the crystal structure of B. burgdorferi LolA homolog BB0346 (BbLolA) to 1.9 Å resolution and identified multiple unique loop domains in comparative analyses to other solved LolA structures. We are currently using an optimized Bb CRISPRi PAM* knockdown/complementation system and collection of strategic BbLolA mutants to determine the functional significance of these additional loops in the localization of lipoproteins within the periplasm. Overall, this research aims to define the molecular events that connect lipoprotein maturation at the cytoplasmic membrane to their emergence on the bacterial surface as bioactive virulence determinants. It will shed light on LolA function in the LolB-deficient system employed by various bacterial divisions and may help identify organism-specific therapeutic targets.

Poster Presentation #98

PopB-PcrV Interactions are Critical for Pore Formation in the Pseudomonas aeruginosa Type III Secretion System

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The type III secretion system (T3SS, "injectisome") is an important virulence factor for *Pseudomonas aeruginosa* during acute infections. This system injects toxic effector proteins directly into the host cell. Despite decades of research, the translocation pore—the T3SS component which makes a pore in the host membrane and attaches to the T3SS needle—remains poorly understood due to its complex assembly process. We took a genetic approach to study how the pore proteins PopB and PopD interact with the needle tip protein PcrV. By mixing and matching segments of these *P. aeruginosa* proteins with their *Aeromonas hydrophila* homologs, we were able to map an interaction between PopB and PcrV. We then used the chimeric proteins to explore the function of the PopB-PcrV interaction. We discovered that this interaction is critical for formation of the translocation pore, but not required for insertion of PopB into the host membrane. Our genetic approach provides information about the architecture of the translocation pore which has evaded other biochemical or structural approaches and has furthered our understanding of this potential anti-virulence drug target.

Poster Presentation #99

Vibrio cholerae Modulates Cyclic di-GMP in Response to Zinc via a Horizontally Acquired Genomic Island

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Ongoing 7th pandemic *Vibrio cholerae* El Tor strains have acquired two genomic islands, VSP-1 and 2, that are hypothesized to be important for their pathogenicity and adaptability. However, the functions of several genes in these genomic islands are unknown. Recently it has been shown that genes *vc0512-vc0515* in the VSP-2 island are repressed by zinc via the Zur repressor and are induced by transcriptional activator VerA *vc0513*. This region includes the predicted cyclic-di GMP phosphodiesterase, *vc0515*. Cyclic-di GMP is an important signaling molecule which is involved in several important biological processes including biofilm formation, motility and secretion systems. The intracellular level of cyclic-di GMP is regulated by diguanylate cyclases (DGCs) that synthesize cyclic-di GMP from GTP and phosphodiesterases (PDE) that degrade cyclic-di GMP.

In this study, we delineated role of zinc in the regulation of phosphodiesterase VC0515 present in VSP-2 island. We have shown that VC0515 is indeed an active PDE. We then determined that the intracellular concentrations of cyclic-di GMP in a Δzur mutant was lower than the WT and *ΔverA* mutant owing to increased expression of VC0515. We also analyzed the presence of *vc0512-vc0515* in several El Tor strains of V. cholerae and found them to be prevalent in the 7th pandemic strains underlining the evolutionary significance of these genes. Our results demonstrate that *V. cholerae* alters cyclic di-GMP levels in response to zinc through transcriptional regulation of VC0515. The impact of this altered cyclic di-GMP on *V. cholerae* physiology are currently being explored.

Poster Presentation #100

Inflammation Stimulated by Listeria Monocytogenes Impairs Wound Healing in Zebrafish Tail Wounds via IL-1 Signaling

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Microbial infection is a major complication in wound healing. Larval zebrafish are a useful model for studying wound healing because the immune system is highly conserved, the tissue is optically transparent, and transgenic lines with cell-specific expression of fluorescent proteins are available, allowing us to visualize immune cells responses to infections and the damaged epithelial layers. When zebrafish transected tail wounds are infected with Listeria monocytogenes (Lm), wound healing is inhibited, and increased numbers of TNFα-expressing macrophages are recruited to the wound compared to uninfected zebrafish tail wounds. Treatment with ampicillin depleted Lm from the infected zebrafish tail wounds. However, persistent inflammation in the absence of Lm still inhibited wound healing. To further understand the impact of inflammation stimulated by Lm on wound healing, we utilized Lm genetical engineered to activate either pro- or anti-inflammatory forms of cell deaths. Lm that induced inflammatory cell death, including pyroptosis and necrosis, inhibited wound healing; whereas, Lm that induced anti-inflammatory cell death, apoptosis, did not inhibit wound healing, further supporting that extensive inflammation impairs wound healing. Finally, to investigate the inflammatory mediators that inhibit wound healing, we performed RNA-sequencing and found that IL-1β was upregulated in Lm-infected zebrafish tail wounds. Strikingly, we found that treatment with an IL-1R antagonist, partially rescued wound healing in infected zebrafish, suggesting that IL-1 signaling driven by Lm infection leads to wound healing defects. Ongoing studies are aimed at further investigating if IL-1 signaling can serve as a potential therapeutic target for treating infected wounds.

Poster Presentation #101

Cation Homeostasis: Coordinate Regulation of Polyamine and Magnesium Levels in Salmonella

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Polyamines are organic cations important in all domains of life. Here we show that, in *Salmonella*, polyamine levels and Mg^{2+} levels are coordinately regulated, and this regulation is critical for viability under both low and high concentrations of polyamines. Upon Mg^{2+} starvation, polyamine synthesis is induced, as is production of the high affinity Mg^{2+} transporters, MgtA and MgtB. Either polyamine synthesis or Mg^{2+} transport is required to maintain viability. Mutants lacking the polyamine exporter PaeA, expression of which is induced by PhoPQ in response to low Mg^{2+}, lose viability in stationary phase and this lethality is suppressed by blocking either polyamine synthesis or Mg^{2+} transport, suggesting that once Mg^{2+} levels are re-established, the excess polyamines must be excreted. Thus, it is the relative levels of both Mg^{2+} and polyamines that are required to maintain homeostasis. Indeed, sensitivity to high concentrations of polyamines in the media is proportional to the Mg^{2+} levels. These results are recapitulated during infection. Polyamine synthesis mutants are attenuated in a mouse model of systemic infection, as are strains lacking the MgtB Mg^{2+} transporter. Loss of MgtB in the synthesis mutant background confers a synthetic phenotype, confirming that Mg^{2+} and polyamines are required for the same process. Mutants lacking PaeA are also attenuated but deleting *paeA* has no phenotype in a polyamine synthesis mutant background. These data support the idea that the cell coordinately controls polyamine and Mg^{2+} concentrations to maintain overall cation homeostasis, critical for survival in the macrophage phagosome.

Poster Presentation #102

The Unfolded Protein Response (UPR) Transcription Factor CHOP as a Virulence Factor in Brucella Infection

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Brucellosis, caused by Brucella bacterial species, is the most prevalent zoonosis worldwide and spondyloarthritis the most common infectious complication. Following inhalation or ingestion, Brucella invade macrophages and traffic to the endoplasmic reticulum where they induce a stress response known as the UPR. The UPR supports Brucella replication and regulates host inflammation and apoptosis. Intriguingly, we found that different Brucella species vary in their capacity to induce UPR signaling axes, with B. melitensis (the most virulent species in humans) inducing greater CHOP than B. abortus or the vaccine strain S19. In other studies, CHOP was found to bind the promoter of a cytokine central to spondyloarthritis IL-23 (IL23A). To further evaluate the role of CHOP in Brucella-induced inflammation, we used CHOP-/- mice. 7 days post infection with B. melitensis, CHOP-/- splenocytes displayed much lower IL-23 induction, with less inhibition of TNF-α. This was not due to lower replication of B. melitensis, as splenic CFU were reliably slightly elevated (~50%) in the CHOP-/- mice. In contrast, B. abortus induced very little IL-23, and neither TNF-α, nor replication were altered in the CHOP-/- mice. In vitro, in bone-marrow derived macrophages, IL-23, but not TNF-α, was highly CHOP-dependent. Together these results suggest the UPR transcription factor CHOP may drive relative virulence and spondyloarthritis complications of B. melitensis infection. The role of the UPR in non-infectious Spondyloarthritis, a disease associated with the misfolding allele HLA-B27, has remained enigmatic. Further dissection of UPR-associated inflammation using the Brucella model may elucidate pathogenic mechanisms in the rheumatologic condition.

Poster Presentation #103

Uncovering PASTA Kinase Dependent-signaling Cascades that Mediate Intrinsic β-lactam Resistance in Methicillin-resistant Staphylococcus aureus

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Stk1, a eukaryotic-like serine/threonine kinase with penicillin-binding-protein and serine/threonine kinase-associated (PASTA) domains plays a central role in regulating intrinsic β-lactam antibiotic resistance in Methicillin-resistant *Staphylococcus aureus* (MRSA). Deletion or pharmacologic inhibition of *stk1* drastically sensitizes MRSA to β-lactams, however, the Stk1-dependent signaling cascades that mediate antibiotic resistance remain undefined. To identify Stk1 substrates required for β-lactam resistance, we combined shotgun phosphoproteomics with a forward genetic screen in the context of sub-inhibitory antibiotic stress. Phosphoproteomic analysis identified 150 phosphopeptides from 141 proteins, revealing distinct populations of Ser/Thr phosphoproteins within WT and Δ*stk1*. Notably, 78 unique phosphoproteins were observed in Δ*stk1*, including drug and antimicrobial peptide transporters and DNA replication and repair proteins, suggesting other Ser/Thr kinases play a role in regulating stress responses when *stk1* is absent. 20 proteins were phosphorylated in an Stk1-dependent manner, of which 10 overlapped with the genetic screen and included proteins involved in cell wall homeostasis, reactive oxygen species detoxification, and purine metabolism. Notably, the enzyme PurA, which provides the first committed step in the AMP/GMP biosynthetic pathway towards AMP biosynthesis was identified both as an Stk1 phosphosubstrate and required for β-lactam resistance. This suggests that Stk1 phosphorylation of PurA represents a key regulatory node of intrinsic β-lactam resistance. Upstream AMP/GMP biosynthesis mutants similarly exhibit increased sensitivity to β-lactams and supplementation with adenosine partially restores β-lactam resistance in Δ*purA*. Ongoing studies are underway to verify Stk1-dependent phosphorylation sites on PurA and determine the impact of phosphorylation of PurA on enzymatic activity, bacterial physiology, and pathogenesis.

Poster Presentation #104

Cryptococcus neoformans Generates an Intracellular Niche in Macrophages in an Immune Signal-dependent Manner

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Cryptococcus neoformans is a fungal opportunistic pathogen responsible for nearly 200,000 deaths yearly. It is acquired by inhalation, and thus alveolar macrophages represent the first line of defense. Interactions between these host cells and the fungi will influence whether the infection is controlled or disseminates to cause lethal disease. One of the main drivers of mortality is the ability of *C. neoformans* to survive and replicate inside host macrophages. Understanding the mechanisms that allow this parasitism of host cells is critical for our fight to control this devastating disease.

The identity of *C. neoformans'* intracellular replicative niche is unclear, and how immune signals affect it is unknown. To better understand these interactions, we aim to decipher the properties of the cryptococcal-containing phagosome (CCP). We find a population of CCPs that display both PI3P, an early endosomal marker, and lysosomal characteristics, a combination not normally observed. We also show that CCPs exhibit altered acidification dynamics, in contrast to *S. cerevisiae*-containing phagosomes, which rapidly acidify and stay acidic. Moreover, several CCP markers are also altered temporally compared to control phagosomes. This suggests that *Cryptococcus* alters its phagosome, providing a potential mechanism for intracellular survival that may be driving pathogenesis. Notably, when macrophages are stimulated with interferon-γ, the CCP acquires lysosomal markers more rapidly, and this correlates with acquisition of Rab20. We believe that *Cryptococcus* actively manipulates its phagosome by targeting Rab GTPases, phosphoinositides, or both, in a way that is dependent on host's immune signals.

Poster Presentation #105

Investigating How MARTX Toxins of Vibrio vulnificus Interact with Host Cells at the Surface

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Multifunctional-autoprocessing repeats-in-toxin (MARTX) toxins are large pore-forming toxins secreted from several Gram-negative bacteria. Importantly, it is the dominant virulence factor of *Vibrio vulnificus*, a highly lethal foodborne pathogen. MARTX toxins contain highly conserved repeats regions at their N- and C-termini, which flank the variable effector domains and a cysteine protease domain (CPD). Previously, we showed that those conserved regions are sufficient to translocate effector domains and the CPD into the cell. Subsequently, CPD autoprocessing releases effectors to the cytosol for cell intoxication. While much is known about MARTX effector mechanisms, how MARTX toxins interact with cells at the surface is not known. The structures of fragments of the toxin translocation regions were predicted by the AlphaFold2 algorithm to inform successful purification of soluble fragments of the toxin. Two purified fragments from the N-terminal conserved region were found to bind to cell surfaces across a spectrum of cell lines susceptible to *Vibrio vulnificus* MARTX toxin. For one of the two fragments, a smaller sub-fragment was observed after incubation with cells, raising the possibility that this fragment is cleaved by a host surface protease. We further used negative staining techniques to show that this fragment exhibits oligomeric states, suggesting that this portion of the toxin may oligomerize at the host surface. Collectively, this study discovered two fragments of the MARTX toxin that bind the host cell surface, providing essential knowledge for future discovery of host receptor(s) for the toxin.

Poster Presentation #106

Listeria monocytogenes Infection Alters Content and Function of Extracellular Vesicles Produced by Trophoblast Stem Cells

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The placenta is a vital organ that develops during the beginning stages of pregnancy and facilitates the exchange of nutrients and gases to the fetus. Pathogens, such as *Listeria monocytogenes (Lm)*, can cross the placental barrier, leading to poor fetal outcomes. A strong cell-mediated immune response is required to control this infection. One mechanism to stimulate such a response relies on signaling through extracellular vesicles (EVs), membrane-enclosed particles that are secreted by every cell type. EVs have been implicated in many inflammatory processes, leading us to hypothesize that EVs produced by *Lm* infected cells might be immunostimulatory, helping to protect against the spread of *Lm*. We used a trophoblast stem cell (TSC) model, as trophoblasts are one of the primary cell types of the placenta. We found that treatment of macrophage-like cells with EVs from *Lm*-infected TSCs induced production of the proinflammatory cytokine TNF-α. Surprisingly, when we infected the macrophage-like cells with *Lm* after EV treatment, the cells became more susceptible to infection. Analyzing EV contents revealed that infection with *Lm* changed EV protein composition. EVs from infected TSCs contained increased levels of ribosomal and other RNA-binding proteins compared those from uninfected cells. RNA sequencing showed that infection also changes the mRNAs loaded into the EVs, with an overrepresentation of genes involving vasculogenesis and morphogenesis. Altogether, we have found that Lm infection alters EVs produced by host cells, these EVs appear to be pro-inflammatory, and they may aid Lm in its spread across barrier tissues.

Poster Presentation #107

S. aureus Membrane Homeostasis is Impaired by the Short Chain Fatty Acids Propionate and Butyrate.

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Chronic rhinosinusitis (CRS) is a heterogeneous inflammatory condition characterized by mucus hypersecretion and infection. While Staphylococcus aureus is recognized as a CRS pathogen, microbiome analyses of CRS sinus mucus have found increased levels of anaerobes, suggesting S. aureus experiences altered metabolic landscapes in CRS relative to healthy airways. We characterized the growth kinetics and transcriptome of S. aureus in supernatants of the abundant CRS anaerobe Fusobacterium nucleatum. There was an extended lag phase, and the transcriptome was profoundly affected, with decreased expression of the agr quorum sensing system. fadX, encoding a putative propionate coA-transferase, was significantly increased, therefore we hypothesized that short chain fatty acids (SCFAs) produced by anaerobes affect S. aureus growth and gene expression. Exposure to propionate and butyrate impeded growth. A ΔfadX mutant was found to be more sensitive than wild type to growth inhibition by propionate, suggesting a role for FadX in propionate metabolism. Spontaneous resistance to butyrate, but not propionate, was detected frequently, and genome sequencing identified SNPs and indels in codY. A codY::tn mutant was resistant to growth inhibition by butyrate but not propionate. Supplementation of SCFA media with isoleucine, but not leucine or valine, increased growth of wild-type S. aureus. Mutants in the branched chain amino acid-to-branched chain fatty acid pathway were significantly more sensitive to SCFAs. Together, these data show that S. aureus pathophysiology in CRS may be affected by co-colonizing microbiota and identify S. aureus lipid membrane homeostasis as a possible target of their activity.

Poster Presentation #108

Microbial Population Dynamics in the *Vibrio*-Squid Symbiosis

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Animal-microbe symbioses are ubiquitous and affect host health and development. Understanding the mechanisms involved at the animal-microbe interface is necessary for development of therapeutics that manipulate host-associated microbiomes. The symbiosis between *Vibrio fischeri* and the Hawaiian bobtail squid *Euprymna scolopes* is an experimentally tractable model to study colonization of host-tissues with high resolution. To characterize putative colonization factors identified previously in *V. fischeri*, we developed a method to generate barcode-tagged gene deletions and perform high-throughput competition experiments with detection of each strain by barcode sequencing (BarSeq). BarSeq experiments measuring various timepoints during colonization using 24 pooled barcoded mutant and WT strains revealed a population bottleneck between 6- and 24-hours post-infection (hpi) that reduces the diversity of the colonizing bacterial population to 5-9 unique strains within the squid light organ (LO). Discrepancy in the literature regarding the number of cells that can enter the LO (6-12 to >80) led us to generate pooled libraries of barcode-tagged WT strains and test the population bottleneck at various inoculum amounts. The results confirmed the population bottleneck of 5-9 unique strains at high inoculum amounts. Current work aims to test the colonization-dependent constriction of an anatomical bottleneck within the LO that occurs at ~18 hpi in causing the observed collapse in population diversity by using pooled barcoded strains in mutant backgrounds known to prevent constriction. Future work will define the timing and the mechanism of the population bottleneck and explore how infection dynamics are modulated by interactions of the host with phylogenetically diverse colonizing microbes.

Poster Presentation #109

Exogenous Saturated Fatty Acids Impede Proliferation of Metabolically Restricted Staphylococcus aureus

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Staphylococcus aureus poses a major threat to human health due to its ability to cause a multitude of ailments as well as the prevalence of antibiotic resistant strains. *S. aureus* is the leading cause of skin and soft tissue infections, osteomyelitis, and endocarditis. To colonize distinct host tissues, *S. aureus* relies on aerobic respiration and fermentation. Fermentation also supports development of the small colony variant (SCV) phenotype, a metabolically altered cell-type that is associated in the clinic with aminoglycoside treatment and persistent infection. We hypothesize that dedicated physiological pathways sustain the SCV phenotype. Identifying these pathways and developing cognate inhibitors has the potential to restrict *S. aureus* metabolic versatility, growth, and its ability to resist aminoglycosides. In an effort to find new therapeutics, we discovered metabolically restricted *S. aureus* downregulated genes associated with acquisition of host fatty acids. To examine this, we screened biologically relevant fatty acids against respiring and fermenting *S. aureus* cells. We show that fermenting *S. aureus* is sensitive to saturated fatty acids (SFA) while respiring cells are not. Exploring SFA sensitivity has revealed that myristic acid is bacteriostatic towards fermenting *S. aureus*. Further experimentation is necessary to identify the precise mechanism of action of SFAs. Overall, these data are consistent with a model that predicts that *S. aureus* transition between different metabolic states relies on dedicated physiological pathways that are sensitive to SFAs. Identifying mechanisms by which SFAs elicit toxicity towards fermenting staphylococci will identify new therapeutic targets for this evasive threat.

Poster Presentation #110

CCR2-dependent Migration of CX3CR1-hi Antigen Presenting cells Facilitates Enterococcus faecalis Dissemination

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Migratory antigen presenting cells (APCs) are critical mediators of innate and adaptive immunity in the gut and coordinate healthy commensalism with the residing microbiome. However, intracellular pathogens such as *Salmonella typhimurium* and *Citrobacter rodentium* are known to hijack migratory APCs to facilitate their dissemination throughout the body. This study aimed to explore the role of migratory APCs in the dissemination of a common commensal bacteria, *Enterococcus faecalis* (EF), which is known to cause opportunistic infections in response to antibiotic-induced dysbiosis. We reveal that clodronate-mediated phagocyte depletion resulted in reduced EF burden in the mesenteric lymph nodes (MLNs) of ceftriaxone treated mice, suggesting that migratory APCs may promote EF dissemination. We also confirmed that EF mutants that lack the gene encoding manganese-containing superoxide dismutase (ΔsodA) are less adapted to intracellular survival within macrophages in vitro and exhibit impaired dissemination in response to ceftriaxone in vivo. This suggests that intracellular APC survival may serve as a mechanism by which EF disseminate in response to ceftriaxone treatment. Finally, we identified an APC subset that expresses high levels of C-X3-C Motif Chemokine Receptor 1 (CX3CR1-hi APCs) and uniquely relies on C-C Motif Chemokine Receptor 2 (CCR2) for migration to the MLNs. Chemical antagonism of CCR2 prevented CX3CR1-hi APC migration to the MLNs, and concomitantly reduced EF dissemination. Together, these data suggest that EF survive within migratory CX3CR1-hi APCs that transport EF to the MLNs in a CCR2-dependent manner, thus promoting EF dissemination during antibiotic-induced dysbiosis.

Poster Presentation #111

Bacterial Intercellular Spread Requires α-catenin

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During infection, several important human bacterial pathogens invade cells and then spread between cells of an epithelial layer. This intercellular spread is enabled at the cell periphery by the reorganization of the plasma membrane into a structure known as a protrusion that is engulfed by the neighboring cell. The molecular mechanisms by which protrusions are formed and engulfed are poorly understood. Here, we show α-catenin is required for efficient intercellular spread. Our data demonstrate that S. flexneri and L. monocytogenes, model intracellular pathogens that spread, require α-catenin to initiate and to stabilize protrusions. In order to form a protrusion, these pathogens polymerize actin for motility and use the forces derived from actin polymerization to push against the membrane. Interestingly for S. flexneri, actin polymerization and bacterial motility were not affected by α-catenin loss, but at the periphery, bacteria more frequently formed protrusions that failed when α-catenin was absent. We show that α-catenin is required to recruit the formin mDia1 to bacteria. Formins enable actin to polymerize in parallel bundles that generate more force. These data show α-catenin dependent mDia1 recruitment is necessary to generate the force required to establish protrusions necessary for bacteria to spread between cells.

Poster Presentation #112

The c-di-AMP Level in *Streptococcus pyogenes* Mutants with Envelope Stress

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Cyclic di-AMP (c-di-AMP) is a signaling molecule that has been shown to play important roles in bacterial physiology and pathogenesis. Since a major role of c-di-AMP is to maintain proper turgor pressure, we investigated if c-di-AMP amount varies in the mutants with cell envelope stress or turgor pressure change. For measuring c-di-AMP level, we employed a competitive ELISA assay using CabP that has high affinity and specificity to c-di-AMP. We quantified both secretory and cellular c-di-AMP levels of mutants that have cell envelope stress potential (Δ*liaR* and Δ*dltX*) or ionic imbalance (Δ*ktrB*) in the wild type or the Δ*ped2* mutant (Δ*pde2*Δ*liaR*, Δ*pde2*Δ*dltX*, Δ*dacA*Δ*ktrB*). *liaR* is the response regulator gene of the three-component system LiaFSR that responds to membrane stress, *dltX* is the first gene of the *dlt* operon that confers D-alanylation of teichoic acids, and *ktrB* is a major potassium transporter gene. We observed a marked difference in the secretory and cytosolic c-di-AMP level between these mutants and wild-type *S. pyogenes*, indicating that these gene products are necessary for maintaining the proper level of c-di-AMP. Measuring c-di-AMP levels in various clinical isolates of *S. pyogenes* is ongoing, which might shed more light on the involvement of c-di-AMP in the pathogenesis of *S. pyogenes*.

Poster Presentation #113

In Vitro Phenotypic and Transcriptomic Variation in *Neisseria musculi* Morphotypes Correlate with Colonization Variability and Persistence in Vivo

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Asymptomatic colonization of the upper respiratory tract is a common trait of the two human restricted pathogens, *Neisseria gonorrhoeae* and *Neisseria meningitidis*. In vivo models of pathogenic neisserial infections are heterologous systems that permit short-term colonization but do not fully recapitulate infections in humans. Studying *Neisseria musculi* (Nmus), an oral commensal, in laboratory mice allows investigation of *Neisseria*-host interactions that avoids host restriction barriers. Nmus produces smooth and rough morphotypes on solid media. We compared the in vitro phenotypes, biofilm transcriptomes, in vivo colonization patterns and burdens of the two Nmus morphotypes. We observed that the two morphotypes differ in biofilm formation, pilin production, transformation frequency, and aggregation in vitro. These phenotypes strongly correlated with differential expression of a set of genes in the Nmus biofilms including those that encoded factors for bacterial attachment. In vivo, the smooth morphotype stably colonized the oral cavities of all inoculated A/J and C57BL/6J mice at higher burdens relative to the rough. Interestingly, both morphotypes colonized the oral cavities of A/Js at higher magnitudes than in C57BL/6Js. Gut colonization by the smooth morphotype was qualitatively higher than the rough. Nasal colonization in the A/Js were transient following nasal inoculations. Collectively, our results demonstrate that colonization by Nmus can be affected by various factors including Nmus morphotypes, inoculation routes, anatomical niches, and host backgrounds. The Nmus-mouse model can use variable morphotype-host combinations to study the dynamics of neisserial asymptomatic colonization and persistence in multiple extragenital niches.

Poster Presentation #114

Probing Inducer Specificity of a Glyoxal-Inducible Operon in Pseudomonas aeruginosa Using a Plasmid-Based Dual-Fluorophore Reporter System

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Reactive electrophilic species (RES), such as glyoxal and methylglyoxal, have gained traction in recent years as important sources of cellular damage and stress. However, the systems that detect, respond, and detoxify RES have been poorly characterized. We have recently identified an antibiotic biosynthesis monooxygenase (ABM) domain-containing protein, PA0709, in the opportunistic pathogen Pseudomonas aeruginosa that is co-transcribed with a key glyoxal-detoxifying gene, gloA2. A high-resolution structure of PA0709 revealed a unique hexameric quaternary arrangement that has not been seen in other ABM domain proteins. In addition, the structure also revealed a glyoxal-derived post-translational modification (PTM) on arginine 49 (R49), which we hypothesize is acting as a novel glyoxal sensor to regulate the activity and downstream signaling of PA0709. To better understand the precise cellular role of PA0709 in the native pathogen, we created a dual-fluorophore reporter system, where sfGFP is expressed constitutively, and mScarlet-I is driven by the PA0709-gloA2 operon promoter. Using this tool, we found that PA0709 is significantly upregulated in the presence of glyoxal and glycolaldehyde, but not by other RES such as methylglyoxal or reactive oxygen species (ROS). Interestingly, a 37-bp fragment within the promoter was required for glyoxal induction, indicating an unknown transcription factor(s) must be sensing glyoxal and directly activating the PA0709-gloA2 operon through this cis-acting element. Future experiments will exploit our reporter system in the background of a transposon library and FACS to identify the aldehyde-sensing transcription factor(s).

Poster Presentation #115

Uropathogenic Escherichia coli Specific Adaptations by the Anaerobic Global Regulator FNR

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We have deciphered the global role of the transcription factor FNR in uropathogenic Escherichia coli (UPEC) strain CFT073. FNR is a well-conserved bacterial transcription factor that plays a key role in adapting to anaerobiosis by activating expression of most anaerobic respiratory pathways, and repressing expression of several aerobic respiratory enzymes as well as promoting usage of fermentative pathways. From analysis of CFT073 RNA-seq and ChIP-seq data, we found that the FNR regulon is largely conserved between this uropathogen and E. coli K12 strain MG1655. Amongst the differences, we observed the surprising finding that melibiose utilization required FNR in CFT073 and only grew with melibiose as a sole carbon source under anaerobic conditions. The switch to FNR dependence can be explained by the presence of a FNR ChIP-seq peak upstream of melA encoding the α-galactosidase that cleaves melibiose into galactose and glucose. Bioinformatic analysis indicates that the CRP binding site upstream of melA in MG1655 is replaced with an FNR site in CFT073. Since previous studies have shown that galactose metabolism is important for formation of intracellular bacterial communities, rewiring expression of this α-galactosidase to be under FNR control may provide an adaptative advantage for this pathogen. Indeed, bioinformatic analysis of several E. coli strains indicates that the FNR site is conserved in UPEC but not in other pathogenic E. coli.

Keywords: Uropathogenic E. coli (UPEC), CFT073, FNR, pathogenicity

Poster Presentation #116

The Novel Lipoxygenase of Toxoplasma gondii is Essential for Host Immune Response Manipulation and Survival in a Mouse Model

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Toxoplasma gondii is a successful parasite with a wide array of mechanisms that allows it to invade, disseminate and evade the host immune response. From these mechanisms, the role of T. gondii lipoxygenases in the production of eicosanoids to manipulate the host immune response has been understudied. We identified and characterized the role of the novel lipoxygenase-1 (TgME49_315970) from T. gondii, herein called TgLOX1. We successfully designed a knock-out and complement strains and assessed its role in an in vitro and in vivo model of toxoplasmosis. The deletion of TgLOX1 (ΔLOX1) showed no significant changes in tissue culture fibroblast cells; however, it resulted in a strong virulence defect in mice. ΔLOX1 was not lethal to mice during acute infection, even with a dose of 106 parasites. By IVIS, ΔLOX1 parasitemia was reduced by 3 days postinfection and largely cleared by 7 days postinfection. At 3 days postinfection, the cytokines IFNγ, IL-6, MCP-1, and TNF-α were significantly reduced in serum of ΔLOX1-infected mice compared to mice infected with parental or complemented strains. This result promoted us to examine ΔLOX1 in IFNγKO mice. We found that IFNγKO mice infected with ΔLOX1 succumbed to acute infection, suggesting the role of TgLOX1 in mice was IFNγ-dependent. In tissue culture fibroblasts, TgLOX1 was localized to the parasite cytoplasm but in leukocytes of infected mice, TgLOX1 was localized in vesicular structures in the host cytoplasm. We hypothesize that TgLOX1 in these vesicular structures modify the host immune response.

Poster Presentation #117

Caspofungin-Induced Transcription Factor ZfpA Governs Antifungal Response and Susceptibility to Neutrophil Killing of Aspergillus fumigatus

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The efficacy of echinocandin drugs for invasive aspergillosis treatment is marred by the ability of *Aspergillus* to mount compensatory alterations in cell wall integrity and stress response signaling. The transcription factor ZfpA is upregulated in response to the echinocandin caspofungin and regulates features of *A. fumigatus* growth reminiscent of echinocandin tolerance mechanisms, including hyphal branching, septa formation, and cell wall composition. We hypothesize that ZfpA regulation of fungal growth and stress resistance shapes fungal interaction with immune cells and echinocandin tolerance, and therefore represents a valuable target for improving aspergillosis therapies. Our work couples the optical transparency of larval zebrafish with RFP-expressing wildtype, ZfpA deletion (*ΔzfpA*), and overexpression (*OE::zfpA*) strains to track fungal development, immune cell recruitment, and host survival during *A. fumigatus* infection. In a wild-type host, ZfpA deletion does not affect germination or leukocyte recruitment but reduces fungal burden and attenuates virulence. Virulence of *ΔzfpA* is re-established in a neutropenic host, suggesting enhanced susceptibility of *ΔzfpA*to neutrophil killing. ZfpA overexpression has no effect on germination, fungal burden, leukocyte recruitment, or virulence in wild-type or neutropenic hosts. Concomitant with in vitro analyses of antifungal tolerance, caspofungin treatment improves survival of animals infected with wildtype and *ΔzfpA* but has no effect on survival of *OE::zfpA* infected animals, suggesting that ZfpA is a determinant of caspofungin tolerance and resistance to host defenses during *A. fumigatus*infection.

Poster Presentation #118

ABC Importers: The Underestimated Contributors to Uropathogenesis

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The majority of uncomplicated urinary tract infection (UTI) are caused by uropathogenic *Escherichia coli* (UPEC). In the host, bacterial pathogens adapt to acquire nutrients from their milieu using a variety of transporter systems. Correspondingly, ~23% of fitness factors identified in UPEC type strain CFT073 by transposon sequencing were involved in transport. ATP-binding cassette (ABC) transporters had the greatest overall contribution during murine UTI, representing 45%, 34%, and 47% of the fitness factors in the urine, bladder, and kidneys, respectively. Transport systems were selected for further study based on phenotypic differences. Loss of the branched-chain amino acid transport system (*liv*) resulted in decreased bacterial motility *in vitro* and reduced colonization in the urine and kidneys. These data suggest that UPEC scavenge amino acids during UTI to support metabolic processes promoting urovirulence. Several metabolic enzymes in the amino acid-fueled TCA cycle require iron as a co-factor, but the urinary tract is an iron-limited environment. Accordingly, the iron and manganese uptake (*sit*) mutant displayed significant defects in the urine and kidneys of mice yet had enhanced motility *in vitro*. Under chemical iron limitation CFT073 had elevated flagella (*fliC*) promoter activity, a response not observed in commensal *E. coli* K12. qRT-PCR and immunoblot showed increased *fliC* transcript and protein in UPEC strains but not in K12. Furthermore, the addition of exogenous iron was sufficient to re-repress *fliC* expression, indicating an iron-specific response. Additional studies will continue to explore the relationship between the transport of nutrients and the molecular mechanisms driving uropathogenesis.

Poster Presentation #119

Trade-offs between Antiseptic Efficacy and Cytotoxicity in a Human Ex Vivo Wound Contamination Model

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Antiseptic activity of wound cleansing agents intends to prevent contaminating microbes from establishing an infection while also raising concerns of cytotoxicity and delayed wound healing. We evaluated the cytotoxicity of five clinically-used wound cleaning agents (saline, povidone iodine, Dove® and Dial® soaps, and chlorhexidine gluconate *CHG*) using both ex vivo and in vivo human wound models, in contrast to classical in vitro models that lack the structural and compositional heterogeneity of human skin. We further established an ex vivo wound contamination model inoculated with ~100 cells of *Pseudomonas aeruginosa* or *Staphylococcus aureus* to evaluate antimicrobial efficacy. Scanning electron microscopy and confocal microscopy were used to evaluate phenotypic and spatial characteristics of bacterial cells in wound tissue. CHG significantly reduced metabolic activity of the skin explants, while all treatments except saline affected local cellular viability. CHG cytotoxicity persisted and progressed over 14 days, impairing wound healing in vivo. Within the contamination model, CHG treatment resulted in a significant reduction of *P. aeruginosa* wound surface counts at 24 hours post-treatment. However, this effect was transient and serial application of CHG had no effect on both *P. aeruginosa* or *S. aureus* microbial growth. Microscopy revealed that viable cells of *P. aeruginosa* reside deep within wound tissue post-CHG application, likely serving as a reservoir to re-populate the tissue to a high bioburden. We reveal concerning cytotoxicity and limited antimicrobial activity of CHG in human skin using clinically-relevant models, with the ability to resolve spatial localization and temporal dynamics of tissue viability and microbial growth.

Poster Presentation #120

A Portable Method for Quantifying Protein N-terminal Acetylation from Pathogenic Mycobacteria

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Protein N-terminal acetylation (NTA) is a common post-translational modification in eukaryotic and prokaryotic organisms. NTA impacts protein stability, localization, and function. In bacteria, the mechanisms underlying NTA and its consequences are largely unstudied. Our previous work has shown that ~10% of the proteins in mycobacteria are acetylated at their N-terminus and NTA is correlated with virulence in Mycobacterium tuberculosis, the causative agent of human tuberculosis. Yet, the N-acetyltransferases (NATs) responsible for mycobacterial NTA have yet to be identified. Existing mass-spectrometry-based proteomics methods to enrich and quantify protein termini and NTA across different species including mycobacteria detected thousands of protein termini and hundreds of acetylation events. However, these methods are massively time-consuming and present significant challenges in sample throughput. These bottlenecks restrict the number of samples, mutants, and growth conditions that can be tested. Here, we report a new strategy with significant improvements in our sample preparation that also take advantage of enhancements in mass spectrometers. This one-pot procedure enabled us to identify >30-fold more unique N-terminal peptides per unit time when compared to our existing methodology. We demonstrate this new proteomic workflow on gene mutants of a putative NAT we previously identified. Our method identified 838 unique protein N-termini from 3,592 detected proteins using only 3 replicate LC-MS injections of digested M. marinum lysate. These findings will facilitate more rapid and in-depth assessment of acetylated substrates, kinetics, and the role of NTA in bacterial virulence.

Poster Presentation #121

Mechanisms of Formate Binding Proteins

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Shigella is a human pathogen that causes shigellosis. *Shigella* is able to invade the colon epithelium and uses the host's nutrients to replicate within the host cell cytosol. During this time, *Shigella* is able to sense and adapt to the changing conditions in the host cytoplasm. Previous studies concluded that *S. flexneri* uses glycolysis and mixed acid fermentation for metabolism of the host cytosolic carbon. One enzyme essential for these metabolic pathways is pyruvate formate lyase (PFL). PFL plays a role in the conversion of pyruvate to acetyl coenzyme A (CoA) and produces formate as a by-product. Previous research concluded that formate regulates *S. flexneri* pathogenesis, acting as a signaling molecule in the host cell cytoplasm; however, this mechanism is not fully understood. There are no studies that have identified formate binding proteins, but in the closely related bacteria *Escherichia coli*, there is a transcriptional regulator called FhlA that regulates the expression of formate hydrogenlyase complex (FHL) in response to formate. To study formate binding proteins in *S. flexneri*, we used a method called differential radial capillary of action ligand assay (DRaCALA). This is a high throughput assay where a small volume of protein mixed with radiolabeled ligand in a binding buffer is applied to a dry nitrocellulose membrane, then radionuclides bound to the membrane are visualized using a phosphor screen. We performed DRaCLA using 14C-labeled formate. We compared the known formate binding protein FhlA, and FhlA with a E183K mutation, which is predicted to be deficient in formate binding.

Poster Presentation #122

Linoleic Acid Induces Key Developmental Switches in Toxoplasma gondii

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The sexual forms of *Toxoplasma gondii* contribute significantly to *T. gondii* transmission, but sexual stage parasites are difficult to study because they grow exclusively in cats. Our lab and others recently developed tools to grow the pre-sexual and early sexual stages of *T. gondii* in tissue culture. With these tools, we uncovered linoleic acid as a critical factor that triggers *T. gondii* sexual development. However, the molecular mechanism(s) by which linoleic acid promotes sexual development remain unknown. Our preliminary data are consistent with linoleic acid acting via epigenetic and host cell-dependent metabolic pathways to promote parasite life stage switching. We are using genetic and pharmacological techniques to further test this hypothesis. The implications and future directions of our work are to improve non-cat models and to better understand how a cat gut-like environment promotes *T. gondii* sexual development.

Poster Presentation #123

Virulence Regulation of Type 1 Pili by the Phase Variable Invertible Promoter, fimS, in Uropathogenic Escherichia coli

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Urinary tract infections (UTIs), are most commonly caused by uropathogenic Escherichia coli (UPEC), affecting over 150 million people worldwide. Currently, there is a great need for new antibiotic-sparing therapeutics to be developed to address the alarming rise in antibiotic resistance.

UPEC strains carry the highly conserved fim operon that encodes for type 1 pili (T1P), which are critical virulence factors for establishing UTIs by binding to mannosylated proteins in the bladder lumen. The fim operon is regulated by an invertible promoter region, fimS, that is affected by a variety of factors. While the fim operon is highly conserved across UPEC strains, the behavior of the fim operon promoter region, fimS, is highly variable.

While the variability in fimS sequence can affect expression of type 1 pili, mutations and chemical compounds that affect the pilus structure are known to induce fimS to the OFF state. Most notably, small molecules termed mannosides bind to FimH, and and induce the OFF state. Determining the mechanism by which these pilus-dependent changes affect the fimS switch is critical to developing targeted therapies.

Through genome-wide protein occupancy data, the impact of mannosides on global transcriptional regulation can be determined. The type 6 secretion system (T6SS) is one putative novel target, suggesting a link between fim regulation and other virulence programs.

Poster Presentation #124

Vesicles Formation is an Orchestrated Process in the Gut Commensal Bacteroides thetaiotaomicron

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Bacteroides is one of the most prominent genera in the densely-populated human gut microbiota, representing nearly one third of the total relative abundance. *Bacteroides spp*, unlike other bacteria, produce large quantities of uniformly-sized extracellular vesicles that present distinct protein composition when compared to the outer membrane (OM). These *Bacteroides* extracellular vesicles (BEVs) are composed mainly of lipoproteins with lytic activity that harbor a negatively rich amino acid motif, $S(D/E)_3$, adjacent to the cysteine residue required for acylation. BEVs have been proposed to have a profound impact on gut homeostasis, however, little is known about vesicle formation and their physiological role.

In this work visualized BEVs production in *Bacteroides thetaiotaomicron* (*Bt*). We developed chimeric fluorescent markers based on specific proteins enriched or excluded from the BEVs. Widefield fluorescence microscopy of *Bt* co-expressing OM-mCherry and BEVs-GFP markers showed that round-shaped structures of size consistent with vesicles only appear in the BEVs marker channel. Moreover, by employing time-lapse fluorescence microscopy we were able to monitor BEVs biogenesis process in live *Bt* cells. Finally, we determined that protein cargo content in BEVs depends on nutrient conditions, as the protein content packed in BEVs changed based on the specific carbon source available. Altogether, our results indicate that, in *Bt*, BEVs formation is the result of a highly orchestrated process based on protein cargo selection of specific proteins, mainly lipoproteins with conserved amino acid motif, dedicated to the degradation of specific polysaccharides and glycosaminoglycans.

Poster Presentation #125

An In-silico Investigation of Vitamin D3 Dependent IL-12 Modulation of NO Production in Macrophages

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Tuberculosis (TB) is an airborne disease caused by the *Mycobacterium* tuberculosis (Mtb) pathogen. Since 2015, one-third of the world's population has been infected and the disease remains a global health problem. While vitamin D3 (1,25(OH)2 D3) is classically associated with calcium absorption, the risk of Mtb infection and progression of TB disease is elevated in individuals with vitamin D3 deficiency. Our lab previously, explored the mechanisms of vitamin D3 modulation of pro-inflammatory (IL-12, IFN-γ, TNF-α) and anti-inflammatory (IL-10) products in murine macrophages (Gough, et.al.2017). The study found that in the presence of vitamin D3, infected macrophages produced cytokines and reactive nitrogen species in a manner that is infection level dependent. We also observed IL-12 plays a key role in the development of an immune response to intracellular bacterial infections. Expanding on our previous model (Gough & May,2017) the current study uses a systems biology approach to capture the mechanistic effects of vitamin D3 deficiency on IL-12 gene expression and intracellular signaling pathways. By coupling empirical data with computer simulations, we develop an integrated mathematical model to investigate how IL-12 modulates nitric oxide (NO) production in macrophages and whether modulation is via an IFN-γ dependent manner. Our model explores how IL12 can promote the endogenous release of IFN-γ in vitamin D3 conditioned murine cells. Validation of the model would provide a platform to predict the in-vitro response of infected host cells and furthers our understanding of the role of vitamin D3 as regulator of effector molecules and protective agent against host cell damage.

Poster Presentation #126

Histidine kinase BinK is a Key Mediator of Biofilm Signaling during Vibrio fischeri Host Colonization

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The symbiosis between the Hawaiian bobtail squid, *Euprymna scolopes*, and its exclusive light organ symbiont, *Vibrio fischeri*, provides a natural system in which to study host-microbe specificity and gene regulation during the establishment of a mutually beneficial symbiosis. Colonization of the host relies on bacterial biofilm-like aggregation in the squid mucus field. Symbiotic biofilm formation is controlled by a phosphorelay system consisting of regulators RscS-SypF-SypG, which together direct transcription of the symbiosis polysaccharide Syp. Previously, we identified the hybrid histidine kinase BinK as a strong negative regulator of *syp* transcription, and here we further explore BinK function. Using a *sypA'-gfp*+ reporter and conducting colonization assays, we demonstrate that BinK functions to inhibit biofilm gene expression at multiple stages of host colonization. In a Δ*binK* background, the positive regulator RscS is no longer necessary for colonization or the initial aggregation phenotype, providing evidence that BinK senses a signal from the host to regulate proper symbiotic development. To determine how BinK interprets such a signal, we used differential scanning fluorimetry with purified BinK periplasmic/Cache domain, which identified ethanolamine as a putative ligand of BinK. Mutation of Cache domain binding pocket residues render BinK unable to inhibit *syp* transcription both on agar and during colonization. Overall, this study provides evidence for opposing activities of RscS and BinK and suggests that BinK interprets a host signal during the colonization process.

Poster Presentation #127

Metaproteomic Profiles of Translationally Active Bacteria in Human Airway Mucus Samples Enriched by Bioorthogonal Non-canonical Amino Acid Tagging

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Microbiota of the airways of patients with cystic fibrosis (CF) have a complex and diverse ecology as demonstrated via culture-independent studies. However, these studies have yet to provide meaningful insight into the dynamics of chronic respiratory infection and the contributions of specific bacterial community structures to CF disease progression. Though 16S rRNA gene profiling of bacterial communities in sputum has yielded new insights into ecological diversity, conventional approaches suffer from their inability to distinguish between live and dead cells and lack detail regarding *in situ* translational activity. Consequently, critical aspects of bacterial pathogenesis are lost. To overcome this barrier, we apply biorthogonal non-canonical amino acid tagging (BONCAT). This approach renders translationally active bacteria susceptible to labeling by a strain-promoted "click-reaction". We used this approach to demonstrate that 16S amplicon sequencing overrepresents the viable fraction of airway microbiota, of which only ~5-55% are translationally active *in vivo*. Building on these data, we now combine BONCAT with targeted proteomics; BONCAT-labeled translationally active bacterial cells are separated from abundant host-derived biomass in human whole mucus samples, followed by the generation of bacterial proteomic profiles using liquid chromatography tandem mass spectrometry (LC/MS). Preliminary studies using this method have yielded MS analyses indicative of a relatively high degree of fidelity and identification of a relatively high percentage of bacterial proteins involved with central metabolism. Together, BONCAT-based genomic and metaproteomic profiling of microbiota promise to generate insights into polymicrobial community functions *in situ* and their specific contributions to disease.

Poster Presentation #128

Streptococcus agalactiae npx is Required for Survival in Placental Macrophages and Full Virulence in a Model of Ascending Vaginal Infection During Pregnancy

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Streptococcus agalactiae, also known as Group B *Streptococcus* (GBS), is a Gram-positive encapsulated bacterium that colonizes the gastrointestinal tract of 30-50% of humans. GBS causes invasive infection during pregnancy that can lead to chorioamnionitis, funisitis, preterm prelabor rupture of membranes (PPROM), preterm birth, neonatal sepsis, and maternal and fetal demise. Upon infecting the host, GBS encounters sentinel innate immune cells, such as macrophages, within reproductive tissues. Once phagocytosed by macrophages, GBS upregulates expression of the gene, *npx*, which encodes a NADH peroxidase. GBS mutants with a *npx* deletion (Δ*npx*) are exquisitely sensitive to reactive oxygen stress. Furthermore, we have shown that *npx* is required for GBS survival in both THP-1 and placental macrophages. In an in vivo murine model of ascending GBS vaginal infection during pregnancy, *npx* is required for invasion of reproductive tissues and is critical for inducing disease progression including PPROM and preterm birth. Reproductive tissue cytokine production was also significantly diminished in Δ*npx* infected animals compared to those infected with wild type (WT) GBS. Complementation in trans reversed this phenotype, indicating *npx* is critical for GBS survival, initiation of proinflammatory signaling, and disease progression in the gravid host.

Poster Presentation #129

A New Ex Vivo Primary Cell Model Allows for Genetic Dissection of Alveolar Macrophage-specific Components Necessary for Uptake of Mycobacterium tuberculosis

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For unknown reasons, 90% of individuals that are infected with *Mycobacterium tuberculosis* (Mtb) never progress to active Tuberculosis infections and remain latently infected or clear the bacteria. To better understand the determinants of tuberculosis disease fate, we must first understand how the host responds to the bacteria. Studies exploring the pathogenesis of Mtb have largely relied on generalized bone-marrow derived macrophage (BMDM) models, even though these are largely distinct from the tissue-resident alveolar macrophage populations that Mtb primarily resides in during early infection. To address this, we created a fetal-liver derived alveolar macrophage like cell culture system (FLAMs) that employs primary cells to closely mimic alveolar macrophage biology. Importantly, these cells are amenable to Crispr-Cas9 genetic editing, which allows for the dissection of alveolar macrophage-specific immune response pathways. To better understand regulation of the initial events of Mtb infection, we performed a genome-wide forward genetic screen in FLAMs to uncover genes responsible for the uptake of Mtb during early infection. In addition to known endocytic pathways, we identified Surfactant Protein D (Sfpd) as one of the most significant genes necessary to phagocytose Mtb. Sfpd is known to modulate alveolar macrophage immune responses and surfactant uptake but has not been implicated as being directly involved in bacterial uptake and is not involved in uptake of Mtb in BMDMs. These results highlight both the differences between AMs and BMDMs in response to infection, and the utility of the FLAM model to uncover AM-specific immune responses pathways in a previously impossible manner.

Poster Presentation #130

M1/M2 Repolarization in Alveolar-Like Macrophages

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Macrophages are immune cells that elicit variable functions that are dependent on the local inflammatory environment. Within the lungs, alveolar macrophages (AMs) control inflammation to protect respiratory function. Importantly, the activation state of AMs can be polarized/re-polarized based on local signals. However, our understanding of polarization in AMs and its role in lung function remains poor due to a lack of ex vivo models. To overcome this technical hurdle, we developed the fetal liver alveolar-like macrophage (FLAM) model that closely resembles AMs. Previous studies suggest that AMs are metabolically distinct from well characterized bone marrow-derived macrophages (BMDMs), yet these differences in metabolism that impact polarization/re-polarization remain unknown. We hypothesize that these FLAMs will regulate polarization/re-polarization differently from BMDMs because of metabolic differences. To test this hypothesis, we will polarize/re-polarize BMDMs and FLAMs to either pro-inflammatory (M1) or anti-inflammatory (M2) states and quantify changes in phenotypic traits. We will quantify the expression of surface marker proteins using flow cytometry, gene expression using RT-PCR, and metabolism using Seahorse assays for macrophages that are resting, polarized to M1/M2, or re-polarized to M1/M2 following initial polarization. Our preliminary data suggest that FLAMs differentially regulate the surface expression of M2-associated markers compared to BMDMs. We will build upon these observations to dissect unique immune regulatory functions of AMs in the future. These regulatory mechanisms are potential therapeutic targets to protect the lungs against inflammation and infection-mediated damage.

Poster Presentation #131

Transcriptional Control of the Pneumococcal Capsule During Infection

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Streptococcus pneumoniae (the pneumococcus) is the primary cause of bacterial pneumonia in the US and worldwide. Its major virulence factor is its capsule, without which it is unable to cause life-threatening infections. Importantly, to survive in different host environments the capsule has to change size. There are over 100 pneumococcal serotypes that possess unique, and therefore antigenically-distinct capsule matrixes. However, in all but two serotypes, the genes that encode the proteins responsible for capsule synthesis are transcribed as a single operon (cps) and the upstream promoter/regulatory DNA is highly similar. To understand how the cps operon is regulated throughout the colonization and invasive process, we used a biochemical approach to identify transcription factors (TFs) that interact directly with the cps promoter. We have identified a distal, 37 base pair cis-acting element (the 37-CE) that dictates pneumococcal infection through the conserved transcription factors SpxR and CpsR. We identify the DNA binding sites for SpxR and CpsR within the 37-CE and, through additional molecular, structural and in vivo infection approaches, show how these two TFs act to repress capsule synthesis in the airways.

Poster Presentation #132

Lysis of Host Cells Promotes S. aureus Growth in the Airway

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Pneumonia caused by Staphylococcus aureus (S. aureus) has a mortality rate of over 30%, which has improved little despite optimal antibiotic usage. S. aureus is known to bind to, invade, and kill the cells of the airway epithelium, but how these properties are impacted by the tremendous genetic diversity of infecting isolates is not completely clear. To model the interaction between S. aureus and the human airway epithelium, we utilized an infection model of pseudostratified polarized human airway epithelial cultures (HAEC). By comparing S. aureus clinical αsolates from pneumonia patients, we found that isolates that harbor inactivating mutations in the agr virulence regulator are less cytotoxic to HAECs compared to isolates with a functional copy of agr. Unexpectedly, we also observed a positive correlation between cytotoxicity and bacterial growth in this model. The Agr system regulates many critical S. aureus virulence factors, including powerful toxins. To investigate the role of these toxins in our model, we next tested isogenic mutants lacking each toxin regulated by Agr and identified α-toxin as the toxin enhancing cytotoxicity and bacterial growth in the HAEC model. Importantly, we confirmed that presence of α-toxin confers a growth advantage in an in vivo model of murine pneumonia. Altogether, our findings support a model in which S. aureus uses α-toxin to release nutrients that the bacteria can then use to promote growth. Further understanding of the mechanisms by which S. aureus thrives in the human airway will be imperative in understanding how best to treat this deadly disease.

Poster Presentation #133

Imaging-based Screening Identifies EIf3 as a New Anti-virulence Target in C. albicans

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Candida albicans is a fungal pathogen which can cause devastating human disease, especially in immunocompromised individuals. With minimal options for antifungal drugs, coupled with high levels of resistance and host toxicity, candidemia infections can be notoriously difficult to treat. This dilemma demonstrates the need for novel antifungal therapies. Promising targets for these new therapies are virulence factors. An important virulence factor in C. albicans is the ability to transition between two morphological states: yeast and hyphae. Using a high throughput automated microscopy and image analysis pipeline which can distinguish between yeast and hyphae, we screened an FDA drug repurposing library for compounds which inhibit the transition from yeast to hyphae in a dose-responsive manner. We identified 13 novel compounds which displayed efficacy and dose-responsive behavior. Three of these compounds shared a similar chemotype, and we therefore focused on these to determine mechanism of action. We evolved resistance to the three compounds and used whole-genome sequencing and allele swap experiments to identify translation initiation complex EIf3 as a novel regulator of filamentation. Future work will include analysis of additional compounds, including a subset of the DART library of diverse drug-like small molecules, to identify additional pathways for limiting C. albicans virulence factors.

Poster Presentation #134

Identification of Novel Colistin Resistance Genes in an Extremely Colistin Resistant Pseudomonas aeruginosa Clinical Isolate

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Pseudomonas aeruginosa (PA), a gram-negative bacterium and opportunistic pathogen, is a leading cause of multidrug- or extensively drug-resistant (MDR/XDR) infections. Increasing numbers of such infections prompted the re-introduction of antimicrobial agents previously retired due to toxicity. Colistin (polymyxin E), one such sidelined antibiotic, is a cationic antimicrobial peptide often used as a "last resort" for the treatment of several MDR/XDR Gram-negative organisms including PA. Concerningly, there has been a rapid increase in colistin resistant infections following its re-introduction. To date, researchers have characterized a variety of bacterial mechanisms responsible for colistin resistance. Nevertheless, our understanding of colistin resistance remains woefully incomplete. Using a clinical isolate of PA, BWH047, with extensive resistance to colistin (minimum inhibitory concentration (MIC) >1280μg/mL, determined by microbroth dilution), our laboratory generated a saturated transposon insertion library. We performed INSeq analysis and identified 27 candidate conditionally essential genes for BWH047 survival in the presence of ½ MIC colistin. Using allelic exchange cloning, we generated clean deletions of 11 gene candidates and assayed their resistance to colistin. Thus far, we have identified two genes which are necessary for BWH047 resistance to colistin—*wapH* and *dedA*. Clean deletions of these genes render BWH047 susceptible to therapeutic levels of colistin (MIC BWH047Δ*wapH* = 1μg/mL, MIC BWH047Δ*dedA* = 0.5μg/mL). Interestingly, neither gene has previously been implicated in colistin resistance mechanisms in PA. Therefore, we have successfully demonstrated the utility of using INSeq to identify novel genes involved in antibiotic resistance in MDR clinical isolates of PA.

Poster Presentation #135

Determining the Mechanism of Flightless 1 Recruitment to the Chlamydial Inclusion Membrane

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Chlamydia trachomatis is an obligate intracellular pathogen, which undergoes its developmental cycle within a membrane-bound, pathogen specific organelle termed the inclusion. *C. trachomatis* recruits host proteins to the inclusion membrane (IM), and these interactions are often mediated by chlamydial proteins, known as inclusion membrane proteins (Incs). Prior affinity purification mass spectrometry studies identified novel host proteins that localized or were proximal to the IM, including Flightless 1 (FLII). Our recent studies demonstrated that FLII binds in complex with its interacting partner LRRF1 and chlamydial Inc protein Ct226. Notably, FLII localizes to the inclusion in the absence of LRRF1 but cannot independently bind to Ct226. Under simultaneous inducible knockdown of the *ct226*, *ct225*, and *ct224*, FLII and LRRF1 no longer localize to the IM. We hypothesize that FLII recruitment to the IM occurs by two distinct mechanisms via interaction with LRRF1 or an Inc. FLII has several cellular functions both in the host cytosol and within the nucleus, where FLII is a co-activator of nuclear receptor-mediated transcription. Therefore, the mechanism of recruitment to the IM could trigger distinct FLII-signaling events. To determine if sequestration of FLII at the IM alters expression of FLII-coactivated host genes, we will measure select FLII-targeted genes after infection with wild type or mutant (single Inc knockdown, including complements) chlamydial strains in the presence or absence of LRRF1 siRNA. This work provides a more detailed understanding of the mechanism of FLII recruitment to the IM and insights into the resulting biological consequences and contribution to chlamydial pathogenesis.

Poster Presentation #136

A Novel Transcription Factor Linking Mycobacterial Gene Expression to the ESX-1 System

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Bacterial pathogens use protein secretion systems to interface with the host and promote bacterial survival. However, an emerging theme is that protein secretion systems also have fundamental, often overlooked roles in the bacterial cell. The ESX-1 (ESAT-6 system 1) secretion system facilitates escape of mycobacterial pathogens from the macrophage phagosome during infection. We discovered that the ESX-1 system also regulates widespread gene expression in the mycobacterial cell. We have identified several transcription factors that regulate gene expression in response to the ESX-1 secretory components. In this study, we identified a transcription factor, EspN, that is unique from other characterized ESX-1-associated transcription factors. Using genetic and biochemical techniques, we found that EspN works in opposition to the existing network of ESX-1 transcriptional regulators to activate expression of the *whiB6* transcription factor gene, specific ESX-1 substrate genes, and ESX-1 secretory component genes. Our study links, for the first time, transcriptional activation of secretory component genes with transcription of specific substrate genes. EspN is the only ESX-1-responsive transcription factor required for the cytotoxic function of ESX-1 in a macrophage model of infection. Ultimately, EspN appears to serve as an essential node, connecting all parts of the ESX-1 system for optimal regulation.

Poster Presentation #137

Mycobacterium tuberculosis Requires the Outer Membrane Lipid Phthiocerol Dimycocerosate for Starvation-induced Antibiotic Tolerance

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Tolerance of Mycobacterium tuberculosis to antibiotics contributes to the long duration of tuberculosis treatment and to emergence of drug-resistant strains. Nutrient restriction induces M. tuberculosis drug tolerance, but the genetic determinants that promote antibiotic tolerance triggered by nutrient limitation have not been comprehensively identified. Here, we show that M. tuberculosis requires production of the outer membrane lipid phthiocerol dimycocerosate (PDIM) to tolerate antibiotics under nutrient-limited conditions. We developed an arrayed transposon (Tn) mutant library in M. tuberculosis Erdman and used orthogonal pooling and transposon sequencing (Tn-seq) to map the locations of individual mutants in the library. We screened a subset of the library (~1,000 mutants) by Tn-seq and identified 33 and 102 Tn mutants with altered tolerance to antibiotics in stationary phase and phosphate-starved conditions, respectively. Two mutants recovered from the arrayed library, ppgK::Tn and clpS::Tn, showed increased susceptibility to two different drug combinations in both nutrient-limited conditions, but their phenotypes were not complemented by the Tn-disrupted gene. Whole genome sequencing revealed single nucleotide polymorphisms in both the ppgK::Tn and clpS::Tn mutants that prevented PDIM production. Complementation of the clpS::Tn ppsDQ291* mutant with ppsD restored PDIM production and antibiotic tolerance, demonstrating that loss of PDIM sensitized M. tuberculosis to antibiotics. Our data suggest that drugs targeting production of PDIM, a critical M. tuberculosis virulence determinant, have the potential to enhance the efficacy of existing antibiotics, thereby shortening tuberculosis treatment and limiting development of drug resistance.

Poster Presentation #138

CT229 Interacts with SNARE-like Domain Containing Incs

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Vesicle trafficking is a tightly regulated process within the cell, requiring precise coordination of cargo organization and movement. Intracellular pathogens have developed specialized strategies to hijack this network to reroute essential nutrients. One pathogen, Chlamydia trachomatis (C.t), interacts with the host through the highly modified inclusion membrane. Numerous C.t effectors, termed inclusion membrane proteins (Incs), are inserted within the vacuolar membrane to facilitate key interactions with the host, however the function of most Incs remain unknown. One Inc, CT229/CpoS, has been shown to recruit specific Rab coated vesicles to the inclusion membrane and deletion of this effector leads to a significant growth defect. While CT229 is important for Rab-recruitment, other host and bacterial factors are likely required to promote efficient tethering and fusion between the Rab-vesicle and the chlamydia-containing inclusion. Several Inc proteins, including CT229, possess a SNARE-like domain (SLD). Here we sought to determine whether CT229 binds to other SLD-containing Incs to coordinate subversion of host vesicular traffic. We show that several SLD-Incs bind to CT229. We further show that CT229 interacts with the SLD-Inc CT813/InaC, and this interaction is necessary for ARF vesicle recruitment. This data suggests a coordinated organization of Incs within the inclusion membrane. Deciphering how Inc-Inc interactions facilitate vesicle recruitment will provide knowledge of how C.t successfully establishes its intracellular niche and what host factors are required.

Poster Presentation #139

Characterization of Chlamydia trachomatis T3SS Protein TmeA Interaction with N-WASP During Host Cell Invasion

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Chlamydia trachomatis (C.t.) infection causes multiple human diseases, most notably the sexually transmitted disease Chlamydia and the eye infection Trachoma, which is the leading cause of infectious blindness worldwide. Untreated Chlamydia infections can lead to lifelong complications including ectopic pregnancies and increased risk of developing cervical or ovarian cancer. As an obligate intracellular bacterium, entry into host cells is essential for *C.t.* survival and reproduction. Type Ill secretion system (T3SS) effector proteins such as TmeA are critical regulators of host cell invasion, but how TmeA promotes invasion of cells is not fully characterized. We have shown that TmeA directly interacts with the host cell protein N-WASP to mediate invasion of host cells. TmeA's GTPase binding domain ligand motif is necessary for this binding interaction and through deletion of portions of N-WASP's GTPase binding domain we have shown that TmeA acts as a Cdc42 mimic, binding to the Cdc42 binding region. Using scanning and transmission electron microscopy we have identified a connection between TmeA and filopodial-mediated uptake of *C.t.* and are working to identify further host proteins involved in this TmeA-mediated invasion pathway. By elucidating this mechanism of *C.t.* invasion, we will be able to gain new insight into pathogen modulation of the host cell cytoskeleton as well as open the door for potential new targets of treatment or prevention of *C.t.* infections.

Poster Presentation #140

Methods for Analyzing *Candida auris* Colonization on Diverse Surfaces

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Candida auris is an emerging fungal pathogen which causes outbreaks in hospitals and skilled nursing facilities across the globe. *Candida auris* transmissibility and persistence during outbreaks is driven by high adhesion to diverse surfaces, making it difficult to control outbreaks and prevent fomite transmission. To understand the genetic circuitry and regulation of this phenotype, we are studying *Candida auris* adhesion on various surfaces, including clinically relevant surfaces such as stainless steel, gloves, silicone, and glass. There is a lack of protocols and methods to measure adhesion of *Candida* species on surfaces/ materials that do not have optical properties. On many surfaces, techniques that require microscopy cannot be employed. Due to this challenge, we are developing methods and protocols which will aid in reproducible analysis of adhesion to these surfaces. We have developed assays to measure *C. auris* adhesion to plastics, gloves, and glass, and are developing new assays for adhesion to stainless steel and antimicrobially-treated paints. Once we have developed these methods, we will analyze our *Candida auris* insertion mutant libraries to identify the *C. auris* genetic circuitry underlying adhesion to various abiotic surfaces. Identifying specific mediators and regulators of adhesion on different surfaces is important in the search of control measures for *Candida auris* outbreaks.

Poster Presentation #141

Retinoic Acid Control of Coronavirus Infection

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The ongoing Coronavirus Disease 2019 (COVID-19) pandemic has revealed the shortfalls in our understanding of how to treat coronavirus infections. With over 6 million case fatalities of COVID-19 globally, the catalog of FDA approved anti-coronaviral therapeutics is limited when compared to other medication types, such as antibiotics. Because of its immunomodulatory properties, we investigated the efficacy of Retinoic Acid (RA), or activated Vitamin A, against coronavirus infection in a mouse cell model. Tested against a mouse coronavirus, Mouse Hepatitis Virus strain A59 (MHV), we demonstrated that RA treatment significantly decreases viral titers in MHV infected mouse L929 fibroblasts and RAW 264.7 macrophages. The reduced viral titers in RA treated cells were associated with corresponding decreases in MHV Non-structural Protein 9 (NSP-9) and nucleocapsid mRNA expression. qRT-PCR analysis revealed that RA treatment 1hr post-infection significantly increased the mRNA expression of antiviral proteins, namely Interferon induced tetratricopeptide repeat protein 1,2,3 (IFIT1,2,3). Using Interferon Regulatory Factor 3 (IRF3) knockout RAW 264.7 cells, we determined that RA induced increases in IFIT1-3 mRNA expression were independent of IRF3 activation. We propose that RA treatment can control coronavirus infection by inducing the antiviral Type I IFN response and upregulating the production of IFIT1,2,3.

Poster Presentation #142

Influence of Tryptophan Biosynthesis on the Formation of Intracellular Bacterial Communities in Nontypeable Haemophilus Influenzae

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Nontypeable *Haemophilus influenzae* (NTHI) is a commensal organism of the human nasopharynx. However, invasion of the middle ear from the nasopharynx by NTHI gives rise to pathogenesis and is a cause of otitis media, a disease with significant impact on child health worldwide. We previously observed that nutritional sequestration results in NTHI adaptation through microevolution. These adaptations include the development of intracellular bacterial communities (IBCs) and differences in metabolism within biofilms which include changes in tryptophan biosynthetic pathways. We hypothesize that bacterial adaptation to nutrient limitation modulates NTHI tryptophan biosynthesis that directly impacts pathogenesis and persistence. To assess this, we introduced mutations in tryptophan biosynthesis genes that impede the ability of *Haemophilus* to synthesize and utilize tryptophan. We found that the loss of the ability to regulate the synthesis of tryptophan and indole significantly inhibits NTHI IBC formation in normal human bronchial epithelial cells *in vitro*. In addition, we predict that the accumulation of indole results in indole toxicity and killing of NTHI. In a pre-clinical chinchilla model of otitis media, the inability to regulate tryptophan synthesis significantly reduced the ability of NTHI to form IBCs. Taken together, these data support our hypothesis that bacterial adaptation to nutrient sequestration modulates NTHI tryptophan biosynthesis, and so directly impacts persistence of NTHI through the formation of IBCs. The implications of these discoveries will help guide the future developments of therapeutics for treatment of otitis media.

Poster Presentation #143

Iron Starvation of Chlamydia trachomatis Leads to Enhanced TNF-a Production in Epithelial Cells

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As an obligate intracellular pathogen, Chlamydia *trachomatis* relies on the host to acquire nutrients for replication. Host-mediated nutritional deprivation induces C. *trachomatis* to enter a viable but non-replicative growth state known as persistence. It remains unclear whether persistence is a direct consequence of nutritional deprivation or a host cell response to stress. To answer this question, we employed host-pathogen dual RNA-sequencing to characterize persistent chlamydial transcriptome and identify host cell contributions during both iron and tryptophan starvation. Data analyses revealed multiple mechanisms classified into two categories that are non-exclusive of each other – nutrient starvation and immune-mediated. We reported the significance of down-regulation of host GTP biosynthesis on persistence. Here we focus on the exclusive TNF-α production by epithelial cells infected with iron-starved C. *trachomatis*. The significance of TNF-α production is unclear, but previous reports hinted on its anti-chlamydial effect by promoting apoptosis. However, these studies relied on exogenously added TNF-α and cycloheximide inhibition of host protein synthesis. Here, we describe a specific situation, i.e., iron starvation and infection where TNF transcription is induced 1000-fold relative to mock-infection. Transcriptional activation of TNF-α and its production required iron starved C. *trachomatis*, but not the host, chlamydial de novo protein synthesis, and the host NFκB transcription factor. In addition, a correlation between TNF-α production and cell death exists, as indicated by the increased release of the cytosolic enzyme lactate dehydrogenase. In summary, we provide the first report of TNF-α acting in a cell-autonomous manner to induce death of cell harboring iron-starved Chlamydia.

Poster Presentation #144

Inflammatory Responses to Listeria Monocytogenes Infection in the Placenta

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The immune defense of the developing fetus is crucial for a successful pregnancy. The placenta plays vital roles in protecting the semi-allogeneic fetus against rejection by the maternal immune system, while ensuring its defense against most pathogens. The mechanisms that orchestrate the placental immune functions are still poorly understood. In particular, little is known about the antimicrobial functions of placental macrophages called Hofbauer cells, or HBCs. HBCs are M2-polarised macrophages that maintain placental homeostasis and fetal tolerance by multiple mechanisms. They also are the only leukocytes residing in healthy chorionic villi forming a critical immune barrier that protects the fetus from infection. The bacterial pathogen Listeria monocytogenes infects the maternal/fetal unit resulting in very poor pregnancy outcomes. We isolated human primary HBCs from healthy term placentas and analyzed for the first time the L. monocytogenes intracellular lifecycle as well as the HBC responses to L. monocytogenes infection (RNA-seq and cytokine arrays). We found that HBCs are less permissive to infection than expected and mount a pro-inflammatory response to L. monocytogenes. Infected HBCs rapidly repolarize towards a pro-inflammatory M1-like phenotype favoring the innate immune responses. However, consistent with their placental homeostatic functions, repolarized HBCs maintain the expression of tolerogenic factors known to prevent maternal anti-fetal adaptive immunity.

Poster Presentation #145

Limiting pABA Secretion by S. gordonii Enhances P. gingivalis Transcription of Virulence Factors and Proteolytic Proteins In Vivo in a Murine Abscess

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Periodontitis is a disease where specific organisms can have an outsized impact on the trajectory of the pathogenicity of the oral polymicrobial community. Interactions among organisms can enhance colonization and pathogenicity of the community as a whole. However, the molecular mechanisms that underpin oral bacterial interactions remain largely uncharacterized.

In this study, we characterized how Streptococcus gordonii (Sg) modulates the transcription of Porphyromonas gingivalis (Pg) virulence factors in a murine abscess model via secretion of para-aminobenzoate (pABA), a precursor for the synthesis of folate. Using RNA sequencing analysis, we examined the transcriptome of Pg in vivo in several contexts: Pg grown to a mid-logarithmic growth phase inoculated subcutaneously as a monoinfection into the thigh of Balb/c mice, as a co-infection with wildtype (WT) Sg, or as a co-infection with a Sg Δcbe mutant unable to synthesize pABA.

When Pg was co-inoculated with S. gordonii Δcbe, we found increased transcription of genes encoding pathogenic factors such as the gingipains RgpA, RgpB, and Kgp. Additionally, Gene Ontology pathways showed a significant enrichment in Proteolysis (GO:0006508) and in Peptidase Activity (GO:0008233). These Pg proteolytic genes were upregulated by Sg Δcbe relative to Sg WT, and in many cases relative to Pg as a monoinfection. We are further investigating the role of pABA availability on regulating Pg virulence factors via deletion of the pabB and pabC pABA synthetic genes in Pg.

Overall, our work contributes to understanding how limiting pABA, and by extension folate, can modulate the virulence of periodontal microbial communities.

Poster Presentation #146

The Broad-spectrum Metallophore Staphylopine Sensitizes *Staphylococcus aureus* to Copper Poisoning during Infection

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During infection, *Staphylococcus aureus* and other pathogens rely on small molecules, or metallophores, to obtain the essential nutrient zinc. The *cnt* locus of *S. aureus* encodes proteins necessary for the synthesis, export, and import of the metallophore, staphylopine. While essential, transition metals are also toxic in excess, and the host harnesses the toxicity of copper to combat invaders. However, the mechanism by which copper enters bacteria remains largely unknown. Staphylopine and other metallophores can bind and import transition metals other than zinc in conjunction with their transporters. While their broad-spectrum metal-binding ability is generally considered beneficial for bacteria, the current studies tested the hypothesis that it sensitizes bacteria to host-imposed copper toxicity. Growth and elemental analyses showed that the Cnt system increases the susceptibility of *S. aureus* to copper intoxication in culture and drives cellular copper accumulation. Loss of the Cnt system nearly eliminated copper accumulation, implying that this transporter is a significant route of copper entry into *S. aureus*. Relying on the Cnt system leads to increased expression of the copper efflux pump, CopA, even at nanomolar levels of copper, indicating that its use significantly increases the threat posed by copper. A *S. aureus* skin infection model revealed that the use of the Cnt system leads to copper import during infection. Collectively, these observations demonstrate that even though metallophore use is crucial to overcome host-imposed zinc starvation, the limited metal selectivity of metallophores can adversely affect bacteria by importing non-zinc metals like copper.

Poster Presentation #147

Listeria Monocytogenes YvcJ and GlmR Contribute to Muropeptide Synthesis to Facilitate Cell Wall Stress Response

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Pathogenic intracellular bacteria encounter a variety of stresses during infection including in the eukaryotic cytosol. Professional cytosolic pathogens including Listeria monocytogenes, Shigella flexneria and Francisella tularensis, among others, have adapted to survive and replicate in the hostile eukaryotic cytosol, however the mechanisms cytosolic bacteria have evolved to survive in the cytosol are not well understood. Identification and characterization of these adaptations could facilitate the development of novel therapeutics against these pathogens. We utilized Listeria monocytogenes, a model cytosolic pathogen, as a tool to further understand the mechanisms cytosolic bacteria use to survive in the cytosol. Previous studies demonstrated that L. monocytogenes requires GlmR, a conserved accessory uridyltransferase, for resistance to cell wall stress, cytosolic survival inflammasome avoidance and virulence. YvcJ, a conserved protein of unknown function, lies in the same operon as GlmR and we found that similar to GlmR, YvcJ is required for L. monocytogenes cytosolic survival. YvcJ is also required for inflammasome evasion, intracellular growth, and virulence. We demonstrate that ΔyvcJ mutants are susceptible to cell wall stress (lysozyme and β-lactam antibiotics) and untargeted metabolomics demonstrated that ΔyvcJ mutants have increased pools of muropeptide precursors essential for peptidoglycan production, most notably uridine diphosphate N-acetylglucosamine (UDP-GlcNAc). Previous studies in B. subtilis demonstrated a UDP-GlcNAc dependent interaction between YvcJ and GlmR. Ongoing studies are focused on characterizing the interaction between GlmR and YvcJ in L. monocytogenes, how this interaction alters GlmR enzymatic activity and finally how this interaction might impact cell wall stress responses, cytosolic survival and virulence.

Poster Presentation #148

Gut Dysbiosis is an Exacerbating Factor on Pathology during Listeriosis in NHP Model of Pregnancy

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Listeria monocytogenes (Lm) is an enteric bacterial pathogen associated with serious pregnancy complications, including miscarriage, stillbirth, preterm birth, neonatal sepsis, and meningitis. However, very little is known about how the microenvironment within the intestines influences infection outcome. Not only is the intestinal tract home to millions of microflora, but also receptors for sex hormones which are increased during pregnancy. In previous studies, both microbial presence and circulating sex hormones have individually been associated with microbial dysbiosis. Dysbiosis of any origin increases susceptibility to Lm implores understanding of the role of the maternal intestinal microbiome during listeriosis.

METHODS
Due to the severe APOs caused by Lm (Wolfe et al, mBio 2017), non-human primate models are utilized. Cynomolgus macaques (Macaca fascicularis) received either an intragastric dose of Lm or saline control. The inoculated dams were monitored closely by ultrasound, with bacteremia and fecal shedding monitored until collection of tissues. From the collected fecal samples, bacterial DNA was isolated and 16S NGS sequencing and analysis performed.

RESULTS
The non-pregnant cohort displayed no sign of listeriosis. Meanwhile, those pregnant and exposed to Lm displayed fecal shedding, bacteremia, and tissue infection. Disruption to intestinal flora is strongly associated with the pregnant state, rather than infection status. Furthermore, pregnancy and listeriosis appear to have a compounded effect on gut microbial diversity.

CONCLUSIONS
The pregnant state is responsible for increased susceptibility to Lm within the intestinal tract tract. Microbial diversity within the maternal intestines is predisposed for disruption, potentially through increased circulating sex hormones during pregnancy.

Poster Presentation #149

Regulation of Phosphate Homeostasis in *Staphylococcus aureus* Differs from Established Models

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Inorganic phosphate (Pi) is essential for life and regulation of phosphate acquisition and homeostasis is necessary for *Staphylococcus aureus* and other pathogens to cause infection. In *S. aureus*, the PhoPR two-component system controls phosphate homeostasis. Differing from *Escherichia coli*, the paradigm for phosphate homeostasis, *S. aureus* possesses an expanded repertoire of regulatory proteins. In addition to PhoU, a negative regulator of the *E. coli* phosphate responsive two-component system, *S. aureus* possesses two additional PhoU homologs of unknown function: PitR and the PhoU-like domain possessed by the phosphate transporter NptA. As an initial step in determining their contribution to phosphate homeostasis, *ΔpitR*, *ΔphoU*, and *ΔpitRΔphoU* were assessed for growth and PhoPR activity. In Pi-replete medium, both *ΔpitR* and *ΔphoU* grew similar to wildtype *S. aureus*, while *ΔpitRΔphoU* had an extended lag phase. Unlike *E. coli*, loss of PhoU did not alter PhoPR activity in Pi-replete medium. However, both *ΔpitR* and *ΔpitRΔphoU* had elevated PhoPR activity, suggesting that PitR is the primary regulator of PhoPR activity. Constitutive expression of *phoU* does not compensate for loss of PitR, indicating that the two PhoU homologs are not functionally redundant and that the importance of PitR is not a matter of expression. While PitR mutants have elevated PhoPR activity in Pi-replete environments, it does not reach that observed in Pi-deplete medium. This implies that *S. aureus* possess an additional negative regulator of PhoPR. These observations indicate that differing from established models, multiple accessory regulatory proteins control PhoPR activity and phosphate homeostasis in *S. aureus*.

Poster Presentation #150

Deciphering the Role in Virulence of the MARTX Toxin Effector DUF1 of V. vulnificus

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Vibrio vulnificus is a Gram-negative pathogen that causes vibriosis through the consumption of contaminated shellfish, which can lead to fulminant septicemia. The key virulence factor produced by *V. vulnificus* is the Multifunctional autoprocessing repeats-in-toxin (MARTX) toxin, a large, secreted pore-forming protein that translocates multiple effector domains into target cells. These effectors are released as individual proteins that target specific host cell components and influence cell signaling. The effector DUF1 is a domain of unknown function present in many clinically relevant strains, but little is known about its role in disease. In this study, a 3D protein structure model of DUF1 was generated using the AI-driven Alphafold modeling software. The resulting model was used to identify potential ligand-binding pockets and to predict catalytic residues using the P2Rank algorithm. After comparing the highest ranked residues with conserved distant relatives, three were selected as putative catalytic residues. We then generated in situ alanine substitutions of all three residues in the native gene on the *V. vulnificus* chromosome. We predicted these three substitutions would create a strain that produces a toxin with DUF1 catalytically inactive, while all other aspects of the toxin remain unchanged. When tested for virulence using the intragastric route of infection, the DUF1 inactivated strain was more virulent than the wild-type strain, suggesting DUF1 might be an anti-virulence factor that reduces the virulence potential of other effectors. This entails a promising start to the unveiling of DUF1 function in context-dependent virulence and how it contributes to pathogenicity.

Poster Presentation #151

Deletion of Core Septin Genes Result in Fungicidal Activity of Caspofungin

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Septins are a family of eukaryotic GTP-binding proteins. Although they are highly conserved, their functions vary across species. In fungal pathogens, septins are involved in adaptation to anti-cell wall stress. In *A. fumigatus*, the etiological agent of invasive aspergillosis, aspB deletion led to a greater sensitivity to caspofungin, a secondary therapeutic for invasive aspergillosis, through an unknown mechanism. Nonetheless, caspofungin typically has a fungistatic effect toward *A. fumigatus*. We hypothesize that septin deletion results in funcidal effect of caspofungin. To test this, we performed cell viability staining post caspofungin exposure and found that *ΔaspA*, *ΔaspB*, and *ΔaspC* showed significant reduction in cell viability. Deletion strains are more susceptible to drugs on solid media, indicating that the septin cytoskeleton is important for *A. fumigatus* survival in the presence of caspofungin. Based on these results, we are validating hits from a quantitative proteomics experiment to elucidate the mechanism of septin-mediated caspofungin response and determine if septin deletion leads to improved caspofungin treatment using a neutropenic murine model of invasive aspergillosis. Taken together, our preliminary findings indicate that targeting both the septins and the cell wall could improve patient outcome.

Poster Presentation #152

Mobile CRISPRi as an Effective Tool for Interrogating Quorum Sensing in Vibrio

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Historically, quorum sensing research focused heavily on studying bacterial model organisms such as Vibrio campbellii BB120 to ask fundamental questions about signaling mechanisms. However, this particular Vibrio strain is genetically tricky to work with in the lab and is not naturally competent. This creates long and laborious strain construction timelines and delays experiments. As such, this work seeks to circumvent the cumbersome genetics of this organism. Mobile Clustered Regularly Interspaced Palindromic Repeats interference (Mobile CRISPRi) allows for rapid generation of knock down mutants. The CRISPRi machinery genes are delivered via a Tn7-transposon plasmid system, and gene-specific guide RNAs are cloned into this plasmid. Thus, the entire system can be rapidly inserted into Vibrio genomes and, upon induction of the system, targeted to knock down genes by a deactivated Cas9 protein to block transcription. This system allows for the reliable generation of useful mutants far more quickly than standard suicide vector strategies. We test CRISPRi efficacy by knock down of genes with defined phenotypes: bioluminescence, quorum sensing, cell division, and transcription. Our data show that gene knockdowns are indistinguishable from deletions of non-essential genes. We aim to exploit this system by synthesizing libraries of randomized CRISPRi guide RNAs to use in screens. Through these libraries, we will explore the influence of essential genes in quorum sensing signaling and mechanisms of quorum quenching in Vibrio species.

Poster Presentation #153

Burkholderia pseudomallei Binds Factor H via a Surface-Exposed Receptor, Designated Protein 1

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Background
Melioidosis is caused by the encapsulated Gram-negative organism Burkholderia pseudomallei (Bp). Due to its low LD_{50}, high infectivity, and antibiotic resistance, Bp is considered a Tier 1 select agent and there is great interest in characterizing virulence factors that may be targets for novel therapeutic agents. Our lab and others previously demonstrated that evasion of the host complement system is critical for successful Bp infection and persistence.

Hypothesis
We hypothesize that Bp serum resistance is mediated by the binding of complement-regulator Factor H (FH) to prevent optimal complement activation on the bacterial surface and evade critical host immune mechanisms. Identification of receptors responsible for FH binding may uncover novel therapeutic targets to prevent and/or treat melioidosis.

Methods and Results
FH binding was demonstrated using a direct binding assay and bound FH was confirmed to be functionally active via cofactor assay. Candidate FH-binding proteins were identified by both in silico analysis and far-western analysis of Bp membrane proteins following 2D SDS-PAGE with mass spectrometry. Evaluation of candidate FH-binding proteins is described.

Conclusions
Bp-bound FH is functional and contributes to Bp endurance in serum. Expression of a Bp surface receptor, designated Protein 1, confers the ability to bind FH. Protein 1 is highly conserved across numerous important Burkholderia pathogens and thus may serve as a multivalent therapeutic target.

Moving Forward
Evaluation of opsonophagocytic killing of Bp following blockage of FH-binding and computational structural modeling of Protein 1 are underway. Mutation of Protein 1 residues predicted to bind FH is planned.

Poster Presentation #154

Staphylococcus aureus Scavenges Diverse Glutathione Metabolites to Satisfy the Nutrient Sulfur Requirement.

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The opportunistic pathogen Staphylococcus aureus proliferates within numerous mammalian tissues. To propagate throughout these dynamic environments, S. aureus must procure the macronutrient sulfur. Maintaining this essential nutrient requires import of sulfur compounds and subsequent assimilation to cysteine (Cys). Yet, how S. aureus scavenges this element from diverse host metabolites is poorly understood. One sulfur source S. aureus encounters is the tripeptide glutathione (GSH), which consists of gamma-glutamate, cysteine, and glycine. GSH reaches millimolar concentrations within the host and has critical roles in mammalian physiology. For instance, GSH acts as a Cys reservoir due to its gamma-peptide bond covalently linking glutamate and cysteine. The gamma-glutamyl cycle either synthesizes GSH or releases Cys through the intermediate cysteinyl-glycine (CysGly). Additionally, GSH contributes to thiol homeostasis, a process which involves disulfide bond formation. Furthermore, the signaling thiol S-nitroso-glutathione (GSNO) impacts host protein function while S-lactoylglutathione (SLG) results from GSH detoxification of methylglyoxal and formaldehydes. We have demonstrated that S. aureus utilizes the GSH import system (GisABCD), gamma-glutamyl transpeptidase (Ggt), and unidentified factors to utilize GSH as a sulfur source. Herein we describe the ability of S. aureus to catabolize metabolites functionally derived from GSH and expand upon mechanisms engaged to access these nutrients as sulfur sources. Moreover, imaging mass spectrometry has identified a novel sulfur source, cysteine-glutathione disulfide (CSSG) that is available to S. aureus within host kidneys. Collectively, these data advance our understanding of the host-pathogen nutritional sulfur relationship for this persistent threat to global health.

Poster Presentation #155

Lipoteichoic Acid-mediated Ion Homeostasis Facilitates Metabolic Versatility and Antibiotic Resistance in Staphylococcus aureus

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Staphylococcus aureus is a public health threat due to the prevalence of antibiotic resistance and its capacity to infect numerous vertebrate organs. To generate energy needed to proliferate within diverse tissues, S. aureus transitions between aerobic respiration and fermentation. Fermentation results in a distinct colony morphology called the small colony variant (SCV) due to decreased membrane potential and ATP production. These traits promote increased resistance to aminoglycoside antibiotics. Consequently, SCVs are associated with prolonged, recalcitrant infections. We hypothesize that dedicated physiological pathways support fermentative growth of S. aureus that represent potential targets for treatment of resistant infections. Lipoteichoic acid (LTA) is an essential component of the Gram-positive cell envelope that functions to maintain ion homeostasis, resist osmotic stress, and regulate autolytic activity. Previous studies revealed that perturbations to LTA reduces viability of metabolically restricted S. aureus, but the mechanism by which LTA supports S. aureus metabolic versatility is unknown. Though LTA is essential, the enzyme that synthesizes the modified lipid anchor, YpfP, is dispensable. However, ypfP mutants produce altered LTA, leading to elongation of the polymer and decreased cell association. We demonstrate that viability of ypfP mutants is significantly reduced upon environmental and genetic induction of fermentation. Anaerobiosis further decreases the ypfP mutant membrane potential, which can be reversed via cation supplementation. Selecting ypfP mutants exhibiting increased anaerobic viability revealed compensatory mutations in the LTA biosynthetic pathway that restore membrane potential. Overall, these results demonstrate that LTA maintains membrane potential during fermentative proliferation and promotes S. aureus metabolic versatility.

Poster Presentation #156

Co-exposure to Polyethylene Microplastics and Salmonellaenterica Typhimurium in Ceca of Broiler Chickens

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Microplastics (MPs) are persistent pollutants that microbes can form biofilms on. Singularly, MPs have been implicated in negative health impacts following exposure. However, MPs are increasingly being found in locations where there could be an interaction with pathogens such as *Salmonella enterica* Typhimurium. The zoonotic nature of S. Typhimurium as well as its ability to form biofilms on biotic and abiotic surfaces makes it a continued public health risk. This study aims to determine the parameters needed to develop a predictive toxicology model for co-exposure to polyethylene (PE) microplastic and S. Typhimurium to prevent adverse health outcomes. Our preliminary data indicates that PE fiber treatment groups have greater changes to ceca microbial composition in response to this co-exposure. Further experiments with this in vitro gut model will indicate if and how a high concentration of PE microplastics and S. Typhimurium will lead to host gastrointestinal tract cell toxicity. This will give insight into human health implications from consumption of or interaction with chicken contaminated with this co-exposure.

Poster Presentation #157

Characterizing CBASS Phage Defense and Activation of Vibrio cholerae El Tor

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Cholera is a serious threat, causing 1.4 to 4.3 million cases every year worldwide especially in developing countries[2]. Vibrio cholerae El Tor differs from the previous pandemic causing classical biotype through its two pathogenicity islands VSP-1 and VSP-2 that offer an advantage in virulence and its epidemiological success[2]. Transposon mutagenesis was done on VSP-1 which isolated an abortive cyclic-oligonucleotide based antiphage signaling system dubbed CBASS. CBASS is not unique to Vibrio cholerae, with it being a well-conserved system in all bacteria phyla and found near 65.5% of other microbial defense systems[1]. Better understanding of the mechanism and gene relationships may reveal novel bacteria control methods amid rising drug resistant microbes. In addition, as a well-conserved phage defense mechanism, further exploitation of this system may prove imperative in growing areas such as phage therapy. One area of exploitation is the initiation of the defense system to induce cell death. CBASS is activated by activation of its dinucleotide cyclase through folate level changes during phage infection. The proposed activation comes from prior protein work indicating CBASS's dinucleotide cyclase is regulated by folate[6]. Work done, found CBASS defense being specific to phages T2, T4, and T5 all which carry viral gene products for dihydrofolate reductases (DHFR)[3] that manipulates host folate levels. The proposed activation mechanism will be tested through viability assays of phage DHFR expression along with DHFR phage mutant testing and mass spectrometry of folate levels within the cell during infection.

Poster Presentation #158

Organ Agar Reveals Physiologically Relevant Urinary Fitness Factors for Proteus mirabilis

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Proteus mirabilis is a Gram-negative bacterium that causes complicated urinary tract infections (UTI). To facilitate studies into virulence determinants of *P. mirabilis*, we built an ordered transposon library containing 1728 unique gene insertions. Due to concerns about bottleneck effects in our mouse model of UTI for *P. mirabilis*, we devised a method to investigate UTI fitness genes that allowed for screening of individual mutants without pooling. The ordered library was stamped onto agar made from homogenized kidneys or liver pooled from five male Swiss-Webster mice or buffered human urine from healthy volunteers, yielding 48 mutants with reproducible defects from one or more conditions (2.8%). Multiple hits were obtained in genes encoding amino acid and nucleotide metabolism, LPS biosynthesis, and metal uptake. We selected eight genes to make stable targetron mutations for follow-up studies. We assessed the fitness of these mutants using a murine ascending UTI model, where mice were transurethrally inoculated with a 1:1 mixture of wild type and mutant. At 7 days post-inoculation, 7/8 mutants had a significant defect, demonstrating that the organ agar screen led to the identification of physiologically relevant mutants. Six of the eight mutants had profound growth defects in minimal medium, indicating that organ agar is especially useful for identifying nutrient availability at different anatomical sites. Organ agar is relatively simple to make and can reduce the numbers of animals needed for screening, and therefore may be useful for studying bacterial fitness in a broad range of model systems.

Poster Presentation #159

Extended Lifespan and Atypical Death of Human Neutrophils Infected by Helicobacter pylori

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Neutrophils (polymorphonuclear leukocytes, PMN) are extremely short-lived innate immune cells. As first responders to threats, their functions include chemotaxis, phagocytosis, and killing of pathogens. PMN are produced at a rate of ~10^{11} daily in humans and undergo a constitutive apoptosis program ~24 hours after entering circulation. Proper clearance of PMN is necessary to maintain homeostasis and prevent release of their proinflammatory contents.

Helicobacter pylori is a human-specific, Gram-negative bacterium that infects PMN. *H. pylori* colonizes the gastric mucosa of >50% of the world's population and infection is characterized by a PMN-dominant inflammatory response in which gastritis can progress to more severe disease including peptic ulcers or gastric cancer. Infection induces a unique phenotype in PMN characterized by nuclear hypersegmentation, extended lifespan, proinflammatory cytokine secretion, and cytotoxicity. *H. pylori*-infected PMN survive ~2-3 times longer than uninfected cells, and infected PMN do not die via apoptosis as shown by nuclear morphology, Annexin V-FITC/PI flow cytometry, and western blotting of apoptotic proteins. Moreover, approximately 48-72 hours post-infection, robust extracellular *H. pylori* growth surrounds PMN and PMN carcasses. Evidence of PMN membrane permeability assays suggest a lytic form of cell death and infected PMN exhibit an abnormal, lobular extrusion of nuclear content that lacks granule components and differs from NETosis. Furthermore, immunoblotting reveals the selective disappearance of key necroptosis proteins, RIPK-1 and RIPK-3, after ~24-48 hours of infection. We therefore hypothesize H. pylori induces an atypical mechanism of cell death in PMN. Currently, we are analyzing components of other death pathways including pyroptosis.

Poster Presentation #160

The Mutation of the Dlt System Conferring D-alanylation to Teichoic Acids Suppresses the SpeB Null Phenotype of the Δpde2 Mutant of Streptococcus pyogenes

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Deletion of a c-di-AMP phosphodiesterase gene, pde2 in S. pyogenes, severely suppresses the SpeB transcription. Through transposon mutagenesis, we identified dltX as a suppressor of the SpeB null phenotype of the Δpde2 mutant. The dlt operon consists of four to five genes dlt(X)ABCD in most G(+) bacteria and primarily incorporates D alanine into lipoteichoic acid. The in-frame deletion of dltX or insertional inactivation of dltA in the Δpde2 mutant restored SpeB expression. These mutations did not affect the growth in the lab media but showed increased sensitivity to polymyxin B, as previously reported. Since Dlt mutation changes cell surface charge and possibly causes cell-envelope stress, we deleted the gene of the response regulator LiaR in LiaFSR that senses cell envelope stress. The Δpde2 ΔliaR mutant also produced SpeB but less than that of the Δpde2 ΔdltX mutant. qRT PCR showed that the cell wall stressor vancomycin did not significantly change the expression of the LiaFSR-regulated gene, spxA2. SpxA2 might compete with the speB transcriptional activator RopB, but overexpression of ropB did not change the SpeB-phenotype of the Δpde2 mutant either. Our results suggest that the Dlt system and LiaFSR influence SpeB expression in the Δpde2 mutant through two separate pathways; further investigation is required to understand how Pde2 and D-alanylation of teichoic acid are linked to SpeB expression in S. pyogenes.

Poster Presentation #161

A Tale of Two Forms of Glycogen and the Cryptococcal Enzymes that Synthesize Them

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The basidiomycete yeast *Cryptococcus neoformans* (Cn) is a devastating opportunistic pathogen, accounting for 15% of AIDS-associated mortality. In immunocompromised people, Cn can proliferate in the lungs and disseminate to cause meningoencephalitis.

Carbohydrate storage molecules are a conserved strategy to address carbon limitation, which Cn encounters both in the environment and the host. One such molecule, glycogen, is comprised of a glycogenin protein dimer surrounded by α-1,4-linked glucose chains with α-1,6-linked branchpoints. A predicted glycogenin, Glg1, is required for cryptococcal virulence. Deletion of *GLG1* results in a glycogen defect, while its expression in a *S. cerevisiae* (Sc) strain lacking glycogenins rescues glycogen synthesis.

α-1,4-glucan has also been reported to be covalently attached to beta glucans in the cell wall of Sc and Candida. Despite a modest literature on this "insoluble glycogen" dating to 1925, no enzyme(s) have been reported to synthesize it. Our preliminary assays of this material suggest that the cryptococcal cell wall contains insoluble glycogen and that a previously uncharacterized glycosyltransferase, Glycogen Priming Enzyme (Gpe1), may participate in its synthesis. Deletion of *GPE1* results in defects in both soluble glycogen (exacerbated when *GLG1* is also deleted) and insoluble glycogen. Moreover, *ΔGPE1* displays phenotypes related to its cell wall: temperature sensitivity; sensitivity to oxidative/nitrosative stress; and a survival defect inside macrophages. Ongoing experiments will explore the biochemical activity of Gpe1 and the possibility of an interaction between Glg1 and Gpe1. In addition to implicating glycogenin activity in cryptococcal virulence, this work has revealed novel biology of a putative alpha-1,4-glucosyltransferase.

Poster Presentation #162

Salmonella enterica Serovar Typhimurium Chitinases Facilitate Invasion of Small Intestinal IPEC-1 Cells

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Salmonella enterica serovar Typhimurium (STM) causes millions of yearly diarrheal disease cases worldwide. To effectively invade the gastrointestinal tract, STM expresses multiple virulence factors. Two chitinases, STM0018 (ChiA) and STM0233, may act as additional virulence factors. Upregulation of *chiA* was detected during the infection of murine macrophages and the chicken gastrointestinal system. However, the presumable target substrate, chitin, is absent in the mammalian gastrointestinal tract. ChiA has been shown to cleave N-acetyllactosamine (LacNAc) subunits, common components of the mammalian glycome. We thus hypothesized that these STM chitinases contribute to the invasion of intestinal epithelial cells. *In vitro* results using intestinal epithelial cell-line (IPEC-1) cells indicated a significant deficit in the invasion of the double gene-deletion strain (Δ*chiA ΔSTM0233*) compared to WT. Complemented strains demonstrated restored invasion levels. This confirms that lack of chitinases was indeed responsible for the observed invasion defect. STM chitinases were also required for adhesion to IPEC-1 cells. Previous results with an *in vivo* mouse model indicated that chitinases are important for invading small intestinal cells. Presence of WT STM also rescued the chitinase mutant invasion defect, supporting the conclusion that chitinases are secreted. Indeed, when FLAG-tagged, both chitinases were detected in the supernatant. Currently, we are expressing and purifying both chitinases to further study their catalytic activity. Altogether these results demonstrate that the two chitinases produced by STM contribute to pathogenesis by enhancing adhesion to and invasion of small intestinal cells.

Poster Presentation #163

Genetic Redundancy Uncovers a Role for Arginine and Ornithine Catabolism in Acinetobacter baumannii Gut Colonization

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Acinetobacter baumannii is a major healthcare associated pathogen of wide concern due to its multidrug-resistance. The gastrointestinal tract has been proposed as a reservoir for *A. baumanni* to develop multidrug resistance. However, mechanisms governing *A. baumanni* gut colonization remain unclear. Previous studies have shown arginine and ornithine metabolic pathways were critical for host colonization of other gut bacteria. Here, we found that *A. baumannii* utilizes the arginine succinyl transferase (AST) pathway to degrade arginine. Using an ortholog analysis, we uncovered a second, partial duplication of the *ast* operon (*ast2*) in the *A.baumannii* genome. Phylogenetic analysis reveals that an *ast2* locus and an associated regulator are present in two clades of*Acinetobacter*: the pathogen-associated *A. calcoaceticus/baumannii* (ACB) clade and the *A. colistiniresistens* clade containing common vertebrate commensals. Clades of environmental *Acinetobacter* do not encode the *ast2* locus. We further determined that *astA2* is required for ornithine catabolism but is dispensable for arginine catabolism. The mutant strain *astA1*H229A Δ*astA2* lost the ability to replicate with arginine or ornithine as the only carbon source. We tested the hypothesis that *astA* duplication contributes to *Acinetobacter* gut colonization. The mutant strains Δ*astA2* and Δ*astA1*H229A Δ*astA2* had a gut colonization defect in antibiotic-treated mice compared to the WT strain. Thus, ornithine catabolism is critical for *A. baumannii* gut colonization. This work suggests that redundancy in arginine catabolism was selected for by acquisition of ornithine catabolism and is essential for gut colonization of *Acinetobacter*.

Poster Presentation #164

Gut Inflammation Supports Population Expansion of C. jejuni in Ferret Gut

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Campylobacter jejuni is a leading global cause of human gastroenteritis caused by bacteria. Population expansion of C. jejuni during infection is not well understood due to lack of a natural disease model. Recent studies demonstrated that facultative anaerobes in the family Enterobacteriaceae, such as Citrobacter rodentium and Salmonella typhimurium, exploit host factors during inflammation for expansion in the gut. To investigate expansion of C. jejuni population during infection, we infected infant ferrets with 109 CFU/ml of C. jejuni strain 11168. After 72 hours of infection, we observed C. jejuni fecal loads of nearly 1010 CFU/gram with moderate to severe gastroenteritis symptoms. Further, we observed cryptic hyperplasia by induction of a tissue repair system and recruitment of undifferentiated colonocytes in the lamina propria of infected colon tissue after day 3. Differential metabolomics analysis of colonic contents with and without C. jejuni infection by mass spectrometry demonstrated significantly higher lactate levels in infected animals than in uninfected animals. This led us to investigate how elevated lactate influences C. jejuni. A deletion-insertion mutant lacking the lactate transporter (lctP::kan) was attenuated for L-lactate dependent growth in vitro. In ferret infections we observed wild type levels of colonization at 24 hours, but by 72 hours when inflammation was high, the mutant strain was significantly less fit for colonization compared with wild type. These data suggest that gut inflammation and the ensuing increase in lactate levels provide a favorable environment for C. jejuni expansion during the acute stage of infection.

Poster Presentation #165

SarS is a Repressor of Staphylococcus aureus Leukocidins

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Staphylococcus aureus is a successful pathogen that produces a wide range of virulence factors that it uses to subvert and suppress the immune system. These include the bi-component pore-forming leukocidins. How the expression of these toxins is regulated is not completely understood. We performed a screen to identify transcriptional factors involved in the regulation of leukocidin expression. The most prominent discovery from this screen is that SarS, a known transcription factor which had previously been described as a repressor of alpha-toxin expression, was found to be a potent repressor of leukocidins LukED and LukSF-PV. We found that inactivating sarS resulted in increased virulence in both an ex vivo model using primary human neutrophils and an in vivo infection model in mice. Further experimentation revealed that this repression is achieved by means of SarS being an activator of Rot, a critical repressor of toxins, as well as direct repression by binding to leukocidin promoters. By studying contemporary clinical isolates, we identified naturally occurring mutations in the sarS promoter that resulted in overexpression of sarS and increased repression of leukocidins in USA300 bloodstream clinical isolates. Overall, these data establish SarS as a repressor of leukocidins and expanded our understanding of how these virulence factors are being regulated in vitro and in vivo.

Poster Presentation #166

Serotype Diversity in Controlling the Pneumococcal Capsule through a Conserved Cis-acting Element

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Streptococcus pneumoniae is the major cause of bacterial pneumonia worldwide. Its ability to effectively colonize and infect the host is largely dependent on the regulation of the capsule. Between colonization and infection, S. pneumoniae is able to quickly adapt to the changing host environment via regulating the thickness of its polysaccharide capsule. Whereas a thinner capsule is most effective when the pathogen needs to colonize and attach to the surface of the nasopharynx, expansion of the capsule volume has been associated with evasion of opsonophagocytosis in the lung (pneumonia) and especially the blood (sepsis). We have identified a 37-base pair inverted repeat region (37-CE) in the capsule (cps) promoter where two transcription factors, CpsR and SpxR, bind and repress capsule synthesis in the host airways. Point mutations in the 37-CE dramatically change the affinity of SpxR and CpsR to this region, and consequently, affect the size of the capsule and its ability to infect (Glanville et al. under review). Extrapolating from these observations, we hypothesize that small changes within the 37-CE between serotypes correlate with certain serotypes being more or less infectious than others. Across 87 different S. pneumoniae serotypes, we identified 13 unique variations of the 37-CE. Here we demonstrate the variation in how SpxR and CpsR bind to the 13 versions of the 37-CE DNA and discuss how these data may translate into the known differences between serotype colonization and infection.

Poster Presentation #167

Alkyl Quinolone Distribution in Pseudomonas aeruginosa Colony Biofilms

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The bacterium *Pseudomonas aeruginosa* is a leading cause of nosocomial infection. One factor important to *P. aeruginosa* pathogenesis is its production of alkyl quinolones, which includes *Pseudomonas* quinolone signal (PQS), the quorum sensing signal that cues regulation of the PQS system. In addition to quorum sensing, PQS and the other alkyl quinolones constitute a diverse class of molecules produced in response to antibiotics and other stresses. Several studies have shown that alkyl quinolone distribution within surface-growing *P. aeruginosa*, such as biofilms, is not uniform. However, the details and resultant impact of this heterogeneity within biofilms has remained unclear. By applying both mass spectrometry imaging and confocal Raman microscopy we can acquire two-dimensional heatmaps identifying the distribution of PQS, 4-hydroxy-2-heptylquinoline (HHQ), 2-heptyl-4-hydroxyquinoline n-oxide (HQNO) and their nine carbon congeners within a colony biofilm. We then compare these heatmaps to confocal fluorescence microscopy images to pinpoint intersections of these quinolones with microbial behaviors. We found that the alkyl quinolone distribution of congeners shifts with changes in alkyl chain length and that the alkyl quinolones present in a region can shift dramatically over short distances. At the surface/air interface quinolones have been found to become supersaturated. We see these molecules are often identified within chemical aggregates of varied shapes, and that some aggregates were found to be spatially associated with localized cell death. More broadly, we hope to apply this correlated strategy of combining chemical and optical imaging to better discern the function and reach of these heterogeneous alkyl quinolone rich regions.

Poster Presentation #168

Bidirectional Sequestration between a Bacterial Hibernation Factor and a Glutamate Metabolizing Protein

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Bacterial hibernating 100S ribosomes (the 70S dimers) are excluded from translation and are protected from ribonucleolytic degradation, thereby promoting long-term viability and increased regrowth. No extra-ribosomal target of any hibernation factor has been reported. Here, we discovered a previously unrecognized binding partner (YwlG) of hibernation-promoting factor (HPF) in the human pathogen Staphylococcus aureus. YwlG is an uncharacterized virulence factor in S. aureus. We show that the HPF-YwlG interaction is direct, independent of ribosome binding, and functionally linked to cold adaptation and glucose metabolism. Consistent with the distant resemblance of YwlG to the hexameric structures of NAD-specific glutamate dehydrogenases (GDHs), YwlG overexpression can compensate for a loss of cellular GDH activity. The reduced abundance of 100S complexes and the suppression of YwlG-dependent GDH activity provide evidence for a two-way sequestration between YwlG and HPF. These findings reveal an unexpected layer of regulation linking the biogenesis of 100S ribosomes to glutamate metabolism.

Poster Presentation #169

Glycogen Dynamics and Survival of Pathogen-Infected Human Neutrophils

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Neutrophils (polymorphonuclear leukocytes, PMN) are short-lived innate immune cells primarily involved in pathogen clearance by phagocytosis. The ability of metabolism to regulate immune cell function is established, but studies have focused on macrophages and T cells rather than PMNs. The bacterial pathogen *Francisella tularensis* replicates in neutrophils, extending cell lifespan. We demonstrated the ability of *F. tularensis* to delay PMN apoptosis was linked to metabolic reprogramming including upregulation of glycolytic enzymes, enhanced glucose uptake and glycolytic flux, and glycogen storage. Dogma states that neutrophils utilize glycogen only during phagocytosis or when extracellular glucose is depleted. However, we discovered complexities in glycogen dynamics that can be modulated by bacterial pathogens to sustain cell survival. We demonstrated that PMN glycogen levels decline progressively after isolation, reaching a nadir at ~ 12 hours, coinciding with progression to constitutive apoptosis. In contrast, glycogen in *F. tularensis*-infected cells declined during initial hours of infection, then were replenished and declined again after 32-48 hours, in parallel with onset of cell death. Similar data were obtained for neutrophils infected with gastric bacterial pathogen, *Helicobacter pylori*. Based on this, we propose that glycogen depletion may be the signal initiating neutrophil apoptosis. Cell longevity could be recapitulated in absence of infection by treatment with CP-91149 which inhibits glycogen phosphorylase, preventing glycogen breakdown. On-going studies are examining the role of serum and prosurvival factors such as GM-CSF in this process. The results of this study underscore the ability of bacterial pathogens to reveal new aspects of neutrophil biology and function.

Poster Presentation #170

Role of Borrelia burgdorferi Extracellular Matrix Binding Proteins in the Colonization of Mouse Dura Mater

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Borrelia burgdorferi (Bb) is an extracellular pathogen and resides primarily within the extracellular matrix (ECM) and connective tissues during mammalian infection. Bb expresses several outer surface membrane proteins, known as adhesins, which are capable of binding to host ECM components. Adhesins give Bb the capability to disseminate to and colonize distal tissues, including the joints, heart, and brain, leading to conditions such as Lyme arthritis, carditis, and neuroborreliosis, respectively.

Although many factors have been shown to contribute to the pathogenesis of neuroborreliosis, Bb adhesins could play a key role in tissue tropism and dissemination to the dura mater.

We hypothesize that Bb utilizes laminin and decorin binding proteins in the colonization of the dura mater, the outer layer of the meninges. Decorin binding proteins, which are also capable of binding glycosaminoglycans (GAGs), and laminin binding proteins have been shown to influence tissue colonization. However, their effect on the ability of Bb to colonize the dura mater has not been characterized.

Our objective is to test the ability of Bb lacking the outer surface lipoproteins BB0406 or DpbA/B to colonize the dura mater by interaction with laminin or decorin and GAGs, respectively. We confirmed decreased binding of the adhesin mutants as determined by ELISA and fluorescent binding assays and observed a decrease in bacterial burden of the adhesin mutants to mouse dura mater. These results confirm the importance of Bb adhesins in early tissue colonization, but further investigation is needed to understand the biological significance of laminin and or decorin/GAG binding.

Poster Presentation #171

Modulation of the Coagulation Cascade Affects Enterococcus faecalis Colonization in Catheter-associated Urinary Tract Infections

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Bladder catheterization is a common healthcare procedure. Despite its benefits, catheterization mechanically damages bladder epithelium and disrupts bladder immunity. During this response, fibrinogen, a member of the coagulation cascade, is recruited to the inflamed bladder aimed at healing the catheter-induced damage. Typically, fibrinogen is polymerized into fibrin to form meshes, allowing clotting and healing. Once the healing has resolved, plasminogen is polymerized into plasmin to degrade fibrin clots and restore tissue homeostasis. In the catheterized bladder, however, fibrinogen is deposited onto the catheter thereby permitting pathogen adhesion and biofilm formation and ultimately leading to catheter-associated urinary tract infections (CAUTIs). In a mouse model of CAUTIs with Enterococcus faecalis, prevention of fibrinogen deposition on the catheter decreases E. faecalis infections. However, how fibrinogen levels modulate bacterial colonization has not yet been investigated. We hypothesized that reducing factors involved in fibrinogen/fibrin clearance (plasmin presence), should increase E. faecalis colonization To investigate whether fibrinogen/fibrin levels influence CAUTI outcome, we examined E. faecalis CAUTI in C56BL/6 wild-type mice and in mice with mutations targeting the coagulation cascade: I) No fibrinogen/fibrin (fibrinogen-/-); II) Soluble fibrinogen (AEK); III) fibrin accumulation (Pg-/-, PGB, UPA-/-, TPA-/-); and IV) Low levels of fibrin (PAI-/-). Fibrinogen-/- mice showed defective bladder and catheter colonization while accumulation of fibrin further enhanced E. faecalis infection. This data suggests that fibrinogen/fibrin has a critical role in CAUTI pathogenesis and that by decreasing fibrinogen/fibrin abundances in the bladder, we can improve patient outcomes.

Poster Presentation #172

Host-derived Sugars Suppress Klebsiella pneumoniae Hypermucoviscosity without Altering Capsule Abundance or Chain Length

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Hypervirulent *Klebsiella pneumoniae* (hvKP) is an important pathotype of *K. pneumoniae* which is known to cause severe infection in immunocompetent individuals. Hypermucoviscosity is a phenotype commonly associated with hvKP. Hypermucoviscosity has been shown to be a different phenotype than overproduction of capsule (CPS) and is also found to affect pathogenesis differently than CPS. We have previously shown that different growth media can alter hypermucoviscosity, but the regulation of hypermucoviscosity by specific nutrient signals is not well studied. We hypothesized that sugars present in glycans on the surface of host cells could induce changes in *K. pneumoniae* hypermucoviscosity. We cultured *K. pneumoniae* strain KPPR1 in medium supplemented with varying concentrations of sugars (mannose, galactose and rhamnose), then measured hypermucoviscosity and capsule production by low-speed centrifugation assay and uronic acid quantification, respectively. Mannose, galactose and rhamnose were all found to significantly suppress the hypermucoviscosity in a dose-dependent manner. However, CPS production was not affected significantly. Previous work from our lab has shown that point mutations in the Wzc tyrosine kinase, which regulates CPS polymerization, can alter hypermucoviscosity. Therefore, we examined if the sugar-mediated suppression of hypermucoviscosity was due to a change in CPS polymerization. However, we found that sugars did not change the capsular polysaccharide chain length as observed by glycostaining of purified CPS. Taken together, our results implicate that hypermucoviscosity and CPS production can be selectively regulated by host-derived nutrient signals. Further defining how host signals regulate *K. pneumoniae* hypermucoviscosity will reveal how this virulence feature is regulated during infection.

Poster Presentation #173

Wzc Activity is Sufficient to Regulate Mucoidy and Capsule Biosynthesis of rmp-negative Klebsiella pneumoniae Clinical Isolates

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Klebsiella pneumoniae (Kp) causes 23% of all urinary tract infections (UTI), 14% of all surgical-site infections, 12% of all pneumonia cases, and 8% of all bloodstream infections. Two challenging lineages are hypervirulent (hvKp) and carbapenem-resistant (CR-Kp) isolates. hvKp are invasive, produce high capsular polysaccharides (CPS) amounts, and carry the regulator of mucoid phenotype (*rmp*) genes that confer hypermucoviscosity (HMV). CR-Kp are frequently isolated from the urinary tract, complicating UTI treatments. CR-Kp are typically non-mucoid, lack *rmp* genes, and have low CPS production. Both CR-Kp and hvKp have a Wzc tyrosine kinase that regulates CPS assembly. We have recently identified Wzc mutations that significantly increase HMV in an *rmp*-encoding strain without increasing CPS biosynthesis. We hypothesized that the overexpression of Wzc activity can differentially regulate HMV and CPS production of clinical UTI isolates independent of *rmp*. Four clinical UTI isolates were selected that are *rmp*-negative and have significantly lower HMV and CPS biosynthesis compared to wildtype (WT) hvKp. We transformed the four clinical UTI isolates with plasmids over-expressing Wzc WT or six other variants. The six different Wzc variants had an array of effects on HMV and CPS production across the four clinical UTI isolates. Three of the four clinical UTI isolates became HMV upon over-expressing one or more Wzc variants. Further, some Wzc variants impacted CPS biosynthesis, although not always accompanied by increased HMV. Combined, these data demonstrate that Wzc activity can regulate HMV independent of *rmp* and suggest that other unknown genetic factors are involved in regulating Kp HMV.

Poster Presentation #174

Metabolic Genes Dissociate Capsule Production from Mucoidy in Klebsiella pneumoniae

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Klebsiella pneumoniae is one of the leading opportunistic pathogens in nosocomial gram-negative bacteremia, only second to *E. coli*. The two pathotypes of *K. pneumoniae* are identified as either hypervirulent (hvKp) or classical (cKp) *K. pneumoniae*. The hvKp strains are typically seen as hypermucoviscous, which has been linked to an increase in capsular polysaccharides. Traditionally, in *K. pneumoniae* elevated levels of capsule polysaccharides have been associated with an increase in the mucoid phenotype. However, our research has shown that specific nutrients and metabolic genes dissociate the mucoid phenotype from capsule production. Culturing *K. pneumoniae* in M9 with casamino acids increases mucoidy and reduces capsule production, while growth in M9 with glucose suppresses mucoidy and increases capsule production. We hypothesized that the nutrient signals act through our identified metabolic genes to differentiate capsule production from the mucoid phenotype. All metabolic mutants were cultured in either M9 with casamino acids or glucose, then mucoidy was measured with a low-speed centrifugation and capsule was measured based on uronic acid quantification. We identified metabolic genes that conditionally regulate mucoidy and/or capsule in the presence of specific nutrients. Our findings indicate that nutrient signals could lead to alterations of capsular polysaccharides and the mucoid phenotype independently of one another through a signaling pathway involving core metabolic genes. Identifying metabolic genes that dissociate capsule and mucoidy in response to nutrient availability will elucidate pathways that could be potential targets for therapeutic interventions.

Poster Presentation #175

The Phenylacetic Acid Catabolic Pathway Regulates Antibiotic and Oxidative Stress Responses in Acinetobacter

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The opportunistic pathogen Acinetobacter baumannii is responsible for a wide range of infections that are becoming increasingly difficult to treat due to extremely high rates of multidrug resistance. Acinetobacter's pathogenic potential is thought to rely on a "persist and resist" strategy that facilitates its remarkable ability to survive under a variety of harsh conditions. The paa operon is involved in the catabolism of phenylacetic acid (PAA), an intermediate in phenylalanine degradation, and is the most differentially regulated pathway under many environmental conditions. We found that, under subinhibitory concentrations of antibiotics, A. baumannii upregulates expression of the paa operon while simultaneously repressing chaperone-usher Csu pilus expression and biofilm formation. These phenotypes are reverted either by exogenous addition of PAA and its nonmetabolizable derivative 4-fluoro-PAA or by a mutation that blocks PAA degradation. Interference with PAA degradation increases susceptibility to antibiotics and hydrogen peroxide treatment. Transcriptomic and proteomic analyses identified a subset of genes and proteins whose expression is affected by addition of PAA or disruption of the paa pathway. Finally, we demonstrated that blocking PAA catabolism results in attenuated virulence in a murine catheter-associated urinary tract infection (CAUTI) model. We conclude that the paa operon is part of a regulatory network that responds to antibiotic and oxidative stress and is important for virulence. PAA has known regulatory functions in plants, and our experiments suggest that PAA is a cross-kingdom signaling molecule. Interference with this pathway may lead, in the future, to novel therapeutic strategies against A. baumannii infections.

Poster Presentation #176

Aspergillus nidulans Inhibitor of Apoptosis-like Protein, AnBir1, is Essential for Survival and Regulates Fungal Development

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All organisms balance the decision of life or death at the cellular level. Programmed cell death (PCD) is the highly conserved mechanism of coordinated cell death. Across kingdoms, including fungi, PCD governs fundamental processes, including growth, development, stress response, & host-pathogen interactions. Despite its importance, much remains unknown about fungal PCD. Inhibitors of apoptosis proteins (IAPs) are negative regulators of PCD and highly conserved regulatory proteins. IAPs are defined by the presence of N-terminally located Baculovirus IAP Repeat (BIR) domain(s). Although IAPs are conserved in fungi, there is very limited research into the processes that IAPs regulate and the mechanism through which they regulate fungal processes. In this study, we identified an IAP-like protein in the model filamentous fungal organism Aspergillus nidulans (AnBir1) and investigated fungal processes it regulates.

We found that AnBIR1 is an essential gene as AnBIR1 deletion was lethal. Moreover, we found AnBir1 is critical in regulating fungal development. Constitutive expression of AnBIR1 resulted in a strong push towards sexual reproduction with asexual reproduction almost completely lost. We are investigating the role that AnBir1 plays in regulating other processes, including PCD, and the biochemical context within which it operates to regulate fungal processes. Moreover, to capitalize on the finding that IAPs are critical in regulating survival, we evaluated whether pathogenic fungal IAPs can be targeted through RNAi for plant disease management and observed that RNAi-mediated hijacking of fungal PCD could be effective to manage disease.

Poster Presentation #177

Investigating Cytoplasmic Survival of Mycobacterium Marinum in the Host Macrophage

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Mycobacterium tuberculosis remains a serious global health threat today in part because it evades host immune macrophages. A key factor in the success of *M. tuberculosis* and nontuberculous mycobacterial pathogens, such as *Mycobacterium marinum*, is the ESX-1 (ESAT-6 system 1) secretion system. ESX-1 mediates phagosomal lysis and drives mycobacterial entry into the macrophage cytoplasm to promote cell to cell spread. While interactions between *Mycobacterium* and the phagosome are widely studied, there is little known about the mechanisms of mycobacterial survival in the harsh cytoplasmic environment after they escape the phagosome. We used *Mycobacterium marinum* as a model organism for *M. tuberculosis* to develop a system to study cytoplasmic adaptation and survival in host macrophages. We adapted a luciferase-based reporter plasmid used in *L. monocytogenes* to measure bacteriolysis in the macrophage cytoplasm. We infected macrophages with WT and ESX-1-deficient *M. marinum* strains. We found that *M. marinum* exhibits bacteriolysis in the macrophage cytoplasm. As expected, the ESX-1-deficient *M. marinum* strain showed reduced bacteriolysis, consistent with its retention in the phagosome. We are leveraging this reporter plasmid in a transposon screen to identify *M. marinum* genes that are required for cytoplasmic survival. Together, this work will allow us to understand for the first time how mycobacterial pathogens survive in the macrophage cytoplasm.

Poster Presentation #178

Biochemical Characterizations of AvcD Phage-Defense Cytosine Deaminases

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Recently we described the enzyme AvcD (anti-viral cytosine deaminase) and the regulatory sRNA AvcI (AvcD inhibitor) as a toxin-antitoxin system from Vibrio which results in abortive phage infection through the depletion of cytosolic dCTP pools. Cytosine deamination is a conserved anti-viral defense mechanism in eukaryotes and homologs of avcD are found in across prokaryotic and eukaryotic domains of life. AvcD consists of two domains required for deamination activity; a P-loop NTPase and a deoxycytidylate deaminase. Previous attempts to purify active AvcD have been unsuccessful yielding soluble but inactive protein. Utilizing an optimized expression and purification strategy I have now obtained pure AvcD enzymes from multiple species for enzymatic characterization. Using this purified enzyme, I am determining the substrate specificity of AvcD, the function of the P-loop NTPase, and characterizing the mechanism by which the AvcI sRNA inhibits the activity of AvcD. This research will enhance our understanding of this widely conserved bacterial phage defense system.

Poster Presentation #179

AefR, a TetR Transcriptional Repressor, is a Regulator of the Pseudomonas syringae pv. Tomato Strain DC3000 Response to the Plant Hormone Indole-3-acetic Acid

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Indole-3-acetic acid (IAA) is a well characterized plant hormone that is also found in several microbial communities. Many bacteria have been shown to sense IAA to alter metabolism, signal stress, and promote virulence. One such organism, *Pseudomonas syringae* pv. tomato strain DC3000 (PtoDC3000), is a gram negative hemi biotrophic extracellular pathogen that infects tomato, *Arabidopsis*, and other brassicas. IAA has been shown by transcriptional profiling to regulate many PtoDC3000 genes, including several known virulence genes. How PtoDC3000 senses IAA to coordinate a response to alter its own biology remains unknown.

To investigate potential IAA sensing mechanisms, we carried out a genetic screen for mutants with altered responses to IAA. One group of mutants of particular interest had disruptions in the *aefR* gene encoding a TetR family regulator. Transcriptional analysis confirmed that the *aefR* mutant has an altered response to IAA. We also investigated which aspects of biology are regulated by AefR and IAA, including epiphytic fitness, antibiotic resistance, and motility. Examining these facets of biology in the PtoDC3000 *aefR* mutant confirmed that AefR regulates a subset of auxin-responsive genes and physiological responses. The PtoDC3000 *aefR* mutant also has altered virulence in *Arabidopsis*, suggesting that the sector of the IAA response regulated by AefR is important during pathogenesis. Overall, our data provide evidence that supports the model of AefR working as a "switch" facilitate entry and the transition from epiphytic to endophytic growth.

Poster Presentation #180

Using a Putative Serine Recombinase as a Proxy for Identifying Novel Antiphage Defense Systems in Diverse Bacterial Hosts

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In the era of increasing antibiotic resistance and decreasing discovery of novel antibiotics, we must turn our attention to alternative mechanisms of fighting bacterial infections. Multi-drug resistant (MDR) enterococci are members of the normal gut microbiome but cause significant human disease following antibiotic-mediated dysbiosis such as bacteremia and endocarditis. While it is accepted that lytic bacteriophage (phage) serve as a potential therapeutic for MDR enterococci, the mechanisms that MDR bacteria use to restrict phage infection are largely understudied. Through phage-resistance experiments, we discovered a novel antiphage defense system (EF_B0059) on a mobilizable plasmid in Enterococcus faecalis. This defense system was found to be co-transcribed with a site-specific serine recombinase (EF_B0058). A bioinformatic search for homologues of this recombinase in other *Enterococcus* species and domain analysis of the resulting loci revealed conservation of antiphage defense systems in surrounding EF_B0058 homologues. Phylogenetic analysis reveals differential relatedness between EF_B0058 homologues and their surrounding loci in Enterococcus, suggesting mobilization of elements independent of the evolution of the recombinase itself. The bioinformatics search for homologues of EF_B0058 was then performed on the phylum of firmicutes as a whole. This phylum contains many bacteria of utmost importance to human and ecological health, including *Clostridium*, *Listeria*, and *Staphylococcus*. Following identification of putative antiphage defense systems from this search, I aim to offer preliminary characterization of these systems using in vitro studies.

Poster Presentation #181

Hybrid Histidine Kinase SypF Confers Strain-Specific Host Colonization in Vibrio fischeri

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Host specificity of many animal-microbe interactions relies on bacterial biofilm production. *Vibrio fischeri*, the mutualistic symbiont of bobtail squids, produces a symbiosis polysaccharide (Syp) that is required for colonization of the juvenile squid light organ. In the canonical strain ES114, activation of the *syp* locus, and host colonization, requires the hybrid sensor kinase RscS. Mediterranean squid isolates SR5 and SA1 lack RscS but maintain the ability to colonize squid. These strains require Syp production for squid colonization like other *V. fischeri* strains, yet how *syp* is regulated in the absence of RscS remains unclear. To determine if native differences in the *syp* regulators between ES114 and SR5 allows colonization of the squid in the absence of RscS, I exchanged the *sypEFG* alleles between the two strains. With the ES114 alleles, SR5 shows a significant reduction in its ability to colonize. Furthermore, the SR5 alleles of *sypEFG* fully rescues colonization of an ES114 Δ*rscS* strain. Therefore, strain-specific differences in the encoded regulators are necessary and sufficient to enable squid colonization in the absence of RscS. When tested individually, only the ES114 allele of *sypF* significantly reduced colonization. SypF is a hybrid sensor kinase, and this result suggests that in the absence of RscS, allele specificity of SypF plays a vital role in the regulation of Syp and subsequent host colonization. Future work will determine how the evolution of SypF impacted strain-specific biofilm production and host colonization.

Poster Presentation #182

Proteo-genetic Analysis Reveals Clear Hierarchy of ESX-1 Secretion in Mycobacterium marinum

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Pathogenic mycobacteria, including *Mycobacterium tuberculosis*, require the ESX-1 secretion system for survival within the host. ESX-1 actively transports protein substrates that are required for lysing the phagosomal membrane. ESX-1-dependent phagosomal lysis is essential for bacterial survival; mycobacteria lacking an ESX-1 system are retained in the phagosome and attenuated. Using *Mycobacterium marinum*, an established model of *M. tuberculosis*, we sought to characterize the role of these substrates in ESX-1 secretion and virulence. We generated a collection of *M. marinum* strains with single deletions of all known ESX-1 substrate genes, along with the relevant complementation strains. We demonstrated that the loss of each individual substrate has differential impacts on ESX-1 function as measured by virulence in a macrophage model of infection and hemolytic activity. We quantified changes to the secreted proteome in each substrate deletion strain as compared to the WT and corresponding complementation strain. We found that each protein substrate differentially impacts the secreted proteome of *M. marinum*. We used statistical analyses to identify distinct substrate groups that had similar secretion profiles, potentially indicating shared or redundant function. We found that ESX-1 substrates are hierarchically secreted, providing a testable model in which ESX-1 substrates make up part of the secretion machinery. Lastly, we generated a collection of *M. marinum* strains with double deletions of ESX-1 substrate genes and identified genetic interactions among the distinct substrate groups. Overall, our study provides a comprehensive understanding of how ESX-1 substrates differentially impact ESX-1 function and secretion in *M. marinum*.

Poster Presentation #183

Cross-kingdom Interaction with Candida albicans Promotes Gut Colonization and Pathogenesis of Salmonella Typhimurium

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Salmonella enterica serovar Typhimurium (STm) infects over 1.35 million people in the United States each year, causing acute intestinal inflammation. The pathogenesis of STm is influenced by host immune status, gut microbiome composition, and co-infections with viruses, parasites, and pathogenic bacteria. Recently, a clinical study described the increased recurrence of *S*. Typhi and *S*. Paratyphi infection when patients were colonized with *Candida albicans*. *C. albicans* is a commensal yeast that colonizes the gut of up to 60% of the humans. STm and *C. albicans* both thrive in an inflamed intestine and are likely to co-occur frequently. Nevertheless, the role of *C. albicans* during STm infection is currently unknown. We found that during co-infection with STm and *C. albicans*, mice showed significantly increased STm colonization in the cecum, spleen, and liver, and higher weight loss compared to STm infection alone. We hypothesize that direct bacteria-fungi and host-dependent interactions result in the increased STm virulence. In support of direct interaction, we show that presence of live *C. albicans* increased STm invasion of colonic epithelial cells in vitro. The host immune response was also altered during co-infection. Phagocytosis of STm by macrophages was drastically increased in the presence of *C. albicans*. In mice, expression of inflammatory genes was significantly reduced during co-infection and resulted in reduced neutrophil recruitment. The lower inflammatory response might lead to inefficient clearance and increased dissemination of STm. This study thus addresses the significant lack of knowledge on an important cross-kingdom interaction between gut commensal fungi and STm.

Poster Presentation #184

Francisella tularensis-infected Neutrophils as Trojan Horses for Infection and Reprogramming of Macrophages

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Francisella tularensis (Ft), a Gram-negative bacterial pathogen that infects macrophages and neutrophils and causes the potentially fatal disease tularemia. Previous studies by our group and others demonstrate that Ft inhibits the respiratory burst, escapes the phagosome, replicates in the cytosol, and prolongs human neutrophil lifespan. However, the fate of infected neutrophils and their bacterial cargo are unknown. Herein we report the unexpected finding that Ft-infected neutrophils interacted more efficiently with macrophages than the aged, control PMNs despite low surface PS. Moreover, we show that infected neutrophils were internalized and functioned as Trojan Horses that supported indirect infection of macrophages. Bacterial escape from efferosomes into macrophage cytosol was apparent by 6 hours and the released bacteria replicated avidly thereafter. Typically, ingestion of apoptotic PMNs switches macrophages from a classical (M1) activation state to a state that favors resolution of inflammation and tissue repair, but effects of Ft on this paradigm are poorly defined. We show here that human macrophages remained unpolarized (M0) upon direct infection with Ft. However, if these cells were M1-polarized by pretreatment with IFNγ and LPS, subsequent uptake of Ft-infected neutrophils elicited more efficient reprogramming to an M2 phenotype than the aged control PMNs as judged by downregulation of CD80. Further studies of PMN-macrophages interactions and activation state are underway.

Poster Presentation #185

Coxiella burnetii Manipulates Host MiT-TFE Proteins to Influence Lysosome Biogenesis and Promote Infection

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Coxiella burnetii is a highly infectious, gram-negative obligate intracellular bacterium and the causative agent of human Q fever. The Coxiella Containing Vacuole (CCV) is a modified phagolysosome which forms through fusion with host endosomes and lysosomes. While an initial acidic pH<4.7 is essential to activate *C. burnetii* metabolic activity, the mature, growth permissive CCV is pH ~5.2. Additionally, further acidifying the CCV to a lysosomal pH (~4.7) causes degradation of *C. burnetii*, suggesting that regulation of CCV pH is important for *C. burnetii* to maintain an infection. We recently demonstrated that *C. burnetii* inhibits host cell lysosome biogenesis by halting the acidification of endosomes into lysosomes. Lysosome biogenesis is primarily controlled by the host cell Transcription Factor EB (TFEB). TFEB is a member of the MiT/TFE family, a highly conserved group of proteins defined by basic helix-loop-helix (bHLH) structure and includes MITF, TFE3, and TFEC along with TFEB. Utilizing knock out cell lines as well as protein overexpression, we determined that TFEB restricts CCV size and *C. burnetii* growth. Further, through the Type IV Secretion System, *C. burnetii* actively blocks nuclear translocation of TFEB, thus decreasing lysosomal biogenesis. Intriguingly, while structurally similar to TFEB, TFE3 appears to promote *C. burnetii* infection based on reduced growth and CCV size in TFE3KO cells. These results suggest that to avoid further acidification through heterotypic fusion between the CCV and lysosomes, *C. burnetii* inhibits TFEB nuclear translocation to limit lysosomal biogenesis.

Poster Presentation #186

Functional Genomics Reveals Novel Pathways Underlying Pseudomonas Aeruginosa Persistence in Human Plasma

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The opportunistic pathogen *Pseudomonas aeruginosa* is a leading cause of bacteremia worldwide. *P. aeruginosa* can persist in blood and plasma through the formation of a tiny persister-like sub-population of evaders. The molecular mechanisms underlying the evaders formation are largely unknown. Using a gain-of-function screen, among other factors, ATP and biotin availability greatly influenced bacterial persistence in plasma; mutants in the purine and biotin pathways showed reduced survival in plasma. Electron microscopy combined with energy-dispersive X-ray spectroscopy (EDX) on different clinical strains revealed the appearance of polyphosphate granules upon incubation in plasma and transposon mutants with insertion in polyphosphate kinases genes had an increased survival to plasma, showing the implication of bacterial response to a stressful environment in the defense against the complement system. Assessing several steps of the complement cascade and responses to an outer-membrane impermeable drug, nisin, we demonstrated an altered membrane attack complex (MAC) activity *per se*. Through this work, we identified novel mechanisms underlying *P. aeruginosa* resilience to plasma and shed light onto the complexity of the interplay between *P. aeruginosa* and the complement system.

Poster Presentation #187

Diversity of LPXTG Surface Virulence Factors of Aerococcus urinae Subspecies

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Among the members within the *Aerococcus* genus, only the species, *Aerococcus urinae*, is associated with lethal disease in humans. An uncommon inhabitant of the human urogenital tract, the Gram-positive bacterium has been implicated in a range of urinary tract diseases that, if left untreated or undiagnosed, can progress to severe disease, such as infective endocarditis. It is hypothesized that adherence plays a major role in colonization and infection. Comparative analysis of 114 *A. urinae* isolates from infection episodes of urinary tract infection, bacteremia, infective endocarditis, overactive bladder, urgency urinary incontinence, stress urinary incontinence, and asymptomatic patients reveal substantial diversity with respect to predicted virulence factors and observed biofilm behavior. No capsule has been observed in any subspecies of *A. urinae*, which suggests that adherent behaviors are conferred via surface proteins. As such, this study analyzes predicted LPXTG surface proteins encoded by each of the *A. urinae* subspecies. Between 11 – 20 LPXTG proteins are encoded within each isolate with variants cladding by subspecies. Most striking is the enormous size of these proteins, some reaching as large as 3500 amino acids in length. One type of LPXTG proteins has been identified as a serine rich repeat family protein whose members are heavily glycosylated and secreted via a non-canonical accessory secretory pathway. These proteins consist of up to 50% serine residues and can be easily identified via a surface trafficking signal peptide containing a KMYKAGKSW motif. Ongoing studies seek to find these proteins' binding substrates and their contribution towards host adherence.

Poster Presentation #188

Estrogen Signaling Contributes to Group B Streptococcal Disruption and Invasion of Brain Endothelial Cells

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Bacterial meningitis is a serious infection of the central nervous system (CNS) that occurs when blood-borne pathogens can disrupt the blood-brain barrier (BBB) and enter the CNS. The BBB is comprised of specialized brain endothelial cells (BEC) that serve to protect the CNS from toxins and pathogens while supporting brain function. Group B Streptococcus (GBS) is the leading cause of neonatal meningitis and mechanisms of how the BBB fails to protect the CNS during infection remain unclear. We have conducted microRNAseq on BECs either infected with GBS or mock infected and strikingly we found that globally microRNAs are downregulated. Estrogen signaling has been demonstrated to contribute to global microRNA downregulation and we hypothesize that estrogen signaling may play a role in GBS – BEC disruption. Our preliminary findings demonstrate that treatment of BECs with the estrogen receptor (ER) antagonist is sufficient to inhibit GBS invasion of BECs and rescue candidate microRNA expression. We find that the ER agonist beta-estradiol is sufficient to reduce microRNA expression, reduce trans-endothelial electrical resistance, and increase rates of bacterial invasion. Our findings suggest that GBS may utilize estrogen signaling to gain access to the CNS and cause bacterial meningitis. These results are supported by population-based observations that mothers on perinatal hormones have higher rates of neonates that experience invasive GBS disease. Future work will elucidate mechanisms of global microRNA failure and determine if rescue of microRNAs can restore BBB function during GBS infection.

Poster Presentation #189

Haemophilus ducreyi Infection Induces Oxidative Stress, Central Metabolic Changes, and a Mixed Pro- and Anti-inflammatory Environment in the Human Host

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Few studies have investigated the immune response to a bacterial pathogen in humans. *Haemophilus ducreyi* is the causative agent of cutaneous ulcers that occur primarily on the lower limbs of children and of the genital ulcer disease chancroid, which is a sexually transmitted infection in adults. We developed a human challenge model in which healthy adult volunteers are infected with *H. ducreyi* on the upper arm until they develop pustules and defined host pathogen interactions on a cellular level. Here we sought to characterize host pathogen interactions in pustules on the molecular level using transcriptomics and metabolomics. In a previous pilot study, we determined the human and *H. ducreyi* transcriptomes and the metabolome of pustule and wounded sites of volunteers. While we could form provisional transcriptional networks between the host and H. ducreyi, the study was too underpowered to integrate the metabolome with the host transcriptome. We used samples from both the pilot study (n=4) and the new volunteers (n=8) to identify 5,495 human differentially expressed genes (DEGs), 123 H. ducreyi DEGs, 205 differentially abundant positive ions, and 198 differentially abundant negative ions. By increasing our sample size, we could form 42 positively correlated and 29 negatively correlated human-*H. ducreyi* transcriptome clusters in addition to human transcriptome-metabolome networks consisting of 9 total clusters, which highlighted changes in fatty acid metabolism and countering of oxidative damage. Taken together, the data suggests a predominantly anti-inflammatory environment and rewired central metabolism in the host and a hostile, nutrient limited environment for *H. ducreyi*.

Poster Presentation #190

Unraveling the Secrets of Cell Killing by the Staphylococcus aureus Toxin LukAB

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Staphylococcus aureus (*S. aureus*) is a serious global human pathogen, whose success can be partly attributed to a family of virulence factors known as the bi-component leukocidins. These pore-forming toxins target and kill phagocytes, promoting pathogenesis by allowing *S. aureus* to evade and dampen host immune defenses. Among these leukocidins is LukAB, which is a critical virulence factor in *ex vivo* and *in vivo* infection models. To function, this heterodimeric toxin utilizes two host receptors: CD11b of the Mac-1 complex, and the proton channel HVCN1. Following receptor engagement and oligomerization, the stem domain, which forms the beta-barrel pore, unfolds to embed the octameric pore into the host cell membrane. While both receptors are essential for the cytotoxicity of most LukAB natural variants, it is unknown what role each receptor plays in the pore-forming mechanism. To begin answering this question, we performed a comprehensive alanine scanning screen with the goal of identifying LukAB variants that can forgo their dependency on CD11b. Utilizing this approach, we identified a series of mutants that allow for CD11b-independent cytotoxicity. Remarkably, all the amino acid mutations clustered at the interface where the stem domain is folded within the core of the soluble dimer. Follow-up crosslinking and negative stain electron microscopy experiments lead to a model in which mutations in this region of the stem domain trigger a conformational change that promotes toxin oligomerization. Further understanding the mechanism of how this potent toxin targets and kills host cells will allow us to develop novel anti-leukocidin therapeutics.

Poster Presentation #191

Staphylococcus aureus TcyA Scavenges Host Cysteine and Methionine Synthesis Intermediates to Satisfy the Requirement for Nutrient Sulfur

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Staphylococcus aureus is an opportunistic pathogen known to cause numerous diseases. To proliferate within host tissues, S. aureus must acquire the essential nutrient sulfur. Previous work demonstrated that *S. aureus* targets cysteine and its analogues, cystine, N-acetylcysteine, and homocysteine using the ABC-transporter TcyABC and TcyP, a high-affinity sodium symporter. A systemic murine infection model established that these transporters promote fitness during host colonization. Given the dynamic range of cysteine-like metabolites that TcyP and TcyABC likely bind, we hypothesized that the cysteine and methionine synthesis intermediates cystathionine and lanthionine are also viable sources of nutrient sulfur for *S. aureus*. Cystathionine is present within numerous human tissues including the brain, heart, and lungs while lanthionine is found in urine. Lanthionine is commonly present as lanthionine ketimine in mammals but its role in the synthesis of cysteine remains elusive. Using a chemically defined medium, we demonstrate that *S. aureus* utilizes cystathionine and lanthionine as sources of nutrient sulfur in vitro. Furthermore, TcyABC, but not TcyP, is required for proliferation on both sulfur sources. Notably, the tcyA transposon mutant displays reduced proliferation in cystathionine- or lanthionine-supplemented medium but a tcyBC mutant exhibits wild-type like growth. These findings support a model whereby the solute binding protein, TcyA, functions in concert with another permease to procure the metabolites. Overall, this work expands the number of metabolites present in host tissues that *S. aureus* is capable of scavenging to fulfill the sulfur requirement, increasing our understanding of nutrient reservoirs this pathogen and likely others exploit during infection.

Poster Presentation #192

Understanding Polymicrobial Interactions in Catheterized Bladders

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Catheter-associated urinary tract infections (CAUTIs) are one of the most prevalent hospital-acquired infections. Unlike uncomplicated UTI, 86% of CAUTIs involve multiple pathogens designating them as polymicrobial infections. These pathogens can form biofilms and interact with each other, potentially protecting microbes from antibiotics and further promote the rise of antibiotic resistance. Studies have shown different types of interactions between these pathogens *in vitro* and *in vivo* but their interactions in the catheterized bladder environment are not well-understood. In this study, we focus on the interactions between the three most common CAUTI pathogens: *Enterococcus faecalis*, *Escherichia coli*, and *Candida albicans*.

In these experiments, we study *E. faecalis*' role in polymicrobial interactions with *E. coli* and *C. albicans*. These microbial combinations were tested *in vitro* (planktonic cultures and biofilms). Our preliminary data shows defective growth of *E. faecalis in vitro* when grown with *E. coli*, both in planktonic cultures and biofilms, suggesting a form of inhibition by *E. coli*. Our data also suggests that the inhibition occurs through a contact-dependent manner. However, *in vivo* no competition was observed between them 24h post infection (hpi) and a decrease in *E. coli* bacterial titer at 72 (hpi). When grown *in vitro* with *E. faecalis*, *C. albicans* showed a slight decrease in growth, whereas *E. faecalis* was not affected. In conclusion, different types of interactions between pathogens exist in environments with limited nutrients and further study needs to be done to determine their interactions on catheterized bladders.

Poster Presentation #193

Identification of Functional Receptors in the SmcR Quorum Sensing Circuit of Vibrio vulnificus

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Bacterial quorum sensing (QS) is a cell-cell communication based on local population density, and typically regulating specific group behaviors. QS is mediated by small molecules excreted by the bacteria, called auto-inducers (AIs). These are recognized by specific sensors either in the bacterial inner membrane or in the cytoplasm. Vibrio species are model organisms for the study of QS systems. However, the QS circuit of Vibrio vulnificus is relatively unexplored, the only confirmed AI for the species being AI-2, sensed by the LuxPQ histidine kinase complex. At low cell density, this signal receptor functions to activate the key regulator LuxO, which leads to the repression of global regulator SmcR. At high cell density, binding of AI-2 to the LuxPQ receptor leads to deactivation of LuxO and the eventual de-repression of SmcR. However, neither deletion of AI-2 (luxS) nor luxPQ is sufficient to abolish LuxO activation and the SmcR dependent phenotype. This suggests that LuxO is activated by additional, unidentified signaling pathways. A bioinformatic analysis of the known QS genes of other Vibrio species predicts the presence of three additional histidine kinases sensors for V. vulnificus; CqsR (AOT11_19315), VpsS (AOT11_21625), and H-nox/HqsK (AOT11_19760/AOT11_19765). We propose that these, in conjunction with LuxPQ, make up the receptors of the QS circuit of V. vulnificus acting through the phosphotransfer protein LuxU, to activate LuxO. We also speculate that the four receptors may be partially functionally redundant to the QS circuit, buffering against major signal perturbation from any one receptor.

Poster Presentation #194

Borrelia burgdorferi Colonization of Cerebrospinal Fluid and Associated Immune Responses in a Tractable Murine Model of Lyme Neuroborreliosis

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Lyme Neuroborreliosis (LNB) occurs in 10–15% of Lyme disease cases. In order to characterize a tractable animal model of LNB, we previously demonstrated inflammatory markers and leukocyte infiltration associated with vascular damage in the meninges and ventricles of *Borrelia burgdorferi* infected mice. In the current study, we set out to characterize the kinetics of *B. burgdorferi* colonization and associated immune responses in CSF of these animals.

Borrelia burgdorferi was readily cultured from murine CSF, with higher sensitivity than from blood or surrounding tissues/fluids. Additionally, *B. burgdorferi* was directly visualized by darkfield microscopy without the need for culture in CSF samples from mice challenged with as little as 100 spirochetes, whereas spirochetes were not seen in equivalent volumes of plasma, serum, or blood with inoculation doses up to 1X106 bacteria. Although no cytokines/chemokines were detectable in CSF samples from any uninfected mice, detectable levels of cytokines were found in CSF from infected animals.

We demonstrate for the first time, that *B. burgdorferi* is capable of colonizing the CSF in laboratory mice, which is correlated with increased inflammatory cytokines and chemokines. To our knowledge, this is the first description of direct visualization of live *B. burgdorferi* in mammalian fluids. Experiments are ongoing to determine the timecourse of CSF bacteria and cytokine levels, as well as the presence of leukocytes and *B. burgdorferi*-specific antibodies. Once these parameters have been established, the tractability of the murine model will allow for future studies on the bacterial, host, and environmental factors contributing to neuroborreliosis.

Poster Presentation #195

Unique Structural Insights into nsp16-nsp10 an Essential Complex for the Replication of SARS-CoV-2 and the Host-immune System Evasion

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Viruses have several mechanisms to protect the viral genome from the host immune system mimicking host mRNAs. SARS-CoV-2 the etiological agent of COVID-19 and other coronaviruses encode the capping proteins in its genome to modify the 5' UTR. Capping viral RNA promotes translation, prevents degradation and activation of immune responses. This process is initiated by the complex nsp9-nsp12-nsp13, which cleaves the 5' phosphate group from the nascent (+)ssRNA and transfers a GDP from GTP to the 5'-ppRNA. The nsp14-nsp10 complex catalyzes the transfer of a methyl group from S-adenosylmethionine to the cap GMP-N-7 position, generating Cap-0. Finally, the nsp16-nsp10 complex utilizes SAM to catalyze the 2'-O methylation of the first ribonucleotide, generating Cap-1. Although, some structures of the nsp16-nsp10 complex were solved previously for SARS-CoV, MERS and SARS-CoV-2, none of them was determined in the absence of the SAM, in the presence of metals or show the RNA-protein interactions. Herein, we determined the structure and activity of SARS-CoV-2 2'-O-methyltransferase in complex with the substrates, metals and products of the reaction, as well as a new structure with an empty SAM binding site, generating the first apo nsp16-nsp10 complex. This study shows unique structural features in the 2'-O-methyl transferase from SARS-CoV-2 that could be used for designing coronavirus-specific inhibitors.

Poster Presentation #196

Carbon Source and DMSO Differentially Activate the DMSO Reductase Promoters of Salmonella

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Facultative anaerobes, such as the pathogen iSalmonella entericai, can utilize a diverse array of electron acceptors to gain a metabolic foothold during gut colonization. One anaerobic electron acceptor that has received little attention is dimethyl sulfoxide (DMSO). The genomes of gastroenteritis-causing iSalmonellai serotypes contain three operons encoding putative DMSO reductases homologous to the idmsABCi operon of iE. colii. Our previous work highlights the importance of two non-redundant DMSO reductases to enhance anaerobic growth in vitro and support intestinal colonization in a calf infection model. We hypothesize that the lack of functional redundancy of the DMSO reductases is due to differential transcriptional regulation. Herein, we begin to identify regulatory elements controlling the expression of each idmsAi homolog iin vitroi. Mutants lacking the global transcriptional regulators ifnri, iarcAi, or ifuri do not gain an anaerobic growth enhancement when DMSO is added to the media, suggesting their activity is necessary for transcriptional regulation of DMSO reduction. Using ilacZYi-transcriptional fusions to measure promoter activity of each idmsAi homolog, we show the magnitude of promoter activity for each DMSO reductase varies under anaerobic conditions. The promoters of iSTM0964i and iSTM4305i also differ in responsiveness to DMSO and the carbon source glycerol. Together, the data suggest that there is/are additional activator(s) working in concert with global regulators of anaerobic metabolism to fine-tune the expression of individual DMSO reductases. Future work will establish the essential regulatory regions and transcription factors necessary to stimulate promoter activity of each DMSO reductase.

Poster Presentation #197

Development and Implementation of an Inducible Type I-C CRISPR-Interference System in Neisseria gonorrhoeae

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CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) are prokaryotic adaptive immune systems against foreign DNA and are regularly utilized as DNA editing tools. While *Neisseria gonorrhoeae* does not have an endogenous CRISPR system, the commensal species *Neisseria lactamica* encodes a functional Type I-C CRISPR-Cas. This system employs a multi-subunit ribonucleoprotein complex termed Cascade to recognize the targeted sequence and a helicase-nuclease fusion enzyme Cas3 to degrade target DNA. We have established an IPTG-inducible, CRISPR-interference platform based on the *N. lactamica* Type I-C CRISPR, where Cascade expression is under the control of IPTG. Notably, the Cas3 nuclease is missing, rendering it unable to cleave target DNA but can serve as a locus-specific DNA binding and transcription repression machinery. As proof in principle for using this CRISPRi system in *N. gonorrhoeae*, we targeted the *opaD* gene as it is highly expressed and generates an opaque colony phenotype. Both the *opaD* gene and protein expression was repressed in an IPTG-dependent manner. We have also found that we can fine-tune the knockdown magnitude, develop an efficient guide-switching method, and knockdown multiple genes simultaneously. We have used CRISPRi to target essential genes to generate conditional lethal strains, demonstrating that we can use CRISPRi to study the function of essential genes that cannot be knocked out. This CRISPRi tool will allow us to interrogate the function of understudied essential genes in the *N. gonorrhoeae*.

Poster Presentation #198

Carbohydrate Metabolism in Clinical Antibiotic-Resistant Enterococcus faecium

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Vancomycin-resistant *Enterococcus* (VRE) can dominate the intestinal microbiota after antibiotic treatment and translocate to the bloodstream, causing deadly infections. Previous research has shown that VRE blood isolates have a higher quantity of sugar transport genes than fecal isolates; additionally, fructose is associated with increased gut permeability, which may contribute to translocation from the gut and dissemination to other organs. We hypothesize that dietary sugars can benefit VRE both directly by providing a novel nutrient source and indirectly by increasing gut permeability, enhancing their competitive fitness and increasing their ability to spread to the bloodstream and cause disease. To test this, we examined the effect of dietary sucrose and fructose on the colonization and dissemination abilities of a clinical *Enterococcus faecium* strain in a mouse model. We found that a fructose diet contributed to increased dissemination, while the mice on regular and sucrose diets had greater colonization in the gut. Interestingly, the mice on a regular diet had increased inflammation in the gut compared to the mice on sucrose or fructose. Additionally, clinical isolates had increased growth on mannose, sucrose, and other sugars compared to fecal isolates. We are using a novel CRISPR-Cas12 system to generate deletions of these sugar metabolism genes in clinical *E. faecium* isolates, and we are developing a targeted *E. faecium* CRISPRi library to repress expression of these genes. Our results will contribute to a greater understanding of VRE infection dynamics and the impact of sugar additives on their competitive fitness, which will inform better treatment options.

Poster Presentation #199

Beta-2 Adrenoreceptors Role in Neisseria meningitidis (Nm) Invasion of Brain Endothelial Cells

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The blood-brain barrier (BBB) is a highly specialized cellular barrier that separates the circulation from the central nervous system (CNS). The BBB is comprised of highly specialized brain endothelial cells (BECs) that serve to maintain brain homeostasis by importing essential nutrients while restricting the passage of toxins and pathogens. *Neisseria meningitidis* (Nm) is a human-specific pathogen and a leading cause of bacterial meningitis, however determining mechanisms of how the BBB fails to protect the brain during infection remains poorly understood. A key challenge in discovering how the BBB fails to protect the CNS during Nm infection are partially due to lack of robust human-based in vitro models. Here we employ an induced pluripotent stem-cell (iPSC) based BEC model to interrogate Nm-BEC interactions. Recent human population based genetic studies have revealed that SNPs in the Beta-2 adrenergic receptor (B2AR) are associated with increased incidence of bacterial meningitis with Nm. The B2AR is a cell surface receptor that is a member of the G protein-coupled receptor (GPCR) family. Using commonly utilized FDA approved beta-blockers, we observe significant reduction in Nm invasion of BECs, and that beta-blockers can rescue Nm mediated Ca2+ signaling and rescue BEC tight junctions during infection. Together these data suggest a critical role of the B2AR in disease progression and BEC dysfunction. Future work will introduce the identified SNPs into iPSCs to model the contribution of these SNPs on bacterial interaction with BECs and BBB dysfunction.

Poster Presentation #200

Dietary Zinc Deficiency Compromises Immunity to Acinetobacter baumannii Pneumonia

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Acinetobacter baumannii is a gram-negative opportunistic pathogen that is a leading cause of ventilator-associated pneumonia infections. *A. baumannii* is critically important due to its widespread multi-drug resistance. Zinc is an essential metal nutrient for animals and bacteria, including pathogens such as *A. baumannii*. Studies show that patients with dietary zinc deficiency have an increased susceptibility to pneumonia infections, yet the mechanisms linking zinc and pneumonia are not well understood. To investigate this link, we developed a murine model of *A. baumannii* pneumonia infections and dietary zinc deficiency. In our murine model, dietary zinc deficiency increases mortality and bacterial burdens in the lungs by 24 hours post infection. Additionally, zinc deficient mice produce more pro-inflammatory cytokines in response to *A. baumannii* infection. Infected zinc deficient mice also produced IL-13, part of the type 2 inflammation that can promote a pro-resolution macrophage phenotype and inhibit bactericidal responses. Neutralization of IL-13 prevented mortality from *A. baumannii* pneumonia in zinc deficient mice. Therefore, zinc deficient mice are compromised in immunity to *A. baumannii* pneumonia due, in part, to the production of IL-13. This model has identified IL-13 as a potential therapeutic target to promote innate immunity to *A. baumannii* pneumonia in zinc deficient patients.

Poster Presentation #201

Systematically Phenotyping Acinetobacter baumannii Essential Genes using Antibiotics

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Acinetobacter baumannii is a Gram-negative bacterial pathogen considered an urgent threat as it can cause multidrug-resistant, hospital-acquired infections. Despite its clinical relevance, much about its core biology in the context of antibiotics remains poorly understood; the function of most A. baumannii essential genes have not been experimentally verified, and many have no predicted function at all. To better understand the network of essential genes in A. baumannii, we knocked down all predicted essential genes using CRISPR interference and screened the knockdown library with a panel of antibiotics and other chemicals. By looking at patterns of drug sensitivity and resistance of these gene knockdowns, we identified previously uncharacterized drug-gene interactions, observed phenotypes for genes of unknown function that hint at their role, and created a preliminary essential gene network for A. baumannii. By building off this initial network, we can better establish the A. baumannii essential gene network, providing insight into antibiotic mechanisms and potential drug targets.

Poster Presentation #202

Catabolite Activator Protein (CAP) and LuxR Co-regulate Quorum Sensing Genes in Vibrio campbellii

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Vibrio species are ubiquitous marine bacteria responsible for infections in a variety of hosts including shrimp, fish, humans, and coral. Vibrios utilize quorum sensing (QS) to globally regulate genes in response to changes in cell densities. At high cell densities, the master QS regulator LuxR controls expression of hundreds of genes, including those involved in pathogenesis and bioluminescence. However, the role of QS in regulation of virulence genes required for causing disease is under-characterized. Our lab studies the bioluminescence operon to dissect QS gene regulatory mechanisms in the model organism Vibrio campbellii BB120. The enzymes required for bioluminescence in V. campbellii are encoded by the genes luxCDABE, and their transcription is controlled by multiple proteins. We identified multiple LuxR and Catabolite Activator Protein (CAP) binding sites in vitro within the lux promoter (PluxCDABE) and upstream intergenic region. We found that while both transcription factors are required for bioluminescence, multiple binding sites are dispensable for activation of the lux operon. Additionally, dRNA-seq analysis revealed a previously unreported transcript mapping to the intergenic region upstream of PluxCDABE. Neon green reporter data suggest that expression of this transcript is highest in the absence of CAP, indicating that CAP may be acting as a repressor. Future experiments will include identification of the transcript length and start site, as well as determining its role in regulation of the lux operon. By characterizing the lux promoter, we gain insight into the complex ways in which V. campbellii controls global gene regulation via QS and CAP.

Poster Presentation #203

Roles of Essential Genes in Pseudomonas aeruginosa Biofilm Formation

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A biofilm is a collection of surface attached microorganisms that exist in an extracellular matrix that serves as a protective barrier against antibiotics and other environmental stressors. Several studies have identified gene pathways that are important for biofilm formation in *Pseudomonas aeruginosa*, but these studies lack the ability to assess the roles of essential genes. Here, I propose the use of a *P. aeruginosa* essential gene knockdown library to discover novel connections between core cellular processes and biofilm formation. My goal is to find essential gene knockdowns that positively or negatively impact biofilm formation in *P. aeruginosa*. These findings may lead to new therapeutic strategies that can simultaneously disrupt biofilm formation and the viability of *P. aeruginosa* by perturbing a single pathway.

Poster Presentation #204

Single-Cell Analysis of Salmonella SPI-2 Induction Reveals Environment Tunable Heterogeneity

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During infection of a host, *Salmonella* must sense and process a diverse range of environmental cues to elicit virulence responses in contexts where they are required and repress them when not advantageous. One example of this is the activation of *Salmonella* Pathogenicity Island 2 (SPI-2) upon entry into a host cell. SPI-2 encodes a type III secretion system and secreted effectors required for intracellular proliferation. Numerous signals have been found to induce SPI-2 upon entry into the intracellular environment, including pH, magnesium concentration, and nutrient levels. However, the extent to which individual bacteria are able to sense changes in these signals to induce SPI-2 has not been explored, nor is it clear how homogenous these responses are across a clonal population. To investigate this, we used smFISH and live-cell promoter-based reporters to measure SPI-2 transcriptional induction at the single-cell level. We have identified bimodal expression of *Salmonella* SPI-2 in *in vitro* conditions and demonstrate that the timing of this response can be tuned through incremental changes in pH as well as the integration of pH and magnesium signals. These results provide insight into the information processing capacity of the SPI-2 regulon and suggest heterogeneity in SPI-2 expression may be advantageous during environmental transitions. Future work will evaluate the mechanistic basis of SPI-2 heterogeneity and explore whether this bimodal expression is functionally significant within the contexts of bacterial physiology and epithelial cell culture infection models.

Poster Presentation #205

Defining Synthetic Interactions between Putative Mycobacterial Acetyltransferases

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Mycobacterial pathogens cause acute and chronic disease in humans and in animals. *M. tuberculosis*, the cause of Tuberculosis, and *Mycobacterium marinum*, a non-tubercular pathogen that causes tuberculosis like disease in ectotherms, share mechanisms used for host interaction. Mycobacteria promote survival in the host through the secretion and modification of protein virulence factors. *M. marinum* shares 23 acetyltransferase (NAT) orthologs with *M. tuberculosis* that likely promote lysine and N-terminal protein acetylation. We are studying the roles of the conserved acetyltransferase proteins using proteo-genetic approaches in *M. marinum*. To define the functional relationships between these acetyltransferases, we generated a collection of double deletion *M. marinum* strains of the twelve most conserved putative NAT genes. We characterized the resulting strain collection by measuring growth *in vitro*, hemolytic activity and protein acetylation. We have identified redundant and suppressive interactions between putative NATs. Single or double deletion of the putative NAT genes *MMAR_1123* or *MMAR_4496* reduced hemolytic activity. In the double deleted strain, each complement is able to restore hemolysis to WT levels. This redundant interaction suggests that these two NATs may share a common pathway where the function of one can rescue the absence of the other. Single deletion of *MMAR_1839* showed reduced levels of the protein acetylation compared to WT or double deletion with *MMAR_1968* and *MMAR_1123*. This suppressive interaction could indicate a specific restoration impact on protein acetylation between two given NATs. Our studies will generate a network map between the NATs, their targets and their role in *M. marinum*.

Poster Presentation #206

Investigating the Role of Lysine Acetyltransferases in Mycobacterial Pathogenesis

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Lysine (K) acetylation is the addition of an acetyl group to an epsilon amino group of a lysine side chain via enzymatic or non-enzymatic mechanisms. Enzymatic K-acetylation is mediated through lysine acetyltransferases (KATs). In eukaryotes, KATs and the acetylation events that they facilitate have been well-studied. In pathogenic mycobacteria, numerous putative acetyltransferases result in K-acetylation of approximately 15 percent of proteins. However, the exact roles of many of these acetyltransferases and their acetylated proteins remain to be uncovered. Utilizing *Mycobacterium marinum*, a non-tuberculous mycobacterial species, we have generated a single deletion mutant library to characterize known and putative lysine acetyltransferases and their contributions to mycobacterial pathogenesis. Mycobacteria employ a conserved Type VII secretion system, ESX-1, to survive within the host. The ESX-1 system is necessary for phagosomal lysis within the host alveolar macrophage, promoting both virulence and survival of the bacteria. Several components of the ESX-1 secretory apparatus are K-acetylated, implicating KATs in mycobacterial pathogenesis. We found that KAT mutant strains display decreased hemolysis, a proxy measurement of ESX-1 function. In a macrophage infection model, overexpression of some KATs results in a hypervirulent phenotype. In bacterial growth assays, many KAT mutant and overexpression strains grow at increased rates. Finally, by thin-layer chromatography, we observed that deletion of specific KATs depletes of PDIM and PGL lipids. Overall, we have begun to uncover a unique connection between KATs and the ESX-1 system, impacting overall mycobacterial virulence and pathogenesis.

Poster Presentation #207

Characterizing New Nitro-containing Compounds that Kill Mycobacterium tuberculosis

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Tuberculosis (TB) chemotherapy is hampered by the evolution of multidrug-resistant *Mycobacterium tuberculosis* (Mtb) and the limited number of leads in the TB drug development pipeline. With the recent FDA approval of pretomanid, a nitroimidazole-based TB drug, nitro-containing molecules have emerged as promising compounds for TB therapy. The goal of this study is to characterize new nitro-containing compounds that target Mtb. Our lab conducted a high throughput screen of more than 300,000 molecules and identified about 800 growth inhibitors of Mtb. Five of these compounds (HC2209, HC2210, HC2211, HC2233, and HC2234) are novel nitrofurans that are bactericidal and highly specific for Mtb. They are also effective against intracellular Mtb and have no observable eukaryotic cytotoxicity in murine bone-marrow macrophages. Forward genetic screening identified HC2209, HC2210, and HC2211 as prodrugs that require a cofactor F420-dependent nitroreductase (Ddn) and an unknown enzyme for activation. Interestingly, HC2233 and HC2234 do not require either cofactor F420 or Ddn for their activity. Our lab is currently in the process of further characterizing the mechanism of action of these molecules and testing their treatment efficacy in a murine model of TB. Overall, these preliminary data show these nitrofurans as promising molecules that can be further developed as drugs for TB treatment.

Poster Presentation #208

ZnuABC is Not Essential for Klebsiella pneumoniae Virulence

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Nutritional immunity is the process used by hosts to sequester transition metals from invading pathogens. To counteract nutritional immunity, bacteria have developed mechanisms to obtain transition metals, which include ABC transporters and siderophores. Klebsiella pneumoniae, a major cause of hospital-acquired pneumonia and sepsis, encodes the siderophore, yersiniabactin (Ybt) within its genome. Ybt is an important virulence factor used by pathogens such as Yersinia pestis to overcome not only iron (Fe) limitation but zinc (Zn) limitation during infection. Ybt's role in Fe acquisition during K. pneumoniae infection has been well characterized. However, the role of Ybt in K. pneumoniae zinc acquisition during infection has not yet been defined. We sought to define the contribution of Ybt and ZnuABC to K. pneumoniae virulence during pneumonia. Mutants lacking YbtX and the high affinity Zn transporter, ZnuBC, were generated. We infected C57BL/6 mice with wildtype, znuBC and znuBC ybtX mutants and monitored survival and determined bacterial burden in the lungs. Mice infected with the znuBC ybtX mutant had similar survival rates as mice infected with a znuBC mutant and wildtype K. pneumoniae. Furthermore, there was no significant difference in bacterial burden in the lungs of mice infected with znuBC ybtX compared to mice infected with wildtype or znuBC mutant. These data indicate K. pneumoniae encodes an additional zinc acquisition system, and the bacterium uses multiple mechanisms to acquire Zn during infection.

Poster Presentation #209

Chlamydia Circumvents Thermal Stress via Tail Specific Protease

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Chlamydiae are ancient obligate intracellular pathogens that have extensively adapted to their niche, yet how these pathogens resist intracellular stresses is unclear. While screening a mutagenized Chlamydia muridarum (CM) library, we identified a mutant attenuated at 40°C in vitro and in mouse urogenital models in vivo. Using a traditional complementation approach and a novel counter-selective lateral gene transfer technique, we linked the temperature sensitive phenotype of the mutant to a nonsense mutation in tc_0725, a gene encoding tail specific protease (TSP). A TSP ortholog has been hypothesized to be involved in cellular immune evasion by C. trachomatis. However, TSP shares homology with proteases that are essential for thermal stress survival in other free-living and intracellular bacteria. Our results indicated that TSP is not involved in the cleavage of host Nfκβ subunits, as previously described. Using temporal temperature shifts at different stages of the developmental cycle we observed that TSP is functional at an early stage of chlamydial development. Electron microscopy indicated that loss of a functional TSP dramatically altered chlamydial morphology throughout development. Consequently, quantitative proteomics indicated that loss of TSP led to shifts in protein levels that are critical for chlamydial pathogenesis and maintenance during the developmental cycle. TSP was also linked to chlamydial infectivity and pathogenesis in the mouse genital tract. Overall, our results TSP might play a canonical role in chlamydial development, virulence, and heat shock response. To resolve the molecular function of TSP, we plan to identify potential TSP interacting partners and substrates in future studies.

Poster Presentation #210

Genetic Determinants of Bacteroides Fragilis Fitness in the Presence of Deoxycholate

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Inflammatory diseases of the colon such as ulcerative colitis (UC) are associated with major shifts in the gut microbiome. For example, *Bacteroides fragilis* constitutes 1-5% of the microbiome in healthy individuals but can bloom to >50% of the population in UC patients. The mechanisms underlying these microbial blooms remain poorly understood. Using a *B. fragilis* cultivar isolated from a UC pouchitis patient, we constructed a barcoded transposon mutant collection containing ~50,000 unique mutant strains to serve as a platform to investigate *B. fragilis* genes that influence its fitness in healthy and diseased patients. The bile acid deoxycholate (DC) is a modulator of microbial fitness and community composition *in vivo*. We cultivated our mutant pool in 0.01% DC, an inhibitory concentration *in vitro* that reflects DC levels in healthy patients. *B. fragilis* genes critical for DC survival affect a range of cell envelope functions and include an alternative ATP synthase, cardiolipin synthase, and surface polysaccharide enzymes. Disruption of beta-oxidation and pentose phosphate pathway enzymes enhanced fitness in DC. We leveraged RNAseq as a complementary approach to assess the *B. fragilis* response to bile. A 6min DC exposure dramatically remodeled the transcriptome with ~20% of transcripts exhibiting significant changes. Chaperones and efflux systems were among the most highly induced genes, while protein synthesis genes -- including tRNAs -- were strongly repressed by DC. These experiments provide a genome-scale view of genes that determine *B. fragilis* fitness in the presence bile and suggest pathways that could be targeted to control *B. fragilis* blooms.

Poster Presentation #211

Defining New Regulatory Mechanisms of Hypoxia-driven Adaptation in Mycobacterium tuberculosis

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Mycobacterium tuberculosis (Mtb) adapts to the host immune response by altering its gene expression in response to environmental cues. When Mtb is enclosed in a granuloma, it experiences hypoxia which arrests its growth because the pathogen is a strict aerobe. To overcome this challenge, Mtb enters a non-replicative persistent (NRP) state, where metabolism is limited until conditions improve. Mtb initiates NRP through a two-component regulatory system called DosRST. DosS and DosT are both membrane-bound heme-containing sensor histidine kinases. DosR is the response regulator; when activated, DosR induces expression of at least 50 genes, including the alpha-crystallin chaperone gene hspX. We hypothesize that additional regulatory networks may provide feedback on the DosR regulon and modulate its expression. To test this hypothesis, we conducted a forward genetic screen for mutants with reduced DosR regulon expression. Using the Mtb (hspX'::GFP) reporter strain, which has fluorescence that is induced by hypoxia in a DosR-dependent manner, we used a flow cytometer to isolate transposon mutants with >50% reduced fluorescence under hypoxia. We identified multiple independent transposon insertions in several Mtb genes, suggesting they play a role in regulating DosR signaling. Follow-up studies are underway to validate and characterize these mutants and their role in hypoxia-driven adaptation. We hope this research will identify additional genes that may participate in DosR regulation and persistence under hypoxic conditions.

Poster Presentation #212

Examining the Interplay between the Distinct Contact Dependent Growth Inhibition Systems within Burkholderia Species

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Contact-dependent growth inhibition (CDI) systems mediate interbacterial competition and are widespread among Gram-negative bacteria, including members of the *Burkholderia cepacia* complex (Bcc). CDI systems consist of a Two-Partner secretion system that delivers a large toxic exoprotein (BcpA) to the surface of the cell through a cognate outer membrane β-barrel protein (BcpB). The C-terminal portion of the exoprotein (BcpA-CT), comprising a nuclease toxin, is ultimately imported into the cytoplasm of a neighboring bacterium upon direct cell-cell contact. The neighboring cell's growth will be inhibited unless it produces a corresponding immunity protein (BcpI). Many bacterial strains encode multiple CDI systems but is unclear what, if any, cross talk occurs between distinct CDI systems within the same cell. *Burkholderia dolosa* AU0158 contains three loci that encode for CDI systems that mediate interbacterial competition. Our data show that for interbacterial antagonism mediated by *B. dolosa* BcpA-1 and BcpA-2, either the BcpB-1 or BcpB-2 transporter is sufficient. These data suggest that BcpA-1 and BcpA-2 toxins can be exported through either their cognate BcpB transporter or through the BcpB protein associated with another CDI system. Our data also show that a distinct BcpA produced by *Burkholderia multivorans* CGD2M can be secreted by non-cognate BcpB transporters. Together, these data suggest that there is an interaction between separate CDI systems produced in *Burkholderia* bacteria. Further understanding the interplay between distinct CDI systems will provide insight into understanding the advantage producing multiple CDI systems provides for CDI mediated competition among *Burkholderia* pathogens.

Poster Presentation #213

Investigating the Role of the tRNA Methyltransferase TrmB in Acinetobacter Stress Responses

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Acinetobacter baumannii (*Ab*) is a major global health threat due to its skyrocketing rates of multidrug resistance and exceptional ability to adapt to hostile environments. Unfortunately, given the extensive diversity among clinical isolates, little is known about *Ab* virulence mechanisms. While recent studies have begun to explore the role of tRNA methyltransferases (trm) in bacterial stress responses, trm have not yet been investigated in *Ab*. Bioinformatic analyses revealed eight putative trm conserved across modern clinical *Ab* isolates and laboratory strains, and the m7G46 methyltransferase, AbTrmB, has been implicated to be involved in antibiotic resistance. Upon generating Δ*trmB* mutants in multiple clinical isolates, we observed that the absence of AbTrmB increased the susceptibility of clinical isolates to a range of antibiotics. In response to ciprofloxacin stress, Δ*trmB* mutants had a growth defect and chaperone-usher pili were repressed, indicating an altered ability to react to stress. To investigate the role of AbTrmB in response to apramycin stress, whole cell proteomics was performed, revealing Δ*trmB* had diminished production of porins and proteins involved in lipid transport and metabolism. Notably, Δ*trmB* mutants were also significantly more susceptible to hydrogen peroxide treatment and acid stress. Accordingly, Δ*trmB* mutants were unable to replicate in macrophages and had decreased virulence in catheter-associated urinary tract infection and pneumonia murine models. These results highlight the therapeutic potential of targeting trm to combat the rise of multi-drug resistant *Ab*.

Poster Presentation #214

Porphyromonas gingivalis Rescues Receptor Polysaccharide Mutants of Streptococcus gordonii from In Vivo and In Vitro Killing

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Oral bacterial communities trigger the development of periodontitis. Streptococcus gordonii (Sg) is a primary colonizer of dental plaque, and Porphyromonas gingivalis (Pg), the main causative organism, is a secondary colonizer. Defining the association between these organisms is, therefore, essential for a fuller understanding of the pathogenicity of periodontitis. Using a Tn-Seq library of Sg in a mouse abscess coinfection model with Pg, we identified multiple genes within the receptor polysaccharide (RPS) operon that showed a fitness defect and could be rescued by Pg. Two clean deletion mutants were made in sgo_2020 and sgo_2024, representing a glycosyltransferase and an extracellular polysaccharide biosynthesis protein, respectively. Both mutants were deficient in survival in the mouse abscess model, more susceptible to neutrophil killing, and failed to produce capsule. Interestingly, Pg rescued the mutants from neutrophil killing and increased their fitness in the abscess model. In vivo mouse imaging showed that Pg reduced neutrophil recruitment during infections with the Sg mutant strains. An elastase assay with human neutrophils found higher mobilization of this granule subtype in response to the mutants; however, the presence of Pg dramatically reduced granule mobilization. Pg also suppressed neutrophil extracellular trap (NET) formation in response to the mutants. Collectively these data show that the RPS of Sg is organized into a capsule which is important to resist neutrophil killing. In the absence of RPS, Pg can protect Sg cells by suppressing elastase release and the formation of neutrophil NETS.

Poster Presentation #215

Coxiella burnetii Exploits Host Protein ORP1 to Optimize Cholesterol Content of Its Intracellular Niche

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Coxiella burnetii causes the somewhat mysterious and potentially debilitating disease Q Fever. The largest recorded outbreak, which caused more than 4,000 acute cases in the Netherlands between 2007 and 2009, makes this bacterium cause for growing concern, especially in absence of a widely-available vaccine. Furthermore, clearance of chronic *Coxiella* infection can require up to 24-48 months of antibiotics. By elucidating how *Coxiella* manipulates its host, we may discover more successful approaches to control infection. Previous research revealed that *Coxiella* is sensitive to elevated host cholesterol in the intracellular niche. Yet *Coxiella*'s host cell, the macrophage, can be lipid-laden: so how does the bacterium avoid cholesterol? Our lab demonstrated that membrane contact sites exist between the host endoplasmic reticulum and the *Coxiella*-containing vacuole (CCV) and require the bacterial Type 4B Secretion System (T4BSS) and a host tether and lipid transfer protein, ORP1. We asked whether *Coxiella* exploits ORP1 to regulate cholesterol content of its intracellular niche. To this end we infected an ORP1-null cell line deficient in both ORP1 isoforms and assessed two different metrics of *Coxiella* proliferation, CCV size and bacterial growth, using fixed cell immunofluorescence microscopy and a colony-forming unit (CFU) assay, respectively. We also assessed CCV cholesterol content using filipin labeling in ORP1-null and wild-type cells. We found that ORP1-null CCVs were smaller in MH-S macrophages and HeLa cells and contained more cholesterol in HeLa cells. We also found that bacteria from ORP1-null infected macrophages grew poorly. These data point to ORP1-modulation of CCV cholesterol.

Poster Presentation #216

Genetic Tradeoffs Underlying Synergistic Antibiotic Function in Acinetobacter baumannii

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Antibiotic-resistant *Acinetobacter baumannii* is an emerging threat to public health, but little is known about how antibiotics interact with their essential gene targets in *A. baumannii*. Here, we systematically probe antibiotic-essential gene interactions in *A. baumannii* using a titratable CRISPR interference (CRISPRi) knockdown library. We identify essential genes that are sensitive to even modest knockdown—these genes may represent novel antibiotic targets. We also comprehensively discover antibiotic-gene interactions including those that underlie the lethal synergy of colistin and rifampicin—two antibiotics of last resort with unrelated targets. Under our model, anticorrelated gene phenotypes reveal distinct mechanisms of resistance to colistin and rifampicin that effectively cancel each other out when the drugs are administered jointly. These tradeoffs can explain the genetic basis of known combination therapies and may represent a new platform to predict drug synergies that overcome resistance mechanisms.

Poster Presentation #217

Role of L-ornithine in Mediating Metabolic Crosstalk between E. faecalis and P. aeruginosa

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Pseudomonas aeruginosa is commonly co-isolated with *Enterococcus faecalis* in UTIs, post-intestinal reconstruction, and prosthetic joint infections. Little is understood regarding how bacteria such as *P. aeruginosa* and *E. faecalis* interact in such infections. We find that when grown in planktonic co-culture, *P. aeruginosa* does not kill *E. faecalis*. Moreover, we find that one species can facilitate growth of the other when non-metabolizable sugars are used as the sole carbon source in minimal medium. We also find that the non-proteinogenic amino acid L-ornithine plays a role in facilitating *P. aeruginosa-E. faecalis* interaction–but the effect of L-ornithine on *P. aeruginosa* growth is slow. In minimal medium, *P. aeruginosa* readily metabolizes glucose when it is the sole carbon source. Contrarily, in L-ornithine, *P. aeruginosa* will grow only at minimal levels with a lag phase beginning between 18 and 24 hours. Growth curve measurements were performed at OD600 for *P. aeruginosa* monocultures in varying conditions involving glucose, L-ornithine and filtrates from *E. faecalis* cultures. The addition of *E. faecalis* culture filtrates to L-ornithine minimal media reproduced growth observed when glucose was present. This effect was lost when the filtrate was treated with 100°C for 10 minutes. This implies there is a heat-sensitive component in *E. faecalis* cultures that impacts growth of *P. aeruginosa* cultures. It remains unknown the structure and identity of this component, but we hypothesize it may be proteinaceous.

Poster Presentation #218

Examining the Role of Diet in Clostridioides difficile Infection

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Background:
Sugary diets, common in the modern world, may affect the gut microbiota, induce inflammation, and exacerbate inflammatory illnesses. Positive selection is occurring in sugar metabolism genes of the intestinal pathogen Clostridioides difficile, indicating their role in C. difficile's lifestyle. It is still unknown how a high sugar diet influences C. difficile disease and carriage.

Methods:
C57B/6 mice were obtained (12m/12f) and fed either a regular chow diet or a diet high in sucrose or fructose for 4 weeks. Following antibiotic treatment mice were gavaged with 1x107 CFUs of CD2015 spores. Four days following infection four mice from each group were sacrificed and necropsied. The surviving mice were monitored for clearance of the disease while on their respective diets. At d=28 the surviving mice were sacrificed and necropsied.

Results:
Mice fed the sugar diet had increased C. difficile carriage compared to regular chow counterparts. Interestingly, mice on the regular chow diet experienced greater weight loss compared to mice on experimental diets. Additionally, mice fed the sucrose chow had shorter colon lengths than mice fed the other chows.

Conclusions:
Our findings suggest a diet high in sugars contributes to elevated C. difficile carriage in the gut. Additionally, we found evidence of increased gut inflammation in mice being fed a diet high in a specific sugar. It is possible that a diet high in sugar leaves mice less vulnerable to weight loss during infection. Given these results, further investigation is necessary to illuminate the role that diet plays in C. difficile infection.

Poster Presentation #219

Loss of Infectivity in Mice after Long-term in Vitro Passage of Relapsing Fever Spirochete Borrelia miyamotoi

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Borrelia miyamotoi (*Bmi*) is a spirochete bacterium that is vectored by hard-ticks and causes Borrelia miyamotoi disease (BMD), an emerging public health threat. It has been previously demonstrated that long-term *in vitro* culture can lead to attenuation of microbial pathogens. In the current study, we set out to investigate infectivity of host-adapted versus culture-adapted *Bmi*; however, during routine culture, we discovered our previously infectious isolate had become non-infectious after long-term *in vitro* cultivation.

Initially, host-adapted and culture-adapted *Bmi* infections demonstrated similar infectivity: SCID mice developing high, persistent spirochetemia detectable by qPCR, blood smears, and culture, and C3H mice with demonstrable relapses detectable by qPCR and culture. The loss of infectivity was discovered when additional infections with culture-adapted long-term *in vitro* passaged *Bmi* were unable to maintain infection in either SCID or C3H mice.

We demonstrate for the first time a loss of infectivity in mice after long-term *in vitro* passage of *Bmi*. Our ongoing experiments are trying to identify the genetic basis for the infectivity loss. To our knowledge, there are currently no identified *Bmi* virulence determinants and our non-infectious strain provides a model to address that. Identifying novel virulence determinants would provide targets for prophylactic or therapeutic BMD treatments.

Poster Presentation #220

Listeria monocytogenes' Metabolic Interaction with the Host

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The metabolic crosstalk between pathogens and host has historically been overlooked in the research of host-pathogen interactions. This is partially due to technical limitations, incomplete genetic toolsets to screen for novel routes of metabolism, and underappreciated importance of metabolism to bacterial pathogenesis. Intracellular bacteria, particularly cytosolic bacteria such as Listeria monocytogenes, must utilize host derived metabolites to fulfill their basic metabolic needs while simultaneously remaining undetected. Further, L. monocytogenes is an exceptional model organism to study shared host-pathogen metabolism given its tractable genetics and well-defined life cycle. Having a complete understanding of L. monocytogenes intracellular metabolism could lead to novel antibiotic targets or the ability to engineer L. monocytogenes' metabolism for therapeutic purposes.

We have determined that L. monocytogenes is capable of near wild-type intracellular growth and virulence despite lacking the ability to use established carbon sources of glycerol and/or hexose phosphates. These findings suggest that L. monocytogenes must be utilizing canonically alternative carbon sources in the macrophage cytosol, which we have taken initial steps to identify using Biolog's phenotypic carbon metabolic arrays and unbiased metabolomics of infected and uninfected macrophages. Multiple physiologically relevant and previously undescribed carbon sources were identified that L. monocytogenes may be using in the host cytosol. Ongoing work includes using intra-macrophage Tn-seq and dual RNA-seq to determine the genetic requirements of L. monocytogenes to consume these carbon sources and the metabolic cross-talk between host and bacterial cells, respectively.

Poster Presentation #221

Phenotypic Differences in Gardnerella Species Colony Variants

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Gardnerella spp. is part of the normal microflora in healthy women, but they are also found at much higher abundance in women with bacterial vaginosis (BV), a polymicrobial dysbiosis that can result in complications including preterm birth, pelvic inflammatory disease, and increased risk of acquiring sexually transmitted infections. While *Gardnerella* spp. plays a seminal role in the development of BV, the factors promoting *Gardnerella* growth, biofilm production, and ultimately dysbiosis remain poorly defined. We have discovered phenotypic heterogeneity in both colony size and opacity among 10 species of *Gardnerella*. The switching frequency between small (SCV) and large (LCV) colony variants (~10^{-3}) is suggestive of high frequency phase variation. Other phenotypic differences between variants include growth rate, hemolysis, and inhibition of urogenital bacteria including *Lactobacillus* and *Neisseria gonorrhoeae*. Further, proteomics analyses showed differential expression of ~70-200 proteins between small and large variants. Higher expression of vaginolysin toxin was detected in SCVs, while higher levels of a putative MocR-like transcriptional regulator were found in LCVs. Whole genome sequencing revealed the presence of several intra- and intergenic poly-G/C tracts with variable lengths between small and large variants, suggesting a role for slipped-strand mispairing in *Gardnerella* phenotypic heterogeneity. Many of these tracts were associated with surface proteins. Collectively, these results suggest that phase variation may have an important role in *Gardnerella* pathogenesis and the development of BV, allowing it to adapt to the dynamic environment of the female genital tract, evade the immune system, and modulate its virulence potential.

Poster Presentation #222

Defining Interactions of Carbon Metabolism and Acidic pH during Mycobacterium tuberculosis Pathogenesis

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Mycobacterium tuberculosis (Mtb) is an ongoing public health problem in many communities including the indigenous community. Mtb is a successful pathogen, in part because it can adapt to environmental cues encountered during infection, including the acidic pH of the macrophage. In response to this acidic environment Mtb modulates its metabolism. Mtb uses a two-component system, PhoPR, that is stimulated by acidic pH and causes differential expression of metabolic genes. Mtb arrests its growth on specific single carbon sources at pH 5.7, such as propionate and succinate. However, Mtb grows well on these carbon sources at pH 7.0. We hypothesized that arrested growth is genetically controlled and that mutants could be selected that gain the ability to grow on these carbon sources at acidic pH. To test this hypothesis, we selected for transposon mutants that grew on propionate or succinate, as sole carbon sources, at pH 5.7. Transposon mutants were identified in PhoR in both propionate and succinate selections, and the PhoPR regulated gene, pks2, mutant was found in the succinate selection. Results of RNAseq studies of the phoR mutant grown on propionate at pH 5.7, showed upregulation of genes involved in the methyl citrate cycle, prpC and prpD. This finding suggests enhanced detoxification of propionate by the methyl citrate cycle in the phoR mutant. We propose a model where at acidic pH, PhoPR normally diverts away carbon from central carbon metabolism and slows growth at acidic pH.

Poster Presentation #223

CRM1-mediated Nuclear Export in Helicobacter pylori Induced Neutrophil Hypersegmentation

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Neutrophils are the first line of defense against microbial invaders and are integral for disease caused by Helicobacter pylori infection. H. pylori is a human-specific Gram-negative bacterial pathogen that infects the gastric epithelium and can cause peptic ulcers, gastritis, and gastric cancer. H. pylori induces a massive recruitment of neutrophils to the infection site where they sustain a chronic inflammatory response. H. pylori also induces nuclear hypersegmentation, increasing the number of nuclear lobes from 3-4 to as many as 17 lobes/cell. The mechanisms by which it occurs are not well understood. We have previously shown that this hypersegmentation can be prevented if either host or pathogen transcription or translation is blocked. For example, treatment of infected cells with cycloheximide or chloramphenicol reduced hypersegmentation by 66.1 ± 24.1% ($n=4$, $p < 0.01$) and 81.3 ± 7.3% ($n=3$, $p < 0.01$) respectively. Further evidence supporting a role in nuclear export is our observation that inhibiting CRM1 using Leptomycin B or Selinexor significantly decreased hypersegmentation induced by H. pylori infection. In keeping with this, RNA-seq transcriptional profiling revealed significant differential expression of nuclear pore proteins and components of the Ran GTPase/CRM1 nuclear export pathway. Current studies are focused on confocal microscopy and Western blot analysis of CRM1 as well as its nuclear exporting partners. Completion of these studies will advance understanding of H. pylori infection as well as mechanisms that regulate neutrophil nuclear morphology.

Poster Presentation #224

Listeria monocytogenes Requires Production of DHNA To Maintain Intracellular Redox Homeostasis, Independent of Cellular Respiration

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Listeria monocytogenes is a ubiquitous facultative intracellular pathogen that can thrive in a wide range of ecological niches. *L. monocytogenes* maximizes its ability to generate energy from diverse carbon sources using a respiro-fermentative metabolism that can function under both aerobic and anaerobic conditions. Cellular respiration maintains redox homeostasis by regenerating NAD+ while also generating a proton motive force (PMF). The end products of the menaquinone (MK) biosynthesis pathway are essential to drive both aerobic and anaerobic cellular respiration. Furthermore, we recently demonstrated that intermediates in the MK biosynthesis pathway are independently required for the survival and virulence of *L. monocytogenes* through a currently unknown mechanism. We show here that exogenous supplementation of the MK biosynthetic intermediate 1,4-dihydroxy-2-naphthoate (DHNA) rescues the in vitro growth and ex vivo virulence of DHNA-deficient mutants of *L. monocytogenes*. The endogenous production or direct supplementation of DHNA restored both the cellular redox homeostasis and metabolic output of fermentation in *L. monocytogenes*, independent of respiration. We further demonstrate that, in the absence of respiration, the virulence defects associated with DHNA-deficiency are due to an impaired redox balance that is rescued upon the heterologous expression of an NADH oxidase (NOX) that functions to specifically regenerate NAD+ similar to endogenous DHNA production. These data suggest that the production of DHNA may represent an additional layer of metabolic adaptability by *L. monocytogenes* to drive energy metabolism in the absence of respiration-favorable conditions.

Poster Presentation #225

Identification and Characterization of Substrates for the Conserved N-terminal Acetyltransferases Between M. marinum and M. tuberculosis

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Acetylation of protein N-termini is catalysed by N-terminal acetyltransferase (NATs) enzymes. It is unclear how N-terminal protein acetylation contributes to mycobacterial metabolism, pathogenicity, transcription, and translation. One way to define the consequences of N-terminal acetylation in *Mycobacterium* is to define the NAT-substrate relationships. *Mycobacterium marinum* (opportunistic pathogen) and *Mycobacterium tuberculosis* (causative agent of tuberculosis in humans) share several conserved putative NATs. To identify the substrates targets, we are taking genetic and biochemical approaches including ascorbate peroxidase (APEX) based proximity labelling, immunoprecipitation (IP) assay, far-western analysis, and protein pull-down assays. We have used E. coli to express and purify four putative *M. marinum* NATs (MMAR_1968, MMAR_0522, MMAR_4467, and MMAR_1341). The purified proteins were used in far-western assays to identify direct interactions, and in protein pull-down assay to identify interacting proteins from *M. marinum* cell lysate. We are identifying the interacting proteins using mass spectroscopy. Putative substrates of the NATs will be further confirmed by targeted genetic approaches and by heterologous expression, purification, and *In vitro* enzymatic assays for both *M. marinum* and *M. tuberculosis* proteins. Our study will provide an approach for the identification of other NAT-substrate pairs in bacteria.

Poster Presentation #226

Evaluating Alternative Metabolic Pathways used for Biofilm Formation and Planktonic Growth of Stenotrophomonas maltophilia

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The multidrug resistant, nosocomial bacterium *Stenotrophomonas maltophilia* is an increasing threat to immuno-compromised patients worldwide. The biofilm formation ability of *S. maltophilia* allows it to cause serious respiratory infections in lungs, especially in cystic fibrosis patients. From our laboratory's previous work, we hypothesized that changes in the metabolism due to different carbon sources may directly impact biofilm formation and planktonic growth of *S. maltophilia*. We found that the wildtype strain of *S. maltophilia* favors amino acid rich media over glucose rich media for both biofilm and planktonic growth. Moreover, while deleting the phosphoglycerate mutase gene, *gpmA*, inhibits growth in amino acid rich condition, adding glucose or ribose in the amino acid rich media fixed the growth defect in this mutant. From Nuclear Magnetic Resonance analysis, we found that wildtype and *gpmA* show different patterns of amino acid uptake. We further defined a minimal set of amino acids required for *S. maltophilia* growth: Alanine, Proline, Leucine and Isoleucine. We speculate that *S. maltophilia* shuttles carbon from these amino acids through gluconeogenesis to a unique metabolite required for growth and biofilm formation. Further analysis will investigate this unique metabolic pathway, which could lead to novel therapies to inhibit the growth of this pathogen.

Poster Presentation #227

Mutations in O-Antigen Biosynthesis Enzymes in Vibrio cholerae Confer Pan-Resistance to ICP Phage Infection

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In its natural aquatic environment, the bacterial pathogen *Vibrio cholerae*, the causative agent of the enteric disease cholera, is in constant competition with bacterial viruses known as phages. My research investigated the manner in which the El Tor biotype of *V. cholerae* resists predation by the three ICP lytic phages. We found that following an ICP-3 infection, crashed cultures would spontaneously recover overnight, suggesting the emergence of resistant escape mutants. Recovered isolates from these regrowth populations were then rechallenged with ICP-3 phage via a liquid reinfection assay as well as a solid plaque assay. In each case, complete resistance was confirmed as cells were able to withstand subsequent infections. The genomic DNA of these cells was analyzed by whole genome sequencing, revealing the presence of two different mutations in enzymes involved in the O-antigen biosynthesis pathway. Eight of the nine sequenced strains had an 11 bp deletion in a phosphomannomutase gene while the remaining mutant had a single nucleotide insertion in a gene encoding a glycosyltransferase. To further elucidate the specificity of the resistance conferred by these mutations, the mutant cultures were challenged with the additional *V. cholerae* specific phages ICP-1 and ICP-2. Despite no prior exposure to these phages, the mutants demonstrated pan-resistance to all three ICP phages. Future experiments will examine how these genes are exploited by bacteriophage to cause infection and cell lysis and the evolutionary tradeoff of these mutations.

Poster Presentation #228

Staphylococcal Type 7b Secretion System: Role in Mouse Colonization and Systemic Infection

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Staphylococcus aureus bears a type 7b secretion system (T7bSS) that assembles in the bacterial envelope and promotes secretion of WXG-like proteins, and effectors bearing LXG domains. Secreted effectors of T7bSS have been associated with the pathogenesis of staphylococcal diseases and interbacterial antagonism. When studied in a mouse model of bloodstream infection, *S. aureus* USA300 mutants unable to activate T7bSS exhibit defects in establishment and persistence of abscess lesions within infected tissues, i.e., kidneys. Recently, we isolated *S. aureus* ST88 strain WU1, which causes outbreaks of skin and soft tissue infections in mouse breeding facilities. WU1 persistently colonizes the nasopharynx and gastrointestinal tract of C57BL/6 mice, which is associated with the risk of invasive disease. Hence, WU1 colonization and disease pathogenesis in mice resembles that of MSSA and MRSA strains in humans, where nasal carriage is a major risk factor for invasive infections. Here, we investigated the contribution of T7bSS to WU1 colonization and bacterial antagonism. We find that WU1 mutants in T7bSS structural components or secreted nuclease effector, were still able to successfully colonize the nasopharynx and gastrointestinal tract of mice and did not exhibit competitive disadvantage when challenged with wild-type strain *in vivo* and *in vitro*. However, unlike wild-type, the WU1 mutant unable to activate T7bSS did not exhibit lethal disease outcomes during bloodstream infection, and was diminished for its bacterial load and formation of abscesses. Together these experiments demonstrate that the T7bSS of *S. aureus* WU1 is a contributor to the pathogenesis of invasive disease in mice.

Poster Presentation #229

Biophysical Analyses of Master Quorum Sensing Regulator LuxR/SmcR Binding with dsDNA

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The marine dwelling genus of Gram-negative, and often pathogenic, bacteria called Vibrios communicate through quorum sensing by secreting autoinducers. At high cell densities, Vibrio senses autoinducers using receptors that function as phosphatases, triggering a dephosphorylation cascade, leading to a derepression of LuxR. LuxR is the master transcription factor that controls quorum sensing genes in Vibrio species. As a TetR-type protein, the homodimer of LuxR contains two helix-turn-helix DNA binding domains that bind to an AT-rich consensus sequence, found at >200 promoters in Vibrio campbellii; this function is conserved in other Vibrio species such as the pathogen V. vulnificus. Binding of LuxR(Vc) to its specific binding sites in promotors allows for the activation or repression of high cell density behaviors, such as bioluminescence and type III secretion, respectively. Crystallographic studies of the LuxR homolog, SmcR from V. vulnificus, identified two conformations of the DNA binding domain, narrow and wide, with a ~2.5Å difference. The N55I mutation was identified resulting in SmcR to only adopt the wide conformation. SmcR and the N55I mutant have yet to be crystallized while complexed with their activated and repressed DNA binding sites. The Wang lab crystallized a variant of LuxR from V. alginolyticus complexed with DNA binding sequences. We first began by repeating the published assays with LuxR(Va) and constructed a LuxR(Va) N55I protein. We aim to use similar crystallization conditions to study the crystal structure of SmcR complexed with various activating and repressing DNA sequences along with NMR data to determine conformations occupied by SmcR.

Poster Presentation #230

A Novel Anti-Sigma Factor that Alters OMV Cargo Selection in Bacteroides thetaiotaomicron

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Bacteroides spp. are Gram-negative bacteria that are abundant in the Western gut microbiota and contribute to intestinal homeostasis by modulating host immunity and degrading dietary fibers normally unavailable to the host. These functions may be facilitated by outer membrane vesicles (OMVs) produced by Bacteroides spp. in the gut because they are known to contain immunogenic and saccharolytic cargo. However, the physiological relevance of Bacteroides OMVs in the gut is not well understood because the mechanism by which OMVs are produced in Bacteroides spp. is currently unknown. Due to this, determining the mechanism of OMV biogenesis is essential to understanding Bacteroides physiology and how they shape the intestinal landscape. To identify genes involved in OMV biogenesis, we developed a high-throughput screening methodology, that utilized a chimeric nanoluciferase fusion protein as a reporter, in Bacteroides thetaiotaomicron. During the screening, we identified a transposon mutant, in BT_4721, that packages significantly more lipopolysaccharides and proteins into OMVs. BT_4721 is encoded in an operon with a putative ECF-type sigma factor, BT_4720, so we hypothesized that BT_4721 is likely the cognate anti-sigma factor of BT_4720. However, a structural prediction by AlphaFold revealed that BT_4721 potentially represents a novel protein structure that spans from the outer membrane into the cytoplasm. Our results suggest that BT_4721 will represent (1) the first single polypeptide to span both membranes of a Gram-negative bacterium, and (2) the first regulatory protein shown to impact OMV cargo selection.

Poster Presentation #231

Investigating How the Staphylococcus aureus Superoxide Dismutases are Regulated in Response to Manganese Starvation and Oxidative Stress

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During infection, pathogens must overcome the dual threats of oxidative stress and manganese starvation. Critical to the ability of *Staphylococcus aureus* to deal with these stressors and cause infection are two differentially regulated superoxide dismutases, SodA and SodM. The two superoxide dismutases contribute differentially to infection; SodA is important for resisting oxidative stress in manganese-replete environments, while SodM is important when *S. aureus* is manganese starved. However, the molecular factors that control their expression in response to the host metal chelator calprotectin and oxidative stress are unknown. The sRNA RsaC represses the translation of SodA in response to manganese limitation, but its broader impact on superoxide dismutase expression in response to calprotectin and oxidative stress is unknown. Expression analysis, conducted in the current work, revealed that the loss of *rsaC* decreased transcription of *sodA* and *sodM* in the presence of calprotectin and the superoxide generating compound paraquat. No significant change in *sodA* or *sodM* expression was observed in the absence of paraquat or calprotectin. The impact that the loss of SodA and SodM has on superoxide dismutase expression was also assessed. In metal replete medium in the presence or absence of paraquat, the loss of *sodA* increased transcription of *sodM*. This observation suggests that superoxide dismutase activity impacts SodM expression. Cumulatively, these studies revealed multiple factors that modulate staphylococcal superoxide dismutase expression, including an unexpected role for RsaC in regulating their transcription.

Poster Presentation #232

E. faecalis Secreted Proteases Modulate Host Inflammatory Response and Complement System for Ideal Colonization Conditions

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Urinary catheterization is a common practice in hospitals that predisposes patients to catheter associated urinary tract infections (CAUTI). *E. faecalis* is one of the most prevalent causative CAUTI pathogens, and its treatment is difficult due to intrinsic antibiotic resistances and the emergence of multidrug resistant strains. Previous studies using our mouse CAUTI model have shown the importance of Fg and fibrin deposition for *E. faecalis* CAUTI and established the importance of *E. faecalis* secreted proteases, SprE and GelE, for its persistence. We hypothesize that the secreted protease SprE modulates host inflammatory response to provide ideal conditions for growth and biofilm development in urine conditions. Comparison of WT, ΔsprE, ΔgelE, or ΔsprEΔgelE strains protease activity indicated SprE degraded Pg and plasmin, host factors that clear fibrin.

Additionally, we found neither protease targets thrombin, the activator of Fg into fibrin.

Furthermore, *E. faecalis* persists in CAUTIs despite robust immune response; however, it is unclear how this immune evasion occurs. In the endocarditis model, GelE degrades C3 to aid in immune evasion. This and SprE's activity against other host factors in urine lead us to hypothesize that in the CAUTI model, SprE modulates the host complement system for immune evasion. We found purified SprE leads to increased bacterial survival when incubated with primary immune cells in urine, degrades complement factors, and inhibits complement mediated opsonization. Overall, this study shows that the *E. faecalis* secreted protease SprE may be key to immune evasion during CAUTI.

Poster Presentation #233

Use of Whole-genome Sequencing to Investigate Antibiotic Resistance and Virulence Genes in Clinical Shiga toxin-producing Escherichia coli Isolates from Wyoming, 2002-2020

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Shiga toxin-producing *Escherichia coli* (STEC) are linked to ~265,000 illnesses per year in the United States. STEC infections can cause enteric symptoms and, in some cases, hemolytic uremic syndrome (HUS), especially in vulnerable populations like children under 5 years of age. Unlike other enteric infections, antibiotic treatment is not recommended because it is associated with increased risk of HUS. Antibiotics can enhance the production of Shiga toxins by stressing the bacterial cells, resulting in the activation of bacteriophages incorporated in the STEC genome that encode for toxin production. Despite the guidance against antibiotic treatment, antibiotic resistance has been reported in STEC strains, which is likely due to the presence of resistance genes acquired via natural selection. Therefore, it is important to enhance understanding of circulating antibiotic resistance genes in clinical STEC strains and define factors that increase the likelihood of infection. Herein, we used whole-genome sequencing and the genomic processing pipelines Terra.bio, Bactopia and DeepARG to analyze 270 strains from Wyoming patients collected between 2002-2020. All patient data have been extracted and genomes have been quality checked, assembled, and annotated to detect resistance and virulence genes as well as genes that dictate the serotype. An analysis of gene frequencies and trends over time is ongoing, while future analyses will examine trends by geographic location and cattle density, as cattle are an important STEC reservoir and ranching/farm work is a major industry in Wyoming.

Poster Presentation #234

The Lytic Transglycosylase LtgC is Necessary for Cell Separation and Survival in Neisseria gonorrhoeae

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Neisseria gonorrhoeae, the gonococcus (GC), is the etiological agent of the sexually transmitted infection gonorrhea and more severe disease states such as pelvic inflammatory disease and disseminated gonococcal infection. GC is a Gram-negative pathogen with seven core lytic transglycosylases (LTs) responsible for breaking down the peptidoglycan sugar backbone that is the structural component of the cell wall. The function of a few of these LTs are known. Two LTs from GC are known to produce disaccharide peptides that are released from the cell and are known to induce inflammation and damage tissue in human Fallopian tubes. The other LT with a known function is LtgC, which is required for normal cell separation. Using a human whole blood model, we found that the absence of LtgC resulted in a dramatic loss of gonococcal survival. Deletion of ltgC alone causes incomplete cell separation and clusters of unseparated cells, but deletion of the Escherichia coli homolog mltA has no detectable effect on cell separation or growth. Peculiar to LtgC is an extra domain, termed domain 3, only present in bacteria closely related to GC. Structural studies have indicated that domain 3 narrows the binding cleft of LtgC relative to MltA. Here we investigate the role of LtgC in cell separation and how the presence of domain 3 changes peptidoglycan substrates and protein-protein interactions that occur during septation and cell separation.

Poster Presentation #235

Towards a Systematic Genetic Approach to Define Regulatory Networks

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Bacteria respond to environmental stresses through signal transduction pathways, where the cell rapidly changes expression profiles of specific sets of genes. Due to the complexities of bacterial signal transduction, comprehensively understanding how the many inputs and regulators work together to modulate gene expression can be difficult. Even in model bacteria we understand how many of the individual components of signal transduction function but lack a detailed understanding of how multiple processes, pathways, and sets of genes work together to respond to stresses. Current methods of studying this regulation are limited, as there is not a good tool for assaying both genetic inputs and transcriptional outputs across multiple signal transduction pathways, or the regulatory network. Here, we describe a new genetic approach to comprehensively identify genes involved in the regulatory network. By combining CRISPR knockdown (CRISPRi) libraries with transcriptional reporters, we can identify signal transduction pathways and define regulatory networks in a high-throughput way. We validated this approach with a proof-of-principle experiment in E. coli and were able to recapitulate known biology as well as uncover new members of the regulatory network. As we currently lack a sufficient method of systematically studying signal transduction and gene regulatory networks, this approach will provide a valuable new tool to the field.

Poster Presentation #236

Investigating Macrophage Inflammatory State Transitions During Salmonella Infection

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Macrophages eliminate bacterial pathogens via phagocytosis and cytokine secretion. Despite these antibacterial properties, several pathogens, including Salmonella Typhimurium (STm), can survive and replicate within macrophages. During bacterial infection, macrophages can be in pro- or anti-inflammatory states. Although recent literature indicates that macrophages with replicating STm express anti-inflammatory genes and suppress pro-inflammatory genes, these studies only profiled macrophage inflammatory state at a late-stage infection timepoint. Therefore, it remains unknown when these transitions occur during infection and if their dynamics play a role in STm replication. To investigate the dynamics of macrophage state transitions during STm infection, we used smFISH and live-cell reporters of common inflammatory state genes. We found the pro-inflammatory gene iNOS was expressed after treatment with pro-inflammatory stimuli but not after STm infection. In contrast, we only observed high expression of the anti-inflammatory gene IL-4Rα in macrophages with high levels of STm replication. We then applied pro- and anti-inflammatory stimuli before, during, or after STm infection to investigate the extent to which the order of stimuli exposure impacts these transitions. When we infected macrophages with STm prior to treatment with anti-inflammatory stimuli, we observed expression of anti-inflammatory genes at levels not observed under any other condition. These results suggest that although STm infected macrophages express anti-inflammatory genes, this cell state may be distinct from that induced by common anti-inflammatory stimuli alone. Future work will use multiplexed smFISH, live-cell reporters, and gene knockout approaches to investigate the dynamics of how STm infected cells enter this distinct inflammatory state.

Poster Presentation #237

Post-transcriptional Regulation of Virulence Factor InlB Leads to Increased Vertical Transmission of Listeria monocytogenes

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Listeria monocytogenes (Lm) is a common environmental bacterium that can also act as a foodborne pathogen. Susceptible individuals such as pregnant women are more likely to be infected and develop severe invasive disease. Epidemiologic data has long supported the observation that certain clades of Lm are much more likely to be associated with epidemics and severe disease, however the reasons for this within species variation are not fully understood. We have previously published the observation that high InlB expression in certain Lm strains confers enhanced vertical transmission in animal models. InlB is a virulence factor that is non-covalently bound to the cell surface and promotes bacterial uptake into host cells. One highly invasive strain, 07PF0776, has approximately 15-fold more surface InlB compared to reference strain 10403S. This difference persists when prfA, a virulence regulator that controls *inlB* transcription, is activated. Despite the difference in protein abundance, there is no difference in *inlB* transcript between the two strains. Similarly, there is no difference in InlB protein stability or cell wall affinity. Switching *inlB* alleles between strains does not impact surface InlB levels. However, expression of *inlB* from a plasmid abolishes the difference in expression. These findings indicate that some Lm strains exhibit increased InlB abundance due to alterations in pre- or co-translational regulation of *inlB*. These data also suggest that expression of *inlB* from its native locus is necessary for the observed regulation.

Poster Presentation #238

The Role of Cyclic Dimeric Guanosine Monophosphate Specific Phosphodiesterases (PDE's) in Regulating Different Phenotypes in Shigella flexneri

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Shigella causes bacillary dysentery, known as Shigellosis. Shigellosis is characterized by fever, abdominal cramps and diarrhoea. Shigellosis affects an estimated 80-165 million persons annually, resulting in 600,000 deaths. Currently, there are no vaccine for shigellosis. Antibiotics are effective; however, Shigella's advancing antibiotic resistances makes treatment challenging. Shigella's success is owed to its low infectious dose and ability to invade epithelial cells.

Shigella uses the second messenger c-di-GMP to regulate various bacterial phenotypes including virulence. C-di-GMP homeostasis within a bacterial cell is maintained by two classes of enzymes: diguanylate cyclases (DGC) which synthesize c-di-GMP and phosphodiesterases (PDE) which hydrolyse c-di-GMP.

Shigella encodes 4 putative DGCs, and 6 putative PDEs. These enzymes contain sensory domains which interacts with environmental cues, and in turn dictates their activities. Deletion of Shigella DGC's results in decreased invasion, plaque size, biofilm, and increased resistance to acid shock. However, we do not know how c-di-GMP specific PDE's regulate these phenotypes.

The objective of my research is to determine how PDEs regulate Shigella's virulence phenotypes. I created Shigella knockouts of the 6 PDEs to characterize their impact on Shigella's behaviour. I have found that Shigella's PDE knockout strains formed larger plaques, had greater invasion frequency in Henle-407 cells and decreased resistance to acid shock.
Shigella PDE mutants behave oppositely than DGC mutants. Here we demonstrate how varying c-di-GMP levels in bacterial signalling can impact phenotypic expression. This study will provide a greater understanding of Shigella's ability to overcome environmental hurdles, which enables it to cause its grave disease.

Poster Presentation #239

Expression of Botulinum-like Toxins in Enterococcus and Weissella

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Botulinum neurotoxin (BoNT) is a potent proteinaceous toxin produced by several species of Clostridium and is the causative agent of botulism, a potentially life-threatening disease typified by flaccid paralysis. Mechanistically, BoNT's act through proteolysis of SNARE proteins within neuronal cells at the neuromuscular junction, resulting in a loss of acetylcholine signaling from the neuron to the muscle cell. Over the past decade bioinformatic studies have revealed the presence of botulinum neurotoxin homologs in genera outside of Clostridium including Paraclostridium, Paeniclostridium, Enterococcus, Weissella, and Chryseobacterium. While enzymatic activity against SNARE proteins in vitro has been demonstrated for many of these homologs, their capacity for causing disease in mammalian species, cell binding preference, and the conditions for their expression by their native hosts remain mostly unknown. Utilizing polyclonal mouse antibody raised against recombinant, synthesized botulinum-like toxins discovered bioinformatically in Enterococcus and Weissella, we determined that both homologs are produced by their native strains under standard growth conditions. We discuss these findings in relation to the broader trend by which bioinformatics increasingly plays a central role in the novel discovery of medically and pharmaceutically relevant biomolecules and provides novel insight into the broader family and the evolutionary past of key virulence factors.

Poster Presentation #240

Investigating the Impact of Mycobacterium tuberculosis Biofilm Phenotype on Antibiotic Tolerance

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Mycobacterium tuberculosis (*M. tb*), the causative agent of tuberculosis (TB), remains one of the leading causes of death by a single infectious agent. Standard treatment for active TB is a 6-9-month course of antibiotics, with further treatment for antibiotic resistant and recurrent infections. As concerns arise about the increasing prevalence of antibiotic resistant TB cases, careful study of the mechanisms by which *M. tb* persists through long-term antibiotic exposure will aid in development of new, more effective treatments. Previous studies have indicated that *M. tb* biofilms may play a role in persistence, by providing a protective extracellular matrix that shields cells from antibiotic exposure. Our work studying biofilms of clinical isolates shows that biofilm phenotype varies widely between strains, and through experimental evolution we have evolved strains with very robust biofilm phenotypes. To investigate the power of biofilms in persistence, we have incubated strains of *M. tb* with different biofilm phenotypes with two first line antibiotics—Isoniazid and Rifampicin—and then performed quantitative cultures to determine survivorship. From this experiment, we hope to gain additional insight into the role that biofilm formation and genetic variance plays in antibiotic tolerance in TB.

Poster Presentation #241

Towards Generalized Platforms for Functional Genomics in Alphaproteobacteria

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Alphaproteobacteria play diverse roles in the environment, human health, and industry, but many of the genes required to carry out these roles have remained enigmatic. Next generation genetic tools, such as CRISPRi-seq, are capable of systematically phenotyping all genes, but have not been broadly deployed in alphaproteobacteria. Building on our success in establishing genome-scale CRISPRi in *Zymomonas mobilis*, we seek to develop generalized platforms for synthetic biology and functional genomics alphaproteobacteria. Here, we lay the groundwork for these platforms by optimizing site specific integration and developing synthetic, inducible promoters for *Novosphingobium aromaticivorans* and *Rhodobacter sphaeroides*. These enhanced genetic tools will enable basic and applied research such as delivery of CRISPRi systems to investigate gene function and expression of heterologous pathways to generate valuable bioproducts.

Poster Presentation #242

Peptidoglycan-degrading Amidase AmiC is Required for Neisseria Infection

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Neisseria gonorrhoeae (Gc) is gram-negative diplococcus that causes the sexually transmitted infection gonorrhea. *N. gonorrhoeae* infects multiple mucosal surfaces and causes cervicitis, urethritis, proctitis, and pharyngitis. Gonococci can also spread through the blood to cause disseminated gonococcal infection. As *N. gonorrhoeae* is a human-specific pathogen, it has been difficult to study gonococcal pathogenesis in an animal model. A recently developed mouse model uses the commensal species *Neisseria musculi* which is a natural colonizer of the pharynx and oral cavity in wild mice. We extended this model to include blood infection and are using it to study effects of peptidoglycan-related proteins in infection. The peptidoglycan-degrading amidase, AmiC, was found to be involved in cell separation in *N. musculi*, as it is in gonococci. Upon deletion of *amiC*, *N. musculi* cells grew in chains. Experiments using *N. musculi* showed a decrease in oropharyngeal colonization as well as in blood infection in mice. I*n vitro* experiments with gonococci infecting human blood also show a decrease in infection with the *amiC* mutant or that of its activator *nlpD*. Additionally, AmiC was shown to interact with another peptidoglycanase, LtgC, which is required for cell separation. While *amiC* or *nlpD* mutation does not cause decreased growth rates *in vitro*, clear defects in infection are seen in mouse colonization or in human blood infection. These results suggest that targeting divisome components such as LtgC, AmiC, or NlpD will be a promising approach for finding new antimicrobials.

Poster Presentation #243

Inhibiting Type-I Interferon Signaling Promotes Memory T-cell Formation following Immunization with a Listeria-based Vaccine

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Vaccines have led to the control, or eradication of numerous infectious diseases, however, an inability to engage the cell-mediated adaptive immune system has allowed other diseases to persist. Listeria monocytogenes (Lm) has emerged as a powerful platform for generating cell mediated immunity and has been employed as a cancer vaccine. The Lm-induced CD8+ T-cell response is modulated by the innate immune response. Thus, to dissect the impact of type I interferon signaling and inflammasome activation on Lm-induced T-cell responses, we immunized IFNAR-/-, Caspase1/11-/-, and novel IFNAR-/-Caspase1/11-/- knockout mice we generated for this study. The phenotype and magnitude of CD8+ T-cell responses were assessed at the peak T-cell response, after contraction and memory formation, and after rechallenge. IFNAR-/- mice developed the largest number of CD8+ T-cells during the peak primary response, contradicting the dogma that Type-I Interferon promotes robust CD8+ T-cell responses. Importantly, these cells had a memory precursor phenotype, suggesting enhanced long-term persistence. These findings extend to the memory and recall stage with more antigen-specific T-cells observed after contraction and upon rechallenge. Casapse1/11 deficiency did not impact T-cell responses. IFNAR-/- mice were significantly more protected from wild-type Lm rechallenge than their wild-type counterparts, demonstrating the functional consequence of the increased memory T-cell pool. Finally, in ex vivo assays, we show that the cell type responsible for sensing type-I IFN are dendritic cells. In conclusion, type-I interferon signaling deficiency leads to enhanced vaccine efficacy through increased memory T-cell formation, potentially informing the creation of more effective Lm vaccines in the future.

Poster Presentation #244

Infection with Mycobacterium tuberculosis Drives Gene Expression Changes in a Novel Alveolar Macrophage Model

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Macrophages are innate immune cells that digest cellular debris, cancerous cells, and foreign substances such as microbial pathogens. Furthermore, specialized tissue-resident macrophages are critical for maintaining homeostasis in tissues including the lungs and liver. Alveolar macrophages (AMs), the tissue-resident macrophages of the lungs, are the first to encounter inhaled pathogens and are responsible for coordinating the lung immune response. Historically, AMs have been difficult to study ex vivo, as they are challenging to isolate and do not maintain AM-like properties long-term in culture. We recently developed a novel genetically tractable fetal liver-derived alveolar-like macrophage (FLAM) model which allows for long-term maintenance of alveolar-like macrophages in culture, enabling mechanistic study of AM function during infection. We hypothesize that FLAMs can be leveraged to better understand host responses to the respiratory pathogen Mycobacterium tuberculosis (Mtb). Mtb uses AMs as a protected niche to drive pathogenesis often resulting in dysregulated inflammation within the lungs which contributes to disease. To test our hypothesis, we examined changes in the transcriptional landscape in resting and IFNg activated FLAMs during Mtb infection. Using fluorescence activated cell sorting we isolated both infected and bystander FLAMs following Mtb infection and compared them to uninfected FLAMs. Our results show that Mtb infection of FLAMs drives a strong Type I IFN signature and recapitulates many aspects of AM innate responses. Our study serves as a springboard for the identification of unique AM inflammatory networks during Mtb infection that can be leveraged to dissect mechanisms of overactive inflammatory responses to lung infections.

Poster Presentation #245

Characterizing the Role of Pseudomonas aeruginosa Polyphosphate in the Killing of Staphylococcus aureus

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Pseudomonas aeruginosa and Staphylococcus aureus are opportunistic pathogens known to colonize different locations of the human body and cause acute infections. Lung colonization with P. aeruginosa is responsible for reducing the life expectancy of patients with cystic fibrosis (CF) by ten years. Interestingly, S. aureus, which colonizes CF lungs prior to P. aeruginosa, has been shown to be prone to killing by P. aeruginosa although the precise mechanism is still under investigation. We recently made the exciting discovery that the bacterial stress defense system, polyphosphate (polyP), is involved in P. aeruginosa's ability to kill S. aureus. P. aeruginosa produces polyphosphate during various stress situations, which protects them from protein aggregations and contributes to biofilm formation. Polyphosphate-deficient strains, lacking the gene encoding polyphosphate kinase 1 (ppk1), demonstrate attenuated stress defense and biofilm formation. Intriguingly, upon exposing S. aureus to P. aeruginosa supernatants, we observed that the wild-type inhibited its growth more than Δppk. We hypothesized that this could either be because of polyphosphate mediated production of virulence factors or a direct effect of polyphosphate on growth of S. aureus. We tested the production of virulence factors in Δppk and Δppx (lacking the enzyme expolyphosphatase which breaks down polyphosphate) mutants and found that the production of pyocyanin was significantly higher in Δppx compared to Δppk cells. By understanding the role of polyphosphate, we can shed more light on how this biopolymer regulates the virulence of P. aeruginosa and this can further help in designing alternative therapeutic treatments for combating P. aeruginosa infections.

Poster Presentation #246

Development and Validation of a Novel 3'3'-cGAMP Monoclonal ELISA

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Second messengers are small molecules that cause a wide variety of cellular changes both transcriptionally, translationally, and post translationally. 3'3'-cGAMP is a bacterial second messenger produced from ATP and GTP by specific dinucleotide cyclases. It is produced and regulated via two different pathways, which use distinct classes of synthases, effectors, and phosphodiesterases (PDEs). 3’3’-cGAMP binds to riboswitches to regulate motility, biofilm formation, virulence, and colonization through gene transcription. The study of cyclic di-nucleotides (CDNs) in bacterial innate immunity is a growing area of research. Current detection methods utilize mass spectroscopy, which can be both costly and timely. In addition to that, the isolation and purification of CDNs can be complicated and tedious. Rapid and accurate detection methods are critical to enable researchers to study and/or identify the relevant biological pathways.

This poster will focus on the development and validation of a novel 3’3’-cGAMP monoclonal enzyme linked immunoassay (ELISA). The data provided will demonstrate a highly sensitive and specific ELISA validated by LC-MS/MS.

Poster Presentation #247

Using Experimental Evolution to Identify the Genetic Determinants of Gardnerella vaginalis Biofilm Formation

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Gardnerella vaginalis (GV) is a prominent member of the vaginal microbiome and is the causative agent of bacterial vaginosis (BV), a common reproductive disease that can present adverse outcomes for pregnant women. BV is characterized by overgrowth of GV and other anaerobes in a multispecies biofilm, which is thought to play a central role in BV pathogenesis. It is hypothesized that GV acts as the initial "scaffold" for biofilm formation; however, little is known about the mechanism underlying the formation and maintenance of the BV biofilm. Therefore, we set out to identify the genetic determinants of *G. vaginalis* biofilm formation. To accomplish this, I have created a novel in vitro system that uses experimental evolution to select for adherent biofilm cells. Nine replicate GV populations were evolved independently with serial passaging, and optical density and crystal violet stain measurements for each biofilm were recorded over time to determine phenotypic change. I then performed whole genome sequencing (WGS) of ancestral and evolved biofilm populations to identify mutations accompanying changes in biofilm phenotype. I discovered that evolved GV strains generally make denser, "clumpier" biofilms, as shown by increased crystal violet stain and scanning electron microscopy (SEM). Additionally, we identified signs of positive selection and convergent mutations within a type I fatty acid synthase (FAS) gene (GAVG_1022), which acquired at least one mutation in all replicate populations. These results indicate that type I FAS may serve an important function during GV biofilm formation, and future directions will interrogate the mechanism of this function.

Poster Presentation #248

Helicobacter pylori Inhibits Phagosome Maturation in Human Neutrophils

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Helicobacter pylori (Hp) is a microaerophilic gram-negative bacterium that resides in the mucus layer of the human stomach. Patients with Hp can develop gastritis, peptic ulcers, and gastric adenocarcinoma. Hp infections are characterized by an influx of polymorphonuclear neutrophils (PMNs); however, it has been found that despite the influx of PMNs Hp can resist phagocytic killing and thrive, surviving in neutrophils for at least 48 hours. Proper phagosome maturation in PMNs is mediated by fusion with three types of granules (azurophil, specific, and gelatinase) that contain antimicrobial enzymes, cationic peptides, iron-binding proteins and degradative enzymes including catalytic subunits of the NADPH oxidase complex (gp91phox/p22phox). Previous studies in our lab have shown that when PMNs are infected with Hp NADPH oxidase subunits are diverted away from phagosomes to the plasma membrane, but whether this reflects a broader defect in phagosome maturation is unknown. In these studies, we aim to understand the mechanisms of which Hp use to inhibit phagosome maturation in human PMNs, thus evading death. To this end we compared unopsonized live Hp to formalin-fixed bacteria and Hp opsonized with IgG and phagosome composition was analyzed by confocal microscopy and multiple time points after bacterial uptake. Compartments containing live, unopsonized Hp excluded markers of all three granules, and this was diminished by opsonization with IgG whereas differences between live and fixed bacteria suggest a role for secretion in modulating bacterial fate. These data advance understanding of Hp pathogenesis and fate of this bacterium in neutrophils.

Poster Presentation #249

Investigating the Antibiotic Synergizing Effects of the Novel Antimicrobial AGXX®

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In light of emerging antimicrobic resistance, drug development has expanded into alternative antimicrobial compounds. Our work focuses on the antimicrobial effects of AGXX, a novel antimicrobial surface coating composed of silver and ruthenium. AGXX exerts its effects through the production reactive oxygen species (ROS). Due to recent connections identified between ROS-stress and antibiotic activity, we hypothesized that AGXX could potentially increase the activity of conventional antibiotics Using the gram-negative opportunistic pathogen *Pseudomonas aeruginosa*, one of the major causes of acute and persistent infections, we investigated possible synergistic effects of AGXX on several antibiotic classes. We discovered that combined treatment of AGXX with aminoglycoside antibiotics, exponentially enhanced their bactericidal activity against *P. aeruginosa*. More importantly, we found that this synergistic combination restored sensitivity in a kanamycin resistant *P. aeruginosa* strain. To explore the underlying mechanism of the synergizing effects of AGXX on aminoglycosides, we utilized redox probes and found that combined treatment of AGXX and aminoglycosides significantly increased oxidative stress levels in *P. aeruginosa* cells which was attenuated with the addition of thiourea, an ROS scavenger. We discovered that the increase in oxidative stress resulted in a disruption of iron homeostasis evidenced by a significant decrease in aconitase activity. We further demonstrate that combined treatment of the two antimicrobials also inflicted significant membrane damage, thus increasing membrane permeability. Our subsequent goal is to delineate the mechanisms that underpin this synergistic effect by exploring how the metabolic processes and cellular targets disrupted by AGXX contributes to the synergistic effects with aminoglycosides.

Poster Presentation #250

Aryl Polyenes Expressed by Uropathogenic *Escherichia coli* Contribute to Pathogenesis in a Mouse Model

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Uropathogenic *Escherichia coli* (UPEC) is a causative agent of urinary tract infections (UTIs), and one of the most common causes of pyelonephritis. Interestingly, UPEC is often found as a non-pathobiont in the gut flora. The transition from gut flora to pathogen for UPEC is dependent upon a flexible tropism, and a number of virulence factors that aid in UPEC's colonization and invasion of the urinary tract. We recently demonstrated a role for the *E. coli* aryl polyenes (APEs) as a potential UPEC fitness factor and hypothesize that APEs contribute to UPEC virulence in the host. APEs are encoded by a biosynthetic gene cluster (BGC) family identified among many Gram-negative bacteria. The products of this BGC family are lipids comprised of an aryl head group and conjugated double bond system, which are structurally reminiscent of established virulence factors found in Gram-positives. Additionally, UPEC APE expression conferred resistance to reactive oxygen species and enhanced biofilm formation. In a UTI mouse model, infection with UPEC that constitutively expressed APEs increased pathogenicity with increased TNF-α and MCP-1 levels observed in collected plasma. Transcriptomics analyses of *in vitro* co-incubation experiments indicate that the presence of APE+ during UPEC exposure influences the macrophage gene transcription profile. Our findings suggest that APE expression aids in bacterial virulence and immune modulation during infection. Therefore, targeting the biosynthetic gene product APE in parallel with immune inhibitors may be a viable therapeutic approach to reduce fitness of pathogenic populations while leaving commensal gut microflora unharmed and minimize tissue damage.

Poster Presentation #251

Pseudomonas Sabotage: Determining How Contact Dependent Growth Inhibition Tips the Scales during Interbacterial Competition

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Pseudomonas aeruginosa is a gram-negative ESKAPE pathogen, typically contracted in healthcare settings. The species sports a broad range of virulence factors that it deploys to colonize hosts and compete with other organisms. Contact dependent growth inhibition, or CDI, is a type Vb secretion system consisting of paired toxin and immunity factors used to confer an advantage over neighboring bacteria. Typically, the system is studied via competition assays that pit wild-type "attacker" strains producing a functional CdiA protein against isogenic, mutant "targets" lacking the toxin and immunity factor. Targets are labelled with an antibiotic resistance cassette for quantification on selective media after competition. However, this method does not inform whether a lack of recoverable target cells following competition is because they have been killed, or whether intoxication limits replication only in the presence of antibiotic selection. To determine the effects of the toxin on vulnerable cells, we employed fluorescent microscopy and flow cytometry to count cells directly after competition. The change in ratio of attacker to target indicated by these methods was more modest than observed by CFU. This suggests either a synergistic effect of antibiotic and CDI toxin, or an effect that leaves intoxicated cells intact following competition but prevents growth. We have begun employing FACS to track intoxicated target cells in isolation to better determine which is the case. A better understanding of CDI intoxication could inform on how CDI promotes P. aeruginosa fitness in complex microbial environments and whether they may be useful as novel antimicrobial therapeutics.

Poster Presentation #252

Investigating Putative Toxin-immunity Pairs from a New Class of Rhs Proteins

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Bacteria compete for limited resources within complex environments and have evolved numerous strategies to enable such competition. One tactic is to directly antagonize the replication of nearby rivals (i.e., interference competition). Protein effectors of interference competition are often composed of multiple domains where the N-terminal regions of the protein guide secretion and delivery of a C-terminal "toxin" domain into a neighboring cell. Siblings of toxin-producing strains are protected because they encode a cognate immunity factor that binds and neutralizes the toxin domain. These proteins are often polymorphic, meaning the same protein in a different bacterial strain may contain a different toxin domain. We recently uncovered a new class of polymorphic effectors in Pseudomonas aeruginosa. Our data suggest that this protein exists as a heterodimer of proteins that have rearrangement hotspot (RHS) elements. We propose that these RHS elements act as two halves of a complete shell that encapsulates a C-terminal toxin domain. As with other polymorphic toxins, we identified a small downstream gene that co-varies with the toxin domain. Here we demonstrate that this variable C-terminal domain is a potent toxin domain that can be neutralized by the specific downstream immunity factor. We also demonstrate that the toxin and immunity factor tightly bind to each other, suggesting the function of the immunity factor is to neutralize the toxin by direct binding. Understanding how bacteria have evolved to kill each other may open the door for the development of innovative antimicrobial therapies.

Poster Presentation #253

The Klebsiella pneumoniae ter operon Enhances Stress Tolerance

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Healthcare acquired infections are a leading cause of disease in patients that are hospitalized or in long-term care facilities. *Klebsiella pneumoniae* (Kp) is a leading cause of bacteremia, pneumonia, and urinary tract infections in these settings. Previous studies have established that the *ter* operon is associated with infection in colonized patients. Rather than enhancing fitness during infection, the *ter* operon increases Kp fitness during gut colonization. The *ter* operon confers resistance to tellurite oxide (K2TeO3), however the biologically relevant function of this operon is unknown. First, using a murine model of urinary tract infection, we demonstrate a novel role for the *ter* operon protein TerC as a bladder fitness factor. To further characterize TerC, we explored a variety of functions, including resistance to metal-induced stress, resistance to ROS-induced stress, and sugar transport, all of which were independent of TerC. Then we determined that TerC is necessary for tolerance to ofloxacin, polymyxin B, and cetylpyridinium chloride. We used an ordered transposon library constructed in a Kp strain lacking the *ter* operon to identify genes required to resist K2TeO3- and polymyxin B-induced stress; the results suggested that K2TeO3-induced stress is experienced at the cell envelope. Finally, we confirmed that K2TeO3 disrupts the Kp cell envelope by measuring levels of Ethidium bromide accumulation following exposure to K2TeO3. Collectively, these studies indicate a novel role for the *ter* operon as a stress tolerance factor, potentially explaining its role in enhancing fitness in the gut and bladder.

Poster Presentation #254

Elucidating the Role of the Host Factor Fibrinogen in Impairing Neutrophil Function and Permitting Pathogen Persistence During Catheter-Associated Urinary Tract Infections

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Catheter-associated urinary tract infections (CAUTIs) are the most common healthcare associated infections worldwide. Urinary catheterization is a necessary and common procedure in healthcare facilities; however, placement of urinary catheters has been shown to predispose patients to the development of CAUTI. *Enterococcus spp.* are among the most common pathogens of CAUTIs; however, their prevention and treatment are difficult due to their wide spectrum of antibiotic resistance. Previous work with our murine CAUTI model shows that urinary catheterization leads to bladder inflammation, edema, and robust neutrophil recruitment. Despite the influx of neutrophils, *E. faecalis* (EF) persists and thrives in the catheterized bladder. This project seeks to characterize the neutrophilic response to EF CAUTI and identify how the host factor fibrinogen (Fg) affects neutrophil behavior, ultimately permitting bacterial persistence. I hypothesize that Fg accumulation during CAUTI drives neutrophils away from bactericidal behavior and dysregulates death programming, further inhibiting pathogen clearance. I further believe that Fg-MAC-1 integrin interactions are driving this altered behavior. I have shown that the presence of Fg enhances bacterial survivability in the presence of primary neutrophils, and that Fg-MAC-1 interactions may be partly responsible for this lack of pathogen killing. Furthermore, Fg presence does not appear to substantially reduce phagocytic uptake of *E. faecalis* in an ex vivo system. I also show that neutrophils bias towards survival when in the presence of both EF and Fg. Better understanding of how necessary host factors can dysregulate the immune system will allow for improved intervention targets in preventing and eliminating CAUTI.

Poster Presentation #255

Discovery of New Phage Defense Systems in Vibrio cholerae

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Phage therapy has been used for almost a century and has experienced renewed interest due to phages ability to lyse and target specific bacteria, avoiding the broad disruption to the microbiome caused by antibiotics. Although phages can infect and lyse bacteria, bacteria have evolved a diverse set of defense mechanisms to protect against phage infection. Thus, it is important to identify and understand the mechanisms by which phage defense systems function. Although many phage defense mechanisms have recently been identified, other mechanisms of phage defense have yet to be studied. I hypothesized that *Vibrio cholerae*, which has two known phage defense systems, encodes other novel phage defense systems. To identify these systems, I screened a *V. cholerae* cosmid genomic library in *E. coli* for segments of *V. cholerae's* genome that protected *E. coli* from T2 phage infection. Two unique cosmids, each encoding approximately 25 kB of V. cholerae DNA, protected *E. coli* from T2 infection. One of these regions have yet to be implicated in phage defense, suggesting these cosmid genes encode novel defense systems that have yet to be studied. I isolated transposon mutants of both cosmids that lost defense and I'm currently cloning and deleting genes that had transposon insertions to determine the genetic basis of these phage defense systems. My studies will lead to the identification of new *V. cholerae* phage defense mechanisms that will increase our understanding of the evolution and ecology of *V. cholerae* while highlighting important mechanisms by which bacteria can resist phage therapy.

Poster Presentation #256

Investigating the Dynamics of phoPQ Expression During Salmonella Infection

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Salmonella Typhimurium (STm) is an intracellular pathogen that can replicate within macrophage phagosomes. To replicate STm must first detect the phagosome environment using two-component systems (TCS); for example, PhoPQ detects and responds to low magnesium. Not all STm replicate intracellularly, and those that do replicate asynchronously and to different levels. While *phoPQ* is required for intracellular replication, it remains unknown if the dynamics of *phoPQ* expression during infection are linked to the variation in intracellular replication. To investigate this, single-cell approaches are needed; therefore, we constructed a fluorescent live-cell *phoP* promoter reporter. We used microscopy and flow cytometry to validate that our reporter is induced under limited magnesium. We observed that under limited magnesium not all STm induced the reporter, and those that did had variable levels of reporter expression, while in high magnesium a small subpopulation expressed the reporter. Before measuring the dynamics of reporter induction in a phagosome, we confirmed that *phoPQ* is required for STm replication during macrophage infection by using gene knockout strains. From preliminary macrophage infection experiments with the reporter strain, we observed expression in the phagosome and are in the process of analyzing the relationship between the timing of reporter induction and STm replication. These results suggest that STm responses to magnesium changes are heterogeneous and that STm with basal expression may be primed to detect the phagosome environment. Future studies will focus on tracking reporter induction over time using live-cell microscopy and varying environmental conditions to measure the effect on *phoPQ* induction dynamics.

Poster Presentation #257

Interrogating the Requirements for Vibrio cholerae Growth on TCBS using a Randomly Barcoded Transposon Mutant Library

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Random barcode transposon-site sequencing (RB-TnSeq) is a fast, cost-effective method of profiling mutant fitness in various environments. My research involves the development of an RB-TnSeq library in the facultative human pathogen *Vibrio cholerae*. I have successfully constructed and mapped a transposon mutant library of more than 130,000 mutants, each with a unique 20nt barcode that is tied to each mutant's transposon insertion site. As a first demonstration, I have quantified the fitness of these mutants in thiosulfate-citrate-bile salts-sucrose (TCBS), a selective medium for *Vibrios*.

While TCBS is routinely used to isolate *Vibrios* from environmental samples, its capacity to inhibit the growth of other gram-negatives suggests that there are physiological requirements for survival and sustained growth in the presence of any (or all) of the medium's selective components. In support of this idea, we serendipitously discovered that the large subunit of exonuclease VII, *xseA*, is required for *V. cholerae* growth on TCBS agar. The surprising finding that a ssDNA exonuclease is required for growth on TCBS led us to question the broader genetic requirements for *V. cholerae* growth on this selective medium.

To this end, I have isolated ~500,000 barcoded transposon mutant colonies grown on TCBS agar. By sequencing the most abundant barcodes in the selected mutant pools, my research gives insights as to which genes are required for cultivation of *V. cholerae* from environmental samples on TCBS and, more broadly, may highlight environmental *Vibrio* mutants that cannot be cultured using TCBS and are currently undetectable using standard methodologies.

Poster Presentation #258

Desiccation Resistance and Persistence in Enterobacter cloacae

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Enterobacter cloacae is a Gram-negative, opportunistic pathogen that causes serious infections in clinical settings. Frequently the cause of hospital associated infections, *E. cloacae* can be transmitted between patients through contaminated hospital surfaces. To survive on fomites, it must overcome the stress of desiccation as well as treatment with disinfectants. We demonstrated that *E. cloacae* strain ATCC13047 remains viable after up to 140 days of desiccation, suggesting that persistence on hospital surfaces could facilitate transmission between patients. We tested the effectiveness of multiple disinfectants against planktonic *E. cloacae* cells and demonstrated that the minimum inhibitory concentration (MIC) of each disinfectant to be 50% for ethanol, 25% for quaternary ammonium compounds, and bleach being the most effective with an MIC of 1.5625%. We are evaluating the bactericidal activity of these compounds against long-term desiccated *E. cloacae* to test the hypothesis that desiccation enhances resistance to disinfectants. Our work will elucidate molecular mechanisms underpinning desiccation tolerance. It will also provide insights into developing better decontamination procedures to break the transmission cycle between patients in hospital settings.

Poster Presentation #259

Leveraging Mycobacterium abscessus and Mycobacterium tuberculosis Resistance Mutations to Drive Rational Drug Development of MmpL3 Inhibitors

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Members of the Mycobacterium genus such as Mycobacterium tuberculosis (Mtb) and Mycobacterium abscessus (Mab), cause serious infectious diseases in humans, requiring long courses of therapy. This long course of therapy is associated with the evolution of drug resistance, necessitating the discovery of new antimycobacterial agents. MmpL3, an inner membrane protein that transports trehalose-monomycolate to the mycomembrane is considered an attractive drug target because it is essential for Mtb and Mab survival. However, targeting MmpL3 introduces a dichotomy due to its structural differences between Mtb and Mab, rendering some compounds more active against Mtb and others against Mab. Accordingly, using a combination of in vitro strategies such as isolation and characterizations of resistant mutants and cross-resistance profiling, and in silico strategies such as molecular dynamic simulations, we are identifying molecular differences of MmpL3 in Mtb and Mab to rationally design more effective drugs against Mtb and NTM (Nontuberculous mycobacteria) infections, respectively. Towards this goal, we have isolated and sequenced 33 unique resistant mutants to four novel MmpL3 inhibitors in Mab. Most of the resistant mutants had mutations in MmpL3. Cross-resistance profiling between the resistant mutants and the different inhibitors shows differential patterns of sensitivity and resistance, indicating differential ligand-protein interactions. Using these genetic data, modeling studies are underway to define the structural features in Mmpl3 associated with resistance and susceptibility in Mtb and Mab.

Poster Presentation #260

Transcriptional Regulator VpsR Regulates Biofilm Composition in Vibrio fischeri During Host Colonization

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Cyclic diguanylate (c-di-GMP) is a signaling molecule known to regulate motility and biofilm formation in bacteria, though its role during beneficial host colonization is not well understood. Using the model symbiosis between *Vibrio fischeri* and the Hawaiian bobtail squid (*Euprymna scolopes*), we previously reported that a strain with constitutively high c-di-GMP levels has increased cellulose production, decreased motility, and a host colonization defect compared to wild type. Further analysis of the *in vivo* biofilm produced by the high c-di-GMP strain showed that the biofilm was high in cellulose and low in symbiosis polysaccharide (Syp) that is required for host colonization, and that this altered biofilm composition likely contributes to poor colonization by the strain. Mutation of the cellulose synthase gene (*bcsA*) decreased cellulose biofilm and restored *syp* transcription, but did not rescue host colonization, suggesting the presence of a regulator that may regulate Syp and cellulose. To identify this factor, we screened transposon mutants of the high c-di-GMP strain for diminished cellulose. The screen revealed that the σ^{54}-dependent transcriptional regulator VpsR activates cellulose production, and a deletion of *vpsR* rescues colonization by the high c-di-GMP strain without lowering c-di-GMP levels. This work demonstrates that maintaining appropriate biofilm composition is vital to host colonization and suggests a role for VpsR in regulating symbiotic biofilm composition under conditions of elevated c-di-GMP.

Poster Presentation #261

Variation in Biofilm Forming Ability of Pathogenic E. coli Isolated from Human Urine

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Pathogenic E. coli exhibit variations in virulence including biofilm-forming ability, and multidrug resistance. High biofilm formation in pathogens has been linked to recurring infections in humans that can lead to severe infections. Therefore, we hypothesized that pathogenic E. coli biofilm forming ability will be correlated with higher virulence. Our goal was to determine factors contributing to higher biofilm-formation and disease severity in patients. For this, we screened virulence genes (*stx* 1 and 2, *eae, cnf1, aggR, pic, astA, bfpB*, and *hlyA*.) in clinical E. coli (N=11) isolates from urine in patients by gene-specific amplification. We also tested their biofilm-forming ability by static crystal violet method and antibiotic resistance by VITEK. Growth on blood (5%) agar resulted in nine isolates exhibiting α-hemolysis. Notably all, except one isolate, were positive for either *cnf1* (N=8) or *astA* (N=2) toxins. Isolates were also found to be positive for *pic* (N=3), *eae* (N=2) and *aggR* (N=1) important for pathogen colonization. High strain-dependent biofilm variation was observed with three isolates being classified as high biofilm-formers (OD595 >3 normalized to 1ml culture). Antibiotic testing revealed resistance to multiple antibiotics including aminoglycosides (N=5) and cephalosporins (N= 6). Clinical pathogens and their virulence were found to be highly variable between isolates with no significant correlation. Virulence genes were therefore highly isolate dependent. Future studies will include comparative genomics to determine genetic differences contributing to these variations. These studies will enable us to determine preventative measures that control transmission of pathogen with high biofilm and virulence properties.

Poster Presentation #262

A Natural Mouse Model for Neisserial Transmission in the Upper Respiratory Tract

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Gonorrhea is considered the second most prevalent sexually transmitted bacterial infection worldwide and is caused by *Neisseria gonorrhoeae* (Ngo). Ngo frequently colonizes asymptomatically the mucosal surfaces of the genital tract, rectum, and pharynx. However, mechanisms of gonococcal transmission between hosts are poorly understood. Studies of *in vivo* transmission of Ngo are challenging due to a lack of natural animal models. We have developed the first murine model of *Neisseria* transmission using the model organism *Neisseria musculi*. We aim to use this model to identify and study neisserial persistence and transmission factors. *In vivo* models are needed to develop approaches to combat reservoirs of *Neisseria* that contribute to persistence and dissemination of disease and antimicrobial resistance. *N. musculi* is a commensal of wild-caught house mice that encodes many orthologs of Ngo virulence factors. For this study we evaluated the ability of *N. musculi* to transmit between colonized and uninoculated laboratory mice. Transmission was detected in two inbred mouse strains, A/J and C57BL/6, following single oral inoculations. Mice were inoculated and three weeks later co-housed with uninoculated mice. Transmission to uninoculated mice was detected as early as one week after co-housing. Transmission kinetics were found to differ depending upon which colonized mouse strain was co-housed with uninoculated mice. We have established a rodent model capable of investigating factors that influence neisserial transmission. Mutation of host and bacterial genes will allow future investigation of molecular determinants that influence transmission between hosts.

Poster Presentation #263

In Vitro Antifungal Activity of Antimicrobial Peptoids against Candida auris

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Candida auris is a recently emerged pathogenic fungus which exhibits high mortality during invasive nosocomial infections. *C. auris* can persist long-term and has shown the ability to develop high levels of resistance against all classes of antifungal drugs. Therefore, development of new antifungal drugs with different mechanisms of action that combat this pathogen is a pressing need. Antimicrobial peptides (AMPs) are membrane-disrupting, naturally occurring broad-spectrum antimicrobial agents that represent promising candidates as therapeutic antibiotics and antifungals, due to the lack of acquired resistance against AMPs. However, AMPs are expensive to produce and are often subject to *in vitro* digestion by proteolytic enzymes. We have been examining the antimicrobial potential of a novel class of N-substituted glycine oligomers that mimic the structure and activity of AMPs while resisting protease degradation, called peptoids. We hypothesize that these compounds will exhibit potent *in vitro* antifungal activity against several clinical isolates of *C. auris*. In standard MIC assays, we observe significant inhibitory and fungicidal activity *in vitro*, with MICs as low as 8µg/mL and MFCs as low as 16µg/mL. Kinetics assays show rapid killing, with 100% fungicidal activity observed in 1 hour at 5x MIC. These results support the development of peptoids as novel antifungal agents for treating *C. auris* infection.

Poster Presentation #264

Antimicrobial Peptoid Activity Against Herpes Simplex Virus In Vitro And In Vivo

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Herpes simplex virus-1 is a ubiquitous infection affecting millions of people annually. Current treatments for HSV-1 rely on nucleoside analog drugs, such as acyclovir, which act by disrupting the viral replication cycle, and have no effect on the virus. Resistance to these drugs has been increasing, necessitating the development of antiviral therapeutics with different mechanisms of action. Antimicrobial peptides, such as Cathelicidin (LL-37) have been examined as novel broad-spectrum antimicrobials due to their ability to perforate microbial membranes, including the viral envelope. However, AMPs are susceptible to protease cleavage and low bioavailability. Antimicrobial peptide mimics,*N*-substituded glycine oligomers, peptoids, were developed to circumvent these disadvantages. The altered chemical structure allows for increased bioavailability and in vivo activity. We have demonstrated effective antiviral killing in vitro against HSV-1 by several peptoid structures by disrupting the viral envelope. We show here that the peptoids only act at the the pre-attachment stage, killing virions before entering the cell. Further, the peptoids are active against acyclovir-resistant strains, demonstrating that they could be an alternative to this standard drug. To demonstrate in vivo activity, we have optimized a lip model of HSV-1 infection in BALB/c mice. Mice are infected after lip scarification. The lip and trigeminal nerves are harvested, plaque assays are used to determine infective particles, and HSV-1 DNA is quantified by qPCR. Topical treatment of lips on day two post-infection led to reduced lesion severity and viral-DNA levels. This suggests that peptoids could be developed as new antiviral drugs to treat HSV-1 infections.

Poster Presentation #265

Cyclic Diguanylate-modulating Enzymes Impact Key Factors Involved in Host-symbiont Colonization

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Cyclic diguanylate (c-di-GMP) is a signaling molecule that inhibits motility and promotes biofilm formation in many bacteria and has been studied extensively in pathogens. *Vibrio fischeri*, the beneficial light organ symbiont of the Hawaiian bobtail squid (*Euprymna scolopes*), requires motility and biofilm for host colonization, and encodes 50 proteins predicted to modulate c-di-GMP levels. Diguanylate cyclases (DGCs) synthesize c-di-GMP while phosphodiesterases (PDEs) degrade c-di-GMP. While a few of the *V. fischeri* proteins have been characterized, how each of them contributes to *V. fischeri* host colonization is not well understood. To investigate the potential roles of the individual proteins, we overexpressed each protein and performed assays to determine their effects on motility, biofilm, and c-di-GMP levels. Additionally, active site mutant analysis was performed on proteins with the most interesting phenotypes to confirm their functions. 20 of the 28 DGCs and 6 of the 10 PDEs behaved as predicted when overexpressed. Most of the predicted dual-function proteins did not have significant phenotypes when overexpressed, but VF_0985 behaved like a DGC while VF_0094 behaved like a PDE. Interestingly, one DGC, VF_0596, had phenotypes opposite from what was predicted. Additionally, 7 DGCs impacted only biofilm or motility when overexpressed, while the other phenotype remained unaffected. This work provides grounds for future research investigating the roles of specific proteins during host colonization.

Poster Presentation #266

Aerosolized Cyanobacterial Toxin Microcystin-LR Induces Type 1 Inflammation of the Airways

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Harmful algal blooms are caused by cyanobacteria and pose serious health concerns due to the release of cyanotoxins. Microcystin-LR (MC-LR) is one of the most frequently produced cyanotoxins and has been detected in aerosols generated from bloom-containing water. However, the human health effects of MC-LR aerosols on pulmonary health remain unknown. Literature suggests that MC-LR exposure has a pro-inflammatory influence on the airways. The objective of this study was to determine the extent of the pro-inflammatory effects of MC-LR on the airways to elucidate the implications of risk in healthy and potentially vulnerable human populations. To address these knowledge gaps, an in vitro 3D primary human airway model was utilized, in which environmentally relevant concentrations of MC-LR were aerosolized and delivered to the apical cell surface. Additionally, mouse inhalation exposures to aerosolized MC-LR at a concentration chosen to mimic the in vitro study in which C57BL/6J mice (prone to Type 1 inflammation) and BALB/c mice (prone to Type 2 inflammation) were compared to further dissect the pro-inflammatory effects. Both models demonstrated significant increases in cytokines associated with granulocytic inflammation. Importantly, this response was observed in the C57BL/6J but not the BALB/c mice, suggesting a specificity for Th1 and Th17 driven Type 1 inflammation. The results of this study are consistent with our hypothesis that aerosolized MC-LR induces pulmonary inflammation and extend our understanding to the type of inflammation involved. Future investigation should seek to evaluate the impact of exposure in at-risk human populations with pre-existing Type 1 inflammatory pulmonary conditions.

Poster Presentation #267

Phenotypic Changes in Experimentally-Evolved Mycobacterium tuberculosis Biofilms

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Mycobacterium tuberculosis (*M.tb*) is the causative agent of tuberculosis, a difficult-to-treat lung infection with a high mortality. A key factor in the persistence of tuberculosis is the ability of *M.tb* to form biofilms during host infection, as these biofilms are resistant to host defenses and antibiotic treatments. However, little is known about the structure and composition of these biofilms. We have experimentally-evolved clinical strains of *M.tb* by imposing selective pressure for growth as pellicle biofilms, which form at air-liquid interfaces. We measured pellicle adaptation using scanning electron microscopy, transmission electron microscopy, and fluorescent microscopy. We observed differences in cell size and extracellular matrix deposition between ancestral and evolved strains. Another goal of this project is determining how adaptation to pellicle biofilm growth affects the capacity of a given strain to form biofilms in other models meant to replicate different physiological niches. To this end, we grew pellicle-evolved *M.tb* clinical strains as lysed neutrophil biofilms, and used scanning electron microscopy and fluorescent microscopy to determine the structure of these biofilms. Future work includes using confocal fluorescent microscopy to determine the biochemical composition of the extracellular matrix, and incubating biofilms alongside isolated human neutrophils to explore how *M.tb* resists host responses. Determining the structure and composition of these biofilms is crucial for understanding how tuberculosis resists host defenses and how we might target that resistance with new treatments. Together, this work will characterize the phenotypic diversity of *M.tb* biofilms in different environmental niches and identify mechanisms of persistence at the host-pathogen interface.

Poster Presentation #268

The Fatty Acid Regulator SREBP1 Controls Rhinovirus-B Replication

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Known to cause the common cold, human rhinoviruses (RVs) cost the United States billions of dollars annually and are implicated in the exacerbation of cystic fibrosis and childhood asthma. Despite this, antiviral treatment options remain absent for RVs. Better understanding of RV host dependencies will allow for the development of novel antiviral treatments for RV infection. Previous studies suggested endoplasmic reticulum (ER) stress modulates RV replication. To further interrogate a specific ER stress pathway, we utilized 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF), a serine protease inhibitor commonly used to suppress the ER stress response protein: activating transcription factor 6 (ATF6). AEBSF blocks ATF6 activation through the inhibition of site-1 (S1P) and site-2 (S2P) proteases. Although AEBSF significantly reduced RV-B replication, ATF6 siRNA and the ATF6 inhibitor Ceapin-A7 had no effect. Sterol regulatory element-binding protein 1 (SREBP1), the master regulator for fatty acid synthesis, is also processed by S1P and S2P, and inhibited by AEBSF. We treated A549 cells with SREBP1 siRNA to knockdown SREBP1 and afterwards observed a decline in RV-B replication compared to the control siRNA. Given this, SREBP1 looks to play a critical role in determining the fate of RV-B replication post-infection. Interestingly, neither AEBSF nor SREBP siRNA affected RV-A or RV-C species replication. Further interrogating the mode in which SREBP1 regulates RV-B will grant new insight into RV species differences in host dependencies, and may have therapeutic implications.

Poster Presentation #269

Bacterial Infection Drives Trained Immunity through Epigenetic Remodeling of Epithelial Stem Cells

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Recurrent bacterial infections are a major health burden worldwide, yet the mechanisms dictating host susceptibility to recurrence are poorly understood. A history of urinary tract infection (UTI) is known to predispose to future infections. In mice, we found that an initial UTI resulted in disease outcome-dependent, bladder epithelial (urothelial) remodeling affecting susceptibility to recurrent UTI (rUTI). Differentiation of urothelial stem cells (USCs), derived from mice with different UTI histories (naïve, chronic/sensitized or self-resolving/resolved) in Transwell cultures, resulted in polarized urothelium that recapitulated distinct remodeling morphologies seen *in vivo*. Epigenomic analysis demonstrated that differential chromatin accessibility in USCs segregated by disease history, correlating with differences in DNA methylation and histone modifications in the USCs and differences in gene expression in the differentiated urothelium. Epigenetic marks in differentiated USCs from Sensitized mice enhanced Caspase-1-mediated inflammatory cell death upon UPEC infection, which is part of a protective response enhancing bacterial clearance seen in acute stages of challenge infection. However, expression of *Ptgs2os2*, a positive regulator of cyclooxygenase-2 (Cox2) expression in *cis* and pro-inflammatory response regulator in *trans*, in the urothelium was significantly enhanced in sensitized mice. This could contribute to an excessive and sustained Cox2 inflammatory response that is known to lead to mucosal wounding and severe recurrent chronic cystitis. Thus, UPEC infection acts as an epi-mutagen to reprogram the urothelial epigenome leading to urothelial-intrinsic remodeling that trains the mucosal immune response to subsequent infection. These findings may have broad implications for the prevention of UTI and other chronic and recurrent bacterial infections.

Poster Presentation #270

The Combined Effect of Vitamin D and Ethanol on Murine PBMC Response to Mycobacterium bovis (BCG) Infection

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Globally millions of people are exposed or infected with Mycobacterium tuberculosis, resulting in nearly a third of the population having latent Mtb infection (LTBI) (Ding C et al., 2022). The likelihood of active disease or LTBI outcomes increases for immune compromised individuals. Host immune response can be impaired due to various factors, including nutritional status such as vitamin D deficiency (Sassi F et al., 2018) and alcohol use disorder. Our previous studies explored the role of vitamin D in mycobacterium infection in presence or absence of exogenous ethanol (Gough, et al. 2019). We expand our prior study to consider the compounding effect of concurrent vitamin D deficiency and alcohol use. Mice were given feed with/without vitamin D, followed by deficient/sufficient feed mixed with/without ethanol. Following the in vivo conditioning, we collected spleen and blood by cardiac puncture. Peripheral blood mononuclear cells (PBMC) from mice were infected with Mycobacterium bovis BCG. Culture supernatant was collected every 24hr from 0 to 96hr and used to quantify extracellular bacteria (ECB) load and cell death. Our results indicate that ethanol-treated mice had reduced levels of circulatory immune cells and splenic cells regardless of vitamin D condition. We observed vitamin D sufficient cells oscillating between higher and lower EBC loads, indicating a modulatory role of in vivo vitamin D conditioning. Addition of ethanol negatively influence the vitamin D dependent responses. In conclusion, ethanol alters the immune cell profile and increased cell death irrespective of vitamin D condition and extracellular bacterial counts.

Poster Presentation #271

Modeling Iron Homeostasis and Oxidative Stress Response in Bacteria

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Iron metabolism in bacteria is dynamic and impacted by the presence of other molecules in the microenvironment, particularly the presence of oxidants. Notably, the iron homeostatic pathways in most bacteria are coordinated with oxidative stress processes. To better understand bacterial iron stress response in environments with dynamic concentrations of oxidants, we constructed an integrated iron – peroxide stress response model in *Escherichia coli* K12. We employed ensemble modeling methods to parameterize the resulting multi-phenotype model and investigated susceptibility of the bacterial iron stress response system given varying levels of iron availability and under various levels of exogenously introduced peroxide stress. Results suggests that siderophore production, growth and peroxide dependent transcriptional regulation are critical processes in bacterial response to dynamic iron stress. Our simulations further indicate that a combination of low peroxide stress and severe iron limitation present the more challenging stress conditions for bacterial persistence and maintenance of iron homeostatic processes. This result seems to suggest that such an environment is the least physiologically favorable. Further investigation into the likelihood of bacteria encountering such conditions within the host environment would be valuable for understanding the intersecting contributions of iron and oxidative stress to host-pathogen dynamics. Such understanding would aid in the identification of potential therapeutic targets or novel therapeutic strategies.

Poster Presentation #272

Understanding Nutritional Immunity at the Macrophage-Pathogen Interface Through a Pooled In Vivo CRISPR Screen

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Acinetobacter baumannii is a Gram-negative respiratory pathogen with increasing antibiotic resistance, requiring urgent research and development of new therapeutics. Metals are required by all living organisms for almost all biological processes; because of this, both host and pathogen have developed numerous mechanisms to sequester and use metals during infection. This struggle for nutrient metals represents a unique interface for understanding host-pathogen interactions. Myeloid cells, including neutrophils and macrophages, are among the first line of defense against A. baumannii. Despite this, there is limited understanding of how these cells handle infection with A. baumannii. To better develop therapeutics, I am taking both a host-directed and pathogen-directed approach. By studying both sides of the infection, I aim to understand how to improve host immunity while targeting pathogen fitness. To define metal handling genes that are important for innate immunity against A. baumannii, I performed an in vivo CRISPR screen. This was accomplished by first creating a pooled CRISPR library containing mutated myeloid metal handling genes in bone marrow cells, adoptively transferring the mutated bone marrow into recipient mice, then infecting mice after a rest period. By recovering macrophages and neutrophils which either contain or do not contain bacteria from infected mouse lungs, we have revealed a number of metal handling genes that are important for macrophage and neutrophil control of A. baumannii. We plan to continue these studies by further understanding how these metal genes regulate immunity.

Poster Presentation #273

Macrophage Aggresome-like Induced Structures are Flexible Organizing Platforms for Immune Signaling

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Macrophages respond to bacterial infections through a variety of signaling pathways to mount an inflammatory response. This often coincides with the formation of transient cytosolic p62/SQSTM1 inclusions containing ubiquitinated proteins in structures known as aggresome-like induced structures (ALIS). Although these are thought to form in response to cellular stress, such as a bacterial infection or misfolded and ubiquitinated proteins, preliminary evidence suggests they instead play a role in intracellular organization of immune responses. Here, we show that these structures are induced in macrophages by multiple stimuli, including bacterial infection and cytosolic double-stranded DNA. Instead of accumulating misfolded proteins, we observed that ubiquitin formed a circular structure around the periphery of these foci. Different microbial stimuli led to ALIS formation with selective recruitment of immune regulators depending on the activating signal. In addition, we found that IFI204, an interferon-inducible DNA-binding protein, facilitates the formation of ALIS. These observations are consistent with a model where ALIS can serve as flexible platforms for inflammatory signaling in innate immune cells, potentially protecting or sequestering signaling intermediates to amplify innate immune responses.

Poster Presentation #274

Salmonella enterica serovar Typhi Activates the Integrated Stress Response during Macrophage Infection

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Cellular stress is a linchpin to metabolic reprogramming and transcriptional changes underlying the innate immune response to bacterial infection. The integrated stress response (ISR) triggers in response to diverse stimuli including starvation, proteotoxicity, and some microbial infections. *Salmonella enterica* serovar Typhi is an important human pathogen with well described virulence factors such as Typhoid toxin, capsule, and two critical type III secretion systems and their associated effectors. These characteristics that make it likely that *S* Typhi drive cellular stress responses during intracellular infection by damaging host cell membranes, nutrient deprivation, or inducing proteotoxicity. Our preliminary data have identified that the integral transcription factor of the ISR, ATF4, is induced during *S* Typhi infection and may drive macrophage-specific responses. We hypothesize this alters infection outcomes by manipulating the secreted cytokines, remodeling cellular metabolism, and altering the redox environment of intracellular infections. By understanding cellular stress modulation by intracellular pathogens like *S* Typhi during infection, this research may lay the foundation to identify future therapeutic intervention points.

Poster Presentation #275

Colonization of Suckling Mice by Carbapenem-resistant Enterobacter hormaechei is Enhanced by Acquisition of N-acetyl-D-glucosamine

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Carbapenem- resistant (CR) *Enterobacter* spp. are growing threats to global public health, particularly for infants. CR *Enterobacter* are vertically acquired from the mother and colonize the infant gut. Once established, *Enterobacter* can disseminate from the gut to the bloodstream and cause sepsis. To explore mechanisms of host colonization, we developed an infant mouse model of *Enterobacter* gastrointestinal colonization. Five- day old CD1 mice were orally gavaged with 105 CFU of the human *Enterobacter* isolate, UM-CRE14. We recovered 107 CFU per gram of intestinal tissue 24 hours post gavage. *Enterobacte*r colonization was restricted primarily to the colon, consistent with human GI colonization patterns. We then examined the metabolic requirements for bacterial colonization. A mutant lacking the pyruvate dehydrogenase complex (Δ*aceE*), which converts pyruvate from glycolysis into acetyl-CoA, showed a 4- log reduction in colonization of the infant gut. UM-CRE14 grew well on mucin, a major carbon source available in the gut, and also utilized six of the sugars that comprise mucin. The *aceE* mutant exhibited a slight growth defect in purified mucin compared to wild type and grew poorly on five of the six identified mucin sugars, with the exception of *N* -acetyl-D-glucosamine (GlcNAc). We hypothesize that *Enterobacter* scavenges acetate from GlcNAc via the nag operon to make acetyl- CoA and circumvent the *aceE* mutation. Of significance is that GlcNAc is a major constituent of breast milk. This could provide a rich environment for *Enterobacter* to colonize infants early in life, increasing the risk for these individuals to develop systemic disease.

Poster Presentation #276

Hyperglycemic Conditions Impair Lung Epithelial Innate Response to *Klebsiella pneumoniae* Infection

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Bacterial pneumonia is a leading cause of mortality in adults with increased disease severity and complications in diabetic individuals. We hypothesize that hyperglycemic conditions impair pulmonary innate immune response to bacterial infection. In this study, we used human bronchial epithelial cells (16HBE and BEAS-2B cell lines) and challenged them with a standard strain of *Klebsiella pneumoniae (KP)* under normal (5mM, NG) and high (25 mM, HG) glucose conditions. Our data showed that *KP* grew more and proportionately caused more cell death under HG conditions. The qPCR and ELISA analyses revealed that *KP* infection increased the expression of inflammatory cytokines (IL-6, IL-8, & TNF-α) at both mRNA and protein levels in lung epithelial cells. However, the inflammatory response was higher under HG conditions which coincided with induced expression of Toll-like receptor 4 (TLR4), a key receptor involved in Gram-negative bacterial infection. The pharmacological inhibition of inflammatory signaling, NF-kB, MEK, and p38 attenuated *KP*-induced inflammatory response in NG but to less extent in HG cells. In addition to inflammatory cytokines, epithelial cells trigger the production of antimicrobial peptides (AMPs) to limit bacterial growth. Unexpectedly, we found that HG cells produced more AMPs (LL-37, HBD2, & HBD3) in response to *KP* infection. Finally, like ATCC strain, clinical isolates of KP evoked increased inflammatory response under HG conditions. Collectively, our study shows that hyperglycemic conditions dysregulate the innate response of lung epithelium by promoting an inflammatory milieu. These findings may assist in the development of therapeutic targets to treat bacterial infections in diabetic patients

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MEMORIAL UNION BUILDING MAP

MEMORIAL UNION