

2022 Mid-Atlantic Microbial Pathogenesis Meeting

FEBRUARY 13 - 15, 2022

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MAMPM

Conference Proceedings

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Meeting Schedule

Robert J. Kadner, Ph.D. (1942-2005)



With the unexpected death of Robert J. Kadner at the age of 63 on August 7, 2005, the scientific, and especially the Microbiology, community has lost one its greatest ambassadors. Bob received his B.S. degree from Loyola and his Ph.D. in 1967 from UCLA, and carried out postdoctoral studies at NYU. In 1969, he joined the faculty of the University of Virginia and rose through the academic ranks being appointed a full Professor in 1980. In 1994, he was named the Norman J. Knorr Professor of Basic Medical Sciences.

Bob was an internationally recognized microbiologist whose research on microbial physiology spanned four decades. Bob made significant contributions to the understanding of two *Escherichia coli* transport systems, the Uhp system for hexose phosphate and the TonB/ExbB,D-dependent system for vitamin B_{12}. He authored over a hundred research articles, reviews, and several book sections including the comprehensive chapter on "Cytoplasmic Membranes" in *Escherichia coli and Salmonella*. He trained more than 30 students and fellows, many of whom are now leading scientists in academia, industry, and government laboratories.

He was a leading figure in fostering graduate and medical education. In 1998, Bob was the first recipient of the American Society for Microbiology (ASM) Graduate Teaching Award. He chaired the reorganized ASM Committee on Graduate Education and helped found the ASM Institute for Graduate and Postdoctoral Scientists in Preparation for Careers in Microbiology, which now bears his name. He held several positions for the ASM and the American Academy for Microbiology, and served on many government and national society advisory boards. At the time of his death, he was an editor of the *Journal of Bacteriology* and chair-elect of ASM-Division K, "Microbial Physiology".

Bob contributions to the field were also felt at conferences. He was the organizer of the ASM National Meeting Symposium on Transcription Regulation by Response Regulators in 1998 and the chair of the BLAST VII International Conference on Bacterial Locomotion and Signal Transduction in 2005. He was also an active participant in the first 3 Mid-Atlantic Microbial Pathogenesis Meetings. We will miss his insights and thoughtful commentaries during the conference.

Bob's passing leaves a huge hole in the field of Microbiology. However, his spirit and passion for science, his composed excitement for understanding the unknown, his high standards of scholarship will remain with us for years to come—inspiring faculty, students and fellows to continue in his footsteps.

Mark Shirtliff, Ph.D (1969-2018)



The University of Maryland, Baltimore community was saddened by the loss of researcher and entrepreneur Mark E. Shirtliff, PhD after a rafting accident in the Yellowstone River near Gardiner, MT on July 12, 2018. Mark received his B.S degree from the University of Texas - Houston and his Ph.D. from the University of Texas – Medical Branch, Galveston in 2001. After a brief postdoctoral fellowship at the Center for Biofilm Engineering at Montana State University, he joined the faculty and was appointed a full Professor in 2016. At UMB, Mark held a primary appointment as professor in the Department of Microbial Pathogenesis in the University of Maryland School of Dentistry and a secondary appointment as professor in the Department of Microbiology and Immunology in the University of Maryland School of Medicine.

Mark's passion and the focus of his scientific career was the study of microbial biofilms in chronic infections. The National Institutes of Health estimates that up to 80 percent of all microbial infections are chronic and others have estimated that chronic infections result in over 500,000 deaths per year in the United States alone. One of the most prevalent causes of chronic, persistent bacterial infections is *Staphylococcus aureus*, due in part to its ability to form antibiotic-resistant biofilms. Being Mark, he took on the most difficult of challenges, specifically understanding the mechanism of biofilm development and maturation in methicillin-resistant *S. aureus* (MRSA). His success in this field is clearly seen through his authorship of more than 120 peer-reviewed scientific papers and book chapters. Recently, Mark's work was aimed at developing a hand-held technology that would be capable of identifying six of the most virulent ESKAPE pathogens, including *S. aureus*.

Mark was a unique scientist in that he saw the outcome of his research through two lenses. Academically he was an outstanding mentor to graduate students, post doctoral fellows, and junior facility where he generously gave of his time to help students work through challenging concepts or experiments and provided unwavering encouragement and support. He deeply cared about his students and valued them as fellow colleagues and as developing scientists. As an entrepreneur and inventor, he and his colleagues developed novel diagnostic and vaccine technology that resulted in the establishment of two UMB start-up companies (CelerDx and Serenta Biotechnology). Through both of these avenues, Mark worked diligently to unite and invigorate the field of biofilm research. He believed that the level of recognition and funding for biofilm-related diseases should reflect the magnitude of people they affect. This would increase biofilm research funding, facilitate training more scientists and physicians, and promote medical advances in biofilm-targeted treatments. While achieving these goals will be more difficult without Mark as a driving force, we as a community owe it to him to succeed in these endeavors.

Starting at the 2022 Mid-Atlantic Microbial Pathogenesis Meeting, an award in honor of Mark was established that recognizes excellence in biofilm research by a graduate student or post-doctoral fellow.

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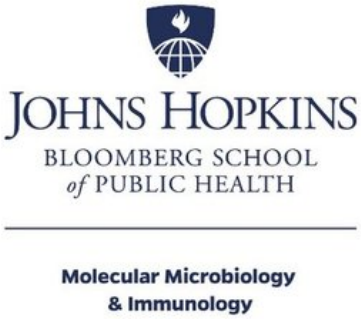


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Division of Microbial Pathogenesis

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2022 MAMPM SCIENTIFIC PROGRAM COMMITTEE



Robert K. Ernst, PhD

MAMPM 2022 Meeting Chair
University of Maryland School of Dentistry

Dr. Robert (Bob) K. Ernst is the Dr. Paul and Mrs. Jean Corcoran Professor and Chair in the Department of Microbial Pathogenesis in the School of Dentistry, and an Adjunct Professor in the School of Medicine at the University of Maryland, Baltimore. In the fall of 2008, Dr. Ernst moved his laboratory from the University of Washington, Seattle, where he was a Research Associate Professor in the Department of Medicine, Division of Allergy and Infectious Diseases and a principal investigator with the Northwest Regional Center of Excellence for Biodefense and Emerging Infectious Diseases Research (NWRCE) to the University of Maryland. He received a BS from the State University of New York at Oswego (biology and chemistry), an MA from SUNY Buffalo (Microbiology), and a PhD from the University of Virginia (Microbiology) studying retroviral RNA transport, where he was also a fellow in the Myles H. Thaler Center for Retrovirus Research. Subsequently, he was a senior fellow at University of Washington in the laboratory of Samuel I. Miller (Allergy and Infectious Diseases) from 1997 – 2002 before joining the faculty in 2002.



Vincent Bruno, PhD

University of Maryland School of Medicine

Dr. Vincent Bruno is Professor of Microbiology and Immunology in the Institute for Genome Sciences at the University of Maryland School of Medicine. Dr. Bruno received a BS in Biological Sciences from Carnegie Mellon University in 1996, and a PhD in Microbiology/Genetics from Columbia University in 2004. His research focuses on utilizing genomics, transcriptomics, genetics and molecular biology to identify and characterize genes and pathways that are important for *C. albicans*, and fungal pathogens in general, to cause disease.



F. Heath Damron, PhD

West Virginia University School of Medicine

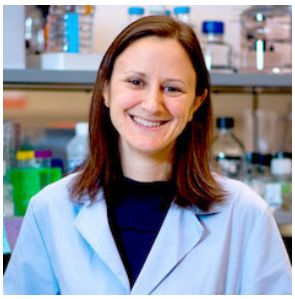
Dr. Heath Damron is an Associate Professor in the the Department of Microbiology, Immunology, and Cell Biology in the School of Medicine at West Virginia University in Morgantown, WV. He is also the Director of the Vaccine Development Center at the WVU Health Sciences Center. The main focus of Dr. Damron's research is to understand *B. pertussis* infections in order to develop a next-generation vaccine.



Kimberly Davis, PhD

Johns Hopkins University Bloomberg School of Public Health

Dr. Kimberly Davis is an Assistant Professor in the W. Harry Feinstone Department of Molecular Microbiology and Immunology at the Johns Hopkins University Bloomberg School of Public Health. Dr. Davis earned a BSc and MSc from the University of Michigan. She then completed her PhD at the University of Pennsylvania in 2011. The goals of her research lab are to better understand how individual bacterial cells within a population contribute to disease, and to determine how to develop more effective therapeutics to target these populations.



Johanna Elfenbein, DVM, PhD, DACVIM

University of Wisconsin-Madison School of Veterinary Medicine

Dr. Johanna Elfenbein earned her undergraduate degree from Harvard College, her DVM from the University of Florida, and her PhD from Texas A&M University. In addition to research training, Dr. Elfenbein pursued advanced clinical training in large animal internal medicine and is a Diplomate of the American College of Veterinary Internal Medicine. Her love for *Salmonella* came from years of clinical practice where she was amazed by the spectrum of disease caused by the organism.



Melissa M. Kendall, PhD

University of Virginia School of Medicine

Dr. Melissa Kendall is an Associate Professor in the Department of Microbiology, Immunology, and Cancer Biology at the University of Virginia School of Medicine. Dr. Kendall received her undergraduate degree in French from Michigan State University in 1995. After college, she entered the Environmental Sciences and Resources: Biology program at Portland State University. Her research explored the physiology and ecology of microbes living in permanently cold, anoxic marine sediments and primarily focused on methanogens, the methane-producing Archaea, and their syntrophic interactions. After receiving her PhD in 2006, she joined Dr. Vanessa Sperandio's lab at the University of Texas Southwestern Medical Center for post-doctoral training. During her fellowship training, Dr. Kendall became interested in how EHEC integrates environmental signals to recognize a host environment and modulate expression of genes important for pathogenesis. This interest remains the driving force behind the research in her lab.



MD A. Motaleb, PhD

East Carolina University Brody School of Medicine

Dr. MD Motaleb is a Professor in the Department of Microbiology and Immunology at the East Carolina University Brody School of Medicine. Dr. Motaleb received his BS and MS from the University of Dhaka in Bangladesh, and his PhD from Osaka University in Japan. His research focuses on *Borrelia burgdorferi* as well as other pathogenic spirochetes.



Rita Tamayo, PhD

University of North Carolina at Chapel Hill School of Medicine

Dr. Rita Tamayo completed her PhD in Microbiology and Immunology at the University of Texas Health Science Center in San Antonio and conducted her postdoctoral research at Tufts University School of Medicine. She is Professor of Microbiology and Immunology at the UNC School of Medicine and a UNC Simmons Scholar. Dr. Tamayo's lab studies the regulatory mechanisms controlling the motility and virulence of *Clostridium difficile*, the most common cause of nosocomial infections in the United States and an urgent public health threat. Her lab has characterized the role of the bacterial signaling molecule c-di-GMP and its RNA-based sensors (riboswitches) in controlling multiple modes of *C. difficile* motility, as well as cytotoxin production. In addition, her group is defining the mechanisms of phase variation and determining the consequences of the resulting phenotypic heterogeneity to *C. difficile* physiology and pathogenesis. Dr. Tamayo's lab is committed to providing scientific and career mentoring in an inclusive research environment.



Matthew Wolfgang, PhD

University of North Carolina at Chapel Hill School of Medicine

Dr. Matthew Wolfgang is an Associate Professor in the Department of Microbiology and Immunology at the UNC School of Medicine. Dr. Wolfgang completed his PhD at the University of Michigan, and conducted postdoctoral research at the University of Washington and Harvard Medical School. Dr. Wolfgang's research focuses on Pseudomonas aeruginosa, a Gram-negative opportunistic pathogen responsible for a variety of diseases in individuals with compromised immune function. This organism poses the greatest risk to the hospitalized population, the elderly, immunosuppressed individuals and those with co-morbid illness, such as heart and pulmonary disease, diabetes, cancer and AIDS. The medical importance of this organism is further underscored by the fact that it is the primary cause of morbidity and mortality in individuals with cystic fibrosis (CF) where it causes chronic lung infection.

Oral Presentations

Sunday, February 13, 2022

Click on title to view abstract

2:00 p.m. - 4:00 p.m.	Check-In	
4:00 p.m. - 4:30 p.m.	**Opening Remarks & Kadner Lecture Introduction**	**Bob Ernst, PhD** - University of Maryland, Baltimore - MAMPM 2022 Chair
4:30 p.m. - 5:30 p.m.	**Keynote #1 - Kadner Memorial Lecture** - "*B. pertussis* Pathogenesis in a Baboon Infection Model"	**Tod Merkel, PhD** - FDA Center for Biologics Evaluation and Research
5:30 p.m. - 6:30 p.m.	**Session #1: Surface Structures in Pathogenesis**	Session Chair: Jennifer Kerr - Notre Dame of Maryland University
5:30 p.m. - 5:45 p.m.	"Adaptation of a *Pseudomonas aeruginosa* Biofilm Deficient Mutant During Chronic Infection"	Erin Gloag - The Ohio State University
5:45 p.m. - 6:00 p.m.	"Phase Variation of LPS and Capsule is Responsible for Stochastic Biofilm Formation in *Francisella tularensis*"	Kevin Mlynek - US Army Medical Research and Materiel Command
6:00 p.m. - 6:30 p.m.	"Biofilms Induce Neutrophil Extracellular Trap Formation Promoting Chronic Inflammation and Tissue Damage"	Jessica Gilbertie - Edward Via College of Osteopathic Medicine
6:30 p.m. - 7:45 p.m.	*Dinner at Copper Mine Bistro featuring "Dine with a Keynote Speaker"*	
8:00 p.m. - 10:00 p.m.	**Poster Session A (Posters 1-48)**	
10:00 p.m. - 11:30 p.m.	*Edge Bar Extended Hours*	

Monday, February 14, 2022

Click on title to view abstract

TIME	DESCRIPTION	SPEAKER
7:00 a.m. - 8:30 a.m.	*Breakfast at Copper Mine Bistro*	
8:30 a.m. - 9:00 a.m.	**Session #1: Surface Structures in Pathogenesis (continued)**	Session Chair: Jennifer Kerr - Notre Dame of Maryland University
8:30 a.m. - 8:45 a.m.	"Planktonic vs. Biofilm: Defining the Sticky Road of Functional Metabolism Shifts in *Gardnerella vaginalis*"	Lillian Dillard - University of Virginia
8:45 a.m. - 9:00 a.m.	"The Genetic and Structural Relationship Between the *Kingella kingae* PAM-galactan and Lipopolysaccharide"	Nina Montoya - University of Pennsylvania
9:00 a.m. - 10:00 a.m.	**Session #2: Evolutions and -omics Approaches to Pathogenesis**	Session Chair: Shaun Brinsmade - Georgetown University
9:00 a.m. - 9:15 a.m.	"A Genome Scale Metabolic Model Reveals Changes in *Neisseria gonorrhoeae* Central Metabolism During Neutrophil Inflammation"	Aimee Potter - University of Virginia
9:15 a.m. - 9:30 a.m.	"Novel Tn-seq Approach Identifies the Primary Secretion Mechanism for Yeriniabactin in *Yersinia pestis*"	Sarah Price - University of Louisville
9:30 a.m. - 10:00 a.m.	"Spatial Lipidomics of *Pseudomonas aeruginosa* in a Mouse Lung Infection Model Identifies New Host-Directed Therapeutic Targets"	Alison Scott - University of Maryland, Baltimore
10:00 a.m. - 10:30 a.m.	*Coffee Break*	
10:30 a.m. - 11:30 a.m.	**Keynote #2** - "Evolution of Transmission Related Characteristics in Foodborne Pathogens: From Foundational Work to Driving Policy Changes"	**Martin Wiedmann, PhD** - Cornell University
11:30 a.m. - 11:45 a.m.	**Session #2: Evolutions and -omics Approaches to Pathogenesis (continued)**	Session Chair: Shaun Brinsmade - Georgetown University

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11:30 a.m. - 11:45 a.m.	"Mini-Bioreactors for the Study of Bacterial Cell-Cell Communication"	Corine Jackman - Carnegie Mellon University
11:45 a.m. - 12:30 p.m.	**Session #3: Host-pathogenesis Interactions**	Session Chair: Yoann Le Breton - Walter Reed Army Institute of Research
11:45 a.m. - 12:00 p.m.	"*Pseudomonas aeruginosa* Lipid A Structures Found in the CF Airway Alter Outer Membrane Porin Expression and Impact *In Vivo* Bacterial Fitness	Casey Hofstaedter - University of Maryland, Baltimore
12:00 p.m. - 12:15 p.m.	"Secondary Infection with *Streptococcus pneumoniae* Decreases Influenza A Virus Replication and is Linked to Severe Disease"	Karina Mueller Brown - Carnegie Mellon University
12:15 p.m. - 12:30 p.m.	"Overcoming Biological Barriers to Eradicate a Chronic Wound Infection"	Sarah Conlon - University of North Carolina at Chapel Hill
12:30 p.m. - 1:30 p.m.	*Lunch at Copper Mine Bistro featuring "Dine with a Keynote Speaker"*	
1:30 p.m. - 3:30 p.m.	*Recreation Time (slopes close at 5:00 p.m.; no night skiing)*	
3:30 p.m. - 4:30 p.m.	**Keynote #3** - "*Salmonella* Under the Microscope"	**Leigh Knodler, PhD** - Washington State University
4:30 p.m. - 5:00 p.m.	**Session #3: Host-pathogenesis Interactions (continued)**	Session Chair: Yoann Le Breton - Walter Reed Army Institute of Research
4:30 p.m. - 4:45 p.m.	"The Type Three Secretion System Effector Protein IpgB1 Promotes *Shigella flexneri* Cell-to-Cell Spread Through Double-Membrane Vacuole Escape"	Erin Weddle - University of Virginia
4:45 p.m. - 5:00 p.m.	"Mucormycosis-Causing Fungi Suppress Nitric Oxide Accumulation During Macrophage Infection"	Alexandra Soare - University of Maryland, Baltimore
5:00 p.m. - 5:30 p.m.	**Session #4: Metabolism, Nutrition, and Virulence**	Session Chair: Kim Davis - Johns Hopkins Bloomberg School of Public Health

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5:00 p.m. - 5:15 p.m.	"Interplay Between PPARγ and GLUT1 is Required for Optimal Resolution of MRSA SSTIs"	Srijon Banerjee - University of Pittsburgh
5:15 p.m. - 5:30 p.m.	"Nitrate is an Environmental Cue in the Gut for *Salmonella enterica* Serovar Typhimurium Biofilm Dispersal Through Curli Repression and Flagella Activation via c-di-GMP Signaling"	Amanda Miller - Temple University
5:30 p.m. - 6:30 p.m.	**Panel Discussion**	**Gregory Carey, PhD** - University of Maryland, Baltimore **Ashley Haines, PhD** - Norfolk State University
6:30 p.m. - 7:45 p.m.	*Dinner at Copper Mine Bistro featuring "Dine with a Keynote Speaker"*	
8:00 p.m. - 10:00 p.m.	**Poster Session B (Posters 49-97)**	
10:00 p.m. - 11:30 p.m.	*Edge Bar Extended Hours*	

Tuesday, February 15, 2022

Click on title to view abstract

TIME	DESCRIPTION	SPEAKER
7:00 a.m. - 8:30 a.m.	*Breakfast at Copper Mine Bistro*	
8:30 a.m. - 9:00 a.m.	**Session #4: Metabolism, Nutrition, and Virulence (continued)**	Session Chair: Kim Davis - Johns Hopkins Bloomberg School of Public Health
8:30 a.m. - 8:45 a.m.	"Branched-Chain Fatty Acids Modulate Two-Component System Activation in *Staphylococcus aureus*"	Augustus Pendleton - Georgetown University
8:45 a.m. - 9:00 a.m.	"Carbon Catabolite Repression and Virulence are Linked in *Staphylococcus aureus*"	Amelia Stephens - University of Pittsburgh

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9:00 a.m. - 9:15 a.m.	**Session #5: Host Innate Immune Responses**	Session Chair: Brittany Johnson - University of North Carolina at Chapel Hill
9:00 a.m. - 9:15 a.m.	"The Role of Colonization Factors CFA/I and CS21 in ETEC Pathogenesis in the Human Enteroid Model"	Emily Smith - University of Baltimore, Maryland
9:15 a.m. - 10:00 a.m.	*Coffee Break & Check-Out*	
10:00 a.m. - 11:00 a.m.	**Keynote #4** - "*Klebsiella pneumoniae* - The Intersection of Capsule, Hypermucoviscosity and Virulence"	**Virginia Miller, PhD** - University of North Carolina at Chapel Hill
11:00 a.m. - 12:00 p.m.	**Session #5: Host Innate Immune Responses (continued)**	Session Chair: Brittany Johnson - University of North Carolina at Chapel Hill
11:00 a.m. - 11:15 a.m.	"C4b-Binding Protein in Human Serum Binds *Neisseria gonorrhoeae* and Suppresses Neutrophil Antigonococcal Activity in a Complement-Independent Manner"	Lacie Werner - University of Virginia
11:15 a.m. - 11:30 a.m.	"The *Orientia tsutsugamushi* Effector Ank5 Targets the Immune Response Regulator NLRC5 for Proteasomal Degradation"	Haley Adcox - Virginia Commonwealth University
11:30 a.m. - 11:45 a.m.	"The Effect of Detoxifying Gonococcal Lipid A Through Inactivation of the msbB Gene on Safety and Outer Membrane Vesicle-Induced Immune Responses"	Lam Thuy Vi Tran Ho - Uniformed Services University of the Health Sciences
11:45 a.m. - 12:00 p.m.	"Utilizing Bacterial Component Adjuvants for COVID-19 Vaccines"	Ting Wong - West Virginia University
12:00 p.m. - 12:15 p.m.	**Meeting Evaluations & Announcement of MAMPM 2023**	**Rita Tamayo, PhD** - University of North Carolina at Chapel Hill - MAMPM 2023 Chair-Elect
12:15 p.m. - 1:30 p.m.	*Lunch at Copper Mine Bistro featuring "Dine with a Keynote Speaker"*	
	End of Meeting - Safe Travels!	

Poster Session A - Sunday, February 13, 2022

Poster Numbers 1-48

Please set up your poster **IMMEDIATELY following** dinner. The room swill be available with boards in place when you arrive.

Posters must be removed at the end of the session.

Click on title to view abstract

POSTER NUMBER	TITLE & PRESENTER	TIME
1	***Rickettsia conorii* Survival in THP-1 Macrophages Requires Host Lipid Metabolic Processes** Paige Allen, Virginia Commonwealth University	8:00 p.m. - 9:00 p.m.
2	**The Zn Finger Protein and Essential Virulence Determinant MucR Acts as an H-NS-like Gene Silencer in *Brucella abortus* 2308** Ian Barton, East Carolina University Brody School of Medicine	9:00 p.m. - 10:00 p.m.
3	**Role of IL-17 in Curli Mediated Autoimmunity** Shingo Bessho, Temple University	8:00 p.m. - 9:00 p.m.
4	**Import of a unique host-derived sugar N-acetylglucosamine 1-P by rickettsiae for biosynthesis of cell envelppe glycoconjugates** Victoria Verhoeve, University Of Maryland Baltimore	9:00 p.m. - 10:00 p.m.
5	**Combining Omics and Challenge Models to Characterize Emerging Clinical Isolates of *Bordetella pertussis* and Evading Vaccine Immunity** Graham Bitzer, West Virginia University	8:00 p.m. - 9:00 p.m.
6	**Interleukin 27 Compromises BCG Clearance and CD4+ T cell Stimulatory Cytokine Production in Neonatal BMDCs** Shelby Bradford, West Virginia University	9:00 p.m. - 10:00 p.m.
7	***Yersinia pestis* Inhibits Host Synthesis of Leukotriene B4, a Potent Mediator of Inflammation** Amanda Brady, University of Louisville	8:00 p.m. - 9:00 p.m.

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8	**Co-Infection with Methicillin-Resistant *Staphylococcus aureus* is Protective Against Infection with Cystic Fibrosis-Adapted *Pseudomonas aeruginosa*** Kristen Brao, University of Maryland, Baltimore	9:00 p.m. - 10:00 p.m.
9	**Colistin Resistance in *Klebsiella pneumoniae*: a Double Edged Sword** Andrew Bray, Wake Forest School of Medicine	8:00 p.m. - 9:00 p.m.
10	**Persister Cell Survival Leads to Treatment Failure During *Yersinia pseudotuberculosis* Systemic Infection** Rezia Era Braza, Johns Hopkins University	9:00 p.m. - 10:00 p.m.
11	**Investigating the Role of Lipooligosaccharide Sialylation in *Neisseria gonorrhoeae*-Neutrophil Interactions** Amaris Cardenas, University of Virginia	8:00 p.m. - 9:00 p.m.
12	**Construction and Characterization of Novel Irradiated Whole-Cell *Neisseria gonorrhoeae* Vaccine** Rhonda Caston, Uniformed Services University of the Health Sciences	9:00 p.m. - 10:00 p.m.
13	**A Chromosomally Encoded Homoserine Lactone Reporter System for *Brucella abortus*** Mitchell Caudill, Virginia Tech	8:00 p.m. - 9:00 p.m.
14	**tusB Overexpression Increases Susceptibility of *Yersinia pseudotuberculosis* to Doxycycline Treatment** Katherine Cotten, Johns Hopkins University	9:00 p.m. - 10:00 p.m.
15	**Is CuxR the Missing Link Between CdG Signaling and *Brucella* Virulence?** Connor Cribb, East Carolina University	8:00 p.m. - 9:00 p.m.
16	**Improving Pertussis Vaccines with Nasal Immunization and the BECC Adjuvant** Megan DeJong, West Virginia University	9:00 p.m. - 10:00 p.m.
17	**Persistence Alters the Interaction Between *Chlamydia trachomatis* and Its Host Cell** Aissata Diallo, Uniformed Services University of the Health Sciences	8:00 p.m. - 9:00 p.m.
18	**Interleukin-27 Opposes Protective Immune Responses Induced by the BCG Vaccine** Ashley Divens, West Virginia University	9:00 p.m. - 10:00 p.m.

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19	**Phospho-Regulation Accommodaes Type III Secretion and Assembly of a Tether of ER-*Chlamydia* Inclusion Membrane Contact Sites** Rachel Ende, University of Virginia	8:00 p.m. - 9:00 p.m.
20	**Monoclonal Antibodies Targeting a Siderophore Receptor and a Porin Involved in Nutrient Acquisition Prevent *Acinetobacter baumannii* Infection in Murine Models of Infection** Mariel Escatte, Walter Reed Army Institute of Research	9:00 p.m. - 10:00 p.m.
21	**SPR206 is Highly Effective *In Vitro* and *In Vivo* Against Important Gram-Negative Pathogens** Timothy Fitzgerald, Walter Reed Army Institute of Research	8:00 p.m. - 9:00 p.m.
22	**Investigation of Spore-Associated Lipoproteins YlaJ, YhcN, YutC, and CoxA Suggests Existence of a Complex That Stabilizes the Germination Apparatus in *Bacillus subtilis* Spores** Matthew Flores, Virginia Tech	9:00 p.m. - 10:00 p.m.
23	**Phenotypic Characterization of *Pseudomonas aeruginosa* CF Isolates Reveals Distinct Clusters Which Represent *In Vitro* Evolution and Predict Performance in a Chronic Infection Model** Taylor Franklin, University of North Carolina at Chapel Hill	8:00 p.m. - 9:00 p.m.
24	**Interrogating Interactions Between *Clostridioides difficile* and the Mucosal Barrier Using a Two-Dimensional Primary Human Colonoid Model** Kathleen Furtado, University of North Carolina at Chapel Hill	9:00 p.m. - 10:00 p.m.
25	**Antimicrobial Tolerance of Synovial Fluid Biofilms Can Be Reversed by Exogenous Metabolic Stimuli** Jessica Gilbertie, Edward Via College of Osteopathic Medicine	8:00 p.m. - 9:00 p.m.
26	**The Role of Bacterial Amyloid Curli, HLA-B27, and Microbiome in *Salmonella*-Induced Reactive Arthritis** Kaitlyn Grando, Temple University	9:00 p.m. - 10:00 p.m.
27	**Mucus Concentration Driven Changes in *Pseudomonas aeruginosa* Virulence Factor Phenotypes and Antibiotic Tolerance** Matthew Greenwald, University of North Carolina at Chapel Hill	8:00 p.m. - 9:00 p.m.
28	**Outer Membrane Protein Bucl8 is a Component of an Efflux Pump and Vaccine Target in *Burkholderia* Pathogens** Megan Grund, West Virginia University	9:00 p.m. - 10:00 p.m.

Click on title to view abstract

29	**Investigating MxiE-Dependent Gene Regulation in *Shigella flexneri*** Chelsea Hall, University of Virginia	8:00 p.m. - 9:00 p.m.
30	**Identification of Lipopolysaccharide Structural Requirements for Toll-Like Receptor 4 and Non-Canonical Inflammasome Activation** Erin Harberts, Towson University	9:00 p.m. - 10:00 p.m.
31	**Connecting Sugar Utilization and Virulence Regulation: RofA as a PRD-Containing Virulence Regulator** Meaghan Hart, University of Maryland	8:00 p.m. - 9:00 p.m.
32	**The Adjacent Pi ATP-Binding Protein-Encoding Genes pstB1 and pstB2 of *Enterococcus faecalis* Have Different Cellular Functions** Christopher Healy, Uniformed Services University of the Health Sciences	9:00 p.m. - 10:00 p.m.
33	**Elucidating the Differential Effects of Capsule Type on *Kingella kingae* Pathogenicity** Kevin Hernandez, University of Pennsylvania	8:00 p.m. - 9:00 p.m.
34	**Aerobactin Enhances Metastatic Infection and Increases Mortality in a Hypervirulent *Klebsiella pneumoniae* Isolate** Giovanna Hernandez, Wake Forest University	9:00 p.m. - 10:00 p.m.
35	**Loss of Flagellar Motility Drives Antibiotic Tolerance by Enhancing Aggregation in *Pseudomonas aeruginosa*** Matthew Higgs, University of North Carolina at Chapel Hill	8:00 p.m. - 9:00 p.m.
36	**Investigating IL-36γ Signaling in Response to *Treponema denticola* Infection** Annie Hinson, Virginia Commonwealth University	9:00 p.m. - 10:00 p.m.
37	**An Uncharacterized Exopolysaccharide is Linked to *Brucella* Virulence** Dariel Hopersberger, East Carolina University	8:00 p.m. - 9:00 p.m.
38	**Development of a Fluorescence Reporter Assay to Study Bacterial Type III Secretion System (T3SS)-Modulating Compounds** Julia Hotinger, Virginia Commonwealth University	9:00 p.m. - 10:00 p.m.
39	**The Role of Fucose Utilization on *Klebsiella pneumoniae* Colonization and Pathogenesis** Andrew Hudson, Wake Forest School of Medicine	8:00 p.m. - 9:00 p.m.
40	**Transformation of *Orientia tsutsugamushi*** Jason Hunt, Virginia Commonwealth University	9:00 p.m. - 10:00 p.m.

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41	**Control of Recombination and Mutation in *Staphylococcus aureus*** Kelly Hurley, University of Pittsburgh	8:00 p.m. - 9:00 p.m.
42	**The Induction of Type 1 Interferon Responses in Primary Osteoblasts Following *Staphylococcus aureus* Infection** Kelli Hutcheson, University of North Carolina at Charlotte	9:00 p.m. - 10:00 p.m.
43	**Investigation of the Acute Virulence of *Pseudomonas aeruginosa* Grown on *Pseudomonas* Isolation Agar Supplemented with Ammonium Metavanadate** Jason Kang, West Virginia University	8:00 p.m. - 9:00 p.m.
44	**Characterizing *Brucella* FtrB: a Novel Cupredoxin** Alexa Kerkan, East Carolina University	9:00 p.m. - 10:00 p.m.
45	***Salmonella*-induced cholesterol accumulation may prevent autophagic clearance of bacteria** Holly Torsilieri, University of Virginia	8:00 p.m. - 9:00 p.m.
46	**Moonlighting Functions of the *Shigella flexneri* Autotransporter IcsA** Volkan Koseoglu, University of Virginia	9:00 p.m. - 10:00 p.m.
47	**A Platform for Genome Mining of Multidrug-Resistant Pathogens to Develop Therapeutic Phages Using Synthetic Biology** Yoann Le Breton, Walter Reed Army Institute of Research	8:00 p.m. - 9:00 p.m.
48	**An Update on the Human Monoclonal Antibody Platform (HMAP): a Programmatic Effort Targeting the Bacteria Responsible for Wound Infections** Yoann Le Breton, Walter Reed Army Institute of Research	9:00 p.m. - 10:00 p.m.

Poster Session B - Monday, February 14, 2022

Poster Numbers 49-97

Please hang your poster before the session starts. The room should be available with boards in place 15 minutes prior to the session beginning.

Posters must be removed at the end of the session.

Click on title to view abstract

POSTER NUMBER	TITLE & PRESENTER	TIME
49	***SARS-CoV-2 Delta Variant Induces Enhanced Pathology, Interferon Responses and a Hyper Inflammatory Profile in K18-hACE2 Mice*** *Katherine Lee, West Virginia University*	8:00 p.m. - 9:00 p.m.
50	**Localized Peptidoglycan Biosynthesis in *Chlamydia trachomatis* Conforms to the Polarized Division and Cell Size Reduction Developmental Models** George Liechti, Uniformed Services University of the Health Sciences	9:00 p.m. - 10:00 p.m.
51	**The *Anaplasma phagocytophilum* Invasin AipA Interacts with CD13 to Facilitate Host Cell Invasion** Mary Clark Lind, Virginia Commonwealth University	8:00 p.m. - 9:00 p.m.
52	**Interbacterial Antagonism Mediated by a Released Polysaccharide** Yiwei Liu, The Ohio State University	9:00 p.m. - 10:00 p.m.
53	**Understanding the Role of Ngo1049 in Subverting Host-Mediated Metal Starvation in *Neisseria gonorrhoeae*** Ian Liyayi, University of Virginia	8:00 p.m. - 9:00 p.m.
54	**Biofilm-Dependent Selection of a Large Chromosomal Duplication in *Burkholderia thailandensis*** Lillian Lowrey, University of North Carolina at Chapel Hill	9:00 p.m. - 10:00 p.m.
55	**Effect of Lipoproteins on the Biofilm Formation and Motility of *Acinetobacter baumannii*** Stephanie Majernik, Walter Reed Army Institute of Research	8:00 p.m. - 9:00 p.m.

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56	**Study of Glucose Supplementation on Antibiotic Efficacy Against *Staphylococcus aureus*** Areej Malik, Old Dominion University	9:00 p.m. - 10:00 p.m.
57	**Investigating the Cytochrome Oxidases of *Bordetella bronchiseptica*** Lily McKay, University of North Carolina at Chapel Hill	8:00 p.m. - 9:00 p.m.
58	**Establishing the Regulon of CmrT, a *Clostridioides difficile* Response Regulator Involved in Motility** Anchal Mehra, University of North Carolina at Chapel Hill	9:00 p.m. - 10:00 p.m.
59	**Analyzing Interactions Between the SpoVA Proteins in *Bacillus subtilis*** Nicole Messner, Virginia Tech	8:00 p.m. - 9:00 p.m.
60	**Copy Number Variability in the Broadly Active, RTX Family Exotoxin Secreted by *Kingella kingae*** Daniel Morreale, Children's Hospital of Philadelphia	9:00 p.m. - 10:00 p.m.
61	***Clostridioides difficile* Biofilm and Spore Production in Response to Antibiotic and Immune Stress** Adenrele Oludiran, Old Dominion University	8:00 p.m. - 9:00 p.m.
62	**Cross-Species Induction of OMV Production in Bacterial Co-Culture** Humberto Ortega, Binghamton University	9:00 p.m. - 10:00 p.m.
63	**The Effects of the Fatty Acid Transportation Protein (FATP) Inhibitor Gr assofermata on Fatty Acid Uptake in *Trypanosoma brucei*** Kristina Parman, Clemson University	8:00 p.m. - 9:00 p.m.
64	**The Role of the Potential Methionine Uptake Transporter MetNPQ in Group A *Streptococcus* Virulence** Aolani Perry, University of Maryland, College Park	9:00 p.m. - 10:00 p.m.
65	**Defining the Molecular Basis of Galactan Homopolymer Synthesis in the Pediatric Pathogen *Kingella kingae*** Eric Porsch, Children's Hospital of Philadelphia	8:00 p.m. - 9:00 p.m.
66	**Interleukin-27-Dependent Regulation of the Transcriptome During Neonatal Sepsis** Jessica Povroznik, West Virginia University	9:00 p.m. - 10:00 p.m.
67	**Metabolic Modulation of Toxin Production in *Clostridioides difficile*** Deborah Powers, University of Virginia	8:00 p.m. - 9:00 p.m.
68	**Unique Features of Alarmone Signaling in *Clostridioides difficile*** Erin Purcell, Old Dominion University	9:00 p.m. - 10:00 p.m.

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69	**B Cell Epitope Peptide-Conjugates as *B. pertussis* Vaccine Antigens** Gage Pyles, West Virginia University	8:00 p.m. - 9:00 p.m.
70	**Investigating the Host- and Pathogen-Related Predisposing Factors for the Development of *Staphylococcus* Infection ssociated Glomerulonephritis** Pranav Rana, The Ohio State University	9:00 p.m. - 10:00 p.m.
71	**Adherence Enables *Neisseria gonorrhoeae* to Overcome Zinc-Limitation Imposed by Nutritional Immunity Proteins** Jocelyn Ray, University of Virginia	8:00 p.m. - 9:00 p.m.
72	***Anaplasma phagocytophilum* Resides in a Host Cell-Derived Multivesicular Body that Exploits ESCRT Machinery for Proliferation** Curtis Read, Virginia Commonwealth University	9:00 p.m. - 10:00 p.m.
73	**Antibiotic Treatment Drives Phenotypic Shift in Heterogeneous *Pseudomonas aeruginosa* Towards Improvement of Virulence** Cristian Roca, University of North Carolina at Chapel Hill	8:00 p.m. - 9:00 p.m.
74	**Investigating the Effect of rpoE Encoding the Delta Subunit of RNA Polymerase on Virulence in the *Streptococcus pyogenes* M1T1 strain 5448** Joseph Rom, University of Maryland	9:00 p.m. - 10:00 p.m.
75	**Acquisition of Hypermucoviscosity in Classical *Klebsiella pneumoniae* – What Does it Take?** Stephen Salisbury, University of North Carolina at Chapel Hill	8:00 p.m. - 9:00 p.m.
76	**Response of *Trypanosoma brucei* to Exogenous Fatty Acids in Low Lipid Conditions** Joshua Saliutama, Clemson University	9:00 p.m. - 10:00 p.m.
77	**Metabolic Plasticity Aids Amphotropism of *Coxiella burnetii*** Savannah E. Sanchez, Virginia Commonwealth University School of Medicine	8:00 p.m. - 9:00 p.m.
78	**Elucidating the Role of Four Neuraminidases on N-glycan Utilization and *In Vivo* Survival of *Bacteroides fragilis*** Nathaniel Seals, East Carolina University Brody School of Medicine	9:00 p.m. - 10:00 p.m.
79	**Requirements for α-(1,3)-glucan Biosynthesis in *Histoplasma capsulatum*** Victoria Sepulveda, University of North Carolina at Chapel Hill	8:00 p.m. - 9:00 p.m.
80	**Detoxification of the Lipid A Component of Live-Attenuated *Shigella* Vaccines Using Bacterial Enzymatic Combinatorial Chemistry** Matthew Sherman, University of Maryland, Baltimore	9:00 p.m. - 10:00 p.m.

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81	**The Production of Neutrophil Chemoattractant Chemokines CXCL1, CXCL2, CXCL3, CXCL5, CCL3, and CCL7 by Osteoblasts Following Infection with *Staphylococcus aureus*** Sophie Sipprell, University of North Carolina at Charlotte	8:00 p.m. - 9:00 p.m.
82	**Development of a Novel Cyst-Deficient *Toxoplasma gondii* Strain: Eno-Dead** Jamison Smiley, University of Virginia	9:00 p.m. - 10:00 p.m.
83	**Fast Lipid Analysis Technique (FLAT): a Rapid, Direct from Urine Diagnostic Assay for Identification of Gram-negative Pathogens** Richard Smith, University of Maryland, Baltimore	8:00 p.m. - 9:00 p.m.
84	**Combatting *Streptococcus suis*: a Combined Sequencing Approach** Tristan Stoyanof, Virginia Tech	9:00 p.m. - 10:00 p.m.
85	**The Effects of Type II Heat-Labile Enterotoxins on Human Respiratory-Specific Immune Responses** Margaret Stroud, Furman University	8:00 p.m. - 9:00 p.m.
86	**Use of a Fast Lipid Analysis Technique (FLAT) to Identify Oral Pathogens via MALDI-TOF MS** Kylie Sumner, University of Maryland, Baltimore	9:00 p.m. - 10:00 p.m.
87	**Investigating the Roles of Eicosanoids and Fatty Acids in Modulating the Inflammatory Response During *Salmonella* Typhimurium Infections** Jessica Taddeo, Lewis Katz School of Medicine at Temple University	8:00 p.m. - 9:00 p.m.
88	**First Steps to Understanding the Mechanism Behind Hypermucoviscosity in *Klebsiella pneumoniae*** Logan Treat, University of North Carolina at Chapel Hill	9:00 p.m. - 10:00 p.m.
89	**Visualizing Interleukin-27 Production in a Murine Model of Neonatal Gram-Negative Bacterial Sepsis** Jordan Vance, West Virginia University	8:00 p.m. - 9:00 p.m.
90	**The Role of Rho actor in Phase Variation of Flagella and Toxins in *Clostridioides difficile*** Mercedes Warren, University of North Carolina at Chapel Hill	9:00 p.m. - 10:00 p.m.
91	**Characterization of Acellular Pertussis Vaccine-Induced Memory Responses and Improvement of Responses by Use of Novel Adjuvant, SWE** Kelly Weaver, West Virginia University	8:00 p.m. - 9:00 p.m.
92	**Investigation of Drug Resistant *Acinetobacter baumannii* Virulence Factors Implicated in Intracellular Survival Within Differentiated THP-1 Cells** Elias Wheibe, University of South Carolina School of Medicine Greenville	9:00 p.m. - 10:00 p.m.

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93	**Characterization of a Phase-Variable Two-Component Signal Transduction System in *Clostridioides difficile*** Caitlin Williams, University of North Carolina at Chapel Hill	8:00 p.m. - 9:00 p.m.
94	**Challenges of Genetic Manipulation within Diverse Species in the Genus *Chlamydia*** Katerina Wolf, University of Kentucky College of Medicine	9:00 p.m. - 10:00 p.m.
95	**Multivalent mRNA Vaccines Provide Protection from *Bordetella pertussis* Infection in urine Models** Allison Wolf, West Virginia University	8:00 p.m. - 9:00 p.m.
96	**The *Kingella kingae* Adhesins PilC1 and PilC2 Have Distinct Functions in Type IV Pili-Mediated Processes** Taylor Yount, University of Pennsylvania	9:00 p.m. - 10:00 p.m.
97	**Two-Component System YesMN Responds to Environmental inc and Contributes Towards Host-to-Host Transmission of *Streptococcus pneumoniae*** M. Ammar Zafar, Wake Forest School of Medicine	8:00 p.m. - 9:00 p.m.

Keynote Speaker Bios



Leigh Knodler, PhD. Associate Professor - Washington State University

Dr. Knodler obtained her PhD from the University of New South Wales in Australia in 1995. Her thesis focused on novel metabolic pathways in the protozoan parasite, Giardia intestinalis. From 1996 to 1999 she was a postdoctoral fellow at the University of California at San Diego studying gene regulation during the developmental cycle of Giardia. In 2000 she moved to Vancouver, Canada for a postdoctoral position in the laboratory of Dr. B. Brett Finlay where she studied the Salmonella enterica pathogenesis. She continued her studies on Salmonella-host cell interactions at the Rocky Mountain Laboratories, NIAID in Montana from 2002-2012, first as a Research Fellow and then as a Staff Scientist. Dr Knodler was recruited to the WSU Paul G. Allen School for Global Animal Health as an Assistant Professor in October 2012.

Personal Statement

I grew up on a cattle farm in Australia and always wanted to be outside, playing in the dirt. At a very young age I was given a magnifying glass and a microscope by my family because I was so interested in all the small bugs crawling around. Science was my favorite subject at school, even though it was not the easiest subject for me. I originally trained as a biochemist, studying parasite metabolism, so there has always been a link to microbes in my research. It was not until I moved to the US for a postdoc and saw Stanley Falkow speak at a regional ASM meeting that I was drawn to the bacterial pathogenesis field. He is considered the "father" of bacterial pathogenesis as a field of study, and his seminar captivated me so much that I ended up doing a second postdoc in Canada so I could work on Salmonella. Then I was hooked.



Tod Merkel, PhD. Principal Investigator
FDA Center for Biologics Evaluation and Research

Dr. Tod Merkel received his undergraduate degree from the University of Illinois and earned his Ph.D. in Dr. Bob Kadner's laboratory at the University of Virginia. Dr. Merkel did his postdoctoral fellowship in the laboratory of Dr. Jerry Keith at the NIH before taking a Principal Investigator position in The Center for Biologicals Evaluation and Research at the FDA. Dr. Merkel has over 20 years' experience developing and utilizing animal models of bacterial infections, including murine and non-human primate models of Pertussis, murine models of Staphylococcal infection, and murine models of Anthrax. His laboratory developed a baboon model of pertussis and used the model make critical breakthroughs in our understanding of the host response to B. pertussis infection and vaccination and to advance our understanding of B. pertussis transmission and pathogenesis.



Virginia Miller, PhD. Professor of Genetics, Microbiology and Immunology
Assistant Dean of Graduate Education, School of Medicine
University of North Carolina

My laboratory has used several Gram negative bacterial pathogens, Yersinia, Salmonella and Klebsiella as model systems to study bacterial pathogenesis. The long-term goals of our work are to understand the bacteria-host interaction at the molecular level to learn how this interaction affects the pathogenesis of infections, and to understand how these pathogens co-ordinate the expression of virulence determinants during an infection. To do this we use genetic, molecular and immunological approaches, in conjunction with the mouse model of infection.

Currently research in the Miller lab focusses on Klebsiella pneumoniae. Klebsiella pneumoniae (Kpn), is a bacterial pathogen that has a remarkable ability to cause a wide range of human diseases including pneumonia often accompanied with systemic spread to other organs. Kpn strains are broadly divided into two classes: Classical strains that are a notable problem in healthcare settings due to multidrug resistance, and hypervirulent strains that are drug sensitive, but able to establish disease in immunocompetent hosts. Alarmingly, there has been an increased frequency of clinical isolates that have both drug resistance and hv-associated genes. One such hv-associated locus is rmpADC that is required for maximal capsule gene (cps) expression and confers hypermucoviscosity (HMV). The capsule is required by all Kpn strains to cause disease and HMV has been tightly associated with hypervirulence. The lab recently found that RmpA autoregulates the expression of the promoter driving expression of the other two genes in the operon, rmpD and rmpC. The lab showed that RmpC is a transcriptional regulator that is required for maximal cps expression and RmpD is a small protein that is required for the HMV phenotype. Importantly, these analyses indicated that HMV and capsule are separable phenotypes and that HMV and capsule likely make distinct contributions to virulence in a mouse model of pneumonia. RmpC appears to regulate expression of ~200 genes in addition to regulating expression of capsule genes. We are currently employing a variety of approaches to investigate how RmpD and RmpC contribute to HMV, capsule and virulence.



Martin Wiedmann, Dr. med. vet, Ph.D.
Gellert Family Professor of Food Safety
Cornell University

Martin received a veterinary degree and a doctorate in Veterinary Medicine from the Ludwig-Maximilians University in Munich, and a Ph.D. in Food Science from Cornell, where he currently is the Gellert Family Professor of Food Safety. His research interests focus on farm-to-table microbial food quality and food safety and the application of molecular tools to study the transmission of foodborne pathogens and spoilage organisms, including translation of the associated research findings into reducing foodborne illnesses and food spoilage. He and his team are also regularly asked to help industry with a range of microbial food safety and quality challenges. Students and staff that were previously associated with his team have pursued successful careers in a range of environments, including industry, government, academia, and non-for profits.

Panelist Bios



Gregory B. Carey, PhD, Executive Director of Student Research and Community Outreach, Office of Student Research; Assistant Dean for Student Research & Education
University of Maryland School of Medicine

Gregory B. Carey (Greg) is Assistant Dean of Student Research and Education, Associate Professor of Microbiology and Immunology and Executive Director of Student Research and Community Outreach at the University of Maryland School of Medicine (UM SOM). He received his PhD in Biochemistry from Virginia Commonwealth University/Medical College of Virginia (VCU/MCV, 1995) and completed postdoctoral studies at the Guthrie Research Foundation, Sayre PA and the Jerome H. Holland Laboratory of the American Red Cross, Rockville, MD.

Dr. Carey's NCI-supported research has investigated signals governing life and death decisions in lymphoma and other cancers. He has been supported on NIH funds as PI and Co-I since 2000. He is a passionate teacher and mentor and is dedicated to broadening diversity, inclusion, achievement and participation in science and medicine. As a lab P.I., he has mentored students and trainees across a continuum from grade school to the junior faculty levels. Forty three percent (43%) are from groups under-represented (UR) in science and medicine. He has served institutionally and nationally to expand diversity, inclusion, participation, excellence and achievement. In addition to his faculty and leadership duties, Dr. Carey has developed, leads and serves on mentored research education pipelines and programs for middle- and high school-, undergraduate pipeline- , post-baccalaureate-, and medical-students. He is active in local and global health education, service and community outreach.



Ashley Haines, PhD. Associate Professor of Biology
Norfolk State University

Dr. Ashley N. Haines, is an Associate Professor in the Department of Biology, at Norfolk State University in Norfolk, VA. Her primary research interest is in the molecular evolution of bacterial virulence factors, with a particular focus on emerging pathogens in fish. With assistance from her undergraduate researchers, she is investigating the impact of host-specific conditions on differential gene expression in Streptococcus parauberis, a pathogen of fish and cattle. Dr. Haines received her B.S. in Biology from Virginia Tech and her Ph.D. in Zoology from Clemson University. She was a post-doctoral fellow in the Department of Microbiology and Immunology at University of Maryland Medical School, Baltimore, MD and Postdoctoral Research Associate in the Department of Environmental & Aquatic Animal Health at Virginia Institute of Marine Science, Gloucester Point, VA. She served as a Visiting Assistant Professor at The College of William and Mary from 2007-2010 and then joined the faculty at Norfolk State University. Since 2010 Dr. Haines has been teaching Microbiology, Integrative Zoology and Immunology courses and mentoring undergraduates through research opportunities in her lab. She is dedicated to the training of minorities in STEM and to improving diversity in the field of Microbiology. This work is supported by a Howard Hughes Medical Institute Inclusive Excellence grant to Norfolk State University, which is focused on institutional transformation.

Oral Presentation Abstracts

The Orientia tsutsugamushi effector Ank5 targets the immune response regulator NLRC5 for proteasomal degradation

Haley E. Adcox[1], Dr. Kyle G. Rodino[2], Dr. Jason A. Carlyon[1]
[1]Department of Microbiology and Immunology, Virginia Commonwealth University School of Medicine, [2]Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania

Orientia tsutsugamushi is an obligate intracellular bacterium and an etiologic agent of scrub typhus, a potentially fatal disease afflicting one million people annually with re-emerging global implications. Mechanisms facilitating O. tsutsugamushi pathobiology and immune response modulation are not well understood. The MHC-I pathway plays a critical role in alerting the adaptive immune response to cells infected with intracellular pathogens. We previously discovered that O. tsutsugamushi modulates this pathway through targeted reduction of the MHC-I gene transactivator, NLRC5. To define how O. tsutsugamushi regulates NLRC5, infected cells were treated with a proteasome-inhibitor, MG132, or a eukaryotic translation inhibitor, cycloheximide. NLRC5 levels were only recovered with MG132 treatment demonstrating a post-translational, proteosome-dependent mechanism. O. tsutsugamushi encodes an arsenal of T1SS effector proteins called Anks that harbor eukaryotic-like ankyrin repeats (ARs). Most O. tsutsugamushi Anks also contain a C-terminal F-box domain capable of co-opting SCF1 (Skp1-Cul1-F-box) ligase machinery. The SCF1 complex is responsible for ubiquitinating target proteins and destining them for proteasomal degradation. Here we identify Ank5, which carries four N-terminal ARs and a C-terminal F-box, as the effector responsible for infection-observed reduction of NLRC5. Yeast two-hybrid assays identified NLRC5 as an Ank5 binding partner. Co-immunoprecipitation of Flag-tagged Ank5 confirmed its interaction with both endogenous and over-expressed NLRC5. Analysis of ank5 expression during infection revealed ank5 transcript was temporally upregulated directly preceding the O. tsutsugamushi expansive replication phase. Ectopically expressed Flag-Ank5 phenocopied infection-induced NLRC5 reduction in a manner that was proteasome-, F-box- and SCF1 complex-dependent. Co-immunoprecipitation assays with Flag-tagged Ank5 mutants delineated that the interaction was independent of the F-box, but dependent on AR4. To define regions of NLRC5 relevant to the interaction, we utilized NLRC5 isoform 3, a naturally occurring variant that lacks the C-terminal leucine-rich repeat (LRR) domain. Flag-Ank5 immunoprecipitated NLRC5 isoform 3, indicating the LRR is irrelevant for interaction. However, unlike full-length NLRC5, levels of isoform 3 were not reduced by Flag-Ank5, which suggests that the LRR is essential for Ank5 to promote NLRC5 proteasomal degradation. Knock out-complementation is not yet possible for O. tsutsugamushi. Importantly, however, ectopically expressed of GFP-Ank5 with a non-functional F-box behaved as a dominant negative inhibitor to block NLRC5 reduction by endogenous Ank5 in O. tsutsugamushi infected cells. Overall, these data confirm that Ank5 is responsible for binding and reducing NLRC5 levels during O. tsutsugamushi infection.

Interplay between PPARγ and GLUT1 is required for optimal resolution of MRSA SSTIs

Srijon Banerjee[1], Lance Thurlow[2], Danielle Lattimer[1], Anthony Richardson[1]
[1]Department of Microbiology and Molecular Genetics, University of Pittsburgh, [2]Department of Microbiology and Immunology, University of North Carolina

Methicillin resistant S. aureus (MRSA) infections are the most common hospital acquired (HA) infections in the US. Additionally, now there are community acquired (CA) strains of MRSA that have spread in persons who have no contact with healthcare settings. Majority of MRSA infections observed in the community are skin and soft tissue infections (SSTIs) which may lead to other systemic manifestations of disease like sepsis. Therefore, the need to discover therapeutic interventions is urgent. The mouse model of MRSA USA300 skin infection shows a distinct inflammatory phase followed by a resolution phase. The inflammatory phase is necessary but not sufficient for initial clearance of the pathogen. Instead, the resolution phase ensures bacterial clearance and healing of the abscess. Wound resolution in mammalian systems is largely dependent on efferocytosis of dead/dying cells by macrophages. Previously, our lab demonstrated that myeloid host factors like peroxisome proliferator-activated receptor gamma (PPARγ) are essential for the onset of the resolution phase in USA300 SSTIs. Further, the absence of a glucose transporter GLUT1 in myeloid cells was found to attenuate the initial inflammatory response to infection. Interestingly, GLUT1 is also known to be essential for efficient efferocytosis by macrophages in an atherosclerosis disease model. Here we propose that both PPARγ and GLUT1 are part of the same regulatory axis that facilitates efferocytosis of apoptotic and necrotic cells found at the site of infection in MRSA SSTIs. Through an efferocytosis assay we demonstrate that both PPARγ and GLUT1 are required for optimal digestion of apoptotic cells by bone marrow derived macrophages (BMMs). We show that the relative expression of the PPARγ regulon during efferocytosis correlates to that of GLUT1. Further, in our attempt to understand the signals triggering PPARγ response in efferocytic macrophages, we find evidence that nitrated mono-unsaturated fatty acids (MUFAs) like 10-nitro-oleic acid and poly-unsaturated fatty acids (PUFAs) like 10-nitro-linoleic acid not only promote digestion of apoptotic cells by BMMs but also induce the expression of the PPARγ regulon and GLUT1. Our results, so far, point towards a putative but crucial relationship between PPARγ and GLUT1 in efferocytic macrophages as well as explore probable methods of enhancing the resolution phase of MRSA SSTIs through topical treatment of abscesses with MUFAs and PUFAs.

Overcoming biological barriers to eradicate a chronic wound infection

Sarah Conlon, Ashelyn Sidders, Phillip Durham, Paul Dayton, Virginie Papadopoulou, Brian Conlon

Chronic wounds, infected with bacterial biofilms, are an expanding global healthcare crisis. Even in the absence of antibiotic resistance, infected wounds do not resolve with antibiotic treatment alone and typically require surgical intervention. Bacterial biofilms within wounds are encased in host-produced matrices which impede penetration of antibiotics. Even when drugs do penetrate effectively, bacterial cells within biofilms display remarkable tolerance to conventional antibiotics and so there is an urgent unmet need for improved treatment strategies.

In this study, we employ palmitoleic acid, an unsaturated fatty acid as a novel aminoglycoside adjuvant, to perturb the membrane of Staphylococcus aureus and facilitate gentamicin uptake. Small colony variants and biofilm cells which typically maintain a proton motive force below the threshold for gentamicin uptake are potentiated several logs in the presence of palmitoleic acid. This novel anti-persister drug combination has much potential but fell short of eradication even in vitro. Improving penetration of gentamicin and palmitoleic acid therapy with ultrasound-stimulated phase change contrast agents (PCCA) eradicated biofilms in a methicillin resistant S. aureus chronic wound infection in diabetic mice. Palmitoleic acid is an attractive antibiotic adjuvant as it has previously shown to accelerate wound healing and reduce inflammation. This therapeutic strategy of utilizing ultrasound to improve penetration of anti-persister drugs has the potential for clinical translation as ultrasound is already used in wound debridement and the PCCA formulation is a variant of FDA approved contrast agents currently used in diagnostic imaging.

Planktonic vs Biofilm: Defining the sticky road of functional metabolism shifts in Gardnerella vaginalis

Lillian Dillard[1], Dr. Jason Papin
[1]*University of Virginia*

Bacterial vaginosis (BV) is the most common vaginal infection in reproductive-age women, and disproportionally effects Black and Hispanic women. The ramifications of BV extend past the discomfort of malodorous vaginal discharge to increased risk of contracting STDs, including HIV, as well as increased risk of pre-term birth. Despite the significant health ramifications BV presents to women, specifically minority women, this area of research remains underfunded and understudied, as noted by the lack of effective antibiotic treatment options. G. vaginalis, the primary pathogen implicated in BV, produces a biofilm which displays increased resistance to antibiotic treatment. These biofilms are a potential causal mechanism for high rates of BV recurrence; however, G. vaginalis biofilm formation has not been well studied.

To address this knowledge gap, I have constructed two genome scale metabolic reconstructions (GENREs) of G. vaginalis based on the annotated genomes of both the type strain (ATCC 14018) and a more recently isolated strain (JCP8151B). Using the algorithm RIPTiDe (Reaction Inclusion by Parsimony and Transcript Distribution) I have integrated transcriptomic data collected from G. vaginalis in both the planktonic and biofilm state into my two respective GENREs. Additionally, I have created an in silico metabolic environment analogous to vaginal conditions during infection, using previously defined synthetic vaginal media conditions, as well as metabolomics data generated from infected cervicovaginal fluid. Through this multi-omic integration I have been able to take into account genetic regulation and extracellular conditions, in regard to their subsequent impact on G. vaginalis functional metabolism. Through subsequent flux balance analysis (FBA), I have analyzed functional metabolic shifts that differentiate G. vaginalis in the planktonic versus biofilm state. These analyses have identified essential metabolic pathways associated with the biofilm state, including increased carbohydrate synthesis. Secondarily, we have run a gene essentiality screening for both the planktonic and biofilm state, in order to identify essentiality shifts between these two states. By identifying a subset of essential metabolic pathways for G. vaginalis biofilm synthesis, as well as genes essential to the biofilm state, we can target these pathways to inhibit biofilm formation and recoup antibiotic susceptibility.

Biofilms induce neutrophil extracellular trap formation promoting chronic inflammation and tissue damage

Jessica Gilbertie[1], Alicia Schubert[2], Katie Sheats[2], Lauren Schnabel[2]
[1]*Edward Via College of Osteopathic Medicine,* [2]*North Carolina State University*

Infection of a joint by Staphylococcus aureus leads to the formation of free-floating biofilm aggregates within the synovial fluid. Unfortunately, these biofilm aggregates are tolerant to antimicrobial therapy, causing treatment failures and persistent inflammation. During synovial infections, an influx of predominately neutrophils into the synovial space occurs. However, neutrophils are not able to combat the infection and are thought to be the main cause of tissue damage. Therefore, we investigated neutrophil function during S. aureus biofilm aggregates or planktonic challenge. We found that planktonic S. aureus was readily phagocytosed, neutrophils had a robust respiratory burst and low extracellular trap (NET) formation leading to a significant decrease in bacterial load. However, when neutrophils were challenged with S. aureus biofilm aggregates, we observed significantly decreased phagocytosis, decreased respiratory burst and prominent NET formation. Although, NETs can be antibacterial in other models, within our model, neutrophils challenged with biofilms exhibited little to no antibacterial activity. To understand if the observed dysfunctional neutrophil response led to increased inflammation and tissue damage, we infected synoviocytes and chrondrocytes with S. aureus in a planktonic or biofilm aggregate phenotype and challenged the infections with neutrophils. We observed significantly increased proinflammatory and catabolic enzyme production in synoviocytes and decreased chondrocyte viability and extracellular matrix production. In conclusion, biofilm aggregates shift the neutrophil response from a productive to non-productive phenotype leading to increased inflammation and tissue damage. Further inquiry is required to understand the pathways responsible for the dysfunctional neutrophil response to biofilms.

Adaptation of a Pseudomonas aeruginosa biofilm deficient mutant during chronic infection

Erin Gloag[1], Christopher Marshall, Vaughn Cooper, Daniel Wozniak
[1]*The Ohio State Univerisity*

Chronic infections persist despite extensive treatment strategies. These infections are commonly caused by bacterial biofilms. Pseudomonas aeruginosa is often implicated in chronic infections, especially in immune-compromised individuals. Further complicating these infections are the ability of bacteria to adapt, usually by evolving variants that are more fit and persistent. Despite our current understanding of the divergent phenotypes of adapted variants, studying their emergence in an infection is challenging, as there are few animal models that mimic what is observed clinically. Our lab has developed a porcine full-thickness chronic burn wound model where bacterial burden and wound healing can be monitored kinetically for up to 35 days. Using this model, we previously demonstrated that P. aeruginosa rugose small-colony variants (RSCVs), with mutations in the wsp pathway, are selected during infection. These wsp mutations led to elevated levels of c-di-GMP, resulting in hyperbiofilm formation. The variant colony morphology, and hyperbiofilm phenotype is dependent on the overproduction of exopolysaccharides, Psl and Pel. We therefore wanted to determine if there are other adaptive benefits of high c-di-GMP levels, independent of exopolysaccharide production, and if other pathways are under selection that promote persistence in the porcine wound model.

To test this, porcine wounds were inoculated with a P. aeruginosa ΔwspFΔpslBCDΔpelA triple mutant. This mutant retains high c-di-GMP levels due to the wspF deletion, however is unable to produce Psl and Pel exopolysaccharides, and as such has a reduced biofilm phenotype. The bacterial burden of the wounds was then examined at days 7, 14 and 35 post infection. At each time point small-colony variants (SCVs) were isolated at a frequency of 5% of the total P. aeruginosa population. Whole genome sequencing revealed that these SCVs had acquired mutations in either the O-antigen lipopolysaccharide (LPS) or type IV pili (T4P). These mutations led to an increase in both c-di-GMP levels and biofilm formation relative to the ΔwspFΔpslBCDΔpelA parent.

Together these results indicate that SCVs can evolve independently to the overproduction of Psl and Pel. We predict that LPS and T4P variants experience selection during the infection due to reduced immune recognition and increased adhesion by these variants, relative to the ancestor. Finally, these data suggest that there may be a hierarchy of mutations under selection during infection (wsp > T4P > LPS).

Pseudomonas aeruginosa lipid A structures found in the CF airway alter outer membrane porin expression and impact in vivo bacterial fitness

Casey Hofstaedter[1,2], Courtney E. Chandler[1], Peter Kim[1], Dr. David Rasko[3,4], Dr. Janette M. Harro[1], Dr Robert K. Ernst[1,3]

[1]Department of Microbial Pathogenesis, University of Maryland, Baltimore, [2]Medical Scientist Training Program, University of Maryland, Baltimore, [3]Department of Microbiology and Immunology, University of Maryland, Baltimore, [4]Institute for Genome Sciences, University of Maryland, Baltimore

Pseudomonas aeruginosa (Pa) is a Gram-negative bacteria that chronically infects individuals with the genetic disorder cystic fibrosis (CF). To establish chronic infection in the CF lung, Pa undergoes functional and structural adaptations that result in increased resistance to antimicrobial therapies. Previously, our group has observed lipid A structural variation in chronic Pa strains isolated from the CF airway that is not seen in other chronic lung diseases. The impact of these structural changes on bacterial fitness, overall outer membrane (OM) composition, and antimicrobial resistance has yet to be explored. To address these gaps, longitudinal Pa isolates were obtained from the airways of patients with CF and lipid A structures were observed using matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Loss of single acyl chain hydroxylation (Δm/z 16) was observed in a subset of isolates. Whole genome sequencing (WGS) was then used to identify genetic drivers of this altered lipid A phenotype. While WGS revealed clonality of these strains within each patient, sequence alignments showed non-synonymous mutations resulting in loss-of-function of lipid A hydroxylation enzymes LpxO1 or LpxO2. These two enzymes each hydroxylate specific lipid A side chains, but their functional impact on overall membrane composition is not yet clear. To further interrogate their role in bacterial fitness, lpxO1 and lpxO2 gene deletions were made in the laboratory-adapted Pa strain PAK and lipid A phenotypes were confirmed using MALDI-TOF MS. Transcriptomic analysis by qRT-PCR revealed decreased expression of oprD, an OM carbapenem porin, when lpxO1 is knocked-out, but not lpxO2. Lastly, Scnn1b transgenic BALB/c mice—a model of CF airway disease—infected with lpxO1- and/or lpxO2-deficient PAK had higher lung bacterial burdens. This work demonstrates that acquired mutations in CF-relevant lipid A biosynthesis genes drive altered lipid A phenotypes and impact OM porin expression. Additionally, we show that mutations in lpxO1 and lpxO2 impact Pa in vivo fitness in a mouse model of CF airway infection. In summary, the impact of lipid A structural variation on OM composition suggests a possible link between lipid A structural variation, carbapenem resistance, and increased bacterial fitness in this vulnerable patient population.

Mini-Bioreactors for the Study of Bacterial Cell-Cell Communication

Corine Jackman[1], Professor Shelley Anna[1], Associate Professor Natalia Luisa Hiller[2]
[1]Department of Chemical Engineering, Carnegie Mellon University,, [2]Department of Biological Sciences, Carnegie Mellon University

Although Streptococcus pneumoniae (Spn) is a major human pathogen that causes over a million annual deaths in young children and the elderly worldwide, it also colonizes the human nasopharynx of many humans without causing any symptoms in its commensal form. After Spn colonizes the upper respiratory tract (URT), it can travel to other tissues in the host, causing mild to severe diseases. Many questions remain regarding the molecular mechanisms associated with the switch from commensalism to pathogenesis. Our overarching goal is to understand whether and how Spn cell-cell communication orchestrates this lifestyle switch.

To answer this question, we have leveraged a droplet-based technology platform that consists of a highly monodisperse water-in-oil emulsion. We have successfully encapsulated Spn cells, and their signaling molecules, in these miniature bioreactors. Spn undergoes multiple division rounds within the droplets, providing an optimal opportunity to quantify the association between cell-cell signaling, cell density, and environmental factors such as nutrient availability, pH, oxygen and temperature. We are investigating the kinetics of signaling of the TprA regulator and its cognate peptide PhrA. This signaling system promotes virulence in animal models of pneumococcal carriage and pneumoniae. To monitor PhrA signaling in real time, we have constructed a reporter where the promoter of PhrA (PphrA) drives the far-red fluorescent protein, mCardinal. Using this system, we have captured cell-density dependent PhrA signaling in a droplet at a single-cell level. We are currently quantifying the association between cell number and signaling, and its dependence on environmental conditions. Further, we are expanding this system to monitor multiple peptide-dependent signaling systems, such as the multiple Rgg/SHP systems widespread across streptococcal species. In the long term, we hope to shed light on the relationship between the kinetics of Spn cell-cell signaling, environmental conditions, and virulence, and to exploit this knowledge for the development of anti-pneumococcal therapies.

An Update on the Human Monoclonal Antibody Platform (HMAP): A Programmatic Effort Targeting the Bacteria Responsible for Wound Infections

Yoann Le Breton[1], Mariel Escatte[1], Stephanie Majernik[1], Timothy Fitzgerald[1], Giovanna Castellanos[1], Dr. Iswarduth Soojhawon[1], Soo Lee[1], Shweta Singh[1], Dr. Schroeder Noble[1], COL Stuart Tyner[2], MAJ Derese Getnet[1], LTC Brett Swierczewski[3], Dr. Molly McLendon[1], Dr. Daniel Zurawski[1]

[1]Walter Reed Army Institute of Research, [2]Military Infectious Diseases Research Program, U.S. Army Medical Research and Development Command, [3]Bacterial Diseases Branch, Center for Infectious Diseases Research, Walter Reed Army Institute of Research

Introduction: Combat wound infections are primarily caused by drug-resistant ESKAPE-E (Enterococcus species, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter cloacae, and Escherichia coli), a major concern for the Military Healthcare System and civilian wound care. Increasing drug resistance and the pivot to prolonged care requires novel antimicrobial solutions to protect the Warfighter. The prophylactic or therapeutic use of human monoclonal antibodies (mAbs) could prevent infection and work with the current standards of care to enhance patient outcomes. The Human Monoclonal Antibody Platform (HMAP) is a programmatic effort established to discover antibody therapeutics to be used for this purpose. HMAP relies on genetics and mutation to identify proteins required by the bacteria for survival and virulence. Proteins are used to immunize humanized mice to develop fully human mAbs after hybridoma fusions. Then, a gated-tiered approach identifies mAbs against these targets that are required for disease. Subsequently, the best candidate mAbs are assessed in vitro and in in vivo animal models to develop new treatments for ESKAPE-E pathogens.

Methods: We generated a series of mAbs against pre-established A. baumannii virulence factors: Hcp, FimA, BauA, OmpW2. The mAbs were first tested in an ELISA assay to establish binding of the native protein on the A. baumannii surface. Then, the mAbs were evaluated in growth assays and in biofilm assays, alone and in combination to further down-select to 1-2 mAbs for testing in animal models. Galleria mellonella was first used to evaluate efficacy before single candidates were tested in murine pulmonary and wound models of infection. Additionally, we cloned six new targets on the A. baumannii surface to make proteins that will be used to immunize humanized mice and enter the pipeline for development and testing.

Results: Native ELISA allowed us to take 5-10 candidate mAbs per protein target and down-select to our two best candidates for further evaluation. Then, two mAbs for each target were determined based on the results in different in vitro assays. For example, αBauA and αOmpW2 reduced bacterial growth in low iron media. Three candidates: αFimA, αBauA and αOmpW2 reduced biofilms over 24-48 hr. All of the antibodies alone and in combination provided increased survival and reduced bacterial numbers in Galleria and murine pulmonary and wound models of infection.

Conclusions: After down-selection and testing, we have identified 4 mAbs, one for each candidate protein as a lead candidate for clinical development. Each antibody has modest efficacy (30-50% survival) in animal models of infection, but we are currently evaluating mAb combinations to drive to 100% survival. We also have 6 promising new candidates at the start of the pipeline now ready for development. We are confident we have the pipeline and tools to construct a mAb cocktail that prevents and remediates A. baumannii infection. As some of these proteins have homologues in other pathogens, we now have a roadmap to develop countermeasures for the other bacteria responsible for combat wound infections, and we now plan to apply this to E. coli, E. faecium, P. aeruginosa, and K. pneumoniae.

Nitrate is an environmental cue in the gut for Salmonella enterica serovar Typhimurium biofilm dispersal through curli repression and flagella activation via c-di-GMP signaling

Amanda Miller[1], Dr. Lauren Nicastro, Shingo Bessho, Kaitlyn Grando, Dr. Aaron P. White, Dr. Yi Zhang, Dr. Gillian Queisser, Dr. Bettina Buttaro, Dr. Cagla Tukel
[1]*Temple University*

Curli, a major component of the bacterial biofilms in the intestinal tract, activates pattern recognition receptors and triggers joint inflammation after infection with Salmonella enterica serovar Typhimurium. The factors that allow S. Typhimurium to disperse from biofilms and invade the epithelium to establish a successful infection during acute inflammation remain unknown. Here, we studied S. Typhimurium biofilms in vitro and in vivo to understand how the inflammatory environment regulates the switch between multicellular and motile S. Typhimurium in the gut. We discovered that nitrate generated by the host is an environmental cue that induces S. Typhimurium to disperse from the biofilm. Nitrate represses production of an important biofilm component, curli, and activates flagella via the modulation of intracellular cyclic-di-GMP levels. We conclude that nitrate plays a central role in pathogen fitness by regulating the sessile to motile lifestyle switch during infection.

Phase variation of LPS and capsule is responsible for stochastic biofilm formation in Francisella tularensis

Kevin Mlynek[1], Beth Bachert[1], Christopher Lopez[1], Ronald T Toothman[1], David Fetterer[2], Janice Williams[3], Joel Bozue[1]

[1]Bacteriology Division, United States Army Medical Research Institute of Infectious Diseases , [2]Division of Biostatistics, United States Army Medical Research Institute of Infectious Diseases , [3]Pathology Division, United States Army Medical Research Institute of Infectious Diseases

Francisella tularensis is a facultative intracellular Gram-negative bacterium found throughout much of the northern hemisphere. As the causative agent of tularemia, Ft is commonly transmitted via arthropod bites, inhalation or direct contact with an infected host. Biofilms have been established as an important lifestyle for bacteria in nature often enabling survivorship and persistence in a multitude of environments, including vectors. However, biofilm formation remains understudied and poorly understood in Ft as non-substantial biofilms are typically observed in vitro by the clinically relevant subspecies F. tularensis tularensis (Type A) and holarctica (Type B). In this study, we found conditions that induce biofilm in a stochastic, but reproducible manner using clinically relevant subspecies. The frequency at which biofilm was observed increased temporally and appeared switch-like as progeny from the initial biofilm quickly formed biofilm in a predictable manner regardless of culture conditions, consistent with a heritable mechanism of biofilm induction. Upon plating biofilm forming cultures, multiple colony morphologies were observed leading to the identification of a particular subset of variants that constitutively produce biofilm. Upon further examination, all biofilm forming variants displayed alterations of the O-antigen that is incorporated into LPS and capsule. Though phase variation of the LPS has been reported in the literature for nearly 70 years, a particular function or role has not been attributed to this phenotype until now. We found not only that purified variants constitutively form biofilm, but variants also delay the onset of a viable non-culturable state. Given that biofilm is a well-known contributor to pathogenesis, we utilized a murine model to study the effects of biofilm formation on virulence in clinically relevant subspecies. For humans, the glandular and ulceroglandular forms of tularemia contracted from an arthropod bite are most common. Though less common, pneumonic forms of tularemia acquired from inhalation of aerosolized bacteria pose the most serious threat to an individual. With this in mind, animals were challenged via subcutaneous and intradermal routes to mimic tick and mosquito bites, respectively, as well as intranasal to mimic aerosol exposure. By all routes of infection, biofilm forming variants of both subspecies were found to be highly attenuated. In this study, we demonstrate that a robust biofilm can be developed naturally by clinically relevant F. tularensis isolates, provide a mechanism for biofilm initiation and examine the role of biofilm formation in virulence.

The genetic and structural relationship between of the Kingella kingae PAM-galactan and lipopolysaccharide

Nina Montoya[1,2], Eric Porsch[2], Courtney Chandler[3], Artur Muszyński[4], Jiri Vlach[4], Parastoo Azadi[4], David K. Hahn[4], Robert Ernst[3], Joseph St. Geme III[1,2]

[1]Department of Microbiology, University of Pennsylvania, [2]Department of Pediatrics, Children's Hospital of Philadelphia, [3]University of Maryland School of Dentistry, Microbial Pathogenesis, [4]Complex Carbohydrate Research Center, University of Georgia

Kingella kingae is a gram-negative, commensal organism of the oropharynx and is the leading cause of bone and joint infections in children between the ages of six months and four years. Critical K. kingae virulence determinants include a polysaccharide capsule and an exopolysaccharide composed of galactofuranose, called PAM-galactan. The PAM-galactan is encoded by the pamABC genes in the pamABCDE locus. Based on homology both by sequence and structure, we predicted that the pamD and pamE genes encode lipopolysaccharide (LPS) glycosyltransferases, prompting us to conduct a structural and functional exploration of the K. kingae LPS. Initial characterization of K. kingae LPS by DOC-PAGE and silver stain suggested a distinctive molecule with an O-antigen, unlike the lipooligosaccharide (LOS) produced by related species. The O-antigen was lost in pamDE mutants. Composition analysis of LPS by mass spectrometry (MS) revealed decreased galactose in mutants lacking pamABC or pamDE, suggesting that LPS and the PAM-galactan are structurally linked. Additional analysis assessing reactivity with a PAM-galactan specific antiserum revealed that pamDE were required for surface localization of PAM-galactan, suggesting that the pamDE-dependent O-antigen may serve to tether the PAM-galactan to the bacterial surface, a mechanism that has not been described previously. To directly evaluate the presence of a covalent linkage between the LPS and PAM-galactan, we used size exclusion chromatography and NMR to separate LPS samples into high molecular weight fractions containing O-antigen and PAM-galactan and low molecular weight fractions containing just O-antigen. Using MALDI-TOF MS we identified K. kingae lipid A in both fractions, confirming a covalent linkage between LPS and PAM-galactan. Future experiments will examine the roles of the individual pam genes in LPS/PAM-galactan biosynthesis as well as the functional implications of this unique structural linkage.

Secondary infection with Streptococcus pneumoniae decreases influenza virus replication and severe disease is linked to reduced airborne transmission

Karina Mueller Brown[1], Valerie Le Sage[2], Andrea French[2], Jennifer Jones[2], Stacey Schultz-Cherry[3], Jason Rosch[3], N. Luisa Hiller[1], Seema Lakdawala[2]

[1]Carnegie Mellon University, [2]University of Pittsburgh, [3]St Jude Children's Hospital

Secondary bacterial infection is a common complication in severe influenza virus infections. During the H1N1 pandemic of 2009, increased mortality was observed among healthy young adults due to secondary bacterial pneumonia, one of the most frequent bacterial species being Streptococcus pneumoniae (Spn). Previous studies in mice and ferrets have suggested a synergistic relationship between Spn and influenza viruses. In this study, the ferret model was used to examine whether airborne transmission of the H1N1 2009 pandemic virus (H1N1pdm09) was influenced by coinfection with two Spn serotypes: type 19F (BHN97) and type 2 (D39). Coinfected animals were found to have increased bacterial colonization of the upper respiratory tract (UTR) and experienced more severe clinical symptoms as compared to animals infected with influenza virus alone. Interestingly, those donor animals that displayed the most severe clinical symptoms had reduced airborne transmission of H1N1pdm09. Infection with Spn after H1N1pdm09 infection consistently resulted in decreased viral shedding in the ferret UTR, suggesting that coinfection with Spn can inhibit H1N1pdm09 replication. Surprisingly, animals precolonized with Spn were equally susceptible to H1N1pdm09 transmission through the air but coinfection in this case did not cause exacerbated disease symptoms. Taken together, our data indicate that coinfection resulting in enhanced disease is due to an acquired infection after H1N1pdm09 as opposed to commensal Spn. Finally, we propose an asymmetrical relationship between these two pathogens rather than a synergistic one since secondary bacterial infection enhances colonization of Spn but decreases replication of H1N1pdm09.

Branched-Chain Fatty Acids Modulate Two-Component System Activation in Staphylococcus aureus

Augustus Pendleton[1], Shahad Alqahtani[1], Dr. WonSik Yeo[1], Dennis DiMaggio[1], Carl Stone[1], Zhaotao Li[2], Dr. Vineet Singh[3], Dr. Christopher Montgomery[2], Dr. Shaun Brinsmade[1]

[1]Georgetown University, [2]Abigail Wexner Institute at Nationwide Children's Hospital, [3]A.T. Still University of Health Sciences

Staphylococcus aureus is a ubiquitous Gram-positive bacterium and an opportunistic human pathogen. S. aureus pathogenesis relies on a complex network of regulatory factors that adjust gene expression. Two important factors in this network are CodY, a repressor protein responsive to nutrient availability, and the SaeRS Two Component System (TCS), which responds to neutrophil-associated factors. Our previous work has revealed that CodY regulates the secretion of many S. aureus toxins indirectly via Sae through an unknown mechanism. We report that S. aureus codY mutant cell membranes contain a higher percentage of branched-chain fatty acids (BCFAs) relative to wild-type membranes, prompting us to hypothesize that changes to the membrane composition modulate the activity of the SaeS sensor kinase. To investigate the role of BCFAs in Sae activity, we disrupted the critical BCFA synthesis gene lpdA, which codes for dihydrolipoyl dehydrogenase. We show that lpdA cell membranes contain fewer BCFAs and exhibit low SaeS activity in vivo and in vitro, resulting in reduced toxin production and attenuated virulence. Sae activity in the lpdA mutant could be complemented genetically and chemically with exogenous short- or full-length-BCFAs, and mutations in the transmembrane domains of SaeS reduce the impact of lpdA mutation. Other TCSs, including AgrABCD and PhoPR, are similarly inactivated in the lpdA mutant, while VraRS activity increased. Taken together, our results suggest a specific BCFA requirement for multiple TCSs. These results reveal a novel method of post-transcriptional virulence regulation via BCFA synthesis, linking CodY activity to multiple virulence regulators in S. aureus.

A genome scale metabolic model reveals changes in Neisseria gonorrhoeae central metabolism during neutrophil inflammation

Aimee Potter[1], Christopher Baiocco[1], Jason Papin[2], Alison Criss[1]
[1]Department of Microbiology, Immunology, and Cancer Biology, University of Virginia, [2]Department of Biomedical Engineering, University of Virginia

Neisseria gonorrhoeae (the gonococcus, Gc) is the causitive agent of the sexually transmitted infection gonorrhoeae. Gc is uniquely adapted to colonize human mucosal surfaces, where it survives despite initiating a robust inflammatory response and influx of innate immune defenses, specifically polymorphonuclear leukocytes (PMNs, or neutrophils). Although PMNs produce reactive oxygen species (ROS) and antimicrobial peptides and proteins, Gc is one of a few pathogens that have the unusual ability to thrive in the presence of PMNs. To investigate these survival mechanisms in an unbiased manner, we recently undertook dual RNA-seq of Gc and human PMNs, alone and in co-culture over time. These data sets revealed an overrepresentation of Gc central metabolism genes following exposure to PMNs. Gc is a fastidious bacterium with limited metabolic capabilities, and is dependent on human nutrient sources for its persistence at mucosal surfaces. The interplay between metabolism and virulence is an emergent concept in microbial pathogenesis, particularly for bacteria with more flexible metabolic strategies. To create a framework for interpreting this data, we have generated a curated genome-scale metabolic network reconstruction (GENRE) of Neisseria gonorrhoeae strain FA1090, which enables the linkage of genetic information to metabolic fluxes, and prediction of biomass production and energy consumption. Data from genome scale transposon mutant screens has been integrated into this model to validate in silico gene essentiality predictions in rich media. Furthermore, using transcriptional data generated from RNAseq, we have contextualized this model using transcriptomic abundances and parsimony of overall flux (RIPTiDe - Reaction Inclusion by Parsimony and Transcript Distribution). This contextualization identifies the most cost-effective usage of metabolism while also reflecting Gc's transcriptional investment. This analysis leads to the prediction that Gc alters its central carbon metabolism (glycolysis, lactate assimilation, TCA cycle) when exposed to PMNs. This contextualized GENRE will serve as a roadmap for important future investigation of pathways that are essential for Gc survival during neutrophilic inflammation. Results arising from these studies will reveal a new way used by Gc to persist in the presence of PMNs and uncover the unique aspects of metabolism in this fastidious bacterium that could be targeted to block infection and reduce the burden of gonorrhea in the human population.

Novel Tn-seq approach identifies the primary secretion mechanism for yersiniabactin in Yersinia pestis

Sarah Price[1], Derek Thibault[2], Taylor Garrison[1], Dr. Tim van Opijnen[2], Dr. Thomas Kehl-Fie[3], Dr. Sylvie Garneau-Tsodikova[4], Dr. Robert Perry[4], Dr. Matthew Lawrenz[1]
[1]University of Louisville, [2]Boston College, [3]University of Illinois Urbana-Champaign, [4]University of Kentucky

Nutritional immunity is the process used by eukaryotic hosts to sequester transition metals, such as iron, zinc, and manganese, from invading bacterial pathogens. To overcome nutritional immunity, bacteria have evolved dedicated metal acquisition systems. Yersinia pestis, a gram-negative bacterium that is the causative agent of plague, acquires iron and zinc by secreting the siderophore yersiniabactin (Ybt). Since Ybt is an essential metallophore required for Y. pestis virulence, understanding the molecular mechanisms for the synthesis and transport of Ybt could reveal novel therapeutic targets to combat plague. The genes required for Ybt synthesis and import are encoded within two genetic loci found within a high pathogenicity island on the Y. pestis chromosome and have thusly been well-defined. However, the genes encoding the mechanism for Ybt secretion are not present within the same loci and have eluded identification. One of the reasons it has been difficult to identify this system is that conventional high-throughput transposon mutagenesis approaches lack the ability to identify genes involved in secretion because of trans-complementation between mutants, also known as “cheating”. To overcome this obstacle, we developed a technique called droplet Tn-seq (dTn-seq). dTn-seq uses microfluidics to isolate individual mutants in oil droplets, eliminating trans-complementation of secreted factors between bacteria, but still allowing for comparisons of bacterial growth in large batch conditions. Because mutants deficient in Ybt and ZnuABC are unable to acquire zinc, we generated a transposon mutant library in the Y. pestis znuBC background and mutants deficient for growth in zinc-limited media were identified by dTn-seq. Primary hits were validated by generating site-directed mutants and measuring changes in growth, trans-complementation, Ybt secretion, and virulence in the mouse model. These primary hits included two genes with homology to an RND efflux system. Independent deletion of the RND efflux system in Y. pestis recapitulated a growth defect in metal limited conditions and showed significantly less yersiniabactin in the supernatant compared to wild-type bacteria. Moreover, the efflux system mutant was unable to trans-complement growth of a mutant unable to synthesize yersiniabactin. Finally, an RND efflux mutant is attenuated for virulence in bubonic and pneumonic plague. Thus, by using dTn-seq, we identified an RND efflux system as the primary mechanism for yersiniabactin export in Y. pestis. Because RND efflux systems are widely conserved, we predict homologs are likely responsible for yersiniabactin secretion in other pathogens, thus these exporters could represent a potential board spectrum, anti-virulence, therapeutic target.

Spatial Lipidomics of Pseudomonas aeruginosa in a Mouse Lung Infection Model Identifies New Host-Directed Therapeutic Targets

Alison Scott[1], Courtney Chandler[1], Shane Ellis[2], Ron Heeren[3], Robert Ernst[1]
[1]Dept. of Microbial Pathogenesis, University of Maryland School of Dentistry, [2]Dept. of Chemistry, University of Wollongong, [3]Maastrict Multimodal Molecular Imaging Institute, Maastricht University

Mass spectrometry imaging (MSI) is a technique for mapping unlabeled spatial distributions of molecules in sectioned tissue and is a powerful tool for describing the host-pathogen lipid interaction. We previously described an MSI-compatible inflation method enabling spatially network analysis of lipids in infected mouse lungs. Lipids and lipid metabolites are potent components of the persistent pulmonary inflammatory response to bacterial infection and their metabolic checkpoints may present unique interventional targets. Using this combination of methods we sought to understand the lipid inflammatory response to an acute lung infection with wildtype Pseudomonas aeruginosa (Pa). Briefly, C57BL/6 mice were infected by the intranasal route with 2 x 108 CFU of Pa strain PAK-1 (or instilled with PBS, vehicle control). Forty-eight hours post-infection (48 hpi) lungs were prepared by post-mortem inflation with 2% gelatin, snap frozen, sectioned at 13 µm thickness, and thaw-mounted to conductive glass slides followed by preparation for matrix-assisted laser desorption/ionization (MALDI)-MSI. MSI was performed on a rapiFlex MALDI-TOF MS in positive and negative ion modes (on serial sections) at 50 µm spatial resolution. Negative ion mode lipid maps were segmented into airway (AW) and parenchyma (PC) regions. Spectra were averaged for each component to build a mass list. Ion presence and abundances were compared between AW and PC, putative ion identities were developed from parallel spatial lipidomic experiments and were converted to closest-match metabolite identities. Metabolic network analyses were seeded using orthologous metabolite-protein interaction networks followed by pathway enrichment analyses. In the AW of infected mouse lungs, two nodes were identified based on phosphatidylserine and phosphatidic acid metabolism and in the PC component of infected mouse lungs a third node emerged, centered on phosphatidylethanolamine metabolism. Analyses of these lipid networks revealed 6 significant and differential lipid metabolic pathways in the PC component and another 6 unique significant and differential pathways in the AW component. The enriched functions in the infected mouse lung PC were predominated by acyl remodeling activities pointing to several predicted functions: bacterial growth, neutrophil-directed acyl remodeling, phospholipid hydrolysis to signaling intermediates, and lipid-directed transport. Lipid metabolic pathways enriched in the AW component of Pa-infected lungs implicated lipid signaling mechanisms, sphingolipid metabolism, and synthesis of phosphatidylinositol. Further, we characterized a series of polyunsaturated phospholipids (PUFA-PLs), specifically phosphatidylinositol lipids linked to lethal inflammation in bacterial infection and mapped them in serial sections of inflated mouse lung. The lipid metabolic functions identified in the PC of Pa-infected lungs are consistent with the cellular response (especially Type II pneumocytes, resident phagocytic cells, and infiltrating neutrophils). We will leverage these findings to identify novel host-based therapeutic targets central to lipid-mediated inflammation and exacerbation of lung disease.

The role of colonization factors CFA/I and CS21 in ETEC pathogenesis in the human enteroid model

Emily Smith[1], Dr. Eileen Barry[1]
[1]*University of Maryland, Baltimore*

Enterotoxigenic Escherichia coli (ETEC) is a primary causative agent of diarrhea in young children in developing countries and of traveler's diarrhea. Following ingestion of contaminated food or water, ETEC adhere to intestinal epithelia via colonization factors (CFs) and secrete heat-stable toxin (ST) and/or heat-labile toxin (LT), causing dysregulated cellular ion transport and water secretion. ETEC isolates often harbor genes encoding more than one CF and are prime targets as vaccine antigens. However, there is no licensed ETEC vaccine. CFA/I is a major CF significantly associated with diarrhea. Many clinical isolates express CFA/I as well as CS21; however, a role for CS21 alone or in combination with CFA/I has not been defined. We hypothesize that expression of both CFA/I and CS21 confers increased adherence and toxin delivery to the human enteroid model. Clinical strains H10407 and CVD30 harboring genes encoding CFA/I (cfaB) and/or CS21 (lngA) were evaluated, and mutants were engineered with deletions in one or both genes. Using Western blotting and immuno-electron microscopy, expression of CFA/I and/or CS21 was confirmed in the wildtype and CF-deficient strains (ΔcfaB, ΔlngA, and ΔcfaBΔlngA) grown on CFA agar and in Terrific broth. To assess the contribution of CFA/I and CS21 to adherence, attachment to human jejunal and ileal enteroid monolayers by CVD30 and its derivative mutant strains was quantified by measuring adherent bacteria and visualized by confocal microscopy. CFA/I was important for adherence to enteroid monolayers as CFA/I-deficient mutants ΔcfaB and ΔcfaBΔlngA adhered significantly less than the wildtype CVD30 strain ($p<0.001$). In contrast, CS21 was not required for adherence as CS21-deficient mutants ΔlngA adhered at similar levels as the wildtype CVD30 strain. Similarly, anti-CFA/I antibody significantly blocked ETEC adherence compared to the wildtype strain, while anti-CS21 did not. These data provide evidence that CFA/I alone is sufficient for adherence in strains with multiple CFs. To test the effect of CFA/I and CS21 on downstream toxin delivery to enteroid monolayers, ST delivery was evaluated by measuring ST-induced cyclic nucleotide cGMP levels by ELISA. Delivery of ST by ETEC and resulting cGMP levels were similar across wildtype CVD30 and CF-deficient strains (ΔcfaB, ΔlngA, and ΔcfaBΔlngA), indicating that CFA/I and CS21 did not affect toxin delivery to enteroid monolayers. These comprehensive data demonstrate that CFA/I alone is important for ETEC adherence in strains with multiple CFs, while CS21 is not required for adherence. Despite the significance to adherence, neither CFA/I nor CS21 is important for ST delivery to human enteroid monolayers, suggesting that other factors may affect toxin delivery. Overall, these data support the role of CFA/I in ETEC pathogenesis and as a main antigen target for ETEC vaccine development.

Mucormycosis-causing fungi suppress nitric oxide accumulation during macrophage infection

Alexandra Soare[1,2], Yiyou Guo[3,4], Amol Shety[2], Carrie McCracken[2], Ashraf Ibrahim[3,4], Vincent Bruno[1,2]
[1]Department of Microbiology and Immunology, University of Maryland School of Medicine, [2]Institute of Genome Sciences, University of Maryland School of Medicine, [3]Division of Infectious Diseases, Los Angeles Biomedical Research Institute, Harbor-University of California, Los Angeles Medical Center, [4]David Geffen School of Medicine at University of California, Los Angeles

Mucormycoses is a NIAID-classified emerging infectious disease caused by Mucorales fungi. The recent surge of cases among COVID-19 patients has thrust the disease and lack of available treatments into the spotlight. Clinical data suggests a lack of inflammatory responses during mucormycosis, despite severe fungal angioinvasion and tissue necrosis. Due to the paucity of knowledge on host-pathogen interactions, we sought to characterize immune evasion mechanisms by Mucorales fungi. Lungs were extracted from mice infected with a wild-type R. delemar strain or an invasion-defective strain of R. delemar for RNA-sequencing and subsequent upstream regulatory analysis (URA). Interestingly, the invasion-defective strain elicited a more robust transcriptional response than the wild-type strain, including many well-known immune pathways. One such pathway included the Nos2 gene. Nos2 (also known as inducible nitric oxide synthase or iNos) is an enzyme expressed by macrophages under pro-inflammatory conditions to produce nitric oxide (NO), a free radical with potent antimicrobial properties and roles in multiple signaling pathways.

To verify our transcriptomic results, we treated an alveolar macrophage cell line (MH-S) with LPS and IFN-γ in the presence or absence of fungal spores. RNA and protein lysates were collected at 8 HPI for RT-qPCR and Western Blots. Supernatants were harvested at 24 HPI to measure nitrite accumulation as an indicator of NO production via Greiss Assay. At 8 HPI, LPS and IFN-γ induced a 2,000-fold induction of Nos2 expression in MH-S cells, which was accompanied by robust NO production at 24 HPI. MH-S cells infected with Mucorales species in the presence of LPS and IFN-γ showed similar levels of Nos2 mRNA. However, these cells showed significantly reduced NO production by >90%. There was no significant difference in NOS2 protein levels between LPS and IFN-γ treated MH-S cells and MH-S cells infected with Mucorales in the presence of LPS and IFN-γ at 8 HPI. These trends were seen with multiple Mucorales species and in other M∅ cell lines and primary cells. M∅s treated with R. delemar conditioned media showed a 70% reduction in NO production. Furthermore, heat-killed conditioned media was able to reduce NO production and showed no significant difference from normal conditioned media. These results suggest that R. delemar secretes a heat-stable molecule that can interfere with NO production during M∅ infection.

Overall, our transcriptomic data and clinical data suggest a repression of important immune pathways during mucormycosis, including Nos2 signaling pathways. We demonstrated that Mucorales species can deplete induced NO production in multiple macrophage models, despite induction of Nos2 transcript and protein. Our results suggest that NO production is blocked a by heat-stable factor that is secreted by the fungus. This suppressive activity represents a potential mechanism by which Mucorales evade the immune response to cause disease.

Carbon Catabolite Repression and virulence are linked in Staphylococcus aureus

Amelia Stephens[1], Dr Lance Thurlow[2], Dr. Anthony Richardson[1]
[1]*University of Pittsburgh,* [2]*University of North Carolina*

Staphylococcus aureus is a major human pathogen and a growing public health issue. S. aureus infections primarily manifest as skin and soft tissue infections (SSTIs) that, if left untreated, can result in more invasive presentations, such as osteomyelitis and sepsis. Previous work from our lab has shown that glucose is imperative for toxin and protease production, and therefore for fully invasive S. aureus infection. We showed that elevated blood glucose levels in individuals with diabetes contribute to more severe infections in these individuals via enhanced toxin and protease production. We also show that the global carbon catabolite protein A, or CcpA, is vital for the full production of these toxins and proteases. CcpA is responsible for the activation of glycolysis in S. aureus, and the absence of CcpA results in increased amino acid catabolism. We tested whether this enhanced amino acid catabolism resulted in reduced amino acid pools thereby limiting AIP production. While the intracellular concentrations of glycine, threonine, and arginine were diminished in the ΔccpA mutant, this did not affect AIP production as supernatants from a ΔccpA constitutively expressing ArgBD stimulated a pRNAIII::YFP reporter construct to similar levels as WT expressing ArgBD. Rather, the preference for gluconeogenesis in the ΔccpA mutant drains energy pools and the subsequent reduction in ATP limits AgrC activity via a previously described mechanism. This finding finally mechanistically explains the indirectly positive effect of CcpA on Agr activity described by many groups over the past few decades.

The effect of detoxifying gonococcal lipid A through inactivation of the msbB gene on safety and outer membrane vesicle-induced immune responses.

Lam Thuy Vi Tran Ho[1], Jacqueline T. Balthazar[2], Dr. Andrew N. Macintyre[3], Dr. Greg D. Sempowski[3], Dr. William M. Shafer[2], Dr. Ann E. Jerse[1]

[1]Uniformed Services University Of The Health Sciences, [2]Emory University School of Medicine, [3]Duke University School of Medicine

Neisseria gonorrhoeae (Ng) is a sexually transmitted bacteria that causes pelvic inflammatory disease and infertility in untreated women. One of the mechanisms by which Ng evades the host immune response is to shed outer membrane vesicles (OMVs), which may act as a decoy for Ng-specific antibodies. The OMVs also contain lipooligosaccharide (LOS), which has inflammatory activity due to its lipid A (endotoxin) component. Here we investigated whether genetic modification of Ng lipid A can reduce endotoxin activity and whether these modifications effect the host humoral and cellular immune response to proteins within the OMV. Using OMVs from Ng mutants with a deleted msbB gene (OMVmsbB) which adds an acyl chain to the lipid A, we found that OMVmsbB induced significantly lower levels of NF-κB activation in a HEK-BLUE TLR4 reporter gene assay compared to OMVWT. A single subcutaneous injection with OMVWT , but not OMVmsbB , caused local skin reactions. Serum and vaginal antibody titers collected after a 2nd and 3rd immunization were similar in all immunization groups, but antisera against OMVmsbB recognized fewer proteins by western blot against OMVWT. Serum bactericidal antibody titers were lower in OMVmsbB immunized sera compared to OMVWT. Splenocyte OMV re-stimulation assays showed that OMVmsbB immunization induced lower IFN-γ, IL-17A and IL-10 cytokine levels in response to antigen stimulation compared to OMVWT. When immunized mice were challenged with FA1090 Ng bacteria in a female lower genital tract mouse model, OMVmsbB – immunized mice were similarly colonized with Ng as OMVWT-immunized mice and control but had no local adverse skin reactions because of the attenuated lipid A. These results reveal interesting lipid A-related differences in the proinflammatory activity of OMVs and in the immune response with respect to, antibody specificity, bactericidal activity and cytokine responses. Future studies should lead to a broader understanding of lipid A biology.

The type three secretion system effector protein IpgB1 promotes Shigella flexneri cell-to-cell spread through double-membrane vacuole escape

Erin Weddle[1], Dr. Volkan Köseoğlu[1], Brittany DeVasure[1], Dr. Hervé Agaisse[1]
[1]*University of Virginia*

S. flexneri is an important human pathogen that causes bacillary dysentery. During infection, S. flexneri invades colonic epithelial cells, hijacks the host cell cytoskeleton to move in the cytosol of infected cells, and spreads from cell to cell through formation of membrane protrusions that project into adjacent cells and resolve into double membrane vacuoles (DMVs). S. flexneri cell-to-cell spread requires the integrity of the bacterial type three secretion system (T3SS). However, the exact role of the T3SS effector proteins in the dissemination process remains poorly understood. Here, we investigated the role of the T3SS effector protein IpgB1 in S. flexneri dissemination. IpgB1 was previously characterized as a guanine nucleotide exchange factor (GEF) that contributes to invasion. In addition to the invasion defect, we showed that the ipgB1 mutant formed smaller infection foci in HT-29 cells. Complementation of this phenotype required the GEF activity of IpgB1. Using live confocal microscopy, we showed that the ipgB1 mutant is specifically impaired in DMV escape. Depletion of Rac1, the host cell target of IpgB1 during invasion, as well as pharmacological inhibition of Rac1 signaling, reduced cell-to-cell spread and DMV escape. In a targeted siRNA screen, we uncovered that RhoA depletion restored ipgB1 cell-to-cell spread and DMV escape, revealing a critical role for the IpgB1-Rac1 axis in antagonizing RhoA-mediated restriction of DMV escape. Using an infant rabbit model of shigellosis, we showed that the ipgB1 mutant formed fewer and smaller infection foci in colons of infected animals, which correlated with attenuated symptoms of disease, including epithelial fenestration and bloody diarrhea. Our results demonstrate that, in addition to its role during invasion, IpgB1 modulates Rho family small GTPase signaling to promote cell-to-cell spread, DMV escape, and S. flexneri pathogenesis.

C4B-BINDING PROTEIN IN HUMAN SERUM BINDS NEISSERIA GONORRHOEAE AND SUPPRESSES NEUTROPHIL ANTIGONOCOCCAL ACTIVITY IN A COMPLEMENT-INDEPENDENT MANNER

Lacie Werner[1], Allison Allcott[1], Asya Smirnov[1], Meagan Belcher Dufrisne[2], Frida Mohlin[3], Linda Columbus[2], Anna Blom[3], Alison Criss[1]

[1]University of Virginia Department of Microbiology, Immunology, and Cancer Biology, [2]University of Virginia Department of Chemistry , [3]Lund University Department of Translational Medicine

Neisseria gonorrhoeae (Gc) is a human-specific pathogen and causative agent of the sexually transmitted infection gonorrhea. Gc is considered an urgent threat to public health with 78 million cases occurring annually worldwide, growing antimicrobial resistance, and the absence of a gonococcal vaccine. A hallmark of gonorrhea infection is the influx of neutrophils, but this response does not clear infection. Phase variation in the Gc population is thought to contribute to the ability of Gc to avoid neutrophil killing. Among these phase-variable components are the surface expressed opacity-associated (Opa) proteins of Gc. Expression of Opa proteins like OpaD, which activates neutrophils by binding to the phagocytic receptor CEACAM-3, significantly decreases Gc survival in the presence of human neutrophils. Neutrophils readily internalize OpaD+ Gc and direct the bacteria to a phagolysosome where they are killed by proteases and antimicrobial peptides. We do not understand why Opa-expressing Gc (Opa+ Gc) survive and even predominate among Gc in neutrophil-rich clinical gonorrhea isolates despite being killed by neutrophils in vitro. Our data provide an explanation for these discordant findings: Opsonization of Gc with human serum, which is found in inflammatory mucosal secretions, enhances Opa+ Gc survival from neutrophils. Moreover, serum opsonization of OpaD+ Gc inhibits neutrophil reactive oxygen species (ROS), indicating a decrease in neutrophil activation. Through biochemical fractionation, C4b-binding protein (C4BP) was identified as the serum component that binds to Opa+ Gc to mediate the phenotypes described above. C4BP is known for inhibiting the classical and lectin complement cascades; however, the effects we observe with human serum are independent of active complement. C4BP was found to be necessary to suppress neutrophil ROS production, reduce internalization of Opa+ Gc by neutrophils, and enhance survival of Opa+ Gc. Purified C4BP was found to be sufficient to mediate these effects. This research identifies for the first time the role of C4BP in enhancing bacterial survival from neutrophils, independently of the effects of complement-mediated lysis. By defining how Opa+ Gc exploits C4BP, we can better understand how Gc survives in neutrophil-rich secretions.

Utilizing bacterial component adjuvants for COVID-19 vaccines

Ting Wong[1,2], Ms Katherine Lee[1,2], Dr. Brynnan Russ[1,2], Dr. Alexander Horspool[1,2], Dr. Jason Kang[1,2], Mr. Michael Winters[1], Dr. Allison Wolf[1,2], Mr Nathaniel Rader[1,2], Ms Olivia Miller[1,2], Morgane Shiflett[6], Dr. Jerilyn Izac[4], Dr. David Varisco[4], Dr. Emel Sen-Kilic[1,2], Mr. Casey Cunningham[1,2], Ms Melissa Cooper[1,2], Dr. Holly Cyphert[5], Dr. Mariette Barbier[1,2], Dr. Ivan Martinez[1,3], Mr. Justin Bevere[1,2], Dr. Robert Ernst[4], Dr. F. Heath Damron[1,2]

[1]Department of Microbiology, Immunology, and Cell Biology, West Virginia University, [2]Vaccine Development Center at WVU, [3]West Virginia University Cancer Institute, [4]Department of Microbial Pathogenesis, University of Maryland of Dentistry, [5]Department of Biological Sciences, Marshall University , [6]Fina Biosolutions

SARS-CoV-2 is a respiratory pathogen responsible for the COVID-19 pandemic. All current WHO approved COVID-19 vaccines are administered through the muscular route. After screening 12 different COVID-19 vaccine combinations, through both intranasal and intramuscular routes, we have developed a prototype two-dose vaccine that combines the Receptor Binding Domain (RBD) conjugated to detoxified Diphtheria toxin (EcoCRM®) adjuvanted with Bacterial Enzymatic Combinatorial Chemistry (BECC) adjuvant (BReC-CoV-2). We hypothesized that the intranasal administration of BreC-CoV-2 will induce a strong localized immune response in the respiratory tissues allowing for protection against SARS-CoV-2. To test this hypothesis, we vaccinated K18-hACE2 mice with BReC-CoV-2 through both intranasal (IN) and intramuscular (IM) routes and challenged mice with a lethal dose of the ancestral strain of SARS-CoV-2 (WA-1). IN BReC-CoV-2 vaccinated mice resulted in increased survival (89%), little to no body weight or temperature fluctuations, and decreased disease manifestations compared to the no vaccine, challenged control (NVC), and IM BreC-CoV-2. A significant decrease in SARS-CoV-2 viral RNA in the lung was observed in the IN BReC-CoV-2 vaccinated mice compared to IM and NVC. IN BreC-CoV-2 induced robust RBD IgG titers in both the serum and lung, as well as significant production of IgA in the lung and nasal wash compared to IM BReC-CoV-2 and NVC. Antibodies produced through IN vaccination were broadly neutralizing against the RBD of the variants of concern (Alpha, Beta, Gamma and Wuhan). Histology of the lung showed recruitment of plasma cells and lymphocytes in the IN vaccinated mice as well as a significant decrease of acute inflammation in both IN and IM BReC-CoV-2 vaccinated mice. Since the Delta variant is the current predominant global strain, we performed a Delta challenge study with K18-hACE2 mice vaccinated with BReC-CoV-2 through the IN, IM, and heterologous IM prime and IN boost. IN administration of BReC-CoV-2 whether administered as a prime and boost (60% survival) or administered as a boost after IM prime (100% survival) provided better protection than IM only administration (40% survival). Overall, these data suggest that IN administration of BReC-CoV-2 induces localized immune responses that can better protect against SARS-CoV-2 than IM administration in the upper respiratory tract, the primary site of infection.

Poster Presentation Abstracts

Rickettsia conorii survival in THP-1 macrophages requires host lipid metabolic processes

Dr. Paige E. Allen[1,2], Dr. Robert C. Noland[3], Katarina Jones[4], Hector Castro[4], Dr. Shawn Campagna[4], Dr. Jason A. Carlyon[2], Dr. Juan J. Martinez[1]

[1]Vector Borne Disease Laboratories, Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, [2]Department of Microbiology and Immunology, School of Medicine, Virginia Commonwealth University , [3]Skeletal Muscle Metabolism Laboratories, Pennington Biomedical Research Center, [4]Biological and Small Molecule Mass Spectrometry Core, Department of Chemistry, University of Tennessee Knoxville

Rickettsia conorii is a Gram-negative, obligate intracellular bacterium that is the causative agent of Mediterranean spotted fever, a disease endemic to the Mediterranean basin and Europe with a mortality rate as high as 30% if left untreated. R. conorii has adapted mechanisms to grow in macrophages during mammalian infection. We have previously shown that infection of these phagocytes shifts the host cell's overall metabolism towards an anti-inflammatory M2 response, defined by an increase in host lipid metabolism and oxidative phosphorylation. Intracellular pathogens have adapted mechanisms of hijacking these host pathways for various functions required for growth and survival. In the present study, we assess the modulation of host lipid droplets (LD) and lipid metabolism during R. conorii infection of macrophages. Oil red O immunofluorescent staining of neutral lipids found within LDs was used to visualize host LD modulation during R. conorii infection in THP-1 macrophages. Host LD changes were initiated early in infection. To determine the processes initiating these defined host LD alterations, R. conorii protein synthesis was deterred, and bacterial-driven initiation of LD changes was assessed. Additionally, pharmacological inhibitors were employed to determine the importance of host lipid catabolic pathways to infection-driven LD modifications and overall R. conorii survival. These data suggest that the initiation of LD modulation early in infection requires an unknown bacterial mechanism, and host lipid catabolism which is necessary for R. conorii survival in THP-1 macrophages. Host LD alterations in macrophages infected with R. conorii are not suggestive of a foam cell phenotype, found to be important for other intracellular bacterial macrophage infections. Therefore, we assessed the effects of lipid loading on rickettsial survival and found that inducing a foam cell-like phenotype has a negative correlation with R. conorii growth in THP-1 macrophages. In addition, we have begun investigating the fate of lipids being released from LDs and lipids being catabolized, by defining significant changes in phospholipid composition during infection through unbiased high throughput lipidomics analysis. Together, these results strongly suggest that the modulation of lipids and lipid catabolism in R. conorii infection of macrophages is an important and underappreciated aspect of infection.

The Zn finger protein and essential virulence determinant MucR acts as an H-NS-like gene silencer in Brucella abortus 2308

Ian Barton[1], Krishna Patel, Brandon Garcia, Daniel Martin, R. Martin Roop II
[1]*Department of Microbiology and Immunology, Brody School of Medicine, East Carolina University*

The 16 kDa Zn finger protein MucR is a global transcriptional regulator found throughout members of α-proteobacteria, regulating a diverse set of functions including virulence, symbiosis, exopolysaccharide production, motility, and cell cycle progression. Studies suggest that MucR predominantly acts as a repressor or gene silencer, preventing the expression of genes when they are not beneficial. For instance, in Agrobacterium tumefaciens and Sinorhizobium meliloti, MucR homologs repress virulence and symbiosis loci, respectively, until extracellular signals lead to their expression through the transcriptional activators VirG and ExpR. Thus, expression of costly virulence or symbiosis functions are restricted to host interaction. MucR has been demonstrated to be an essential virulence determinant in Brucella abortus and Brucella melitensis, where a mucR mutant exhibits decreased survival in macrophages and is severely attenuated in mice. Despite MucR homologs being well-appreciated as important transcriptional regulators, surprisingly little is known about how they function at the molecular level or how their regulatory activity is coordinated with other transcriptional regulators. We have previously shown that MucR binds with low affinity to AT-rich regions in Brucella promoters in a fashion reminiscent of the prototypic gene silencer H-NS. Brucella and related α-proteobacteria lack functional H-NS homologs, so it is tempting to speculate that MucR is performing similar regulatory roles in these systems. Here, we show that MucR binds within the promoter regions of several Brucella genes that have been linked to virulence (e.g. btaE, babR, and bpdB) and silences the expression of these genes and present strategies for dissecting the regulatory role of MucR in the coordination of virulence functions during host interaction and pathogenesis.

Role of IL-17 in curli mediated autoimmunity

Shingo Bessho[1], Kaitlyn Grando, Dr. Amanda Miller, Dr. Cagla Tukel
[1]*Temple University*

Reactive arthritis (ReA), also known as post-infectious arthritis, is an autoimmune condition that develops in 5% of patients after gastrointestinal or genitourinary infection with pathogens such as Salmonella, Shigella, Yersinia, Campylobacter, or Chlamydia trachomatis. Symptoms starts as early as one to four weeks post infection and patients can remain asymptomatic for 5 or more years. Although the disease is a sequela of bacterial infection, antibiotic treatment is ineffective to relieve patient's symptoms. Some studies showed that cultures of joint fluids yield no growth of organisms, suggesting that ReA is a "sterile" arthritis. Curli is the major proteinaceous component of enteric biofilms. Curli fibers can incorporate extracellular DNA, creating highly immunogenic curli/DNA complexes. Intraperitoneal (i.p) injection of curli/DNA complexes into mice leads to the generation of anti-double-stranded (ds) DNA antibody and joint inflammation. Similarly, i.p. injection or oral delivery of Salmonella enterica serovar Typhimurium (STm) but not the isogenic curli mutant also shows elevated levels of anti-dsDNA antibody and joint inflammation. Earlier studies in our lab, showed that the curli/DNA complexes are recognized through the TLR2/1 heterocomplex and 9 leading to the generation of autoantibodies. However, the role of these receptors in eliciting curli-dependent joint inflammation remains unknown. Interleukin (IL)-17, an important pro-inflammatory cytokine has been implicated as critical for autoimmunity. Infections with wild-type STm have been shown to express significantly higher levels of IL-17 when compared the curli mutant. Elevated expression of il17a is blunted in TLR2 deficient mice infected with wild-type STm, indicating TLR2 activation contributes to the expression of this cytokine. Here, we hypothesize that IL-17 activated through the recognition of curli/DNA complex by the TLR 2/1 heterocomplex and TLR9 leads to the generation of autoantibodies. We plan to test our hypothesis by investigating TLR2 and IL-17 mediated inflammation orchestrates the autoimmune response and joint inflammation elicited by curli/DNA complexes. This research is necessary for guiding the development of therapeutic tools that can prevent or ameliorate autoimmunity associated with gastrointestinal infections.

Combining omics and challenge models to characterize emerging clinical isolates of Bordetella pertussis and evading vaccine immunity

Graham Bitzer[1,2], Dr. M. Allison Wolf[1,2], Megan DeJong[1,2], Dr. Jesse Hall[1,2], Casey Cunningham[1,2], Ting Wong[1,2], Matthew Warden[1,2], Justin Bevere[1,2], Jonathan Petty[1,2], Dr. Michael Weigand[3], Dr. Mariette Barbier[1,2], Dr. F. Heath Damron[1,2]

[1]Department of Microbiology, Immunology, and Cell Biology, West Virginia University, [2]West Virginia University Vaccine Development Center, West Virginia University, [3]Centers for Disease Control

Whooping cough (pertussis) has been listed as an emerging pathogen by the NIAID even though the CDC categorized pertussis as a vaccine preventable disease. The reasons for the re-mergence are unclear, but genomic analysis has suggested the circulating strains have evolved due to vaccine pressure. We utilized transcriptomics, proteomics, and naïve/immunized mouse models, to determine the virulence factors and advantages these strains have that allow them to infect vaccinated hosts. Mutations or loss of antigens present in the current DTaP and Tdap vaccines, namely pertactin, are the most apparent genomic changes that could facilitate fitness. RNAseq was performed on in vitro growth of 12 circulating strains collected by the CDC pertussis team and compared to three control strains: UT25, Tohama I, and D420 (baboon infection strain).The CDC clinical isolates had higher (between 5-20-fold change) expression of the ptx operon that encodes the pertussis toxin subunits. In the emerging clinical isolates, we observed low expression of an operon of genes (lmgABC) that encode enzymes involved in lipid A modification. Lower expression of lmgABC would decrease TLR4 activation during infection. To properly model infection, we utilized three different intranasal challenge doses ($2x10^7$, $2x10^6$, and $2x10^5$ CFU), and based on the dose study we concluded that $2x10^6$ provided the bacteria opportunity to increase in burden over the first three days post challenge. Naïve challenge studies ($2x10^6$ CFU dose) in outbred mice were performed with all fifteen strains but bacterial burden was not significant when comparing strain to strain at day 3 post challenge. However, I762 was observed to induce un-characteristically high cytokine production including: interferon gamma, TNF alpha, IL-6, and others. Our studies have suggested that the circulating isolates do not have obvious differences in ability to subvert innate responses. Currently, studies are underway to characterize these strains ability to infect following DTaP acellular or whole cell pertussis vaccine immunization.

Interleukin 27 compromises BCG clearance and CD4+ T cell stimulatory cytokine production in neonatal BMDCs

Shelby Bradford[1,2], Jessica Povroznik[1,2], Dr. Cory Robinson[1,2]
[1]Department of Microbiology, Immunology and Cell Biology; West Virginia University, School of Medicine, [2]Vaccine Development center; West Virginia University, School of Medicine

Vaccination is one of the most important medical advancement in modern history; however, despite their significance, few vaccines are delivered during the neonatal period. Worldwide, only HepB and BCG are administered in the first weeks following birth. While the HepB series provides adequate protection against hepatitis B, BCG, a live-attenuated strain of Mycobacterium bovis, is poorly protective against Mycobacterium tuberculosis, the causative agent of tuberculosis (TB). Globally, there were 10 million cases of TB and 1.5 million deaths in 2020. One of the barriers to stopping TB incidence is the poor protective nature of the BCG vaccine; though protective against disseminated forms of TB in early childhood, it does not provide lasting protection against pulmonary TB in older children and adults. Our hypothesis centers around the age at which BCG is given and the immune architecture in that time. BCG is most commonly given within the first 10 days of life, a time when the immune system is programmed in a more tolerant phenotype. One of these characteristics is elevated levels of interleukin (IL)-27, which our lab and others have shown has immune suppressive activity. Our central hypothesis is that elevated IL-27 negatively impacts the immune response during BCG vaccination resulting in insufficient immunological protection. To investigate this, we have developed a neonatal mouse model of BCG vaccination and, because of their importance in vaccine responses, have focused on the role of dendritic cells (DCs) and the effects IL-27 has on this cell population. We have observed an increase in circulating IL-27 that remains elevated at least 5 weeks in neonatally-vaccinated C57BL/6 mice. This increase in IL-27 production is consistent with failure to clear BCG over the same time frame. To study the effect of IL-27 on bone marrow derived dendritic cells (BMDC), we utilized mice deficient for IL-27Rα (KO), a component of the IL-27 receptor which renders the cells unresponsive to IL-27. We have observed greater BCG killing and elevated IL-12 production in the neonatal KO BMDCs compared to WT. Since both of these events are import during antigen presentation to lymphocytes, we have investigated the ability of these neonatal BMDCs to stimulate CD4+ T cells from WT adult-vaccinated mice. In these experiments, we observed greater interferon-γ production by CD4+ T cells stimulated with KO BMDCs than their WT counterparts. These results highlight the suppressive role of IL-27 on antigen presenting cells in the neonatal period and the impact on immune responses to live-attenuated vaccines such as BCG.

Yersinia pestis inhibits host synthesis of Leukotriene B4, a potent mediator of inflammation

Amanda Brady[1], Dr. Amanda Pulsifer[1,2], Sarah Price[1], Dr. Krishna Maddipati[3], Dr. Haribabu Bodduluri[1], Dr. Silvia Uriarte[1], Dr. Matthew Lawrenz[1]
[1]University of Louisville, [2]Tufts University, [3]Wayne State University

Yersinia pestis, the bacterial agent of human plague, subverts the host immune response to inhibit inflammation. Because the generation of this non-inflammatory environment is required for acute infection, we are interested in defining the molecular mechanisms used by Y. pestis to evade immune recognition. Leukotriene B4 (LTB4) is one of the most potent lipid mediators of inflammation and is required for effective responses to bacterial infections. Despite the importance of lipids in mediating inflammation, the role of inflammatory lipids during plague has not been previously studied. BLT1 mice, which lack the primary receptor for LTB4, are not more susceptible to infection than WT mice, suggesting either: 1) LTB4 signaling does not contribute to controlling Y. pestis infection, or 2) Y. pestis subverts LTB4 responses. To help differentiate between these two possibilities, we determined the kinetics of LTB4 production in the lungs of mice during pneumonic plague. In contrast to mice infected with K. pneumoniae, LTB4 production did not significantly increase in Y. pestis infected mice. Moreover, ex vivo experiments with neutrophils suggest that Y. pestis actively inhibits LTB4 synthesis in a T3SS dependent manner. Together, these data represent the first characterization of the LTB4 response during pneumonic plague and suggest that the lack of LTB4 synthesis by neutrophils, and potentially other host cells, contributes to the non-inflammatory response associated with plague. Future studies will explore the molecular mechanisms of how Y. pestis inhibits LTB4 synthesis, and the consequences of LTB4 synthesis inhibition on interactions between leukocytes and Y. pestis.

Co-infection with Methicillin-Resistant Staphylococcus aureus is Protective against Infection with Cystic Fibrosis-Adapted Pseudomonas aeruginosa

Kristen Brao[1], Casey Hofstaedter[1], Brendan Wille[1], Peter Kim[1], Amanda Fischer[1], Robert Ernst[1], Janette Harro[1]
[1]*University of Maryland, Baltimore*

Patients with cystic fibrosis (CF) develop thick mucus in their lungs that predisposes them to recurrent and chronic infections. Young patients tend to be infected with Staphylococcus aureus (SA); however, as patients age, Pseudomonas aeruginosa (PA), which is responsible for much of the morbidity and mortality associated with CF, becomes the predominant cause of lung infections. In the process of adapting to the CF lung environment, PA often over-expresses the polysaccharide alginate (mucoidy), which contributes to resistance to phagocytosis, and develops lipid A modifications and antibiotic resistance. Infection with SA has been identified as a risk factor for future PA infection, and a significant proportion of patients are co-infected with both pathogens, permitting the species to interact, either directly or via the immune system. Although SA and PA interactions have been extensively studied in vitro, the role that interspecies interactions play in modulating virulence in vivo is less clearly understood.

To determine the effects of SA on the ability of PA to infect the CF lung, we intranasally infected Scnn1b-Tg (β-ENaC) BALB/c mice with both organisms simultaneously. Scnn1b-Tg mice overexpress epithelial sodium channels (ENaC) in the lungs, resulting in dehydrated mucus, reduced mucociliary clearance, and neutrophilic inflammation similar to CF lung pathology, and they have been used previously as CF-associated PA infection models. When Scnn1b-Tg mice were co-infected with a USA300 methicillin-resistant SA (MRSA) and an early PA CF isolate (three-month-old patient), we found a positive correlation between the number of MRSA and PA colony-forming units (CFU) in the lungs on day three post-infection. In contrast, when Scnn1b-Tg mice were co-infected with MRSA and a mucoid PA isolate (18-month-old patient) that is adapted to the CF lung environment, the infection burden of the adapted PA isolate was reduced (geometric mean of $2.8x10^8$ vs $1.4x10^5$ CFU/g lung tissue, $p<0.001$ by Mann-Whitney test). The adapted PA isolate was also less likely to disseminate during co-infection with MRSA. We replicated these findings using a minimally passaged MRSA CF isolate. In contrast to the murine infection study results, the early CF PA isolate inhibited the growth of MRSA in vitro, whereas the adapted PA isolate did not. On examination of H&E-stained lung slides, we found that infection with the adapted PA isolate alone was associated with increased neutrophilic infiltration into the bronchioles, with minimal involvement of the alveolar spaces, whereas coinfection with both MRSA and the adapted PA isolate was associated with less neutrophilic infiltration and more macrophage activity. Infecting Scnn1b-Tg mice with the adapted PA isolate alongside heat-killed MRSA resulted in a decrease in PA CFUs in the lungs ($2.1x10^9$ vs $1.4x10^6$ CFU/g lung, $p<0.001$ by Mann-Whitney), but live MRSA was significantly more protective.

These experiments suggest that in vitro studies remain a useful way to study direct bacterial interactions, although they do not always predict the results of polymicrobial studies in vivo. We have demonstrated that MRSA can play complex roles in the CF lung, potentially both aiding PA in initially colonizing the lung and inhibiting the pathogenicity of adapted PA isolates.

Colistin resistance in Klebsiella pneumoniae- A double edged sword

Andrew Bray[1], Richard Smith[2], Andrew Hudson[1], Taylor Young[1], Robert Ernst[2], M. Ammar Zafar[1]
[1]Wake Forest School of Medicine, [2]University of Maryland- Baltimore

Due to its high transmissibility, Klebsiella pneumoniae (Kpn) is one of the leading causes of nosocomial infections. Here, we studied the biological cost of colistin resistance, an antibiotic of last resort, of this opportunistic pathogen using a murine model of gut colonization and transmission. Colistin resistance in Kpn is commonly the result of inactivation of the small regulatory protein MgrB. Without a functional MgrB, the two-component system PhoPQ is constitutively active, leading to increased lipid A modifications and subsequent colistin resistance. Using an engineered MgrB mutant, we observed that MgrB-dependent colistin resistance is not associated with a fitness defect during in vitro growth conditions. However, colistin-resistant Kpn colonizes the murine gut poorly, which may be due to the decreased production of capsular polysaccharide by Kpn with non-functional MgrB. The colistin-resistant mutant of Kpn had increased survival outside the host when compared to the colistin-sensitive strain. We attribute this enhanced survivability to dysregulation of the PhoPQ two-component system and accumulation of the master stress regulator RpoS. The enhanced survival of the colistin resistant strain may be a key factor in the observed rapid host-to-host transmission in our model. Together, our data demonstrate that colistin- resistant Kpn experiences a biological cost in gastrointestinal colonization. However, this cost is mitigated by enhanced survival outside the host, increasing the risk of transmission. Additionally, it underscores the importance of considering the entire life cycle of a pathogen to truly determine the biological cost associated with antibiotic resistance.

Persister cell survival leads to treatment failure during Yersinia pseudotuberculosis systemic infection

Rezia Era Braza[1], Dr Kimberly Davis
[1]*Johns Hopkins University*

Persister cells are a bacterial subpopulation that survive the course of antibiotic treatment. Once antibiotic concentrations wane, persisters resume growth within the host, leading to chronic bacterial infections. It is imperative to understand and prevent persister cell formation, especially since cycles of treatment, persister cell survival, and resumption of growth can increase the chances for antibiotic resistance to emerge. Here, we use the pathogen Yersinia pseudotuberculosis (Yptb) to study how persister cells emerge during doxycycline (Dox) treatment of murine systemic infection. Dox is an effective antibiotic for a wide range of infections, including Yptb; its mechanism arrests bacterial growth so the host response can take over to clear the infection. Previously, our lab found that one dose of Dox treatment at 2 days (d) post-infection in our Yptb systemic infection model resulted in prolonged murine survival, but this was not enough to fully clear the infection. Dox concentrations in host tissues were also found to wane 48 hours (h) after treatment, suggesting that limited bacterial clearance may be due to transient Dox exposure within host tissues. We then examined if maintaining high levels of Dox exposure within host tissues for a longer timeframe would better clear infection. A 1-week treatment of Dox was administered, similar to human treatment regimens, wherein one dose of Dox was administered every 48h for a total of 3 doses to maintain high levels of Dox within tissues. Mice were observed for signs of morbidity over 14d. Interestingly, this weeklong treatment was also not sufficient to clear infection. Although 3 doses of Dox significantly prolonged mouse survival, mice began to succumb to infection 14d post-infection. Bacterial burdens in tissues at this timepoint were significantly higher compared to both untreated mice and 1-dose treated mice. We also found that 4h after 1 dose of Dox, activated neutrophils were recruited to the site of infection, which coincided with a decrease in bacterial counts; yet, this initial neutrophil-mediated killing was insufficient to fully clear the infection. Since we know very little about what promotes persister cell formation during treatment of ongoing in vivo infections, future experiments in this model of treatment failure will seek to understand how persister cells emerge by determining bacterial and host factors that promote antibiotic persistence. We hypothesize that bacteria undergo transcriptional and genetic changes within the host that promote their survival during antibiotic treatment, allowing them to evade host immune responses. Since this study's long-term goal is to understand and limit the emergence of persister cells, knowledge gained will aid in the development of more effective therapeutic strategies against bacterial infections.

Investigating the role of lipooligosaccharide sialylation in Neisseria gonorrhoeae-neutrophil interactions

Amaris Cardenas[1], Dr. Noel Ferraro, Dr. Marcos Pires, Dr. Alison Criss
[1]*University of Virginia*

Gonorrhea, caused by Neisseria gonorrhoeae (Gc), is characterized by neutrophilic influx to infection sites. Gc has many mechanisms to resist killing by neutrophil antimicrobial components. In neutrophil-rich male exudates, LOS becomes sialylated by host derived sialic acid using the enzyme LOS sialyltransferase (Lst). Leukocytes like neutrophils have been shown to be sources of the sialic acid substrate, CMP-NANA, used by Lst. While sialylation has been shown to be protective for Gc against serum-mediated killing and in various models of infection, how it affects the ability of Gc to resist neutrophils is unclear. We are testing two hypotheses: 1) sialylation enhances survival of Gc after neutrophil challenge, and 2) the sialylation state of Gc changes during the course of exposure to neutrophils. To address these hypotheses, we have generated isogenic Gc strains that produce single, physiologically relevant, oligosaccharide chemotypes and express, or are deficient for, Lst. We have also developed a method that for the first time permits direct, real-time monitoring of Gc sialylation during infection. Our preliminary results show that sialylated Gc significantly reduces the oxidative burst of primary human neutrophils, suggesting modulation of neutrophil anti-gonococcal properties. Using an adherent, IL-8 primed primary neutrophil model, sialylation was found to decrease early (within 30 minutes) neutrophil phagocytosis and killing of Gc. Moreover, opacity protein-expressing sialylated Gc induced a less potent oxidative burst than isogenic unsialylated bacteria. Additional mechanisms we are exploring to explain enhanced survival of Gc after neutrophil challenge include increased resistance to neutrophil antimicrobial components and limiting the production and/or release of these components. In the course of these studies, sialylation was found to block binding of antibodies against various Gc surface components, which has implications for development of gonorrhea vaccines. Together these findings underscore the diverse ways Gc exploits sialylation to persist in its obligate human host.

Construction and characterization of novel irradiated whole-cell Neisseria gonorrhoeae vaccine

Rhonda Caston[1], Rok Tkavc[1], Elena Gaidamakova[2], Vera Matrosova[2], Michael Daly[2], Ann Jerse[1]
[1]Department of Microbiology and Immunology, Uniformed Services University Of The Health Sciences, [2]Department of Pathology, Uniformed Services University of the Health Sciences

Background: Seventy- eight million individuals are infected with Neisseria gonorrhoeae (Ng), the causative agent of gonorrhea, every year with disease disproportionately affecting individuals from disadvantaged populations and low-and middle- income countries. Within the affected populations, women make up the majority of the estimated 2,300 deaths from gonorrhea-associated morbidity. This is caused by silent ascending infections, which can result in pelvic inflammatory disease (PID), a serious condition that is associated with chronic pelvic pain, infertility and ectopic pregnancy. A gonorrhea vaccine is urgently needed because of the global disease burden and the rapid emergence of antibiotic resistant Ng strains. Inactivated whole-cell vaccines are attractive because of the relatively low production costs and ability to be lyophilized for storage. A limitation to inactivated whole-cell vaccines is that they are usually prepared using heat or ultraviolet C (UVC) radiation, which damage protective surface antigens.
Objective: To resolve this obstacle, we are using the process of "bathing" the microbes in a manganous decapeptide peptide complex (MDP) of the radiation-resistant bacterium Deinococcus radiodurans, which leaves surface antigens intact following sterilizing doses of ionizing radiation. This MDP- irradiated vaccine platform has been successfully applied to drug-resistant bacteria (MRSA, Acinetobacter baumannii) (Gaidamakova 2012 and Dollery 2021), and several RNA viruses (VEEV, CHIKV, polio) (Gayen 2017 and Tobin 2020). The objectives of this initial study were to construct a Ng vaccine strain, optimize growth conditions for expression of iron-repressed surface antigens, and measure the susceptibility of the strain to gamma irradiation in the presence or absence of MDP.
Methods: Vaccine strain, USU1090, was constructed from wild-type strain Ng FA1090 by insertionally inactivating the rmp gene to prevent production of blocking antibodies, a known mechanism by which Ng evades humoral responses. Growth of USU1090 in liquid media containing iron-chelator was optimized by monitoring the optical density, viable counts and the expression of iron-regulated proteins as detected by SDS-PAGE and western blot. After 4 hours of growth in liquid culture, USU1090 cultures were washed with PBS, aliquoted, and irradiated on wet ice with a cobalt-60 source for varying lengths of time. Following irradiation, samples were serially diluted and plated for CFU.
Results: Sequencing confirmed the presence of the chloramphenicol resistance gene into the rmp gene. USU1090 expressed iron-repressed proteins, including ferric binding protein (Fbp) and TbpA, a component of the transferrin receptor. Immersion of the vaccine strain in MDP conferred greater resistant to killing by irradiation compared to immersion in PBS alone. However, the vaccine strain was fully inactivated when irradiated with a dose of 400 Gys in both the MDP+ and MDP- conditions.
Conclusion: We have successfully established culture conditions for reproducible expression of iron-repressed proteins in a candidate gonococcal vaccine strain. The strain is protected by MDP but is ultimately inactivated by gamma irradiation suggesting that USU1090 will make an ideal vaccine strain for this platform. In on-going studies mice are being immunized with various concentrations (107, 106 or 105) of irradiated MDP+ or MDP- bacteria to determine optimal vaccine dose and examine whether MDP protects vaccine targets.

A Chromosomally Encoded Homoserine Lactone Reporter System for Brucella abortus

Dr. Mitchell Caudill[1], Dr. Clayton Caswell[1]
[1]*Virginia Maryland College of Veterinary Medicine, Virginia Tech*

Brucella species are the causative agent of brucellosis, a worldwide zoonosis that debilitates millions and severely impacts agriculture production. Brucella uses a repertoire of virulence factors, including the tightly regulated Type IV secretion system, to establish a replicative niche within macrophages and evade immune-mediated destruction. One of the regulatory components of the Type IV secretion system are the homoserine lactone responsive transcriptional regulators, VjbR and BabR. While it is known that Brucella synthesizes C12 homoserine lactone at low levels, the mechanisms of this synthesis and the manner by which Brucella alters homoserine lactone concentration during the macrophage infection have remained obscure. Due to this gap in knowledge, the environmental stimuli altering confirmation of VjbR and BabR, and ultimately expression levels of the Type IV secretion system, are unknown. To identify the mechanism of homoserine lactone synthesis within Brucella, we have constructed a chromosomally encoded homoserine lactone reporter system. In this system, the lux operon of Photorhabdus luminescens is placed under the control of the traI promotor of Agrobacterium tumefaciens. Additionally, the homoserine lactone responsive TraR of Agrobacterium tumefaciens is constitutively expressed, resulting in TraR binding of the traI promotor in the presence of homoserine lactone. This system will be used to detect alterations of homoserine lactone production by Brucella, and may aid in identifying the mechanisms of Brucella's homoserine lactone synthesis.

tusB overexpression increases susceptibility of Yersinia pseudotuberculosis to doxycycline treatment

Katherine Cotten[1], Hector Alvarez Manzo[1], Dr. Kimberly Davis[1]
[1]*Johns Hopkins University*

Treatment of bacterial infections can fail for many reasons, but an underappreciated phenomenon to explain treatment failure is antibiotic tolerance, where bacteria develop phenotypic changes in response to stress that allow them to survive antibiotic treatment. Phenotypic changes in response to antibiotic stress are likely to occur during prolonged antibiotic exposure, which is very common during treatment of bacterial infections, especially ones that are difficult to treat. To study this phenomenon, our lab utilized RNA-seq to understand what changes occur in our model bacterium, Yersinia pseudotuberculosis, in response to doxycycline, a bacteriostatic antibiotic that targets the ribosome. One of the significant hits from this RNA-seq analysis was a downregulated gene called tusB, which promotes a tRNA modification that is thought to increase translation fidelity. We hypothesize that downregulation of tusB decreases translation fidelity allowing for faster translation of proteins that help bacteria respond to the antibiotic stress. To study this hypothesis, we created an inducible overexpression strain using the Tet-On system, which allows us to express tusB at high levels when doxycycline is present. Using this strain, we have found that overexpression of tusB significantly impacts the viability of Yersinia in response to doxycycline. Doxycycline is thought to be a bacteriostatic antibiotic but our results with the overexpression of tusB have revealed conditions where the activity of doxycycline is bactericidal against Yersinia. To study the role of tusB in antibiotic susceptibility further, we have created a tusB deletion strain and constitutive overexpression strain. We have found that deletion of tusB significantly reduces the growth rate of bacteria in untreated conditions, but does not impact susceptibility to doxycycline, consistent with the hypothesis that downregulation of tusB allows bacteria to respond better to the antibiotic stress. We are currently assaying the sensitivity of the constitutive overexpression strain, but we expect it to be more susceptible to doxycycline than our wild-type strain, consistent with what we found using the inducible strain. Future experiments will be done to mechanistically define the role of tusB in translation fidelity and antibiotic susceptibility by looking at how the proteome of Yersinia changes in response to doxycycline treatment and differential tusB regulation. Our results will provide important insight into what phenotypic changes allow bacteria to survive antibiotic treatment and approaches that could be taken to modulate doxycycline activity to improve clearance of bacterial infections.

Is CuxR the missing link between CdG signaling and Brucella virulence?

Connor Cribb[1], Dr. Marty Roop[1]
[1]*East Carolina University*

Bis-(3',5')-cyclic diguanylic acid, also known as cyclic-di-GMP (CdG) is an endogenous signaling molecule that plays important roles in the basic physiology and virulence of many bacteria. CdG functions in three ways, by binding to effector proteins, binding to transcriptional regulators, or binding to riboswitches and modulating their activity. The latter two effect gene transcription while the former modulates protein function. Diguanylate cyclases (DGCs) produce CdG from two GTP molecules, while phosphodiesterases (PDE) degrade it. The regulated activities of these enzymes allow CdG to serve as a powerful second messenger in bacteria.

Previous work has demonstrated a link between CdG signaling and Brucella virulence (Petersen et al., 2011 J. Bacteriol. 193:5683-5691), but the genetic regulators that mediate this link have not been identified. The recent discovery and characterization of the CdG-responsive UDP-xylose regulator (CuxR) in Sinorhizobium meliloti, a close phylogenetic relative of Brucella, has provided a possible clue in this regard (Schäper et al., 2017. Proc. Natl. Acad. Sci. USA 114:E4822-E4831). The DNA-binding activity of this AraC-type transcriptional activator is dependent on CdG-binding and CuxR controls the expression of genes encoding an arabinose-based exopolysaccharide known as APS in S. meliloti in a CdG-responsive manner.

The genes encoding CuxR and those involved in the biosynthesis of APS reside in a single genetic locus in S. meliloti that is conserved in Brucella. Derivatives of virulent strain B. abortus 2308 with null mutations in the cuxR and aps genes are currently being constructed to determine if – a) CuxR mediates CdG signaling in Brucella; b) if CuxR regulates expression of the Brucella aps genes, and c) if the cuxR and/or aps genes are required for the wild-type virulence of B. abortus 2308 in mice and cultured mammalian cells. In addition to providing important insight into the specific role that CdG signaling plays in Brucella virulence, these studies also have the potential to define a link between exopolysaccharide (EPS) production and virulence in these bacteria. This is an exciting possibility because although Brucella strains have been shown to have the genetic capacity to produce EPSs, it is unknown if these extracellular polymers play a role in the pathogenesis of the brucellae in their mammalian hosts.

Improving pertussis vaccines with nasal immunization and the BECC adjuvant

Megan DeJong[1,2], M. Allison Wolf[1,2], Graham Bitzer[1,2], Jesse Hall[1,2], Heath Damron[1,2]
[1]West Virginia University, [2]Vaccine Development Center

Bordetella pertussis is a respiratory pathogen that causes a disease known as whooping cough. The initial vaccine developed for pertussis is known as the whole cell (wP) vaccine; however, due to reactogenicity issues, the wP formulation was replaced by an acellular (aP) vaccine. While initially thought to offer protection on par with that of wP, research has shown that the immunity afforded by aP is waning. With that, there is a need to develop a novel pertussis vaccine such that the longevity of protection can be improved. Pertussis vaccines are currently administered intramuscularly (IM); however, given that it is a respiratory pathogen, we propose that an intranasal (IN) vaccine would afford more localized protection and stimulate mucosal immune responses. With the knowledge that most children are IM vaccinated as early as 2 months of age, if an IN vaccine were to be implemented, we would need to understand how these routes interacted with one another. Therefore, we proposed that utilizing an IM primary vaccine followed by an IN booster would work to address the issue of waning immunity by offering both systemic and localized protection at the site of infection. Specifically, the IM vaccine would induce cellular immune responses (the "push"), while the IN boost would bring these cells to the site of infection/vaccination (the "pull"). Additionally, we implemented an LPS mimetic—BECC438b (bacterial enzymatic combinatorial chemistry)—into the aP vaccine in the hopes that it would create a more Th1-skewed immune response, which is similar to what is seen with a natural infection. In this study, we gave aP-BECC vaccines IM only, IN only, or both. We first found that combining aP-BECC vaccine routes was able to reduce bacterial burden within the respiratory tract (nasal lavage, lung/trachea). Additionally, IM/IN aP-BECC was able increase antibody production as well as increase the production of cells that produce B. pertussis specific antibodies. Our data suggest that, while aP-BECC is a vaccine that provides protection over DTaP, the protective capacity of this novel formulation increases when IM and IN routes are combined. We will continue to evaluate the immune responses elicited by both aP-BECCs and route combinations in terms of cytokine analysis, T cell analysis, and single cell sequencing in an effort to determine the most efficacious pertussis vaccine.

Persistence Alters the Interaction between Chlamydia trachomatis and It's Host Cell

Aissata Diallo[1], PI George Liechti
[1]*Uniformed Services University*

In response to stress, the obligate intracellular pathogen Chlamydia trachomatis stops dividing and halts its biphasic developmental cycle. The infectious, extracellular form of this bacterium is highly susceptible to killing by the host immune response, and by pausing development, Chlamydia can survive in an intracellular, "aberrant" state for extended periods of time. The relevance of these aberrant forms has long been debated, and many questions remain concerning how they contribute to the persistence and pathogenesis of the organism. Using reporter cell lines, fluorescence microscopy, and a dipeptide labeling strategy, we previously demonstrated that all aberrance-inducing conditions affect chlamydial peptidoglycan and that some actually halt the biosynthesis pathway early enough to prevent the release of an immunostimulatory peptidoglycan component, muramyl tripeptide, and its subsequent recognition by Nod-like receptor 1 (NOD1). Here, we expand on these initial observations and explore whether persistence affects the ability of C. trachomatis to signal through other innate immune receptors. We used reporter cell lines to determine how iron-restriction, interferon gamma-induced tryptophan depletion, and the βeta-lactam antibiotic ampicillin affect the ability of C. trachomatis to synthesize and release lipoproteins, lipooligosaccharides, and genomic DNA into host cells, which are then recognized by Toll-like Receptors TLR2, TLR4, and TLR9, respectively. Our findings demonstrate that the immunoevasive features inherent to a subset of aberrant chlamydial forms is broader than previously reported, potentially affecting several pathogen antigen recognition receptors known to be stimulated during Chlamydia infections.

Interleukin-27 Opposes Protective Immune Responses Induced by the BCG Vaccine

Ashley Divens[1], Jordan Vance[1], Dr. Cory Robinson[1,2]
[1]Department of Microbiology, Immunology, and Cell Biology; West Virginia University School of Medicine, [2]Vaccine Development Center; West Virginia University School of Medicine

Tuberculosis (TB) remains a serious public health concern in many regions of the world. In these high impact areas of Mycobacterium tuberculosis infection, the bacillus Calmette-Guérin (BCG) vaccine is commonly administered to newborn infants. While this typically provides protection against disseminated infection early in life, the BCG vaccine does not provide long-term protection against pulmonary tuberculosis later in life. Our laboratory has shown that neonates express the immunosuppressive cytokine interleukin (IL)-27 at elevated levels compared with adults. We hypothesize that early life levels of IL-27 oppose protective immune responses in response to BCG vaccination. IL-27 interferes with bacterial clearance and this may push the cell mediated immune response toward a continual effector response and away from the development of a protective central memory response. To test this hypothesis, T cells were isolated from the spleens and peripheral lymph nodes of C57BL/6 wildtype (WT) and IL-27 receptor (IL-27R)-α knockout (KO) mice vaccinated with BCG as neonates. The T cells were then immunolabeled with a memory T cell panel and BCG antigen-specific tetramers and analyzed via flow cytometry. This analysis revealed that CD4+ T cells from IL-27R-α KO mice are more responsive to BCG antigen than WT mice following 5 weeks of vaccination. Suboptimal antigen processing may also limit the diversity of the T cell response. To address this possibility, we utilized a T cell stimulation assay. Dendritic cells were stimulated with BCG whole cell lysate and then 24 h later cultured with T cells isolated from either WT or IL-27R-α-deficient mice vaccinated as neonates for 5 weeks. Supernatant was then collected at various timepoints through 72 h and an IFN-γ ELISA was performed. This assay revealed that T cells from vaccinated IL-27R-α KO mice were more responsive than T cells from vaccinated WT mice. These results give a preliminary view into the effect of IL-27 on T cell responses following BCG vaccination during the neonatal period. Overall, findings from our laboratory may enhance our understanding of the failures of BCG as a vaccine and inform approaches to improve vaccination for TB.

Phospho-regulation accommodates Type III secretion and assembly of a tether of ER-Chlamydia inclusion membrane contact sites

Rachel Ende[1], Isabelle Derré[1]
[1]*University of Virginia*

Membrane contact sites (MCS) are crucial for non-vesicular trafficking-based inter-organelle communication. ER-organelle tethering occurs in part through the interaction of the ER resident protein VAP with FFAT-motif containing proteins. FFAT motifs are characterized by a seven amino acidic core surrounded by acid tracks. We have previously shown that the human intracellular bacterial pathogen Chlamydia trachomatis establishes MCS between its vacuole (the inclusion) and the ER through expression of a bacterial tether, IncV, displaying molecular mimicry of eukaryotic FFAT motif cores. We show that multiple layers of host cell kinase-mediated phosphorylation events govern the assembly of the IncV-VAP tethering complex. CK2-mediated phosphorylation of a C-terminal region of IncV enables IncV hyperphosphorylation of a phospho-FFAT motif core and serine-rich tracts immediately upstream of IncV FFAT motif cores. Phosphorylatable serine tracts, rather than genetically-encoded acidic tracts, accommodate Type III-mediated translocation of IncV to the inclusion membrane, while achieving full mimicry of FFAT motifs. Thus, regulatory components and post-translational modifications are integral to MCS biology, and intracellular pathogens such as C. trachomatis have evolved complex molecular mimicry of these eukaryotic features.

Monoclonal Antibodies Targeting a Siderophore Receptor and a Porin Involved in Nutrient Acquisition Prevent Acinetobacter baumannii Infection in Murine Models of Infection

Mariel Escatte[1], Yoann Le Breton[1], Stephanie Majernik[1], Molly McLendon[1], Timothy Fitzgerald[1], Yonas Alamneh[1], Rania Abu-Taleb[1], Iswarduth Soojhawon[1], Schroeder Noble[1], Derese Getnet[1], Brett Swierczewski[1], Daniel Zurawski[1]

[1]*Walter Reed Army Institute of Research*

Acinetobacter baumannii is one of the ESKAPEE-E pathogens, which are opportunistic, disease-causing bacterial species, which have an uncanny ability to acquire antibiotic resistance genes. Recently, the WHO identified A. baumannii as an urgent threat for antibacterial development due to the increase in antibiotic resistance and the lack of treatment options. Infection with this pathogen can lead to pneumonia, severe illness, amputations, sepsis, and death, which is often due to treatment failure against multidrug-resistant and sometimes even pandrug-resistant strains. Small molecule approaches, while being investigated, are still many years from approval, and thus, paves the way for alternative therapeutics to also be considered when combatting this pathogen.

We developed a pipeline (the Human Monoclonal Antibody Platform - HMAP) that identifies targets on the surface of a bacterium required for virulence, and subsequently, monoclonal antibodies (mAbs), which are made against these targets to disrupt bacterial infections. For A. baumannii, we identified two proteins, BauA (a siderophore receptor) and OmpW2 (porin required for amino acid and iron transport) that are essential for pathogenesis and contribute to bacterial virulence. We made murine mAbs against BauA and OmpW2, and after screening and down-selection, identified one antibody for each protein that could recognize the native surface of A. baumannii in our model strain, AB5075. Both αBauA and αOmpW2 also recognized the native surface of A. baumannii in >80% of all strains of a 100-strain diversity set suggesting the targets are well-conserved. Second, we assessed growth in both Cationic Müeller-Hinton Broth (CAMHB) and in iron-deficient CAMHB to mimic physiological conditions. The addition of αBauA or αOmpW2 limited growth of AB5075 grown in iron-deficient CAMHB when compared to untreated culture by ~10-15%, but there was additive synergy when the mAbs were used together (~20%-30% growth inhibition). Further, both mAbs had a deleterious effect on biofilm formation when compared to untreated samples or samples treated with isotype control mAb. Lastly, we tested the antibodies in vivo: in Galleria mellonella, and in two murine models of infection. In G. mellonella, the mAbs were injected 30 min before inoculation and promoted 95% survival compared to 0% survival of untreated controls. In the murine pneumonia model, we injected mAbs one day before infection and achieved 70% protection vs. 0% survival of untreated controls. Lastly, the combination of both mAbs reduced bacterial burden and promoted wound healing in the murine wound model. Overall, we postulate that the combination of αBauA and αOmpW2 disrupts iron acquisition, which negatively impacts growth and bacterial functions in vivo leading to the clearance of bacteria. We hypothesize that the addition of a third mAb to this cocktail could further enhance protection and efficacy, and we are currently exploring antibody development against other protein targets that are required for virulence.

SPR206 is highly effective in vitro and in vivo against important Gram-negative pathogens

Timothy Fitzgerald[1], Yoann Le Breton[1], Giovanna Castellanos[1], Stephanie Majernik[1], Malik Raynor[3], Michael Pucci[2], Derese Getnet[1], Troy Lister[2], Brett Swierczewski[4], Daniel Zurawski[1]
[1]Walter Reed Army Institute Of Research, Wound Infections Department, [2]Spero Therapeutics, [3]Walter Reed Army Institute Of Research, Experimental Therapeutics Branch, [4]Walter Reed Army Institute Of Research, Bacterial Disease Branch

Background - The ESKAPE-E (Enterococcus, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter cloacae, and Escherichia coli) are the primary cause of wound infections in the Military Healthcare System (MHS). These bacteria are often resistant to antibiotics, form biofilms in the wound bed, and can disseminate into the bloodstream causing sepsis. The Gram-negatives of this group are particularly problematic. To this end, we evaluated the efficacy of SPR206, a novel polymyxin, against these pathogens. SPR206 is a clinically relevant drug , as shown by 3 Phase I clinical trials, 1 of which has completed.

Methods - We used a battery of in vitro and in vivo assays to evaluate SPR206. MICs of SPR206 were initially determined against a panel of ESKAPE-E pathogens. Then, MICs were determined against diversity sets of the Gram-negatives: A. baumannii, P. aeruginosa, Escherichia coli, and Klebsiella pneumoniae. Time-kill assays were done according to standard methods. Biofilms were grown in 96-well MBEC peg plates and measured by crystal violet staining. Galleria mellonella infection was used to determine initial efficacy in vivo against 1.0 x 105 colony forming units of AB5075. Then, in a murine lung model, we infected mice intranasally with 5.0 x 106 colony forming units of AB5075. Mice are then treated with SPR206 q12h for three days and monitored for clinical scores and survival.

Results - We found that SPR206 resulted in MICs ranging from 0.015625 - 8 µg/mL against the Gram-negative ESKAPE-E pathogens. SPR206 was also active against all A. baumannii, P. aeruginosa, Escherichia coli, and Klebsiella pneumoniae from the diversity sets. Time-kills show a rapid and potent bactericidal effect, but with a resurgence of persister cells in the later hours of the assay. At 4x the MIC and clinically relevant dosing, biofilms were eradicated by SPR206. In the Galleria infection model, SPR206 treatment resulted in 100% survival. In the murine lung model, SPR206 provided 100% survival when compared to untreated controls.

Conclusion - In conclusion, we have identified a drug, with high activity against important Gram-negative pathogens that could have an expanded clinical indication. The results of the murine lung model suggest that this compound could be efficacious in a hospital acquired/ventilator associated bacterial pneumonia (HABP/VABP) clinical scenario. The forthcoming wound infection experiment could show efficacy in an ABSSSI scenario. Animal modeling suggests SPR206 is a potent, new polymyxin for clinical use against Gram-negative pathogens.

Disclaimer
Material has been reviewed by the Walter Reed Army Institute of Research. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense. Research was conducted under an approved animal protocol in an AAALACi accredited facility in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 2011 edition.

Investigation of spore-associated lipoproteins YlaJ, YhcN, YutC, and CoxA suggests existence of a complex that stabilizes the germination apparatus in Bacillus subtilis spores

Matthew Flores[1], Dr. David Popham[1]
[1]*Virginia Tech*

In order to return to a vegetative state, dormant bacterial spores must undergo the process of germination, which can be divided into two stages. In most species, stage I is triggered by sensing of nutrient germinants and is characterized by the release of calcium-dipicolinic acid (DPA) and various cations, while stage II is characterized by spore cortex degradation and full rehydration of the spore core. All of these steps are mediated by membrane-associated proteins, including germinant receptors, the major DPA channel, and a key cortex lytic enzyme, SleB, and all of these proteins have exposure on the outer surface of the membrane, a hydrated environment where they are potentially subject to damage during dormancy. A family of spore lipoproteins, including YlaJ, which is expressed from the sleB operon in some species, are present in all Bacillus and Clostridium species that express SleB. B. subtilis possesses four proteins in this family, and prior studies have demonstrated that two of these are required for the most efficient spore germination. Genetic studies of strains lacking all combinations of these four genes now reveal that all four play roles in ensuring efficient germination, and that they affect multiple steps in this process, including germinant sensing, DPA release, and SleB stability. Bacterial 2-hybrid and in vivo crosslinking data indicate that the lipoproteins are able to self-associate, interact with their paralogues, and interact with SleB. These results are consistent with predicted multimerization domains within the lipoproteins. In vitro analyses of purified lipoproteins indicate the spontaneous formation of protein multimers. All of these data suggest a model in which the lipoproteins form a macromolecular structure on the outer surface of the inner spore membrane, where they act to stabilize and/or promote the function of multiple components of the germination machinery. Continuing studies are examining the assembly of such a structure and potential effects on membrane structure and germination protein function.

Phenotypic characterization of Pseudomonas aeruginosa CF isolates reveals distinct clusters which represent in vitro evolution and predict performance in a chronic infection model

Taylor Franklin[1], Suzanne Meinig[1], Crystal Walden[1], Kristen Wilkinson[1], Allison Volmer[1], Corey Jania[1], Hunter White[1], Stephen Klawa[2], Micah Papanikolas[2], Shreeya Bhonge[2], Quincy Snyder[2], Jacqueline Shin[2], Dr. Ralph Baric[3,4], Jonathan Schisler[5], Dr. Ronit Freeman[2], Dr. Alessandra Livraghi-Butrico[1], Dr. Matthew Wolfgang[1,4]
[1]Marsico Lung Institute/CF Center, School of Medicine, University of North Carolina at Chapel Hill, [2]Applied Physical Sciences Department, University of North Carolina at Chapel Hill, [3]Epidemiology Department, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, [4]Department of Microbiology and Immunology, School of Medicine, University of North Carolina at Chapel Hill, [5]Department of Pharmacology, University of North Carolina at Chapel Hill

Opportunistic pathogens thrive within the thick mucus that accumulates in the dehydrated airways of people with CFTR mutation. Chronic infection in this complex environment results in a wide range of phenotypic diversity among clinical isolates of P. aeruginosa. Strains colonize lungs over a period of many years, acquiring adaptations which allow them to persist and tolerate common therapeutics.
While it is widely known that clinical isolates exhibit a vast array of phenotypic diversity, the implications that these phenotypes collectively have on chronic infection is poorly understood due to the absence of robust animal models. We performed phenotypic characterization of 50 clinical isolates of P. aeruginosa. In addition to 12 phenotypes associated with virulence, we assessed resistance patterns for seven commonly-used antibiotics and tolerance to two of those antibiotics. We then applied latent cluster analysis and multiple correspondence analysis to discriminate the isolates based on phenotype. The isolates clustered into three distinct groups along key phenotypes of biofilm forming ability, pyoverdine production, twitching motility, and mutation frequency which correlated with clinical indicators; from each group, representative isolates were selected for in vivo studies to identify strains capable of developing chronic airway infection. For infection studies bacteria were embedded into agar beads which were administered intra-tracheally. Mice were monitored for 7 days post infection, at which point lungs and spleens were harvested to enumerate bacterial burden and detect systemic infection, respectively. After an initial drop in weight, mice either 1) improved and cleared infection (avirulent), 2) did not recover and showed high mortality (cytotoxic) or 3) mice recovered but maintained persistent lung infection (persistent). A subset of strains which supported persistent 7-day infection were more rigorously evaluated in 28-day infection studies. For longitudinal studies, lungs were harvested every 7 days for 28 days. At each time point, bacterial burden, cytokine profiling, immune cell counts, and tissue damage was assessed. Compared to the control group injected with sterile beads, lungs from infected mice exhibited characteristic features of chronic CF infection such as elevated neutrophil and lymphocyte counts, tissue damage, and elevated pro-inflammatory cytokines.
The majority of strains capable of causing chronic murine infection mapped into the same phenotypic cluster, indicating that the phenotypic profile of an isolate can predict its performance in a chronic infection setting. Correlating the phenotypic and genotypic characterization of strains with their ability to cause chronic infection will shed light on the implications of diversity among CF clinical isolates. Establishing a murine model for chronic lung infection will allow us to study the pathogenesis of chronic disease and provide a platform for antimicrobial and host directed therapeutic testing.

Interrogating interactions between Clostridioides difficile and the mucosal barrier using a two-dimensional primary human colonoid model.

Kathleen Furtado[1], Dr. Rita Tamayo[1]
[1]*University of North Carolina at Chapel Hill*

Clostridioides difficile is the leading cause of hospital-acquired infections, resulting in antibiotic-associated disease ranging from diarrhea to potentially fatal pseudomembranous colitis. Community-acquired infections continue to rise, and recurrent disease is common, greatly impacting quality of life and illustrating a vital need to understand mechanisms of C. difficile pathogenesis. However, little is known about the early steps of C. difficile colonization. C. difficile possesses multiple mechanisms to facilitate adherence to host cells, and previous studies have shown C. difficile interacts directly with the intestinal epithelium in humans and in animal models. But to reach the epithelium, C. difficile must first overcome innate immune barriers in the gut, namely mucus. In a healthy human colon, the mucus barrier consists of two fractions: a luminal layer of secreted mucins that is colonized by the microbiota, and a layer of mucins attached to the epithelium that remains relatively sterile. It has been shown by others that C. difficile decreases expression of secreted colonic mucins (MUC2) and preferentially interacts with mucins attached to host cells (MUC1), and that mucin oligosaccharide structures are altered during CDI. Altogether, these findings indicate that manipulation of host mucins is a key step in establishing infection. However, it remains unclear how C. difficile interacts with mucus, nor how C. difficile metabolism or virulence is affected by the presence of mucus as a potential nutrient source. Using an innovative strategy to co-culture C. difficile with primary human intestinal epithelial cells and an intact mucus barrier, we developed an in vitro model to examine C. difficile's interactions with physiologic mucus. Using fluorescence microscopy and bacterial cell counts, we found that C. difficile translocates the mucus barrier to reach the epithelium. Further, we show that C. difficile can use host-derived mucins for growth, both in our IEC co-culture system and in mimimal media supplemented with purified mucin, indicating that mucus may serve as a nutrient source. We've also found that the transcriptomic profile of C. difficile is significantly altered in the presence of mucus relative to minimal media alone, indicating mucus may impact metabolic pathways in C. difficile. Our work is crucial to understand the dynamics of C. difficile-host interactions in the context of the human gut, which will be important for the development of therapeutics to hinder disease development.

Antimicrobial tolerance of synovial fluid biofilms can be reversed by exogenous metabolic stimuli

Jessica Gilbertie[1], Brian Conlon[3], Lauren Schnabel[2]
[1]Edward Via College of Osteopathic Medicine, [2]North Carolina State University, [3]University of North Carolina-Chapel Hill

Staphylococcus aureus is the most common bacteria isolated from cases of orthopedic infections like infectious arthritis. Within the synovial fluid of articular joints, S. aureus subverts treatment by forming macroscopic free-floating biofilm aggregates which demonstrate high levels of antimicrobial tolerance. We found that key to biofilm aggregate antimicrobial tolerance is altered metabolism due to nutrient availability within synovial fluid. More specifically, synovial fluid contains an overabundance of the polysaccharide hyaluronic acid (HA) relative to the monosaccharide glucose. Because S. aureus metabolism of HA is slow and taxing compared to the rapid and efficient metabolism of glucose, S. aureus grows more slowly on HA resulting in increased antimicrobial tolerance. Due to the altered metabolism of synovial fluid biofilms, we hypothesized that exogenously supplied metabolites native to the synovial environment could increase S. aureus metabolism and potentiate antimicrobial activity. We found that adjunctive treatment of synovial fluid biofilm aggregates with glucosamine restored antimicrobial activity, while adjunctive treatment with glucose or other metabolites did not. As glucosamine is the smallest metabolizable unit of HA, its supplementation circumvents the demanding breakdown of HA required by S. aureus to gain access to glucosamine. As most antimicrobials act on actively growing bacteria, we speculate that the antimicrobial tolerance of synovial fluid biofilms could be due to decreased cellular processes and energy generation by the electron transport chain. We further validated that speculation by exogenously increasing the metabolism of these aggregates by synovial-specific metabolic stimuli. Future investigation on the use of aminosugars as adjuvants to aminoglycoside treatment in vivo could lead to a promising therapeutic for patients suffering from infections related to the synovial space such as periprosthetic joint infection.

The role of bacterial amyloid curli, HLA-B27, and microbiome in Salmonella-induced reactive arthritis

Kaitlyn Grando[1], Shingo Bessho[1], Edison Kim[1], Dr. Amanda Miller[1], Dr. Lauren Nicastro[1], Dr. Çagla Tükel[1]
[1]*Temple University, Lewis Katz School of Medicine*

Salmonella enterica serovar Typhimurium (STm) is the most common Salmonella serotype responsible for gastroenteritis, a diarrheal disease. Approximately 5-10% of STm infections progress to reactive arthritis (ReA), an autoimmune condition causing swollen and painful joints. STm can form biofilms in the gut, multicellular aggregates of bacteria adhered via an extracellular matrix composed of polysaccharides, extracellular DNA, and the bacterial amyloid curli. Our lab has previously shown that curli triggers an autoimmune response in wild-type C57BL/6 and 129/SvJ mice including anti-dsDNA autoantibody formation and inflammation of the joints that resembles ReA. Human leukocyte antigen-B27 (HLA-B27) is a genetic risk factor present in 60-90% of patients with ReA and other spondyloarthropathies. HLA-B27 pathology is hypothesized to be due to (i) specific arthritogenic peptides preferentially binding to HLA-B27 and triggering a CD8+ T cells response and/or (ii) misfolding of the HLA-B27 heavy chain into homodimers which induce the unfolded protein response (UPR) and activate a CD4+ Th17 response or can be expressed on the cell surface and recognized by lymphocytes. We aimed to determine whether HLA-B27 transgenic mice exhibit exacerbated autoimmunity after curli stimulation or STm infection; to characterize the immune response, namely activation of the UPR and Th17 response, associated with ReA and curli-induced autoimmunity; and to examine the synergistic effects of a pro-inflammatory microbiome. HLA-B27tg mice exhibited similar autoantibody levels and knee inflammation to C57BL/6 mice after curli injection or chronic STm infection with a type-3 secretion system-2 mutant (spiB). After an acute infection with wild-type STm and when colonized with a pro-inflammatory microbiome, these mice had elevated pro-inflammatory cytokines in the serum and somewhat elevated UPR markers in the cecum compared to wild-type mice. Further characterization of the immune response, including examination of the UPR and antigen presentation to T cells, is needed to provide a mechanistic link between STm infection, dysbiosis, and genetics in the pathogenesis of ReA.

Mucus concentration driven changes in Pseudomonas aeruginosa virulence factor phenotypes and antibiotic tolerance.

Matthew Greenwald[1,2], Suzanne Meinig[2], Matthew Wolfgang[1,2]
[1]Department of Microbiology and Immunology,University of North Carolina at Chapel Hill, [2]Marsico Lung Institute

Generating a reproducible, biologically relevant model of disease is essential for the accurate translation of laboratory findings to clinical outcomes. Traditional laboratory in vitro broths lack disease relevant concentrations of metabolites and do not recapitulate observed diseased phenotypes. Our lab studies Pseudomonas aeruginosa infections in people with cystic fibrosis (CF). CF is a genetic disorder resulting in dysfunctional ion transport that leads to the accumulation, dehydration, and thickening of airway mucus. CF mucus provides a nutrient-rich protective environment for the colonization of bacterial pathogens. People with CF experience recurrent or chronic infection. Within the adult CF population, P. aeruginosa is the dominant pathogen associated. Chronic respiratory infection by Pseudomonas is the leading cause of death in CF patients. Chronic Pseudomonas lung infections are characterized by high rates of antibiotic treatment failure. Often, the inability of an antibiotic to clear infection is not linked to in vitro antibiotic susceptibility. Tobramycin, an aminoglycoside antibiotic used in the treatment of CF lung infections, exhibits high rates of clinical treatment failure. Tobramycin-sensitive Pseudomonas are able to persist in the lung despite the use of inhaled tobramycin therapy, which allows for direct delivery of antibiotics into the lungs and for concentrations to reach an excess of 300 µg/ml, well above the clinical definition of resistance (16 µg/ml). Our lab utilizes a well-characterized in vitro synthetic sputum medium (SSM), with which we are able to interrogate aspects of Pseudomonas biology that contribute to pathogenesis and antibiotic tolerance. Importantly, we utilize varying concentrations of mucus that reach disease relevant concentrations and represent the heterogeneity observed across different people with CF. We have demonstrated that increasing mucus concentration drastically influence multiple Pseudomonas virulence factor phenotypes including biofilm formation, aggregation, and quorum sensing (QS).

QS systems have been demonstrated to be critical for establishment of chronic infection and have clinical relevance to patient outcomes. We have observed that increased mucus concentrations drive increased Las QS. Furthermore, in our system, we are able to recapitulate the emergence of QS deficient isolates. High levels of QS induction in the presence of mucus selects for Las QS mutants, “social cheaters”, which are common in CF patients. Las QS deficient isolates have increased fitness in the CF lung and their presence in people with CF is associated with a more rapid progression of disease and declined lung function. Additionally, we have observed that antibiotic tolerance is mucus concentration dependent. We have demonstrated the ability to utilize our in vitro system to recapitulate ex vivo sputum tolerance of Pseudomonas clinical populations. Across a panel of multiple patient samples 2% mucus solids concentration in our SSM best recapitulated the majority of ex vivo antibiotic tolerance outcomes. These data highlight the importance of utilizing a defined in vitro model to investigate the biology of a microorganism in disease. Moreover, these data signal the mucus concentration is a driving force in defining Pseudomonas virulence potential and persistence in the lungs of people with CF.

Outer membrane protein Bucl8 is a component of an efflux pump and vaccine target in Burkholderia pathogens

Megan Grund[1], Soo Jeon Choi[1], Dr. Slawomir Lukomski[1]
[1]*West Virginia University*

Burkholderia spp. are Gram-negative, intrinsically-resistant bacteria that are responsible for environmental and nosocomial infections of clinical and biodefense concern. Currently there is no licensed vaccine. Our laboratory previously identified in silico Burkholderia collagen-like protein 8 (Bucl8) in the pathogens Burkholderia pseudomallei and Burkholderia mallei. We hypothesize that Bucl8, which contains two predicted tandem OEP domains, is an outer membrane component of a putative efflux pump. Unique to Bucl8, as compared to other outer membrane proteins, is the presence of an extended extracellular region containing a collagen-like (CL) domain and a non-collagenous C-terminus (Ct). Molecular modeling and circular dichroism spectroscopy with a recombinant protein, corresponding to this extracellular CL-Ct portion of Bucl8, demonstrated that it adopts a collagen triple helix, whereas functional assays screening for Bucl8 ligands identified that the CL-Ct binds to fibrinogen. Bacterial efflux pumps extrude a variety of xenobiotics, including antimicrobials; therefore, they are important pathogenicity traits. Bioinformatic analysis of the bucl8 locus revealed it resembles a classical efflux-pump operon. The bucl8 gene is co-localized with downstream fusCDE genes, presumably encoding fusaric acid (FA) resistance, and with an upstream gene, designated as fusR, encoding a LysR-type transcriptional regulator. Here, we defined the boundaries and transcriptional organization of the fusR-bucl8-fusCDE operon. Next, we found exogenous FA induced bucl8 transcription over 80-fold in B. pseudomallei, while deletion of the entire bucl8 locus decreased the minimum inhibitory concentration of FA 4-fold in its isogenic mutant. We furthermore showed that the Bucl8-associated pump confers FA resistance in the heterologous Escherichia coli host. At the same time, it did not confer resistance to a panel of clinically-relevant antimicrobials tested in Burkholderia and E. coli. However, we found that the deletion mutant grew at a significantly reduced rate, suggesting that under laboratory conditions the pump is important for the cell's physiology. We hypothesize the Bucl8-pump acts more as a metabolic efflux valve; this was substantiated by the upregulation of Bucl8-pump expression by the FA-derivative p-hydroxybenzoic acid, which is involved in regulation of balanced metabolism in E. coli. While Bucl8 is specific to B. pseudomallei and B. mallei, orthologs are present in members of the Burkholderia cepacia complex (BCC) species. These bacteria are known to cause antimicrobial-resistant respiratory infections in patients with cystic fibrosis or those who are immunocompromised. Here, we describe a previously unreported efflux pump with unique structure and functional implications in the biology of Burkholderia species, which confers FA resistance, fibrinogen binding, and optimal growth. Future studies will identify common pump substrates and explore host-relevant functions. Additionally, we established that the CL-Ct and surface-exposed loops of Bucl8 are immunogenic and offer unique vaccine targets; surface-exposed epitopes of outer membrane proteins are common targets for antibody-based therapeutics.

Investigating MxiE-dependent gene regulation in Shigella flexneri

Chelsea Hall[1], Hervé Agaisse[1]
[1]*University of Virginia*

Shigella flexneri is a human-specific pathogen that infects and spreads throughout the colonic mucosal epithelium causing bloody diarrhea, also known as bacillary dysentery. S. flexneri uses a type 3 secretion system (T3SS) apparatus to inject virulence effector proteins into the host cell cytosol. Effectors are translocated into the host cell cytosol in two waves, with the first wave occurring upon initial bacteria-cell contact and the second wave occurring once the bacteria are inside the host cell. The second wave of effectors has been previously shown to be activated by MxiE, a S. flexneri AraC-like transcriptional regulator. The overall objective of this study is to characterize MxiE-dependent regulation, both in defining the full regulon and the mechanism by which MxiE regulates this set of genes.

Using an RNA-seq approach, we identified two chromosomal genes (SF1005 and SF2423) that are positively regulated by MxiE. Following validation of these results, we have found that the decreased gene expression levels of the novel genes of interest (GOI) observed in a mxiE mutant can be rescued by overexpression of mxiE from a plasmid. This data indicates that MxiE is necessary for the transcriptional activation of the GOI. Both of the GOI contain a regulatory element known as the MxiE box, which is found in the 5' UTR of MxiE-regulated genes and is known to be required for transcriptional activation. To test the functionality of the MxiE boxes upstream of the GOI, we mutated two nucleotides in the box sequence in a CFP reporter system. The result was loss of CFP expression, indicating that the putative MxiE boxes upstream of the GOI are functional.

H-NS is a global repressor of AT-rich DNA in gram-negative bacteria. Horizontally acquired genes, such as those on the virulence plasmid, are often distinguished by being AT-rich. Analysis of the GC content of the MxiE regulon revealed that these genes are all AT-rich, including the two novel GOI on the chromosome. Based on this, we hypothesized that MxiE-regulated genes are repressed by H-NS. To test this hypothesis, we generated a construct for the overexpression of a truncated H-NS that contains only the oligomerization domain, such that it binds and sequesters endogenous H-NS, resulting in a dominant negative effect. Using this construct in S. flexneri, we found that the mRNA expression of both MxiE-regulated genes and the novel GOI increased significantly when H-NS was depleted, suggesting that H-NS is repressing MxiE regulated genes. Future directions will include ChIP-qPCR to provide direct evidence that H-NS is binding the DNA of MxiE-regulated genes.

VirB, the transcriptional activator of T3SS genes, is known to de-repress H-NS silenced genes by binding upstream of H-NS bound DNA and dislodging H-NS. However, VirB is not required to activate MxiE-regulated genes as shown by overexpression of mxiE in a virB mutant background. Therefore, we hypothesize there is a novel mechanism for counteracting H-NS silencing involving MxiE and we plan to test this by performing ChIP of H-NS bound DNA in the mxiE mutant compared to WT.

Identification of Lipopolysaccharide Structural Requirements for Toll-like Receptor 4 and Non-canonical Inflammasome Activation

Erin Harberts[1], Daniel Grubaugh[3], Jasmine Alexander-Floyd[4], Antonia Bass[4], Daniel Akuma[3], Joseph Buxbaum[5], Robert Ernst[2], Sunny Shin[4], Igor Brodsky[3]

[1]Towson University, [2]University of Maryland School of Dentistry, [3]University of Pennsylvania School of Veterinary Medicine, [4]University of Pennsylvania Perelman School of Medicine, [5]Ichan School of Medicine at Mount Sinai

Immune sensing of the Gram-negative bacterial membrane glycolipid lipopolysaccharide (LPS) is both a critical component of host defense against Gram-negative bacterial infection, and a contributor to hyper-inflammatory response, leading to sepsis and death. Innate immune activation by LPS is due to the lipid A moiety, an acylated di-glucosamine molecule that can activate inflammatory responses via the extracellular sensor TLR4/MD2 or the cytosolic sensor murine caspase-11 (Casp11) and human Caspase-4/5 (CASP4/5). The number and length of acyl chains present on bacterial lipid A structures, as well as the length of the core oligosaccharide, vary across bacterial species and strains and affect the magnitude of immune activation. In the murine system, TLR4 and Casp11 are thought to respond similarly to various lipid A structures because tetra-acylated lipid A structures have not been found to activate either sensor, while hexa-acylated structures activate both sensors. However, direct analysis of extracellular and cytosolic responses to the same sources and preparations of LPS/lipid A structures have been limited, and the precise features of lipid A that determine the differential activation of each receptor remain poorly defined. To address this question, we used rationally engineered lipid A isolated from a series of bacterial acyl-transferase mutants that produce, structurally defined molecules. Intriguingly, we find that the location of specific secondary acyl chains on lipid A resulted in differential recognition by TLR4- or Casp11. This indicates that TLR4 and Casp11 sense non-overlapping areas of lipid A chemical space. Using the same extraction lots of lipid A, in the human system we found that TLR4 and CASP4/5 detect both shared and non-overlapping LOS/lipid A structures. Specifically, human macrophages required both lipid A and the core oligosaccharide to mount a robust CASP4/5 inflammasome response while the lipid A component was sufficient to activate TLR4. While there are unique structures detected by all four systems, our findings collectively indicate that each single host species possesses a self-contained endotoxin sensing pathway that comprehensively surveys lipid A structures and limits the capacity of microbial pathogens to evade detection.

Connecting Sugar Utilization and Virulence Regulation: RofA as a PRD-Containing Virulence Regulator

Meaghan Hart[1], Dr. Joseph S. Rom[1], Dr. Kevin S. McIver[1]
[1]*Cell Biology & Molecular Genetics, Maryland Pathogen Research Institute, University of Maryland*

The group A Streptococcus (GAS) is a human pathogen estimated to cause 727 million cases of mild superficial infections and 18.1 million severe life-threatening invasive infections per year. In the United States incidence of invasive GAS infections is increasing. This highlights the urgent need to understand how the pathogen senses host tissues and responds to promote infection. One mechanism that GAS uses to detect and import carbohydrates is the phosphoenolpyruvate transferase system (PTS), which mediates both carbohydrate uptake and metabolic gene regulation. Regulation by the PTS is often achieved through phosphorylation of transcriptional regulators at conserved PTS regulatory domains (PRDs). Interestingly, GAS has several stand-alone regulators with PRDs whose regulons expand beyond metabolic genes such as RofA, which has been shown to regulate the expression of genes important for adherence and virulence in multiple GAS serotypes (M6, M2 and M1). RofA is predicted to contain two internal PRDs, suggesting that RofA is regulated by the PTS and would represent a new member of the family of regulators termed PRD-Containing Virulence Regulators (PCVRs). In this work, we found that purified RofA-His from S. pyogenes serotype M1T1 strain 5448 is phosphorylated in vitro by the PTS in an HPr dependent manner. In vivo, transcripts of rofA can be detected throughout growth and are most abundant in the transition from exponential growth to stationary phase. During exponential growth, a RofA mutant exhibited significantly reduced expression of pilus-associated collagen binding protein, cpa, and positively autoregulated its own expression, but did not impact the expression of other genes that were found to be RofA-regulated in serotypes M6 and M2. Finally, a RofA mutant showed modified aggregation potential depending on growth phase. Together, these data support that RofA is a PCVR that may directly couple the sensing and utilization of sugars to regulation of the GAS virulence repertoire.

The adjacent Pi ATP-binding protein-encoding genes *pstB1* and *pstB2* of *Enterococcus faecalis* have different cellular functions

Christopher M. Healy, Biko McMillan, Candace N. Rouchon, and Kristi L. Frank

Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD

Enterococcus faecalis is a gram-positive gastrointestinal commensal that has emerged as a leading healthcare-associated pathogen. Clinical isolates are often resistant to antibacterial agents, readily form biofilms, and are highly adaptable to many environments, making infections difficult to treat. Understanding genetic mechanisms responsible for *E. faecalis* adaptation and virulence is critical for efforts in finding novel methods to treat infections.

Phosphate homeostasis contributes to virulence and biofilm formation in many bacterial species. Bacteria import inorganic phosphate (Pi) through a conserved, membrane-associated, high affinity ABC transporter complex called the phosphate specific transport (Pst) system. Bacteria sense and respond to environmental Pi concentrations through a signal transduction cascade involving the Pst system. *E. faecalis* contains a 6-gene operon comprised of *pstS*, *pstC*, *pstA*, *pstB2*, *pstB1*, and *phoU*. Both *pstB1* and *pstB2* are annotated as encoding ATP-binding proteins; however, the two proteins only share 60% identity and 79% similarity at the amino acid level. Furthermore, while the organization of the *pst-phoU* operon is well-conserved across bacteria, the presence of two annotated ATP-binding protein-encoding genes in the locus is restricted to a limited number of Gram positive species. The functions of *pstB1* and *pstB2* have never been addressed in any of the species that contain both genes. We have generated in-frame deletions of *pstB1* and *pstB2* in *E. faecalis* in order to characterize the roles of these genes in broth culture growth, membrane stress, Pi uptake, and biofilm formation.

Relative to WT, the Δ*pstB1* strain exhibited increased susceptibility to bile salts and SDS on solid medium, while the Δ*pstB2* mutant was even more susceptible. Growth kinetics were measured in chemically defined medium (CDM) containing either low (0.2mM) or high (80mM) Pi concentrations. Δ*pstB1* growth showed a lag compared to WT, and Δ*pstB2* was unable to grow at either concentration of Pi. The Δ*pstB1* strain had a defect in Pi uptake in that was rescued by ectopic expression of *pstB1* and by over-expression of *pstB2*. Interestingly, the Δ*pstB2* strain appeared to lose Pi into the medium. Ectopic expression of the WT *pstB2* gene partially restored Pi uptake. Over-expression of *pstB1* in this mutant did not restore Pi uptake. Finally, in biofilms grown for 6 hours in either 0.2mM or 80mM Pi CDM, Δ*pstB1* had less stainable biofilm biomass compared to WT. Both ectopic expression of *pstB1* and over-expression of *pstB2* restored the phenotype. Biofilm formation of Δ*pstB2* could not be measured since the strain did not grow in CDM. However, ectopic expression of WT *pstB2* rescued growth and biofilm formation in CDM.

These findings demonstrate that *pstB1* and *pstB2* play important yet separate roles in *E. faecalis* growth, stress response, and Pi uptake.

Aerobactin enhances metastatic infection and increases mortality in a hypervirulent Klebsiella pneumoniae isolate

Giovanna Hernandez[1], Taylor Young[1], Alicia Costa-Terryll[1], Dr. M. Ammar Zafar[1]
[1]*Wake Forest School of Medicine*

Klebsiella pneumoniae (Kpn) is an opportunistic gram-negative enteric bacterium of global concern, due to its increasing prevalence of antibiotic resistance and cause of healthcare-associated infections (HAI). Kpn commonly colonizes the mucosal surfaces found in the gastrointestinal (GI) tract and the oropharynx. Currently two distinct pathotypes have been identified, classical K. pneumoniae (cKpn) and hypervirulent K. pneumoniae (hvKpn). In contrast to cKpn, hvKpn strains can infect immunocompetent individuals in a community setting, resulting in metastatic spread and increased mortality. Prior epidemiological data suggests that hvKpn can translocate from the GI tract and cause invasive disease. However, the exact mechanism of translocation remains unknown. To provide a mechanistic understanding of the translocation process, we used our murine model of Kpn GI colonization. We observed that an hvKpn isolate (hvKp1; WT) was able to colonize the GI tract, translocate and cause systemic disease. hvKp1 was observed to translocate within 24 hours of gastric inoculation, with 50% mortality observed within 120 hours post-infection. A distinguishing feature of hvKpn isolates is the presence of the iron-acquisition molecule (siderophore) aerobactin. In comparing an isogenic aerobactin synthesis mutant (iucA-) of hvKp1 to the parental strain, we determined that despite comparable colonization, the iucA- has a defect in translocation and decreased virulence. Consequently, to understand the role of aerobactin in the translocation process, we developed a cell culture assay, utilizing the human colorectal adenocarcinoma cell line Caco-2, to test adhesion, invasion, and translocation of WT and iucA- to determine if translocation occurs paracellularly, transcellularly or as a combination of both. Our preliminary data suggest that iucA- behaves as the parental isolate in its ability to adhere to the epithelial cells. However, it has reduced capacity to invade and translocate compared to the parental isolate, suggesting that the defect observed in translocation in vivo is because of the mutants poor ability to translocate and not clearance by the host. Taken together, we have identified for the first time an hvKpn factor that promotes translocation. Ongoing studies are trying to provide a molecular mechanistic understanding of the translocation process, and further identify new translocation determinants.

Elucidating the differential effects of capsule type on Kingella kingae pathogenicity

Kevin Hernandez[1], Dr. Eric Porsch[2], Dr. Joseph St. Geme III[1]
[1]University of Pennsylvania / Children's Hospital of Philadelphia, [2]Children's Hospital of Philadelphia

Kingella kingae is a gram-negative, encapsulated bacterium that has emerged as the leading cause of bone and joint infections in children between 6 months and 4 years of age. Additionally, this bacterium causes bacteremia, endocarditis, and a variety of other invasive diseases in this age group. K. kingae is a common commensal organism in the oropharynx in young children and is able to breach the oropharyngeal epithelium in the setting of viral respiratory infection or stomatitis, invading the bloodstream, evading serum-mediated and neutrophil-mediated killing, and disseminating to distal sites to cause disease. There are 4 structurally distinct polysaccharide capsules in the K. kingae population, designated as types a, b, c, and d. Based on analysis of a large collection of asymptomatic carrier and invasive disease isolates, our lab has found that 96% of invasive isolates produce either the type a or type b capsule, and a disproportionate percentage of carrier isolates produce the type c or type d capsule. In studies with a prototypic strain that produces the type a capsule, our lab has established that this capsule interferes with adherence to human epithelial cells by masking a key adhesin, yet promotes resistance to serum-mediated killing, opsonin deposition, and neutrophil-mediated killing. The capsule is also necessary for full virulence in a juvenile rat infection model of K. kingae disease. Studies from our lab have shown that capsule type does not affect the ability of K. kingae to resist serum-mediated killing. However, no studies to date have sought to determine the role of capsule type on additional aspects of K. kingae pathogenicity. Using our juvenile rat infection model of K. kingae disease with isogenic strains of K. kingae that differ only in the capsule type produced, we show here that strains producing the type a or type b capsule are more virulent than strains producing the type c or type d capsule. Additionally, preliminary data suggest that the four capsule types have similar effects on adherence to human epithelial cells and resistance to neutrophil-mediated killing. Ongoing studies are addressing how the type a and type b capsules promote increase virulence.

Loss of Flagellar Motility Drives Antibiotic Tolerance by Enhancing Aggregation in Pseudomonas aeruginosa

Matthew Higgs[1], Dr. Matthew Wolfgang[1]
[1]*University of North Carolina at Chapel Hill*

Pseudomonas aeruginosa is an opportunistic pathogen responsible for a wide range of infections. In patients with muco-obstructive lung diseases, such as cystic fibrosis (CF), P. aeruginosa is an apex pathogen and causes chronic lung infections that are notoriously difficult to treat. However, studies suggest that antibiotic tolerance contributes heavily to treatment failure. The mechanisms that drive antibiotic tolerance in the context of muco-obstructive lung diseases are poorly understood. Chronically adapted isolates of P. aeruginosa often exhibit loss of flagellar motility, a trait commonly associated with the switch from an acute, virulent lifestyle to a chronic, sessile lifestyle in the lung. Additionally, we show that as mucus concentration increases, P. aeruginosa becomes increasingly tolerant to antibiotics. In this study, we sought to investigate specific advantages of the loss of flagellar motility in chronically adapted patients. Specifically, we assessed whether loss of motility provided a selective advantage through increased antibiotic tolerance. To assess how loss of flagellar motility influences antibiotic tolerance, we generated a mutant of fliC, which encodes flagellin, and assessed tolerance through an antibiotic killing assay. Bacteria were inoculated into synthetic sputum medium containing 2% mucus to recapitulate the diseased lung environment. Bacteria were treated with a single dose of 300 μg/ml tobramycin for 24 hours. The flagellar mutant exhibited a significant decrease in bacterial killing compared to WT. To begin to investigate why flagellar mutants are more tolerant, we utilized confocal microscopy to assess aggregation in the flagellar mutant. Using the same time point as the antibiotic killing assay, we observed a significant increase in aggregation in the flagellar mutant compared to WT. These results suggest that in a disease relevant in vitro model system, loss of flagellar motility provides an adaptive advantage that increases antibiotic tolerance. The results of this study provide insight into the evolutionary pathway taken by P. aeruginosa towards a more chronic lifestyle during pulmonary infection in individuals with muco-obstructive lung disease. Future studies will focus on investigating mechanisms of how loss of motility enhances aggregation and how aggregation drives antibiotic tolerance.

Investigating IL-36γ signaling in response to Treponema denticola infection

Annie Hinson[1], Christopher Blake[1], Principal Investigator Daniel Miller[1]
[1]*Virginia Commonwealth University*

Periodontitis is a chronic inflammatory disease characterized by advanced degradation of the tissues that support and surround the tooth. With periodontal disease affecting more than 750 million people worldwide and ranking as the third most expensive disease to treat, following diabetes and cardiovascular disease, this is a global health burden that is far from disappearing. Additionally, the prolonged inflammation associated with periodontitis is a systemic health concern as this disease is a risk factor for developing sequalae such as rheumatoid arthritis, cardiovascular disease, and more recently associated with oral cancers and Alzheimer's disease. Dysbiosis of the oral microbiome progresses in periodontitis, inducing robust and unproductive inflammation which drives substantial tissue damage and alveolar bone resorption. The subgingival community in healthy individuals largely consists of Gram-positive, saccharolytic, facultative bacteria such as oral Streptococci. Microbial community shifts occur with disease progression, as Gram-negative and spirochetes become more abundant which are highly proteolytic microorganisms often associated with triggering dysregulated inflammation. Treponema denticola, a subgingival spirochete classically associated with periodontal disease, contributes to dysregulated inflammatory responses and tissue degradation. It is found in low abundance in healthy subgingival sites, but is more prevalent in periodontitis, accounting for ~40% of the total oral bacterial community. T. denticola binds to gingival keratinocytes, fibroblasts and periodontal ligament cells, however the inflammatory responses of these gingival barrier cells when challenged with T. denticola remains understudied. Transcriptomics was utilized to characterize the response of gingival epithelial cells (GECs) following T. denticola infection and identified IL-36γ as the most differentially expressed cytokine or chemokine. IL-36γ is a recently characterized member of the IL-1 superfamily and over-induction of IL-3γ is associated with other chronic inflammatory disease such as psoriasis and rheumatoid arthritis. IL-36γ is elevated in saliva and gingival crevicular fluid in patients with periodontitis and induced expression occurs in GECs during disease. Here, we sought to understand how IL-36γ expression by GECs is regulated in response to T. denticola challenge. First, IL-36γ induction was observed in all strains of T. denticola tested and the response was reproducible in multiple GEC cell lines and primary human gingival keratinocytes. Analysis of isogenic mutants of T. denticola implicate a surface lipoprotein may be leading to IL-36γ induction in a TLR2-dependent manner. Use of pharmacological inhibitors indicate IL-36γ regulation is influenced by NFκB, p38-MAPK, and PI3K signaling pathways, downstream of TLR2. Additional RNA interference studies failed to demonstrate IL-36γ transcriptional regulation downstream of p38-mediated signaling, leading us to investigate MSK1 which is activated by p38 and stimulates NFκB p65 activation in the nucleus. We have shown MSK1 expression is important for IL-36γ induction following T. denticola infection. Collectively, we have identified parallel cell signaling pathways leading to IL-36γ expression which is also of general interest as the regulatory mechanisms controlling IL-36γ expression are poorly understood. Future studies will seek to examine the role of p65 activation in regulating the IL-36γ promoter and the potential role of MSK1 as it also phosphorylates H3S10, potentially leading to epigenetic regulation in addition to p65 activation.

An Uncharacterized Exopolysaccharide is Linked to Brucella Virulence

Dariel Hopersberger[1], Joshua Pitzer, Dr. R. Martin Roop, Dr. Daniel Martin, Dr. Clay Fuqua, Dr. Xavier De Bolle
[1]*East Carolina University*

Brucella abortus is an intracellular pathogen that causes spontaneous abortion in cattle and undulant fever in humans. To facilitate evasion of the host immune system, B. abortus colonizes macrophages and replicates within these cells; thus, the capacity of B. abortus to survive within the host relies on its ability to successfully infect macrophages. As a member of the a-proteobacteria, B. abortus is closely related to the bacterial species Caulobacter crescentus and Agrobacterium tumefaciens, which have been shown to produce a holdfast and unipolar polysaccharide (UPP), respectively. In both cases, a polysaccharide is generated at one pole of the bacterial cell and aids in its attachment to solid surfaces. While Brucella is known to produce exopolysaccharides (EPS), the function of these EPSs remains largely undetermined. Brucella genes homologous to the Agrobacterium UPP biosynthesis genes have been identified, and these genes can restore UPP production in Agrobacterium upp mutants. Moreover, production of a unipolar polysaccharide that reacts with the same lectin that recognizes the Agrobacterium UPP has recently been observed in B. abortus 2308. A mutant derived from this strain lacking two of the putative UPP biosynthesis genes, uppC and uppE, exhibits significant attenuation in mice and an altered intracellular replication profile in cultured murine macrophages. Studies are underway to determine if the uncharacterized EPS produced by the Brucella upp genes is an authentic UPP and define the mechanism(s) by which this EPS contributes to virulence.

Development of a Fluorescence Reporter Assay to Study Bacterial Type III Secretion System (T3SS)-Modulating Compounds

Julia Hotinger[1], Dr. Heather A. Pendergrass[1], Dr. Aaron E. May[1]
[1]*Virginia Commonwealth University*

Enteropathogenic Escherichia coli (EPEC) is a major cause of infantile diarrhea worldwide. EPEC and the closely related murine pathogen, Citrobacter rodentium, utilize a type III secretion system (T3SS) to propagate infection.[1] As the T3SS is only present in pathogenic bacteria, inhibiting or prematurely activating the T3SS could treat an infection without causing harm to commensal bacteria. This lower selective pressure should result in a slower rate of resistance formation than traditional antibiotics. Assays used to study the T3SS usually require a BSL-2 laboratory designation and do not indicate the mechanism of action.[1,2] We have designed and developed a BSL-1 fluorescence reporter assay that does not require mammalian cell culture using the enzyme carboxypeptidase G2 (CPG2) expression system in C. rodentium for screening potential T3SS modulators.[3]

CPG2 was conjugated to the secretion tag of an EPEC T3SS effector protein, EspF, and expressed on a pBAD plasmid under arabinose induction to allow for CPG2's secretion through the T3SS. After secretion, CPG2 cleaves the yellow substrate, Glu-CyFur, to release a purple and fluorescent CyFur (λex = 563 nm, λem = 610 nm). We validated this assay by evaluating known T3SS inhibitors, including (-)-epigallocatechin-3-gallate (EGCG), regacin, and aurodox.[4] We screened multiple natural product libraries, ~1,000 compounds total, and discovered the quorum sensing (QS) inhibitor tannic acid (TA), a natural product found in red wines, is a potent T3SS inhibitor with an IC50 of 0.65 ± 0.09 μM.[4] We additionally tested compounds with the same QS inhibitory mechanism and found they were unable to inhibit the T3SS, indicating a separate mechanism for TA's T3SS inhibition.

Aberrant, or premature, T3SS activation may result in the wasting of effector proteins that, in turn, decreases the infection potential of the pathogen.[5] The viability of this method for infection treatment or prevention has yet to be studied as current assays are not designed to detect T3SS activation. The incomplete induction of the T3SS in our system allows us a unique opportunity to observe T3SS activation. Multiple non-cytotoxic compounds with similar scaffolds (not shown for IP purposes) were serendipitously found to dose-dependently increase the activity of the T3SS. Further experimentation to determine their mechanism of action and structure-activity relationship is ongoing.

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The Role of Fucose Utilization on Klebsiella pneumoniae Colonization and Pathogenesis

Andrew Hudson[1]
[1]*Wake Forest School of Medicine*

Klebsiella pneumoniae (Kpn) is one of the leading causes of nosocomial infections in the United States, owing to its resistance to a broad range of antibiotics and its many potential sites of infection, including the liver, lungs, and urinary tract. While these infection sites are associated with disease, Kpn generally colonizes the human gastrointestinal (GI) tract asymptomatically, from where it can translocate to sterile sites within the host or transmit to another through the fecal-oral route. Kpn gut colonization is poorly understood, but knowledge of this first step toward infection and spread is critical for combatting its pathogenesis and transmission. To establish itself within the gut, Kpn must overcome colonization resistance (CR) provided by the host microbiota. One such mechanism of CR is through nutrient competition. Enteric pathogens have been shown to catabolize fucose, a component of the glycosylated protein mucin - a major component of intestinal mucus. Fucose metabolism allows such pathogens to bypass nutritional competition and successfully infect the GI tract. Kpn possesses a fuc operon homologous to that of E. coli, which we confirmed is essential for Kpn fucose metabolism. Using our murine model for Kpn GI colonization in conjunction with quantitative real-time PCR, we observed a dramatic increase in expression of the fuc operon within the mouse cecum, suggesting fucose utilization in the gut. Further, a mutant defective in fucose metabolism poorly colonized the murine GI tract, identifying the importance of fucose on Kpn's ability to acquire a metabolic niche, bypass CR, and establish itself in the GI tract. Because alternative nutrient sources can allow pathogenic bacteria to coordinate virulence, we investigated whether fucose utilization promotes the expression of several established virulence phenotypes of Kpn. We observed that fucose positively modulates hypermucoviscosity, autoaggregation, and biofilm formation, but not capsule biogenesis. Enzymatic treatment of mature biofilms revealed that fucose promotes biofilm formation by increasing one or more proteinaceous biofilm components. Together, these insights lend a stronger understanding of the role of alternative carbon sources on Kpn gut colonization, as well as the complex relationship between metabolism and virulence in this species.

Transformation of Orientia tsutsugamushi

Jason Hunt[1], Dr. Paige E. Allen[1], Dr. Jason A. Carlyon[1]
[1]*Department of Microbiology and Immunology, Virginia Commonwealth University School of Medicine*

Orientia tsutsugamushi is an obligate intracellular bacterium and etiologic agent of scrub typhus, a potentially fatal rickettsiosis. Currently, no genetic tools for this neglected pathogen exist. As a first step in addressing this technical gap, we constructed a plasmid for mutating O. tsutsugamushi via random Tn5 transposon-mediated insertion of cassettes encoding a fluorescence reporter and selectable marker. We began by identifying O. tsutsugamushi promoters for driving cassette gene expression. Such promoters would need to be highly conserved among strains, expressed throughout infection, and exhibit strong activity. We analyzed the 5' untranslated regions of O. tsutsugamushi genes encoding outer membrane protein A (ompA), 22-kDa type-specific antigen (tsa22), and tsa56. All three were transcribed during O. tsutsugamushi infection of monocytic, endothelial, and epithelial cells. Putative ribosome binding sites were identified in the upstream noncoding regions of each along with one set of predicted -10 and -35 sequences for ompA and two sets of -10 and -35 sequences for tsa22 and tsa56. Comparing these sequences among geographically diverse O. tsutsugamushi patient isolates revealed nucleotide identities ranging from 84.8 to 100.0%. Examination of the promoter candidates for the ability to drive green fluorescence protein expression in Escherichia coli yielded varying activities with one of the tsa22 promoters exhibiting the strongest. Using this information, we constructed a Tn5-mutagenesis plasmid, pJHOT-mCherry. This plasmid is comprised of Tn5 mosaic ends flanking a chloramphenicol resistance cassette and a mCherry reporter gene under control of the strongest tsa22 promoter. After empirically evaluating a range of electroporation conditions, we successfully introduced the plasmid into O. tsutsugamushi, as verified by detection of mCherry-positive bacteria via flow cytometry, Western blot, and immunofluorescence following multiple days of post-transformation antibiotic selection. Together, these data demonstrate the first transformation of O. tsutsugamushi and open many avenues for the genetic manipulation of the bacterium, including construction of a transposon mutant library and site-directed mutagenesis.

Control of Recombination and Mutation in Staphylococcus aureus

Kelly Hurley[1], Gideon Hillebrand[1], Dr. Anthony Richardson[1]
[1]*University of Pittsburgh*

Control of Recombination and Mutation in Staphylococcus aureus
Kelly E. Hurley, Gideon H. Hillebrand, Anthony R. Richardson
Department of Microbiology and Molecular Genetics, School of Medicine, University of Pittsburgh

Staphylococcus aureus is a major human pathogen that causes a variety of illnesses ranging from minor skin and soft tissue infections (SSTIs) to more severe systemic infections such as endocarditis, osteomyelitis, and sepsis. Although the primary host immune response can typically efficiently clear pathogenic bacterial infections, S. aureus is uniquely resistant to this environment. Our lab has determined that nitric oxide (NO), an important component of the innate immune response that plays a role in both immunomodulatory and antibacterial processes, is the effector to which S. aureus is specifically resistant. Additionally, nitric oxide and its derivatives can lead to damage of S. aureus DNA, more specifically, deamination of DNA bases, however, regulation and repair mechanisms of DNA in S. aureus is understudied. Recent evidence suggests S. aureus DNA repair pathways may contribute to the survival of this pathogen in the host environment as well as modulate mutagenesis. Thus, we hypothesize several DNA repair mechanisms may account for the replication fidelity of S. aureus and may contribute to fitness in the presence of nitric oxide. Here we show the role of several DNA repair mechanisms in S. aureus. More specifically, we found recombinational repair genes, recG and recJ, may play a role in the repair of nitric oxide induced replication fork crashes. We also show a role of base excision repair pathway proteins, MutY and Ung, in reducing nitric oxide mediated mutagenesis. Lastly, we show the role of the mismatch repair pathway in preventing illegitimate recombination. It is known that MMR proteins prevent RecA-mediated recombination between divergent sequences. S. aureus has three mismatch repair MutS homologues that work alongside an endonuclease, MutL. Only one has been studied and was shown to limit spontaneous mutagenesis but did not appear to have a role in preventing illegitimate homologous recombination. Here we confirm only one MutS homologue, MutS1, contributes to mutagenesis in S. aureus and no other single or combinatorial homologues play a role in preventing illegitimate recombination between divergent species. Overall, our results suggest nitric oxide leads to DNA damage, which subsequently induces activity of several DNA repair pathways, contributing to the replication fidelity and fitness of S. aureus. Although one mismatch repair homologue contributes to mutagenesis, no other combinatorial homologues play a role.

The induction of type 1 interferon responses in primary osteoblasts following Staphylococcus aureus infection

Mrs. Kelli Hutcheson[1], Dr. Brittany Johnson[1], Dr. Whitney Leach[2], Dr. Sam Furr[1], Dr. Ian Marriott[1]
[1]University of North Carolina at Charlotte, [2]Stowers Institute for Medical Research

Osteomyelitis is a serious bacterial infection of bone that is associated with progressive inflammatory tissue damage. This condition commonly affects the long bones of children along with the vertebrae and pelvis of adults. Staphylococcus aureus, is the principal causative agent of osteomyelitis and can enter bone via the bloodstream or surrounding tissues following injury or surgery resulting in disease that is often refractory to therapies such as debridement or antibiotic treatment. Furthermore, the increasing incidence of infections associated with antibiotic resistant strains of S. aureus has compounded this problem such that new treatment strategies are needed urgently. Osteoblasts can be infected by S. aureus and serve as an intracellular bacterial reservoir for chronic infection. However, this infection can be perceived via an array of microbial pattern recognition receptors (PRRs) that are known to be expressed by osteoblasts, triggering changes in their function. For example, these bone-forming cells play a critical role in the control of the formation and activity of bone resorbing osteoclasts, and we have shown that they can exacerbate inflammation at sites of infection by producing an array of cytokines, chemokines, and growth factors. As such, infected osteoblasts play an important role in the abnormal bone formation and inflammatory damage associated with S. aureus infection. In the present study, we have demonstrated via RNAseq analysis that osteoblasts express mRNA encoding the type-I interferon (IFN), IFN-□, following challenge with a clinical S. aureus isolate (strain UAMS-1). IFN-□□□is released in the presence of pathogens and has been shown to negatively impact intracellular bacterial growth. This could prove beneficial to the host to control such infections. Importantly, we have confirmed the ability of bacterially infected osteoblasts to produce and release IFN-□ in a dose and time dependent manner by specific capture ELISA. In contrast with their well-established protective roles in viral infections, type I IFNs can have either beneficial or detrimental effects during bacterial challenge due to their complex roles in the regulation of immune and tissue homeostasis. However, our RNAseq analysis also revealed an upregulation in the expression of IFN stimulated genes (ISG), IFIT1, IFIT3, PLSCR1, SLFN2, IFI205, and IRGM2, genes that encode products that can confer protection to cells following infection, in S. aureus-challenged osteoblasts. Furthermore, we have confirmed elevated levels of IFIT1 protein in osteoblasts following S. aureus infection by immunoblot analysis, consistent with the upregulation in expression of mRNA encoding this ISG. Together, these data indicate that osteoblasts produce type-I IFNs following infection with S. aureus and demonstrate altered expression of key ISGs. It remains to be determined whether such responses function to limit bacterial burden or, rather, exacerbate the inflammatory bone damage associated with osteomyelitis.

Investigation of the Acute Virulence of Pseudomonas aeruginosa grown on Pseudomonas Isolation Agar Supplemented with Ammonium Metavanadate

Jason Kang[1], Sarah Miller[1], Annalisa Huckaby[1], Kelly Weaver[1], Gage Pyles[1], Emel Sen-Kilic[1], Mariette Barbier[1], Fredrick Damron[1]
[1]*West Virginia University*

Pseudomonas aeruginosa is a Gram-negative, opportunistic pathogen capable of causing a wide range of life-threatening infections, particularly among immunocompromised patients. P. aeruginosa is one of the leading causes of nosocomial infections among hospitalized patients and chronic lung infections in people living with cystic fibrosis. The prevalence of P. aeruginosa in these settings can be attributed to several mechanisms that the bacterium employs to persist in the host. One adaptation that P. aeruginosa undergoes is the secretion of exopolysaccharide alginate, with a colony morphology and phenotype referred to as mucoid. Previous work has demonstrated that P. aeruginosa grown on Pseudomonas isolation agar supplemented with ammonium metavanadate (PIAAMV) induces a mucoid phenotype. In addition, extensive in vitro characterization of P. aeruginosa grown on PIAAMV revealed a diverse set of genes that positively upregulates this phenotype. Based on these findings, we wanted to investigate if growth of the P. aeruginosa strain PAO1 on PIAAMV would have an acute effect on its virulence in vivo. Furthermore, we wanted to investigate the antibody response induced by PIAAMV as a potential therapeutic agent against both mucoid and non-mucoid phenotypes. CD-1 mice were challenged with a sub-lethal dose of PAO1 grown on PIA or PIAAMV by the intranasal route and initial results demonstrated an increase in bacterial burden in the lung and nasal lavage at early time points with a clearance of infection by one-week post-infection for both groups. However, no statistical difference was observed in the weight, temperature, bronchoconstriction patterns, bacterial burden, and myeloid cell populations of these mice. Interestingly, mice challenged with PAO1 grown on PIAAMV were protected from re-challenge with a lethal dose of PAO1 grown on PIA, which indicates an interplay of antibodies shared between strains. Murine monoclonal antibodies were developed from mice challenged with PAO1 grown on PIAAMV and screening revealed a couple of promising antibody candidates against the outer membrane protein of PAO1. Ongoing work involves further characterization of these monoclonal antibodies, evaluation of their cross-protective potential among P. aeruginosa and other Gram-negative species, and evaluation of their therapeutic potential against lethal challenge in mice.

Characterizing Brucella FtrB: A novel cupredoxin

Alexa Kerkan[1], Dr. Sourav Roy[2], Dr. Sambuddha Banerjee[1], Dr. Daniel Martin[2], Dr. Brandon Garcia[2]

[1]Department of Chemistry, East Carolina University, [2]Department of Microbiology and Immunology, East Carolina University

Brucella spp. is a Gram-negative pathogen that can cause reproductive infection in farm animals and can infect humans zoonotically. Although the infection in humans is not life-threatening, in the endemic regions it can add extra stress on the health care system. The infection in ruminants, however, is more detrimental, although can be controlled with vaccination. As an intracellular pathogen, Brucella acquires its nutrients, including iron, from the host system by expressing dedicated transporters. To combat the low iron concentration inside the cells, imposed by mammalian nutritional immunity, Brucella expresses several iron uptake systems. FtrABCD from Brucella spp. is one such system required for infection in the mouse model and takes part in Fe2+ utilization. Similar FtrABCD mediated iron uptake is also observed in Bordetella and Burkholderia spp. and these are characterized by an inner-membrane permease, FtrC, that is a homolog of the yeast Ftr1p permease. In the yeast system, Ftr1p mediated iron utilization is dependent on the expression of a ferroxidase, Fet3p. None of the components of the FtrABCD transporter represent a ferroxidase, however, based on a bioinformatics study, periplasmic FtrB has been predicted as a novel cupredoxin. Cupredoxins are small redox proteins with conserved β-sheet structure and contain a Type-1 copper site described by His-His-Cys residues. Both the protein fold and the copper coordinating residues contribute to the ability of cupredoxins to carry out the electron-transport property. Although FtrB is classified as a novel cupredoxin, it lacks the Type-1 copper-binding residues and there is no biophysical data indicating it can oxidize Fe2+. The aim of this project is to characterize the Cu2+ affinity and the ferrous oxidizing activity of recombinant wild-type FtrB. Using circular dichroism, isothermal titration calorimetry, size exclusion chromatography, and a ferrous oxidation assay using ferrozine, we report that wild-type FtrB purifies as a monomer (10 kDa) and can bind Cu2+ with low μM affinity in a 1:1 ratio. We also report that the global structure of wild-type FtrB is β-sheet and natively folded FtrB shows Fe2+ oxidizing property when compared with denatured wild-type FtrB. FtrB being an essential part of the virulence factor, FtrABCD, understanding the molecular mechanism of function of this protein (also found in two other pathogens) is crucial. Our finding that FtrB can act as a ferrous oxidizing protein is also the first experimental evidence of such property in any novel cupredoxins that do not conserve the classical Type-1 Cu site.

Moonlighting functions of the Shigella flexneri autotransporter IcsA

Dr. Volkan Köseoğlu[1], Dr. Hervé Agaisse[1]
[1]*University of Virginia*

Shigella flexneri causes bacillary dysentery (bloody diarrhea), the second leading cause of diarrheal death globally. S. flexneri is an intracellular bacterial pathogen that uses the type III secretion system to deliver effector proteins into the cytosol of colonic epithelial cells. The collective activity of effectors enables S. flexneri to invade cells. Following the invasion process, S. flexneri expresses the autotransporter protein IcsA that binds to the host actin nucleation promoting factor, N-WASP. Subsequently, N-WASP recruits actin nucleation factor Arp2/3 complex, mediating actin polymerization at the bacterial pole. Forces generated by the actin polymerization mobilize intracellular S. flexneri, referred to as actin-based motility. IcsA-driven actin-based motility is imperative for S. flexneri dissemination in colonic epithelium and disease pathogenesis in infant rabbits. While most research has focused on intracellular infection, there is a gap of knowledge about the extracellular phase of S. flexneri infection. Previously, we showed that the essential virulence factor IcsA facilitates extracellular S. flexneri biofilm formation in the presence of bile salts. Therefore, S. flexneri IcsA is a moonlighting protein that plays niche-specific roles during infection. We set out to genetically uncouple extracellular and intracellular IcsA functions. Towards this, we generated chimeric constructs using IcsA and an Escherichia coli homolog protein (M605) that shares 75% sequence similarity with IcsA. We found that M605 is expressed on the bacterial cell surface, functioning as an adhesin. Structural predictions revealed a structural homology between IcsA and M605 extracellular domains that adopt an L-shaped β-helix structure with conserved β-strands and associated loops. We showed that IcsA-M605 chimera harboring the IcsA 1-433 region (Chimera-433) in M605 backbone rescues actin-based motility and biofilm formation defects of ΔicsA strain while M605 alone cannot complement these phenotypes. Using Chimera-433 as a platform, we sought to dissect IcsA structural motifs that promote niche-specific functions. To this end, we generated IcsA-M605 chimeras with β-strand deletions in the N-terminus of IcsA that support biofilm formation but not actin-based motility. Furthermore, genetic swaps substituting IcsA β-strands with M605 β-strands revealed D275, N276, and I280 as critical IcsA residues for S. flexneri actin-based motility. We will perform in vitro infection experiments to test interactions between IcsA-M605 chimeras and N-WASP. In the future, we will characterize how modulated interactions between IcsA and N-WASP affect disease pathogenesis in the infant rabbit model of bacillary dysentery.

A Platform for Genome Mining of Multidrug-Resistant Pathogens to Develop Therapeutic Phages Using Synthetic Biology

Timothy Fitzgerald[1], Giovanna Castellanos[1], Dr. Ashton Belew[2], Dr. Katie Margulieux[1], Dr. Najib El-Sayed[2], LCT Brett Swierczewski[1], MAJ Damon Ellison[1], MAJ Derese Getnet[1], Dr. Kevin McIver[2], Dr. Hervé Tettelin[3], Dr. Daniel Zurawski[1], Yoann Le Breton[1,4]

[1]Walter Reed Army Institute of Research, [2]University of Maryland, [3](3)University of Maryland Baltimore School of Medicine, [4]The Geneva Foundation

Multidrug-resistant [MDR] pathogens represent a global health threat and a challenge for modern medicine; and, as bacterial resistance to new antibiotics is now outpacing the antibiotic development effort, it is critical to explore new antimicrobial alternatives. The antimicrobial resistance crisis is bringing new interest worldwide for phage-based therapies. Over the last decade, efforts in the U.S. to produce phage therapeutics targeting different bacterial pathogens have shown promising results, including successful treatments of life-threatening infections in human patients. The safety of phage therapy is still a concern; however FDA has highlighted new requirements for phage preparation: they need to be safe, pure, potent, exclusively lytic, non-transducing, and lacking undesirable genes (antibiotic resistance, virulence factors) and bacterial endotoxins.

If bacteriophages for therapy have historically been isolated from natural environments, recent progress in phage genetics and genome engineering have proven successful to generate synthetic, strictly lytic derivatives targeting pathogens. The development of synthetic phages against MDR-pathogens would require pipelines to accelerate our knowledge on newly discovered phages and their potential for synthetic biology. Critical insights into their biology, i.e. genome structure, phage replication cycle, genetic content (essential genes versus dispensable [antibiotic resistance and virulence genes]), interaction with the target host, are a prerequisite.

Here, we propose a platform to (i) mine the genomes of MDR-pathogens, a gold mine to identify dormant lysogenic phages directly from within their natural host; and (ii) develop high-throughput pipelines to quickly gain knowledge on the phage biology to guide our efforts to engineer synthetic phages as therapeutics. We are using the Group A Streptococcus (GAS), a “Concerning Threat” on the 2019 CDC “18 MDR pathogens” Watch List, as our proof-of-concept model. We showed that Tn-seq could identify functional lysogenic phages from cryptic ones in GAS genomes, and mutations to reboot dormant prophages into their lytic cycle. We are currently developing a synthetic-biology pipeline to gain critical knowledge to guide the decision on the selection of phages for therapeutic potential. The pipeline includes steps to experimentally assess phage genome organization, phage replication/transduction mechanisms, host range and cell surface receptor(s). It will also implement a “design-build-test-learn cycle” workflow to optimize the synthetic biology effort, i.e. deletion of undesirable genes and addition of “payload” genes, to enhance their potential as a therapeutic intervention for bacterial infection. Our overarching goal is to develop the tools and experience to develop an agnostic synthetic biology phage-engineering platform applicable to other MDR-pathogens.

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SARS-CoV-2 Delta variant induces enhanced pathology, interferon responses and a hyper inflammatory profile in K18-hACE2 mice

Katherine Lee[1,2], Ting Wong[1,2], Brynnan Russ[1,2], Alexander Horspool[1,2], Olivia Miller[1,2], Nathaniel Rader[1,2], Jerome Givi[3], Michael Winters[1], Zeriel Wong[1], Holly Cyphert[5], James Denvir[6], Peter Stoilov[7], Mariette Barbier[1,2], Shahrier Amin[3], Ivan Martinez[1,3], Justin Bevere[1,2], Dr. F Heath Damron[1,2]

[1]Department of Microbiology, Immunology, and Cell Biology, West Virginia University, [2]Vaccine Development Center at West Virginia University Health Sciences Center, [3]Department of Pathology, Anatomy, and Laboratory Medicine, West Virginia University, [4]West Virginia University Cancer Institute, School of Medicine, [5]Department of Biological Sciences, Marshall University, [6]Department of Biomedical Sciences, Marshall University, [7]Department of Biochemistry, School of Medicine

The COVID-19 pandemic has been fueled by novel variants of concern that have enhanced transmissibility, receptor binding, and other properties that enhance disease. Alpha, Beta, Gamma variants appeared in late 2020 all with concerning attributes including ability to affect efficacy data of Phase III trials. K18-hACE2-mice are used as pre-clinical models of COVID-19. Delayed access to VOC strains has perpetuated use of Wuhan-like strains of SARS-CoV-2. Due to lack of information regarding potentially enhanced virulence of the VOC strains, we performed challenge dose studies and observed that VOC strains such as Alpha and Beta are 100 times more lethal than WA-1, Wuhan-like ancestral virus. The Delta variant of SARS-CoV-2 is the fourth major variant of concern (VoC) identified first emerging in India and then rapidly spread globally. Based on its enhanced transmission and virulence, we would have expected it to cause greater morbidity; however, our dose study revealed that the LD100 of Delta is 104 PFU. In order to compare Alpha and Delta VOC strains we performed a time-course experiment to evaluate: histopathology, viral burden, cytokine production, and gene expression in K18-hACE2-mice challenged with 103 PFU. Histopathological analysis revealed high tissue inflammation and cell infiltration in Delta challenged mouse lungs at day 6. qRT-PCR analysis demonstrated no significant difference between Alpha or Delta RNA burden in lung, nasal wash or brain. Pro-inflammatory cytokines were highest at day 6 in Delta challenged mice. Total RNA-sequencing analysis was performed on lungs of Alpha or Delta challenged mice at days 2 and 6 and compared to non-challenged mice. This approach allowed us to measure the total amount of viral RNA in the lungs but no major differences were observed between the strains. Alpha challenged mice have more total genes differentially activated, but Delta challenged mice have a higher magnitude of differential gene expression. In particular, Delta challenge results in higher interferon dependent gene expression. Cytokine analysis further supported that Delta challenge causes increased interferon gamma as well as increased T cell signatures. We hypothesize Delta has evolved in such a way to engage interferon responses that potentially enhance transmission. Furthermore, Delta clearly induces more pneumonia than all other strains we have evaluated making it a more ideal model strain for evaluating vaccines and therapeutics. The in vivo and in silico observations of this study underscore the need to use VOC strains to best model COVID-19.

Localized peptidoglycan biosynthesis in Chlamydia trachomatis conforms to the polarized division and cell size reduction developmental models.

George Liechti[1]
[1]*Uniformed Services University*

Cell size regulation in bacteria is a function of two basic cellular processes: the expansion of the cell envelope and its constriction at spatially defined points at what will eventually become the division plane. In most bacterial species, both cell wall expansion and restriction are dependent on peptidoglycan (PG), a structural polymer comprised of sugars and amino acids that imparts strength and rigidity to bacterial membranes. Pathogenic Chlamydia species are unique in that their cell walls contain very little PG, which is restricted almost entirely to the apparent division plane of the microbe's replicative forms. Very little is known about the degree to which PG affects the size and shape of C. trachomatis during its division process, and recent studies suggest the process is initiated via a polarized mechanism. We conducted an imaging study to ascertain the dimensions, orientation, and relative density of chlamydial PG throughout the organism's developmental cycle. Our analysis indicates that PG in replicating C. trachomatis can be associated with four, broad structural forms; polar/septal disks, small/thick rings, large rings, and small/thin rings. We found that PG density appeared to be highest in septal disks and small/thick rings, indicating that these structures likely have high PG synthesis to degradation ratios. We also discovered that as C. trachomatis progresses through its developmental cycle PG structures, on average, decrease in total volume, indicating that the average cell volume of chlamydial RBs likely decreases over time. When cells infected with C. trachomatis are treated with inhibitors of critical components of the microbe's two distinct PG synthases, we observed drastic differences in the ratio of PG synthesis to degradation, as well as the volume and shape of PG-containing structures. Overall, our results suggest that C. trachomatis PG synthases differentially regulate the expansion and contraction of the PG ring during both the expansion and constriction of the microbe's cell membrane during cell growth and division, respectively.

The Anaplasma phagocytophilum invasin AipA interacts with CD13 to facilitate host cell invasion

Dr. Waheeda Naimi[1], Mary Clark Lind[1], Dr. Jason Carlyon[1]
[1]*Virginia Commonwealth University*

Human granulocytic anaplasmosis (HGA) is a potentially fatal tick-transmitted disease that is emerging in the United States at an alarming rate and for which no vaccine exists. The causative agent of HGA is Anaplasma phagocytophilum, an obligate intracellular bacterium with a tropism for neutrophils. Investigating the microbial-host interactions that facilitate A. phagocytophilum cellular invasion will advance understanding of this bacterium's molecular pathogenesis and identify potential targets for protecting against HGA. Invasins are bacterial surface components that interact with specific receptors to facilitate invasion of host cells. AipA is one of three known A. phagocytophilum surface proteins that are critical for infection. A yeast two-hybrid screen indicated that the invasin, AipA, potentially interacts with a C-terminal domain of CD13, a multifunctional protein that is expressed on most leukocytes. CD13 exhibits alanyl aminopeptidase activity and mediates signaling events that lead to receptor-mediated endocytosis and phagocytosis. The AipA-CD13 interaction was confirmed by co-immunoprecipitation assays for both recombinant and native AipA. Treating human promyelocytic HL-60 cells and neutrophils with antibody targeting the CD13 C-terminus failed to impair A. phagocytophilum cellular adhesion, but reduced infection by 35% to 38%. Overexpression of CD13 in HEK-293T cells resulted in a 40% increase in susceptibility to A. phagocytophilum infection. Inhibiting CD13 catalytic activity had no effect on the bacterium's ability to bind and enter host cells. However, host cells treated with mAb 452, which crosslinks CD13 to activate Src and Syk kinase cascades known to culminate in cytoskeletal rearrangement, rescues the ability of AipA antibody-treated A. phagocytophilum to infect. Thus, AipA likely crosslinks CD13 to invoke signaling that promotes infection. Indeed, AipA9-21 binding to CD13 elicits Syk signaling. Finally, infection of peripheral blood neutrophils in CD13-/- mice is reduced by 40%, indicating A. phagocytophilum requires CD13 to productively infect neutrophils in vivo. Together, these results indicate that A. phagocytophilum AipA interacts with CD13 to contribute to A. phagocytophilum infection.

Interbacterial antagonism mediated by a released polysaccharide

Yiwei Liu[1,2], Dr. Erin Gloag[2], Preston Hill[2], Dr. Daniel Wozniak[1,2]
[1]Department of Microbiology, Ohio State University, [2]Department of Microbial Infection and Immunity, Ohio State University

Polymicrobial interaction is dynamic and has a significant impact on human diseases, especially chronic infections. Pseudomonas aeruginosa and Staphylococcus aureus are two common pathogens colonizing the lungs of people with cystic fibrosis (CF) and wounds, indicating they can co-exist in vivo. However, P. aeruginosa utilizes various mechanisms to kill S. aureus in vitro. Our objective was to address this conundrum by studying the interactions between these two pathogens. Psl, one of the exopolysaccharides produced by P. aeruginosa, is important for biofilm formation and has both cell-associated and cell-free forms. Our initial screening indicated a role for Psl in killing S. aureus. When grown in planktonic co-culture with a P. aeruginosa psl mutant, S. aureus had increased survival, compared to when grown with wild-type P. aeruginosa. We found that cell-free Psl was critical for the antagonism, as purified cell-free Psl was sufficient to kill S. aureus. This was independent of known mechanisms used by P. aeruginosa to kill S. aureus, including Pseudomonas quinolone signal, pyoverdine, rhamnolipid, and protease. Transmission electron microscopy of S. aureus treated with Psl revealed disrupted cell envelopes, suggesting that cell-free Psl causes S. aureus cell lysis. Preliminary studies using purified Psl revealed that the exopolysaccharide may be inducing S. aureus autolysis, leading to increased cell death. Cell-free Psl could also promote S. aureus killing in in vivo-like conditions, including coculture in synthetic CF sputum media and biofilms. We also found that P. aeruginosa CF clinical isolates with reduced or no Psl production showed reduced killing towards S. aureus. This could be a result of P. aeruginosa co-evolution with S. aureus in CF lungs. In conclusion, this study defines a novel role for P. aeruginosa Psl in killing S. aureus and potentially impacting the co-existence of these two pathogens in vivo. A better understanding of how these two pathogens co-exist and cooperatively infect patients will shed light on the establishment of polymicrobial infections and lead to the development of novel antimicrobial therapies.
Keywords: Pseudomonas aeruginosa, Staphylococcus aureus, exopolysaccharide, Psl, polymicrobial, cystic fibrosis, wound

Understanding the role of Ngo1049 in subverting host-mediated metal starvation in Neisseria gonorrhoeae

Ian Liyayi[1], Jocelyn Ray[1], Alison Criss[1], Aloke Bera[2], Nicholas Noinaj[2], Cynthia Cornelissen[3]
[1]University of Virginia; Department of Microbiology, Immunology, and Cancer Biology, [2]Purdue University; Department of Biological Sciences, [3]Georgia State University; Institute for Biomedical Sciences

Neisseria gonorrhoeae (Ngo) is an obligate human bacterial pathogen that causes the sexually transmitted infection gonorrhea. Due to increasing rates of gonorrhea and increased antibiotic resistance, vaccines and new therapies are urgently needed. A promising strategy is targeting nutrient metal acquisition systems since they are usually conserved, expressed during infection, and essential for bacterial survival in vivo. Ngo undermines host metal restriction mechanisms by expressing outer membrane transporters to acquire essential metals from human metal-sequestering proteins such as iron from lactoferrin and transferrin, and zinc from calprotectin and psoriasin. However, many gene products that support Ngo growth in metal-limiting conditions remain uncharacterized. The open reading frame Ngo1049 encodes a conserved, functionally uncharacterized protein. We found the product of the ngo1049 gene is strongly upregulated in Ngo grown under zinc-limiting conditions. Ngo1049 is conserved among pathogenic Neisseria, and bioinformatic analysis predicts that Ngo1049 is a periplasmic metal binding component of an ABC transport system. We identified a Zur binding motif upstream of ngo1049, suggesting expression is regulated by Zur (zinc uptake regulator), which represses expression in high zinc concentrations. ngo1049 transcripts are highly induced during Ngo infection of human ectocervical cells, indicating a potential role for Ngo1049 in Ngo pathogenesis. Preliminary analysis of Ngo1049 by X-ray crystallography and small angle X-ray scattering revealed Ngo1049 is a dimer with one zinc binding site per monomer. Based on these data, we hypothesize Ngo1049 is a Zur-regulated, zinc binding protein that facilitates zinc acquisition in metal-limited conditions at inflamed epithelial surfaces. To test this hypothesis, we are determining the localization and regulation mechanism(s) of Ngo1049, examining the contribution of Ngo1049 to zinc acquisition in media and in models of Ngo infection, and defining how Ngo1049 enables Ngo survival at mucosal surfaces. By defining this new member of the Ngo metal regulon, this work will potentially point to new therapies for this antibiotic-resistant bacterium.

Biofilm-dependent selection of a large chromosomal duplication in Burkholderia thailandensis

Lillian Lowrey[1], Angelica Ocasio[1], Leslie Kent[1], Bridgett Rios[1], Peggy Cotter[1]
[1]*University of North Carolina at Chapel Hill*

Chromosomal rearrangements, including inversions, deletions, insertions, and duplications, are commonplace in bacterial genomes. Large duplications occur nearly 100 times more frequently than single nucleotide changes and can confer several advantages. In the short term, duplications double the amount of protein produced by the duplicated coding sequences. In the long run, the second copy of each gene can accumulate mutations that confer new functionality to the proteins they encode, while the original copy is preserved.

We have observed that Burkholderia thailandensis strain E264 commonly contains a 210kb tandem DNA duplication. The duplicated region is flanked by two nearly identical IS2-like elements. When we passage cells with a duplication on LSLB agar, a portion (~2.5%) of the resulting colonies lose the duplication. We set out to answer three questions related to the process and purpose of the duplication: 1) What is the mechanism of duplication? 2) What are the conditions that favor loss or formation of the duplication? And 3) Why is duplication of the region advantageous under certain conditions?
To determine the contribution of different genes to duplication of the region, we constructed strains with deletion mutations in the genes encoding the transposases of the IS2-like elements and recA. We found that recA, but not the transposases, is required for duplications of the region to occur. These data suggest that homologous recombination between the IS2-like element sequences, and not unique transposase activity, duplicates the region. Using a gusA reporter strain, we showed that duplication loss is favored in bacteria grown in LSLB and M63 broth and agar. By contrast, a PCR assay for detecting the unique sequences present in strains containing tandem copies of the region demonstrated that duplication is favored when the bacteria are grown in colony biofilms. These results suggest that possessing two copies of one or more genes within the region confers a growth advantage in this dense, nutrient-limited condition. We are currently conducting experiments to determine the genes that confer this fitness advantage. This recurring duplication is a strong example of large DNA duplications providing immediate advantages to cells.

Effect of Lipoproteins on the Biofilm Formation and Motility of Acinetobacter baumannii

Stephanie Majernik[1], Ms Annika Kulkarni[1], Tim Fitzgerald[1], Mariel Escatte[1], Dr. Yoann LeBreton[1], Dr. Daniel Zurawski[1]
[1]*Walter Reed Army Institute of Research*

Acinetobacter baumannii is a Gram-negative, nosocomial bacteria that causes 45,000 infections in the United States and one million global deaths yearly due to its extensive drug resistance and virulence factors, such as biofilm and motility. Due to recent advances in A. baumannii genetic manipulations, we are just now unraveling the virulence factors responsible for its pathogenesis. The ability of A. baumannii to form a biofilm on surfaces and in wounds enhances antibiotic resistance, and motile A. baumannii can spread rapidly through an organism, leading to sepsis and poor patient outcomes.
With respect to biofilms, conserved lipoproteins found on the cell surface have been shown in other bacteria to play a role in biofilm formation, and these genes are upregulated in biofilms made by Gram-positive Staphylococcus aureus. Lipoproteins are lipid-anchored molecules also found in the outer membrane of Gram-negative pathogens, potentially can be virulence factors, and are required for the secretion of other virulence factors; but the connection to infection is still rather understudied. Forty-six putative lipoprotein mutants were selected from an Ordered-Sequenced 3 Allele Transposon Library and parsed down to a set of 23 mutants for further analysis based on the insertion of the transposon within a given gene, if they grew under selection, if they exhibited 1 or 2 colony morphologies, and if homologues to the gene had extracellular facing domains in related Gram-negatives. The strains were then tested in a biofilm formation assay and motility assay against the wild type (WT) control, AB5075. Certain strains exhibited poor biofilm across 3 replicates, indicating that there was a decline in biofilm formation as a result of disrupting the target lipoprotein gene. In terms of motility, most strains were non-motile compared to WT, demonstrating a strong correlation between lipoproteins and motility. Additionally, a virulence screen was run in Galleria mellonella and a subset of strains demonstrated either attenuation or reduced virulence when compared to WT supporting lipoproteins connection to virulence. From this data we have now narrowed down to two targets for future study: rplA and vacJ. rplA was selected due to it being a widely-conserved outer membrane lipoprotein and experimentally demonstrating reduced biofilm production, motility and virulence in Galleria mellonella. vacJ was selected due to experimentally demonstrating reduction in biofilm production and motility and because in other bacteria, it is a virulence factor. Because lipoproteins play such an important role in the secretion of other virulence factors, and because of their extracellular location on the bacterial surface, these proteins are attractive targets for future study and for the development of human monoclonal antibodies, which can be used to prevent infection.

Study of Glucose supplementation on Antibiotic Efficacy against Staphylococcus aureus

Areej Malik[1], Alexandra Chittams-Miles[2], Claudia Muratori[2], Erin Purcell[1]
[1]Department of Chemistry and Biochemistry, Old Dominion University, [2]Frank Reidy Research Center for Bioelectrics, Old Dominion University

Staphylococcus aureus (S. aureus), a Gram-positive biofilm-forming bacterium, is a leading cause of skin and soft tissue infections (SSTIs) in the United States. The public health impact of S. aureus has been increased by the emergence of Methicillin-resistant Staphylococcus aureus. Patients with diabetes experience a greater rate of SSTIs. It is known that hyperglycemia (high blood sugar) from diabetes reduces immune system function. This research explores the effect of glucose supplementation on the bacterial response to multiple classes antibiotics to determine whether hyperglycemia could contribute to treatment failure of diabetic S. aureus SSTIs via multiple mechanisms.

Investigating the cytochrome oxidases of Bordetella bronchiseptica

Lily McKay[1], Dr. M. Ashley Sobran, Dr. Sara Marlatt, Dr. Peggy Cotter[1]
[1]*University of North Carolina at Chapel Hill*

Bacterial pathogens have evolved to survive in diverse microevironments within hosts, moving from the initial site of infection to colonization sites. From a metabolic standpoint, survival depends not only on nutrient availability, but also on the oxidization substrates available for generating ATP. Little is currently known about how bacterial pathogens alter their physiology in response to the microenvironments they encounter in the host. Bordetella bronchiseptica is a natural respiratory pathogen of most mammals, including mice, colonizing both the upper and lower respiratory tracts. B. bronchiseptica is an obligate aerobe that depends on electron transport-coupled oxidative phosphorylation to generate energy and encodes seven cytochrome oxidases with either high or low affinity for oxygen. By studying the roles of the different cytochrome oxidases, we will learn how these bacteria respond to the different respiratory tract environments, where oxygen levels range from ambient air to microoxic, while also learning more about the environments themselves. We constructed strains in which one or more cytochrome oxidase-encoding genes were deleted, then compared the deletion strains to wild-type bacteria during growth either using in vitro laboratory culturing conditions or following nasal infection of mice to determine the necessity and sufficiency of each gene. We found that loss of single cytochrome oxidases does not impact the growth rate during in vitro growth in ambient air, indicating that no single cytochrome oxidase is necessary for growth under these conditions. However, strains with a deletion of either of two loci encoding high affinity cytochrome bd oxidases (named cydAB1 and cydAB3) had a lower bacterial burden relative to the wild-type strain in the mouse trachea but not the nasal cavity or lung, indicating that these cytochrome oxidases are important for colonization and persistence specifically in the trachea. Deletion of genes encoding all four high affinity cytochrome oxidases or all three low affinity cytochrome oxidases resulted in a similar lower bacterial burden in the trachea. These results are surprising since high-affinity and low-affinity cytochrome oxidases are optimized for low and high oxygen conditions, respectively. These data suggest that the trachea is an important transitional environment between higher and lower oxygen levels, requiring both high- and low-affinity cytochrome oxidases. Our results will advance our understanding of how bacterial pathogens adjust their energetic machinery to adapt to different microenvironments, which may inform the development of therapeutics to prevent colonization at specific sites.

Establishing the regulon of CmrT, a Clostridioides difficile response regulator involved in motility

Anchal Mehra[1], Dr. Rita Tamayo[1]
[1]*University of North Carolina at Chapel Hill*

Clostridioides difficile is an urgent public health threat, causing symptoms ranging from mild diarrhea to fatal pseudomembranous colitis in ~500,000 people annually in the U.S.[1] C. difficile can overcome external stressors via phase variation, a mechanism to introduce phenotypic diversity in a heritable, reversible manner within a population.[2] In C. difficile, phase variation occurs via site-specific recombination, in which a site-specific recombinase mediates inversion of an intergenic DNA sequence containing regulatory features, here termed a 'switch'. Switch inversion results in the ON/OFF modulation of expression of the associated gene or operon. Our lab has been characterizing several C. difficile phase variable switches, one of which is the cmr locus (for 'colony morphology regulator').[3,4]

The cmr switch lies upstream of the cmrRST operon, which encodes the putative histidine kinase CmrS and response regulators CmrR and CmrT. Inversion of the cmr switch leads to phase variation of several phenotypes. We showed that the ON orientation of the cmr switch confers a rough colony morphology and increases surface migration, cell chaining, and cell length, whereas the OFF orientation corresponds with a smooth colony morphology, increased swimming motility, and biofilm formation. A ΔcmrR mutant displayed attenuated virulence in the in the hamster model of infection, suggesting a role for cmr-ON in CDI. [4]

How CmrRST contributes to the multiple observed phenotypes is not known. CmrR and CmrT are predicted to regulate gene expression, as both proteins contain phosphoreceiver and helix-turn-helix DNA-binding domains. Our data suggest that CmrR and CmrT have different roles – while CmrT is essential for the in vitro cell and colony morphology phenotypes, CmrR affects biofilm formation and intestinal colonization. Current work is focused on defining the regulons of both CmrR and CmrT. In this study, we describe an approach to find CmrT-regulated genes essential for the rough colony and surface migration phenotypes. As a ΔcmrT strain does not display the rough colony morphology or surface migration, we screened for suppressor mutations in the ΔcmrT strain that restore rough colony formation to identify genes involved. We show that mutations in multiple genes restore CmrT-regulated phenotypes, revealing genes that have not yet been shown to be involved in C. difficile motility. Establishing how CmrRST coordinates several phenotypes, especially surface versus swimming motility, will reveal the importance of phase variation and the cmr switch in C. difficile virulence.

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Analyzing Interactions Between the SpoVA Proteins in Bacillus subtilis

Nicole Messner[1], Dr. David Popham[1]
[1]*Virginia Tech*

Bacillus subtilis is a Gram-positive bacterium that sporulates in a state of nutrient deprivation. In spore form, these bacteria are extremely resistant to environmental stress factors through many adaptations that make them metabolically inactive and relatively impermeable. Upon re-exposure to nutrients, spores resume a vegetative life cycle through the process of germination. One step in the process of sporulation requires uptake of dipicolinic acid (DPA) and Ca2+ ions into the spore core, and a reverse step in germination occurs where DPA and Ca2+ are released from the cell. Prior evidence has shown that the spoVA operon encodes proteins that are essential for both of these processes. The spoVA operon is a seven-gene operon that codes for a lipoprotein and integral membrane proteins associated with the inner spore membrane. The expected soluble domains for five its proteins, SpoVAA, SpoVAD, SpoVAEB, SpoVAEA, and SpoVAF, were used in this study to look at protein-protein interactions between them. PCR was used to amplify the coding regions of soluble domains of each protein, and the resulting DNA was ligated into pUT18c and pKT25 plasmids for bacterial two-hybrid analyses. Plasmid DNA was isolated and sequenced to ensure fully correct spoVA sequences were inserted into the plasmids. Co-transformation into E. coli using complementary pUT18c and pKT25 constructs allowed testing of protein interactions. Transformants were plated on MacConkey agar, a differential medium that causes colonies that utilize lactose to turn pink and colonies that do not utilize lactose to remain white. If the pUT18c- and pKT25-encoded SpoVA-adenyl cyclase domain subunits interact, cAMP will be produced which will allow for lactose utilization, providing evidence of the interaction of the two SpoVA protein domains. Results testing interactions between the two soluble SpoVAA domains with all the other SpoVA proteins have not shown any positive interactions, but the bacterial two-hybrid analyses of the remaining SpoVA protein are continuing. In the future, secondary screening on minimal media containing lactose and a rich media containing X-Gal will be used to further check for interactions. Further studies will involve testing the SpoVA two-hybrid strains with other lab strains containing domains of various inner spore membrane proteins known to participate in spore germination. With the findings of this study, the long-term goal is gain the knowledge to give us a greater ability to stimulate the Bacillus germination apparatus to improve decontamination.

Copy number variability in the broadly active, RTX family exotoxin secreted by Kingella kingae

Daniel Morreale[1,2], Dr. Eric A. Porsch[1], Dr. Paul J. Planet[1,2], Dr. Joseph W. St. Geme[1,2]
[1]*Children's Hospital of Philadlephia,* [2]*Department of Microbiology, University of Pennsylvania*

Kingella kingae is an important pediatric pathogen and is commonly found colonizing the oropharynx of children less than 48 months of age. While most children tolerate colonization without complications, a subset develop severe invasive disease typified by septic arthritis, osteomyelitis, bacteremia, or endocarditis. K. kingae is known to secrete a broadly active, highly conserved exotoxin called RtxA. RtxA is a member of the repeats-in-toxin family, which includes toxins in diverse species. RtxA effectively lyses erythrocytes, leukocytes, synovial cells, and epithelial cells in vitro. In this study, we examined a collection of 124 clinical isolates and identified 37 strains (30%) with a duplication of the toxin locus, resulting in two copies of the rtxA gene. This genotype was strongly correlated with severe, invasive disease. In experiments with eight diverse strains, we examined the impact of the rtxA duplication on toxin production and cytotoxic activity in vitro and on the efficiency of bacterial epithelial breach in a differentiated airway epithelial cell model. Compared with strains with a single copy of rtxA, strains with two copies of rtxA did not express more RtxA, were not more hemolytic, and did not breach epithelial barriers more rapidly. Taken together, these data suggest that, while toxin is essential for epithelial breach, invasion is multifaceted and likely utilizes additional factors.

Clostridioides difficile Biofilm and Spore Production in Response to Antibiotic and Immune Stress

Mr Adenrele Oludiran[1], Dr. Erin Purcell[1], Dr. Myriam Cotten[2]
[1]*OLD DOMINION UNIVERSITY,* [2]*COLLEGE OF WILLIAMS AND MARY*

Clostridioides (Clostridium) difficile, an anaerobic, spore-forming Gram-positive pathogenic bacterium, is a major cause of hospital-acquired infections and can persist as surface-attached biofilms for protection from antibiotic and immune stress. C. difficile can form biofilms as a single species or with other anaerobic intestinal bacteria. The environmental signals that cause individual cells to secrete toxins, form biofilms, or develop into spores that can spread the infection to new patients are unknown. In these studies, we investigate bacterial biofilm formation and sporulation in response to stress.
Antimicrobial host-defense peptides (HDPs) produced by animal immune systems are promising candidates to develop novel therapies for bacterial infection because they cause oxidative stress that damages multiple targets in bacterial cells, so it is difficult for bacteria to evolve resistance to these attacks. We investigate antibiotic treatments, metal ions and sugars, and antimicrobial peptide treatments to determine how C. difficile reacts to multiple environmental stresses like those from antibiotic treatment or the human immune system. In our investigation of C. difficile and HDPs in an anaerobic environment, we found that the interaction of piscidin and copper is different in different oxygen environments. Antibiotics and oxidative stresses from other sources cause the cells to form spores and/or biofilms to protect themselves, but piscidins kill vegetative C. difficile cells without triggering these protective responses. Piscidins are highly active against C. difficile and could be a good candidate for drug development.

Cross-species induction of OMV production in bacterial co-culture

Humberto Ortega[1], Dr. Jeffrey Schertzer
[1]*Binghamton University*

Outer membrane vesicles (OMVs) are derived from the outer membrane of Gram-negative bacteria. They are integral to the transport a wide variety of cargo to target cells and serve as versatile tools for interaction with the environment. While much is known about their functions, especially their roles in virulence and cell-cell interaction, there is currently no unifying model explaining how OMVs are produced. Previous work from our group and others has shown that the Pseudomonas Quinolone Signal (PQS) is capable of inducing OMV formation not only in P. aeruginosa (the producing organism), but also in other Gram-negative recipient bacteria such as E. coli, K. pneumoniae, and P. mirabilis. Our team has shown that spent supernatants from these other species are also capable of stimulating OMV formation in the P. aeruginosa ΔpqsA mutant, which cannot produce PQS. This suggests that many species secrete OMV-inducing factors that can act on the producer and on other species. The current study has extended the list of PQS-responding organisms to include medically relevant species that are often found with P. aeruginosa at infection sites. We further tested whether different species pairs could mutually stimulate OMV production when actually grown together. We report that co-culture growth of P. aeruginosa with several other species results in increased OMV production per cell vs. what would be expected if production was simply additive. This result supports the hypothesis of cross-species induction of OMV biogenesis and provides a model to help understand pathogen synergy at multi-species infection sites.

The Effects of the Fatty Acid Transport Protein (FATP) Inhibitor Grassofermata on Fatty Acid Uptake in Trypanosoma brucei

Kristina Parman[1], Mufida Ammar[2], Dan Spangler[3], Kimberly Paul[1]
[1]Clemson University, [2] University of Tripoli, [3] University of SC School of Medicine-Greenville

The protozoan eukaryotic pathogen, Trypanosoma brucei, is the causative agent of Sleeping Sickness in humans and Nagana in livestock and is found predominantly in Sub-Saharan Africa. The pathogen lives extra-cellularly in the blood and other tissues of its mammalian hosts and is transmitted by tsetse flies. T. brucei must adapt to these different host environments throughout its life cycle, including immune evasion and the acquisition of nutrients such as fatty acids (FAs). Mediating T. brucei's evasion of host defenses are the tsetse fly procyclic form (PF) and mammalian bloodstream form (BF) major surface glycoprotein coats, both of which are glycosylphosphatidylinositol (GPI)-anchored. Interestingly, while the FA moiety of the PF GPI-anchor is a mix of abundant long-chain FAs, the FAs on the BF GPI-anchor are exclusively myristate, a scarce FA in its host. Thus, BFs appear to use de novo FA synthesis to generate myristate for its GPI-anchors, while relying on uptake to acquire longer, more common FAs from the host for its other needs. While FA synthesis has been studied in T. brucei, little is known about the molecular mechanisms governing FA uptake. We are taking a biochemical approach to examine the effect of various uptake inhibitors on T. brucei. One such inhibitor is Grassofermata (GF), which targets Fatty Acid Transport Proteins (FATPs) in yeast and mammals. FATPs couple FA translocation across the membrane with its esterification to Coenzyme A (CoA), catalyzed by an intrinsic acyl-CoA synthetase (ACS) activity. T. brucei lacks predicted FATP homologs, but do possess six ACSs. Therefore, we hypothesized T. brucei might be sensitive to GF. We found that GF treatment inhibits uptake of both medium and long chain fluorescently-labeled FAs (BODIPY-C12 and BODIPY-C16, respectfully). The EC50s for GF inhibition of BODIPY-C12 uptake in BFs and PFs were found to be ~ 157µM and 95µM, respectively, while for BODIPY-C16 uptake the EC50s were slightly lower. GF treatment did not completely block FA uptake, as we observed maximal inhibition at 70-75%. The EC50s for cell killing after 48 hr were found to be much lower, ~18µM and ~4.5µM for BF and PF, respectively. GF-treated cells incubated with BODIPY-FAs caused the emergence of a second population of "dim" cells visualized by flow cytometry that increased with increasing GF concentration. Inhibition of endocytosis by incubation at 4°C did not affect the emergence of the dim population upon GF treatment. We conclude that GF kills T. brucei and partially inhibits its uptake of BODIPY-FAs, with residual uptake due to other non-endocytic uptake mechanisms. Currently, we are utilizing fluorescence microscopy to analyze the patterns of BODIPY-FA incorporation in GF-treated BFs and PFs to further characterize this second dim population.

The Role of the Potential Methionine Uptake Transporter MetNPQ in Group A Streptococcus Virulence

Aolani Perry[1], Luis Vega, Sarah Davis, Yoann Le Breton, Kevin McIver
[1]*University of Maryland*

Streptococcus pyogenes (Group A Streptococcus, GAS) is a Gram-positive, -hemolytic bacterium that serves as a human specific pathogen. GAS causes non-invasive diseases (e.g., strep throat) as well as more life-threatening invasive diseases (e.g., necrotizing fasciitis). As a pathogen that is auxotrophic for at least 15 amino acids, GAS facilitates pathogenesis through the sequestration of amino acids and other nutrients from the host biological fluids. Methionine is one such amino acid that plays an important role in the S-adenosylmethionine pathway that is essential for phospholipid and nucleic acid synthesis as well as cellular repair. GAS encodes two types of methionine transport systems- a primary methionine ATP binding cassette uptake transporter (MET) and a secondary branched chain amino acids transporter. The putative methionine ABC transporter is expressed as an operon, metQNP, which encodes the hisJ histidine binding periplasmic protein, the metQ substrate binding protein, the metN ATP binding protein, and the metP permease. In a transposon sequencing (Tn-seq) screen in the clinically relevant M1T1 GAS strain 5448, we found that the loss of metQ and metN resulted in increased susceptibility to neutrophil killing. In published studies from our lab, the metNPQ operon was implicated as important for fitness murine soft tissue and in human blood. Yet, the exact role of methionine and metNPQ in GAS neutrophil survival and its broader role as a potential virulence factor in infection is unknown. We have generated a non-polar deletion mutant of metN (ΔmetN) and a double mutant of metNP (ΔmetNP) in GAS 5448. During growth in nutrient rich Todd Hewitt media, the mutants have a loose aggregation phenotype when compared to the dense pellet wild type 5448 and the metN and metNP rescues. Biofilm assays using crystal violet staining showed that ΔmetN mutant formed a poor biofilm comparable to wild type strain 5448 and metN rescues whereas the ΔmetNP double mutant formed a robust biofilm comparable to a known high biofilm producing GAS strain 5448-AP. These results suggest that loss of the MetP permease leads to increased expression of cell surface proteins involved in biofilm production. Ongoing studies are underway to characterize the role of MetQNP in innate immune survival and biofilm production as well as defining methionine as it's substrate.

Defining the molecular basis of galactan homopolymer synthesis in the pediatric pathogen Kingella kingae

Eric Porsch[1], Nina Montoya[1,2], Daniel Morreale[1,2], Joseph St. Geme[1,2]
[1]Department of Pediatrics, Children's Hospital of Philadelphia, [2]Department of Microbiology, University of Pennsylvania

Kingella kingae is a common commensal bacterium of the posterior pharynx in children ages six months to four years and is increasingly recognized as a major etiology of osteoarticular infections in this population. K. kingae elaborates a variety of surface polysaccharides, including a polysaccharide capsule, lipopolysaccharide (LPS), and a galactofuranose (Galf) homopolymer called the PAM galactan with the structure of [->5)-β-Galf-(1->] (type 1) or [->3)-β-Galf-(1-6)-β-Galf-(1->] (type 2). As the galactan has been shown to mediate resistance to a variety of anti-microbial immune functions and is the target of an antibody response in convalescent patient serum and normal human serum, we sought to gain a more thorough understanding of the genetic requirements and biochemical mechanism of galactan synthesis. While originally thought to be a freely secreted polysaccharide, recent evidence suggests that the galactan is physically linked to LPS, allowing us to develop a testable model of galactan biosynthesis. In initial experiments, we established that the pamA, pamB, and pamC genes are sufficient for synthesis of galactan when expressed in E. coli. In addition, we found that there are two pamC alleles designated pamC1 and pamC2, which correlate with production of the type 1 vs. the type 2 galactan. Based on these observations and bioinformatic analyses, we are currently exploring the hypotheses that (1) pamB encodes a UDP-galactopyranose mutase responsible for generating the activated sugar substrate (UDP-Galf) for galactan synthesis, (2) pamA encodes a UDP-Galf transferase involved in transferring the first Galf to LPS, and (3) pamC encodes an additional UDP-Galf transferase that serves as the synthase for generating the Galf homopolymer.

Interleukin-27-dependent regulation of the transcriptome during neonatal sepsis

Jessica Povroznik[1,2], Halima Akhter[1], Dr. Gangqing Hu[1], Dr. Cory Robinson[1,2]
[1]Department of Microbiology, Immunology, & Cell Biology, West Virginia University School of Medicine, [2]Vaccine Development Center, West Virginia University Health Sciences Center

Human newborns exhibit increased vulnerability and risk of mortality from infection. This includes sepsis, for which there is an approximate rate of one case per 1,000 births amounting to greater than 75,000 cases per year in the U.S. alone. Elevated disease burden exists in less developed geographic areas across the globe. Increased susceptibility to infection during the neonatal period reflects key differences in the innate and adaptive immune responses relative to those in adult cells. Both phenotypic and functional differences exist in the neonatal and adult immune systems. For example, increased levels of Th1 cytokines, anti-inflammatory cytokine production, and Th2-specific responses characterize the neonatal immune profile. Furthermore, we have previously shown an increase in the pleiotropic immune suppressive cytokine, IL-27, in macrophages derived from human umbilical cord blood compared with those obtained from adult peripheral blood. Similar findings exist in the neonatal murine system, as well as elevated splenic transcripts and serum levels compared to adult mice. IL-27 is a heterodimeric cytokine within the IL-12 family consisting of the Epstein-Barr virus-induced gene 3 (EBI3) and IL-27p28 proteins expressed from distinct genes. Its dimeric receptor complex is comprised of IL-27Rα and the glycoprotein gp130. Activation of the IL-27 receptor complex results in an induction of JAK/STAT, p38 MAPK/ERK, and NF-κβ signaling cascades.

Recently, we established a murine model of neonatal sepsis to explore the impact of early life IL-27 levels on the host response to infection. In this model, neonatal mice deficient in IL-27 signaling exhibit reduced mortality, increased weight gain, and better control of the bacterial burden with reduced systemic inflammation. These results suggest that there is a reprogramming of the host response in the absence of IL-27 signaling. To explore this hypothesis, we utilized RNA-Seq to analyze the neonatal transcriptome of the spleen in the presence or absence of Escherichia coli-induced sepsis in wild-type (WT) and IL-27 receptor alpha (IL-27Rα)-deficient (KO) mice. The spleen, a known site of infection, exhibits increased IL-27 expression and bacterial burdens in WT pups that are significantly lower in IL-27Rα KO pups during infection. There were 874 genes that were differentially expressed between WT and KO neonates during infection; 441 were significantly upregulated and 433 were downregulated with IL-27Rα deficiency. The genes most upregulated in WT mice are associated with inflammation, cytokine signaling, and G protein coupled receptor ligand binding and signaling. These genes fail to increase in IL-27Rα KO mice. These findings highlight a less inflammatory environment in KO pups that is not expected given the anti-inflammatory/suppressive activity of IL-27. However, it is consistent with a lower bacterial burden. This suggests that improved bacterial clearance in the absence of IL-27 signaling does not require a heightened inflammatory state, and instead, there is a direct relationship between IL-27 signaling and bacterial killing. An improved response to infection that is not reliant upon heightened levels of inflammation offers new promise to the potential of antagonizing IL-27 as a host-directed therapy for neonates without concern for driving inflammatory responses that are pathological to host tissues.

Unique Features of Alarmone Signaling in Clostridioides difficile

Asia Poudel[1], Dr. Astha Pokhrel[1], Adenrele Oludiran[1], Erin Purcell[1]
[1]*Old Dominion University*

The challenge of designing therapies against Clostridioides difficile infection (CDI) is to develop something potent enough to kill the notoriously resilient enteropathogen, but specific enough to spare the commensal gut microbiota that protect their hosts from CDI. The bacterial stringent response (SR) is a conserved stress-induced pathway mediated by nucleotide alarmones that contributes to virulence and to survival of antibiotic and immune stress in many pathogens. Even though the SR contributes to infection persistence, it has not been a strong target for antibiotic development because it is so widespread among diverse bacteria; anti-SR therapies would likely be very broad spectrum. However, we have characterized RSH and RelQ, the two alarmone synthetase enzymes that mediate the SR in C. difficile, and have found that they both diverge from canonical alarmone synthetases. Their mechanism of synthesis and their nucleotide alarmone product are distinct from those of homologous synthetases from other organisms. This could allow the design of SR inhibitors specific to C. difficile that could increase antibiotic efficacy in a targeted way.

Metabolic modulation of toxin production in Clostridioides difficile

Deborah Powers[1], Dr. Glynis Kolling[1], Dr. Matthew Jenior[1], Dr. Jason Papin[1]
[1]*University Of Virginia*

Clostridioides difficile is an anaerobic, gram-positive bacterium and the causative agent in approximately 73% of healthcare acquired gastrointestinal infections, resulting in a significant healthcare burden. C. difficile infection (CDI) pathogenesis is mediated through its toxins, TcdA and TcdB, which induce intestinal epithelial cell death and inflammation. A small but established body of literature has demonstrated alterations in C. difficile toxin production following the enrichment of certain metabolites within the extracellular environment. However, while there are several known extracellular metabolites that can influence toxin production, it is unknown which intracellular metabolic pathways are involved and how they regulate toxin production. To investigate the response of intracellular metabolic pathways to the nutritional environment, we have used a combination of experimental and computational methods. First, we measured variables such as growth, toxin production, and gene expression of C. difficile grown in defined media supplemented with a panel of individual metabolites. Next, we used genome-scale metabolic modeling to predict changes in intracellular metabolic flux in response to these experimentally measured variables. Together, these methods have enabled us to characterize common intracellular metabolic pathways that connect the extracellular environment with toxin outcomes in C. difficile.

B cell epitope peptide-conjugates as B. pertussis vaccine antigens

Gage Pyles[1], Kelly Weaver, William Witt, Annalisa Huckaby, Fredrick Damron, Mariette Barbier
[1]*West Virginia University*

B cell epitope peptide-conjugates as B. pertussis vaccine antigens
Gage M. Pyles, Kelly L. Weaver, William T. Witt, Annalisa B. Huckaby, F. Heath Damron, Mariette Barbier
Vaccine Development Center, Department of Cell Biology, Immunology and Microbiology, West Virginia University, Morgantown, West Virginia, USA
Bordetella pertussis is the causative agent of whooping cough. The transition from whole cell to acellular pertussis vaccines in many developed countries has triggered an increased frequency of pertussis outbreaks and disease incidence. Currently, whooping cough is the 2nd leading cause of vaccine-preventable death in the United States. Despite wide-spread vaccine coverage, acellular vaccines fail to provide optimal protection against B. pertussis. This can be attributed to short-lived immunological memory, the emergence of vaccine-escape strains, and the inability of acellular vaccines to prevent asymptomatic colonization and transmission. Included in current acellular vaccines are several purified virulence factors of B. pertussis. Antibodies are crucial for protection against B. pertussis as they can opsonize the pathogen, prevent adhesion and colonization of host respiratory tissue, and neutralize the systemic symptoms caused by pertussis toxin. Antibodies generated following acellular vaccination, however, target a limited repertoire of chemically denatured antigens leading to a narrow, sub-optimal humoral response. Additionally, antibody levels quickly wane below functional levels following vaccination, necessitating the use of frequent booster doses. To improve acellular vaccines, we sought to test new and redesigned antigens in a murine model to determine their potential as vaccine antigens to provide protection against B. pertussis. Toward this goal, immunopeptidomics and in sillico prediction tools were employed to identify new antigens and predict B cell epitopes from outer membrane proteins to test as novel, peptide-based antigens. Synthetic peptides were conjugated to CRM197 as carrier, formulated with aluminum hydroxide as adjuvant and used for the vaccination of CD-1 mice. Using ELISA, we observed that various peptides selected were immunogenic in CD-1 mice as antibodies raised against several of these peptides bound both native antigen as well as B. pertussis. However, none of these peptide antigens alone led to a decrease in bacterial burden in the airways 3 days post-challenge and provided protection against B. pertussis. This work led to the identification of antigenic epitopes which are now being combined and re-designed to be administered as a multivalent sub-unit vaccine to evaluate efficacy in mice.
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Investigating the host- and pathogen-related predisposing factors for the development of Staphylococcus infection associated glomerulonephritis

Pranav Rana[1], Jihad Aljabban[1], Amy Lehman[1], Joan-Miquel Balada-Llasat[1], Preeti Pancholi[1], Samir Parikh[1], Amy Webb[1], Liang Chen[2], Melanie Prarat[3], Yan Zhang[3], Sergey Brodsky[1], Tibor Nadasdy[1], Shu-Hua Wang[1], Daniel Wozniak[1], Michael Olson[4], Anjali Satoskar[1]

[1]The Ohio State University, [2]Hackensack-Meridian Health Center for Discovery and Innovation, [3]Ohio Department of Agriculture, [4]Southern Illinois University School of Medicine

Glomerulonephritis (GN) is a broad term used to define inflammation of the glomerulus and other parts of the kidney that may lead to renal failure and the development of other chronic kidney diseases. The inflammation is caused by an interplay between environmental triggers, genetic predisposition, and immune system dysregulation of an individual. Bacterial infection is a key trigger for the development of an infection associated GN, which can occur either after an infection has been cleared or during an infection. In the last few decades, the number of cases of Staphylococcus infection associated GN (SAGN) has increased, particularly with the rise in incidences of hospital- and community-acquired methicillin resistant Staphylococcus aureus. Both host- and pathogen-related predisposing factors for the development of SAGN are largely unknown. We performed a retrospective pathology-microbiology study to identify potential host- and pathogen-related factors predisposing patients with Staphylococcal infection to GN sequela. The demographics data indicated male predominance in SAGN cases. Individuals with SAGN also showed higher serum creatinine levels, compared to non-GN individuals, suggesting a worsening of renal function. To identify pathogen-related factors, whole genome sequencing (WGS), Multiple Locus Sequence Typing, Pulse Gel Field Electrophoresis, and in vitro virulence assays were performed on S. aureus isolates cultured from GN (n=6) and non-GN individuals (n=12). Isolates in both groups belonged to several clonal complexes (CC), suggesting that the clinical isolates were non-epidemic Staphylococcal strains. WGS revealed mutations in the accessory gene regulator (agr) operon in 5 out of 6 GN isolates. In vitro analyses revealed a significant reduction in β-hemolytic activity of GN isolates, compared to non-GN isolates. The reduced activity was observed in GN isolates belonging to both CC5 and CC59. Among the isolates belonging to CC5, GN isolates exhibited increased biofilm production than the non-GN isolates. Overall, our study shows a higher occurrence of mutations in the agr operon and reduced β-hemolytic activity in the majority of the GN isolates. These could potentially serve as markers to predict whether an individual has an increased risk of developing SAGN.

Adherence enables Neisseria gonorrhoeae to overcome zinc-limitation imposed by nutritional immunity proteins.

Jocelyn Ray[1], Stavros Maurakis[2], Dr. Walter Chazin[3], Simone Harrison[3], Eugene Ke[1], Dr. Cynthia Cornelissen[2], Dr. Alison Criss[1]

[1]University of Virginia, [2]Georgia State University, [3]Vanderbilt University

Neisseria gonorrhoeae (Gc) infects the cervical mucosa in women and must overcome host limitation of metals such as zinc in order to persist. The zinc-binding proteins calprotectin and psoriasin are abundant in human cervicovaginal lavage fluid. Gc possesses TonB-dependent transporters (TDT), TdfH and TdfJ, that specifically bind human calprotectin and psoriasin respectively. These TDTs enable Gc to acquire zinc directly from these proteins, which are able to sequester zinc from other pathogens. However, the role of zinc acquisition in epithelial infection by Gc was unknown. In order to investigate this, we used the immortalized cervical cell line Ect1 as a model of female genital tract infection. We found that TdfH and TdfJ were dispensable for survival of Ect1-associated Gc when zinc was limited by calprotectin and psoriasin. In contrast, suspension-grown Gc were unable to grow under the same conditions. Exposure to mouse calprotectin, which Gc cannot use as a zinc source, similarly reduced survival of suspension-grown Gc, but not Ect1-associated Gc. We ruled out epithelial cells as a contributor to the ability of cell-associated Gc to survive zinc sequestration. Instead, we found that attachment to glass was sufficient to enhance Gc growth when zinc was sequestered. Using RNAseq, we compared the transcriptional profiles of WT Gc adherent to glass coverslips or in suspension, when zinc was sequestered with mouse calprotectin or provided in excess. We identified a subset of ORFs upregulated by zinc sequestration in adherent Gc. One of these, ZnuA, was necessary but not sufficient for survival of Gc under zinc-limiting conditions. We conclude that adherence elicits a transcriptional change, which protects Gc from zinc-dependent growth restriction by host nutritional immunity proteins.

Anaplasma phagocytophilum resides in a host cell-derived multivesicular body that exploits ESCRT machinery for proliferation

Curtis Read[1], Dr. Jason Carlyon[1]
[1]*Virginia Commonwealth University*

Anaplasma phagocytophilum is a vacuole-adapted obligate intracellular bacterium responsible for the emerging and debilitating tick-transmitted disease, human granulocytic anaplasmosis. For its entire intracellular life cycle, the pathogen resides in a host-derived vacuole called the A. phagocytophilum-occupied vacuole (ApV) that interfaces with and acquires lipid-rich vesicular traffic from multiple organelles. This nutritional parasitism strategy is essential for A. phagocytophilum to replicate, transition to its infectious morphotype, and thereby complete its infection cycle. How host-derived vesicles are sorted into the ApV is unknown. Multivesicular bodies (MVBs) are a type of acidic late endosome defined by the presence of intraluminal vesicles (ILVs), lysobisphosphatidic acid (LBPA), and involvement of ESCRT (endosomal sorting complex required for transport) machinery. We confirmed that the ApV is an acidic compartment containing ILVs, LBPA, and is decorated by ESCRT proteins. ESCRT-dependent and -independent pathways are two separate, yet essential mechanisms responsible for sorting lipids and proteins into MVBs. While using siRNA to individually silence genes of critical subunits in either pathway failed to negatively impact A. phagocytophilum, targeting both significantly hindered bacterial proliferation and infectious progeny generation. Knockdown of CHMP4 (charged multivesicular body protein 4), an ESCRT-III subunit at the convergence of the ESCRT-dependent and -independent pathways, also reduced bacterial growth and infectious progeny formation. Furthermore, we will also evaluate whether a CHMP4 knockdown drastically reduces delivery of the host cell transmembrane protein Niemann-Pick disease type C1 (NPC1), which was previously shown to transport cholesterol into the ApV and to be indispensable for optimal bacterial growth, into the ApV. These data demonstrate that A. phagocytophilum occupies MVBs during infection of mammalian cells, permitting its exploitation of ESCRT machinery to facilitate acquisition of metabolites that are essential for fitness and conversion to the infectious form. In contrast to other vacuole-adapted obligate intracellular bacteria that have been shown to interface with host cell MVBs, our finding that the ApV is itself an MVB is a new paradigm in intracellular bacterial pathogenesis.

Antibiotic treatment drives phenotypic shift in heterogeneous Pseudomonas aeruginosa towards improvement of virulence.

Cristian Roca[1], Suzanne Meinig[1], Dr. Matthew Wolfgang[1]
[1]*University of North Carolina at Chapel Hill*

Pseudomonas aeruginosa (Pa) is the leading pathogen associated with chronic infection of the airways in individuals with cystic fibrosis (CF). Although new therapies with high dose of inhaled antibiotics have reduced the burden of bacteria, failure to eradicate infection is the norm. Pa populations show high phenotypic diversity within individual patients which may contribute to treatment failure due to antibiotic tolerant subpopulations. The mechanisms by which bacterial diversity contributes to treatment failure and therefore to persistent airways infections is poorly understood. This study aims to identify the impact of antibiotic treatment on in-patient Pa population dynamics. Fresh sputum samples, obtained from individuals with CF, were treated with antibiotics or vehicle control. After treatment, Pa burden was quantified, and individual colonies arrayed into isoclonal populations. We assayed these arrayed populations for various bacterial phenotypes including biofilm formation, antibiotic minimal inhibitory concentration (MIC), quorum sensing (QS), and antibiotic tolerance as potential phenotypic contributors to antibiotic treatment failure. Our results demonstrate that a single round of antibiotic treatment of ex vivo sputum selects for a phenotypic shift in the patient evolved Pa populations, including the enrichment of antibiotic tolerant subpopulations.

Investigating the Effect of rpoE Encoding the Delta Subunit of RNA polymerase on Virulence in the Streptococcus pyogenes M1T1 strain 5448

Dr. Joseph Rom, Dr. Yoann Le Breton, Emrul Islam, Spencer Lewis, Anna Phan, Dr. Kevin McIver
[1]*University of Maryland*

Streptococcus pyogenes, also known as the Group A Streptococcus (GAS), is a Gram-positive bacterial pathogen of major clinical significance. Despite remaining relatively susceptible to conventional antimicrobial therapeutics, GAS still causes millions of infections and half a million deaths each year worldwide. Thus, a need for prophylactic and therapeutic interventions for GAS are in great demand. We used a transposon library screen of the M1T1 serotype GAS strain 5448 in a murine subcutaneous model of skin infection and identified Spy_1611 (rpoE), encoding the delta subunit of the GAS RNA polymerase, as required for virulence in soft tissue. A defined 5448 mutant inactivating rpoE was found to show reduced competitive fitness when co-infected wild type 5448 in the mouse soft tissue model. Furthermore, the rpoE mutant was defective for survival in whole human blood and was attenuated for both disseminated lethality and lesion size upon mono-culture infection in mouse soft tissue. Transcriptome analysis using RNA-seq of GAS grown in THY to late log phase found that the rpoE mutant significantly impacted (> 4-fold) expression of 119 genes (83 activated, 36 repressed) including a number of know virulence loci. Preliminary findings suggest that the in vivo defects of the rpoE mutant may be, in part, due to enhanced sensitivity to reactive oxygen species based on observations that the mutant's growth in H2O2 was substantially decreased by comparison to the WT. The mutant also exhibited reduced survival upon infection of a neutrophil-like HL-60 cell line compared to wild type 5448 GAS. Taken together, these findings demonstrate that the Delta subunit of RNA polymerase encoded by rpoE is an important component of the GAS virulence regulon, and highlights it as a potential target for new therapeutic approaches.

Acquisition of hypermucoviscosity in classical Klebsiella pneumoniae – what does it take?

Stephen Salisbury[1], Logan Treat[1], Alexander Tufano[1], Professor Virginia L. Miller[1,2], Research Assistant Professor Kimberly Walker[1]

[1]*Department of Microbiology & Immunology, University of North Carolina School of Medicine, Chapel Hill, NC 27599,*
[2]*Department of Genetics, University of North Carolina School of Medicine, Chapel Hill, NC 27599*

Klebsiella pneumoniae is one of the most common causes of hospital-acquired pneumonia. The rise of carbapenem-resistant Klebsiella pneumoniae (Kpn) strains complicates treatment and contributes to a mounting global health threat: the CDC considers the increase of carbapenem-resistant Enterobacteriaceae to be an urgent problem. K. pneumoniae has two pathotypes, hypervirulent and classical. Hypervirulent strains (hvKp) are community acquired and can cause high-mortality rate infections in immunocompetent individuals. Classical strains (cKp) typically cause nosocomial infections in immunocompromised individuals and often display multi-drug resistance. Hypervirulent strains (hvKp) are distinguished by their hypermucoviscosity (HMV), a key virulence factor. RmpA is a transcriptional regulator required for HMV and elevated capsule expression. We showed that its primary role in these processes is as an autoregulator of the operon that encodes RmpA, RmpD and RmpC. RmpD is a small polypeptide that confers HMV, and RmpC is a novel transcriptional regulator that regulates capsule expression as well as a broad array of additional genes. The rmp locus is present either on the chromosome or on virulence plasmids of hvKp strains and typically absent in cKp. In our wild-type hvKp strain, RmpD and capsule production are necessary for HMV. HMV-positive hvKp strains are virulent in our mouse pneumonia model whereas classical strains are not.

Recently antibiotic-resistant cKp strains with hypervirulence traits have emerged. It is not known how classical strains can become hypervirulent, or if acquisition of HMV is sufficient for hypervirulence. We are addressing these questions by introducing rmp genes into a collection of diverse clinical isolates. We have found that some, but not all, cKp clinical isolates can become HMV-positive when transformed with plasmids that express rmpADC. Current experiments are focused on identifying the genetic requirements for HMV in cKp beyond the rmp locus and determining if acquisition of HMV increases the virulence of cKp. To better assess the virulence of HMV-positive cKp, we are introducing the rmp genes in single copy at the Tn7 site to model natural rmp acquisition. This will allow us to explore virulence with both in vitro assays and in our mouse pneumonia model without relying on antibiotic selection.

Response of Trypanosoma brucei to Exogenous Fatty Acids in Low Lipid Conditions

Joshua Saliutama[1], Dr. Kimberly Paul[1]
[1]*Clemson University*

Trypanosoma brucei is an extracellular parasite that causes African Sleeping Sickness and is endemic to the sub-Saharan African region. The parasite's extracellular nature forces the parasite to adapt to its host's environment. One of the regulatory mechanisms is to regulate fatty acid synthesis enzymes in response to exogenous fatty acids. Our previous study showed that the insect-host procyclic form (PF) T. brucei regulates the activity and expression level of acetyl-CoA carboxylase (ACC) when grown in media containing different lipid levels. PF in low lipid media has an elevated ACC activity but lower ACC protein level than in high lipid media. We also observed that the addition of C18:0 to PF in low lipid media reduced ACC activity and increased growth rate, which showed an environmental response to the exogenous fatty acid level. Here, we observe the growth and the ACC level of low lipid-adapted PF T. brucei after treatment with fatty acids of different chain lengths. We did not observe a significant change in the ACC protein level; however, we observed significant growth differences with the addition of different fatty acids, and some of these effects were cell density-dependent. These data suggested that adding fatty acids changes ACC's activity and pattern of PF growth in low lipid but does not change PF's ACC expression level. Future studies will investigate whether different fatty acids regulate ACC activity by phosphorylation, allosteric regulation, or a combination of both mechanisms.

Metabolic plasticity aids amphotropism of Coxiella burnetii

Savannah E. Sanchez[1,2], Jason A. Carlyon[1], Anders Omsland[2]
[1]Department of Microbiology and Immunology, Virginia Commonwealth University School of Medicine, [2]Paul G. Allen School for Global Health, College of Veterinary Medicine, Washington State University

Coxiella burnetii is a Gram-negative bacterial obligate intracellular parasite and the cause of Query (Q) fever in humans. Q fever typically manifests as an acute, self-limiting febrile illness; however, ~1–5% of infected individuals develop chronic Q fever that can lead to severe diseases (e.g., hepatitis and endocarditis). Despite extensive genome reduction during adaptation to an obligate intracellular lifestyle, C. burnetii encodes for a near complete central metabolic machinery and has retained the ability to utilize glycolytic and gluconeogenic carbon sources. Moreover, the pathogen naturally infects a broad range of host organisms—including domestic and wild mammals, reptiles, birds, and arthropods— and cell types. This amphotropic nature of C. burnetii, in combination with its ability to utilize both glycolytic and gluconeogenic carbon substrates, suggests that the pathogen relies on metabolic plasticity to replicate in nutritionally diverse intracellular environments. Amphotropism is exhibited by other clinically relevant intracellular pathogens (e.g., Francisella tularensis and Rickettsia prowazekii) consistent with broad relevance when considering bacterial virulence mechanisms. To test the significance of metabolic plasticity in C. burnetii amphotropism, C. burnetii intracellular replication in seven distinct cell lines (i.e., four mammalian and three arthropod) was compared between a metabolically competent parental strain and a mutant, CbΔpckA, unable to undergo gluconeogenesis. Both the parental strain and CbΔpckA exhibited host cell-dependent infection phenotypes, which were influenced by alterations to host glycolytic or gluconeogenic substrate availability. CbΔpckA was also attenuated during infection of an animal host, suggesting metabolic plasticity plays a role in pathogen virulence during natural infections. This study demonstrates a requirement of C. burnetii core-metabolic plasticity for optimal replication and virulence in physiologically and evolutionary diverse cell types (i.e., amphotropic fitness). Overall, our results indicate that consideration of C. burnetii amphotropism is critical when attempting to resolve virulence mechanisms in this pathogen.

Elucidating the role of four neuraminidases on N-glycan utilization and in vivo survival of Bacteroides fragilis

Nathaniel Seals[1], Anita Parker[1], Dr. Edson Rocha[1]
[1]*East Carolina University Brody School of Medicine*

Bacteroides fragilis is a commensal organism of the large intestine that can cause an infection of the peritoneal cavity following intestinal perforation that culminates in the formation of an abscess; these abscesses typically require antibiotic therapy and surgical drainage, and often respond poorly to antibiotics due to an increase in multi-drug resistance in B. fragilis strains. To survive in the peritoneal cavity, B. fragilis has developed the ability to utilize host N-linked glycoproteins as a source of carbon and energy at extraintestinal sites. One of the many strategies B. fragilis employs to accomplish this is the encoding of four neuraminidase homologs: NanH1, NanH2, NanH3, and NanH4. Intestinal Bacteroides species encode more neuraminidases than the average neuraminidase encoding bacteria. Neuraminidases are responsible for the cleavage of terminal sialic acid residues from sialylated glycans and it represents a committed initial step in the systematic degradation of N-linked glycans. To understand the role of B. fragilis NanHs in pathophysiology, enzymatic activities were measured using purified recombinant NanHs. The four recombinant neuraminidases, named rNanH1, rNanH2, rNanH3, and rNanH4 were shown to have differing activities that were pH, Ca2+, and sialyl-linkage specific. Neuraminidase activity was determined using a fluorometric assay using 2'-(4-Methylumbelliferyl)-α-D-N-acetylneuraminic acid (MUNANA) as substrate. Sialyl-linkage specificity was measured by quantifying free sialic acid after neuraminidase treatment by a TBA-based assay. rNanH1 was shown to have approximately 6-, 60-, and 6000-fold higher specific activity than rNanH3, rNanH2 and rNanH4 respectively. Several known neuraminidase inhibitors were tested against these enzymes and it was found that the plant derived curcuminoids bisdemethoxycurcumin and demethoxycurcumin showed inhibition of most of these enzymes at IC50 levels of <30 μM.
In an attempt to further establish the importance of these enzymes, null mutant strains ΔnanH1, ΔnanH2 and ΔnanH3/4, and ΔnanH1-4 were constructed. In order to establish the most important genes for survival in vivo, these strains were grown in Bacteroides defined medium with rat serous fluid (DMSerum) and bovine fetuin (DMFet) as the sole source of carbon in each culture condition, respectively. In both DMSerum and DMFet, NanH1 was found to be indispensable for growth, growing similarly to the strain lacking all four neuraminidases (ΔnanH1-4). Similarly, the complementation of NanH1 into the ΔnanH1-4 strain rescued the growth phenotype. While curcuminoids were found to inhibit the enzymatic activity of NanH1-4 in the above assays, they do not show significant, if any, inhibition of growth on these media. In our mouse intestinal colonization model, the ΔnanH1-4 exhibited a 2-log-fold growth defect when grown in competition with the wild-type strain. Interestingly, only a modest growth defect was seen in for the ΔnanH1 mutant strain and modest growth increases were seen for the ΔnanH2 and ΔnanH3/4 when in competition with the wild type strain. These results point to a potential synergistic cooperation between the neuraminidases encoded by B. fragilis.

Requirements for α-(1,3)-glucan biosynthesis in Histoplasma capsulatum

Victoria Sepulveda[1], Grant Jones[1], Christopher Marion, William E. Goldman[1]
[1]*Department of Microbiology and Immunology, University of North Carolina at Chapel Hill*

Histoplasma is one of the classic dimorphic fungal pathogens, undergoing a temperature-induced transition from a saprophytic mold form to a yeast form that grows in mammalian hosts. Once inhaled into the lungs, Histoplasma proliferates and persists as an intracellular parasite in phagocytes. Although histoplasmosis is most often seen in immunocompromised patients, Histoplasma is a primary pathogen that can cause severe complications in healthy individuals. α-(1,3)-glucan is a constituent of the cell walls of many human fungal pathogens and has been linked with virulence in several fungi including Histoplasma. Recently we showed that Histoplasma isolates from the Western Hemisphere can be grouped into four species: H. capsulatum sensu stricto, H. mississippiense, H. ohiense, and H. suramericanum. All of these require α-(1,3)-glucan for virulence except for one of the North American species, H. ohiense.

Using a genetic screen for Histoplasma capsulatum mutants deficient in α-(1,3)-glucan production, we identified an important role for AMY1, which encodes a putative α-(1,4)-amylase. amy1-null mutants of H. capsulatum lack cell wall α-(1,3)-glucan and are also attenuated for virulence. We used site-directed mutagenesis to evaluate if the highly conserved catalytic residues required for enzymatic activity in other α-amylases are also important for α-(1,3)-glucan production. We found that all the Amy1p catalytic residues required for amylase activity are also required for α-(1,3)-glucan production in Histoplasma. We performed in silico analysis and identified three additional genes encoding putative α-amylases in the genome of H. capsulatum (AMY2, AMY3 and AMY4). Using RNAi or allelic replacement to construct strains deficient for each enzyme, we evaluated the importance of each in α-(1,3)-glucan biosynthesis. These experiments demonstrated that α-(1,3)-glucan production does not require the function of any α-amylase other than AMY1.

Since α-(1,3)-glucan has been shown to be an important fungal virulence factor, the biochemical processes and genetic elements required for α-(1,3)-glucan biosynthesis and production represent important targets for the development of new antifungal drugs. We are currently evaluating how the absence of α-(1,3)-glucan promotes clearance of the organism after pulmonary infection and how this impacts overall immunity.

Detoxification of the lipid A component of live-attenuated Shigella vaccines using bacterial enzymatic combinatorial chemistry

Matthew Sherman[1], Jane Michalski[1], Dr. Alison Scott[1], Dr. Robert Ernst[1]
[1]*University of Maryland-Baltimore*

Shigella spp. are Gram-negative bacteria that cause severe diarrheal disease, contributing significantly to the global disease burden. Since current treatments are unable to address the high transability and increased antibiotic resistance of this pathogen, there is a large impetus to develop vaccines that prevent infection. Amongst the many vaccine candidates to date, live-attenuated Shigella vaccines have the benefit of closely mimicking a natural infection to induce protective immunity, but face the challenge of balancing immunogenicity and adverse reactogenicity. Attempts at reducing the reactogenicity have included targeting the enterotoxins present within the bacteria, but adverse reactions still remained during clinical phases. To reduce the reactogenicity of the live-attenuated Shigella strains developed by Walter Reed Army Institute of Research (WRAIR), we utilized bacterial enzymatic combinatorial chemistry (BECC), which employs ectopic expression of lipid A modifying enzymes, to generate targeted lipid A modifications that decrease lipid A-mediated stimulation of the innate immune response.

Initially, we focused on two lipid A modifying enzymes, LpxE and PagL, a phosphatase and deacylase that act at positions 1 and 3 on the diglucosamine backbone, respectively. Plasmid-based expression of these enzymes within both WT and the live-attenuated Shigella vaccine strains confirmed the ability to selectively modify the lipid A component. We demonstrated that the base peak at m/z 1797 in the unmodified Shigella, representing the native lipid A structure, was no longer present within the BECC-modified Shigella. Instead, a base peak at m/z 1717 and 1570 appeared in the LpxE- and PagL- modified Shigella strains, representative of the dephosphorylated and deacylated lipid A species, respectively. The lipid A was further characterized by GC-MS to quantitate the terminal phosphate moieties on lipid A.

To assess whether the BECC-modified LPS impacted innate immune signaling, NF-kB reporter cell lines were stimulated over a 5-log scheme using the extracted LPS. It was found that LpxE-modified LPS had an increase in EC50 compared to the unmodified LPS, suggesting a dampened innate immune response. PagL-modified LPS revealed no significant change in EC50 compared to unmodified LPS. We conclude that dephosphorylation by LpxE is a powerful mechanism to reduce lipid A-mediated signaling through TLR4/MD-2. Ultimately, a successful reduction in reactogenicity through this research would provide evidence that BECC may be used as means to reduce reactogenicity in any whole-cell or live-attenuated Gram-negative bacterial vaccine candidate.

The Production of Neutrophil Chemoattractant Chemokines CXCL1, CXCL2, CXCL3, CXCL5, CCL3, and CCL7 by Osteoblasts following infection with Staphylococcus aureus

Sophie Sipprell[1], Dr. M. Brittany Johnson[1], Dr. Whitney Leach[2], Dr. Ian Marriott[1]
[1]University of North Carolina at Charlotte, [2]Stowers Institute for Medical Research

Osteomyelitis is a serious bacterial infection of bone that is associated with progressive inflammatory tissue damage. Staphylococcus aureus is the principal causative agent of osteomyelitis and can enter bone via the bloodstream or surrounding tissues following injury or surgery resulting in disease that is often refractory to treatment such as debridement or antibiotic treatment. Furthermore, the increasing incidence of infections associated with antibiotic resistant strains of S. aureus has compounded this problem such that defining the pathogenesis of staphylococcal osteomyelitis has become urgent.

Resident bone cells including bone-forming osteoblast are important for initiating and maintaining the progressive inflammation associated with osteomyelitis. Osteoblasts have been demonstrated to release inflammatory mediators, chemoattractants, and factors that promote osteoclastogenesis following bacterial challenge. The release of chemoattractants further drives inflammation due to the subsequent infiltration of leukocytes such as neutrophils. Neutrophils are the first cells recruited to sites of infection and are a vital component in innate immune responses to microbial infection, possessing a variety of antimicrobial abilities including, phagocytosis, production of reactive oxygen and nitrogen species (ROS and RNS), release of neutrophil extracellular traps (NETs), and the synthesis of antimicrobial proteins. However, the accumulation of activated neutrophils can exacerbate inflammatory damage attributable to infection. Neutrophil produced mediators, such as IL-6 and CXCL8, exacerbate such damage by recruiting other leukocytes and by promoting osteoclastogenesis, which contributes to the inflammatory bone loss associated with osteomyelitis. Furthermore, neutrophils can inadvertently mediate host tissue destruction via excessive ROS and RNS production initiated following bacterial phagocytosis, and can even serve as an intracellular reservoir for S. aureus thereby contributing to chronic infection.

In the present study, we have employed RNAseq analysis to determine that osteoblasts show marked increases in the expression of mRNA encoding the neutrophil chemoattractants, CXCL1, CXCL2, CXCL3, CCL5, CCL3, and CCL7 following challenge with a clinical S. aureus isolate (strain UAMS-1). Such a finding is consistent with a prior report demonstrating elevated CXCL2 and CCL3 mRNA levels at sites of osteolysis in human osteomyelitis tissue samples. Importantly, we have determined that these cells also release CXCL1, CXCL2, CXCL3, and CXCL5, protein in a dose dependent manner, and also produce CCL3 and CCL7 proteins, the first demonstration that such cells produce these potent neutrophil attracting chemokines in response to staphylococcal infection. The ability of S. aureus-infected osteoblasts to produce an array of chemokines that act via the CXCR2 and CCR1 receptors expressed by neutrophils provides another mechanism whereby these resident bone cells could contribute to host responses to bacterial infection. However, it remains to be determined whether the production of these potent neutrophil chemoattractants contributes to the generation of protective host responses that limit bacterial burden or, alternatively, exacerbate the inflammatory damage associated with staphylococcal osteomyelitis.

Development of a novel cyst-deficient Toxoplasma gondii strain: Eno-dead.

Jamison Smiley[1], Xiaoyu Zhao[1], Imani Sanders[1], Assistant Professor Sarah Ewald[1]
[1]*Department of Microbiology, Immunology and Cancer Biology, Carter Immunology Center, UVA School of Medicine*

T. gondii is a ubiquitous parasite of warmblooded animals, including humans, that results in lifelong chronic infection. The infection is typically well-tolerated by immunocompetent individuals, but can prove lethal to the immunocompromised or the developing fetus. Infection occurs when ingested parasites invade the host gut, differentiating into the rapidly-growing tachyzoite form. Tachyzoites dominate the acute stage of the infection by invading and replicating in a broad array of host cell types. The transition to chronic infection occurs as the host initiates an acquired immune response against the parasite. The CD8 T cell response clears the tachyzoites and drives the differentiation of the remaining parasites into slow-growing, cyst-forming bradyzoites. These cysts evade the immune response by infecting long-lived cell types, primarily muscle cells and neurons, which shelter the parasites from immune clearance and facilitate persistent infection. Currently, no tools exist to clear an established infection from the body. The purpose of this project is to develop a strain of T. gondii that is sterilized at the onset of tachyzoite-to-bradyzoite conversion, rendering the parasite incapable of sustaining chronic infection while allowing a normal acute infectious stage. Our approach is to generate a self-sterilizing strain which capitalizes on the changes in gene expression that drive parasite stage transition. Specifically, we have fused the Eno1 gene, whose expression is required for bradyzoite metabolism but is repressed in tachyzoites, to Cre recombinase and a fluorescent marker using teschovirus translation skip sites. This construct replaces the parasite's endogenous Eno1 gene to drive expression of Cre at the onset of bradyzoite-specific gene expression. In parallel, we have introduced loxP sites around a gene essential to parasite survival. This system allows for selective elimination of that essential gene as Eno1 expression is upregulated, ensuring cell death upon bradyzoite conversion. We expect Eno-dead to undergo typical tachyzoite proliferation, but fail to form cysts after receiving differentiation signals in vitro and in murine hosts. The remaining, undifferentiated tachyzoites are expected to be cleared during the course of infection by the CD8 T cell response. As a strain of the parasite that cannot stably infect animal hosts, Eno-dead represents a novel research tool that will allow us to distinguish the long-term effects of acute inflammation from pathology caused by chronic infection. Moreover, as a self-limiting strain which retains typical tachyzoite proliferation and migration, Eno-dead offers a unique opportunity to develop a safe and effective active immunotherapy and cancer vaccine tool capable of inducing a robust but well-tolerated immune response.

Fast Lipid Analysis Technique (FLAT) a rapid, direct from urine diagnostic assay for identification of Gram-negative pathogens

Richard Smith[1], Hyojik Yang[1], Anuj Joshi[1], David Goodlett[1], J. Kristie Johnson[1], Robert Ernst[1]
[1]*University of Maryland, Baltimore*

Background:

Urinary tract infections (UTIs) pose a major public health burden. The vast majority of UTIs are caused by Gram-negative bacteria, most commonly Escherichia coli, Klebsiella and Proteus species. Current pathogen identification methods require culture which can take 24-48 hours. In this study, we developed and evaluated a method Gram-negative pathogen identification direct from urine, without the need for ex vivo growth, via matrix assisted laser/desorption ionization-time of flight (MALDI-TOF MS) in approximately one hour.

Methods:

Urine samples were collected (n=96) from the University of Maryland Medical Center clinical microbiology laboratory (HP-00064919). Samples were known to be positive, (105 CFU/ml) and pathogens were identified via culture and protein-based MALDI-TOF MS; ten negative urines were collected as negative controls. To identify bacteria direct from urine, two methods were evaluated. First, 1 µL of urine was directly spotted on the MALDI target plate and second, 1 mL of urine was centrifugation at 8000g for 5 minutes before FLAT processing. FLAT (fast lipid analysis technique) is a lipid-based microextraction which was followed by MALDI-TOF MS analysis (Sorensen et al., 2020). Mass spectra were acquired on Bruker MALDI-TOF MS instruments using the matrix norharmane in the negative ion mode. Pathogen identifications were performed using mass-spectrum (www https://mass-spectrum-9fqaa.ondigitalocean.app/) and subsequently verified via manual identification.

Results:

In a comparison to standard clinical practices for UTI pathogen identification, FLAT correctly identified the pathogen(s) as follows: when directly spotting 1 µL of urine, positive agreement was 86.3% (82/95) and after centrifugation, 94.7% (90/95). For both methods, negative agreement was 100% (10/10). Time to results for both FLAT methods was approximately one hour. Furthermore, in one specimen, a marker for colistin resistance was identified from E. coli by directly spotting 1 µL of urine. This specimen was confirmed positive for mcr-1 by polymerase chain reaction.

Conclusions:

UTIs are common infections that produce a substantial workload for a clinical microbiology laboratory. The ability to identify pathogens without need for culture allows for faster turnaround times, reduced time to appropriate antimicrobial therapy, and, potentially, improved patient outcomes. Overall, FLAT provides a reproducible and accurate method to rapidly identify urinary pathogens.

Combatting Streptococcus suis: A Combined Sequencing Approach

Tristan Stoyanof[1], Dr. Kevin Lahmers[1], Dr. Kevin McIver[2], Dr. Clayton Caswell[1]
[1]Virginia Tech, [2]University of Maryland

Streptococcus suis poses as a looming economic threat to the pork industry and a growing health concern for those working in close proximity to the infected pigs. Although outbreaks have been recorded across the world, with many instances displaying high lethality in both pigs and humans, S. suis remains a relatively understudied bacterium. In response, this project utilized multiple high-throughput sequencing methods to better understand the conditional expression and importance of certain genes, and to locate potential therapeutic targets within S. suis. This was done via transposon sequencing (Tn-seq) and RNA sequencing (RNA-seq) approaches.

The RNA-seq experiments demonstrated that specific genes are differentially expressed in the presence of either pig mucin, cerebral spinal fluid, serum, or within a biofilm. We hypothesize that an operon encoding four YhgE/Pip domain-containing proteins, which is upregulated when the bacteria are exposed to mucin, plays a critical role in the overall virulence of S. suis. In addition to measuring total gene expression, multiple novel small RNAs (sRNAs) were predicted and confirmed via northern blot analyses. Among these sRNAs, three appear to be more abundant when isolated from a biofilm.

The Tn-seq experiments established a list of genes essential for survival by first isolating total genomic DNA from an unstressed library of mutants, then sequencing the pool to map transposon insertional sites. This same Tn-seq library was then grown in either mucin or was allowed to form a biofilm, which led to the characterization identification of genes that contributed to the overall fitness of S. suis in those conditions. Combined, these experimental findings begin to lay the groundwork for better defining virulence mechanisms in S. suis.

Title: The Effects of Type II Heat-Labile Enterotoxins on Human Respiratory-Specific Immune Responses

Presentation Type: Poster
Subject Type: Gram Negative

Authors and Affiliations: Margaret C. Stroud[1], Andrea R. Rojas[1], Mary-Peyton Knapp[2], Adam Okinaga[3], Terry D. Connell[4], Steven E. Fiester[2], Sergio Arce[2], Jennifer T. Grier[2]

[1] Furman University, [2]University of South Carolina School of Medicine Greenville, [3]University of South Carolina [4]University at Buffalo Jacobs School of Medicine

Heat-Labile Enterotoxins (HLTs) are bacterial secreted proteins with unique immune properties. Type II HLTs are produced by certain strains of *E. coli* and have been found to act as mucosal and systemic adjuvants. The goal of this study was to determine the potential of three Type II HLTs (LT-IIa, LT-IIb, and LT-IIc) to function as adjuvants to boost respiratory specific immune responses while avoiding cell cytotoxicity. A549, a human lung epithelial cell line, was exposed to HLTs in the presence or absence of lipopolysaccharide (LPS), a known immunostimulant. The impact of enterotoxin exposure on cell structure and viability was measured by flow cytometry. Changes to expression of the cytokines IL-1β and IL-6 were observed via quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) to detect mRNA or enzyme linked immunosorbent assay (ELISA) to measure cytokine secretion. The addition of HLTs resulted in no significant changes to viability with or without LPS, although cytoplasmic inclusions were visible via microscopy in enterotoxin-treated cells. Analysis of gene expression over a short period of time found that immune responses triggered by HLTs significantly differed from that induced by LPS. Secretion of IL-1β was decreased in the presence of HLTs and reduced expression of IL-6 was observed on an mRNA level. Based on these results, we conclude that Type II HLTs drive an altered immune response without significant cellular cytotoxicity. Further study is needed to determine the mechanisms by which the HLTs modulate immune signaling in the respiratory tract, particularly in the presence of other immune adjuvants, and how these effects change over longer periods of time. This study demonstrates that HLTs trigger immune responses that differ from standard adjuvants, which may have therapeutic potential in treatments for targeted drug or vaccine delivery to the respiratory tract.

Use of a Fast Lipid Analysis Technique (FLAT) to Identify Oral Pathogens via MALDI-TOF MS

Kylie Sumner[1], Richard Smith, Robert Palmer, Robert Ernst
[1]*University of Maryland, Baltimore*

Background
The oral microbiome is an environment of high biodiversity, with an abundance of bacteria. Typically, oral microbes are nonpathogenic, but in certain conditions, they can cause dental caries, periodontal diseases, or dental abscesses in extreme cases. Diagnosing the organisms responsible for an oral infection allows for proper treatment and improved patient outcomes.

Methods
Seventeen common oral pathogens were collected from the National Institute of Dental and Craniofacial Research (NIDCR) and processed via Fast Lipid Analysis Technique (FLAT) following methods published in Sorensen et al. (PMID: 33299017). Mass spectra was acquired in negative ion and linear mode on a Bruker Microflex. Fifteen replicates were collected for each of the seventeen oral pathogens.

Results
Distinct spectrum and lipid structures were observed for each of the seventeen pathogens. FLAT was able to identify different species of the oral pathogens. No differences were observed within species when grown at different conditions. Collected spectra were used to develop a reference library for future identification of oral pathogens.

Conclusion
The use of MALDI-TOF MS to diagnose oral pathogens is a technique that could have species level identification of a pathogen in less than one hour. FLAT has potential to diagnose infections or to analyze the complex biodiversity of the healthy oral microbiome. Future directions include identifying pathogens from dental plaque, without need for cult

Investigating the roles of eicosanoids and fatty acids in modulating the inflammatory response during Salmonella Typhimurium infections

Jessica Taddeo[1], Dr. Vincent Tam, Dr. Joris Beld
[1]*Temple University LKSOM*

Salmonella enterica serovar Typhimurium (STM) is a gram-negative intracellular pathogen that causes gastroenteritis and diarrhea in humans. STM invades the intestinal epithelial cells and, in some cases, is internalized by phagocytes, where it can disseminate into other tissues. The inflammatory responses during a bacterial infection must be well-regulated to ensure both clearance of the pathogen and prevention of sustained tissue damage. Eicosanoids-derived bioactive lipids play critical roles in both the induction and resolution of inflammation. A subset of these lipids are endogenous ligands for the nuclear receptors/transcription factors Peroxisome proliferator-activated receptors (PPAR), composed of PPARα, PPARδ, and PPARγ subtypes. PPARs play major roles in fatty acid metabolism, modulation of immune cell functions, and formation of subcellular lipid bodies (sites of eicosanoids metabolism). PPAR also plays a role in macrophage polarization in which microenvironment, cytokines, and bacterial products influence the functional status. Previous studies have shown that STM preferentially infects and persists in M2 (alternatively activated) macrophages and can induce M2 polarization upon infection. The mechanisms by which fatty acids, eicosanoids and PPAR modulate the inflammatory responses during bacterial infections are not well understood. Using Liquid chromatography mass spectrometry (LC/MS), we have defined the fatty acid and eicosanoid profiles of the cecum during in vivo STM colitis and Typhoid fever infection models. We determined that the cecal lipidomic profiles were significantly modulated in STM colitis mice compared to Typhoid fever mice and naive controls, with significant increases in eicosanoid species such as PGD2, LTB4, 13-HODE, and Arachidonic Acid. These lipid mediators have specific pro-inflammatory and anti-inflammatory effects on various immune cells. Specifically, LTB4 is a potent agonist of PPARα, while 13-HODE activates PPARγ. We have determined that PPARα significantly modulates the fatty acid profiles and lipid body formation in macrophages during TLR stimulation or STM infection in vitro, suggesting its role in modulating the immune response and fatty acid metabolism during infection. Lastly, we determined that the fatty acid profiles of macrophages differ depending on the polarization state. Induction of fatty acid oxidation participates in the programming of M2 polarization, indicating that fatty acid metabolism affects the metabolism of immune cells and their functions. Investigating the roles of fatty acids, eicosanoids and PPAR during STM infections will allow us to define the role of lipid-immune signaling axis in microbial infections and gain significant insights into how lipids mediate inflammation and host-pathogen interactions during bacterial infections.

***Salmonella*-induced cholesterol accumulation may prevent autophagic clearance of bacteria**

Holly Torsilieri[1], Adam Greene[1], James Casanova[1]
[1]*University of Virginia*

Salmonella enterica serovar Typhimurium is a Gram-negative bacillus that infects the host intestinal epithelium and resident macrophages through the coordinated activities of two Type III Secretion Systems (T3SS) and their associated effector proteins. Upon internalization, the bacteria remain within a modified endosomal compartment known as the *Salmonella* Containing Vacuole (SCV). While many of the host proteins targeted for SCV formation and maintenance have been well characterized, the role of host lipids during infection remains largely unknown. Previous research has demonstrated that *Salmonella* infection induces cholesterol accumulation within host macrophages and epithelial cells, and that a fraction of this cholesterol is localized in and around the SCV. Recently, we determined that cholesterol accumulation is important for *S.* Typhimurium survival, however the specific role for cholesterol remains unknown. We have previously demonstrated that the T3SS-2 effector SseJ induces cholesterol accumulation through a signaling cascade involving focal adhesion kinase (FAK) and protein kinase B (Akt). Additionally, we found that *Salmonella*-induced signaling through Akt activates mTORC1 (mammalian target of rapamycin complex 1), suppressing the host autophagic machinery and enhancing bacterial survival. Autophagy is closely linked to cholesterol levels, as mTORC1 can sense increased cholesterol levels in the outer leaflet of lysosome membranes, leading to hyperactivity of mTORC1 and the suppression of autophagy. Here we show that mTORC1 activation is modulated by cholesterol accumulation during *Salmonella* infection, suggesting elevated cholesterol may promote *S.* Typhimurium survival through mTORC1-mediated suppression of autophagy. We also demonstrate that mTOR itself localizes to the SCV and lysosomes during infection, in a cholesterol-dependent manner. We postulate that *S.* Typhimurium induces cholesterol accumulation through SseJ in order to activate mTORC1 and suppress autophagy, which ultimately prevents autophagic clearance of bacteria.

First steps to understanding the mechanism behind hypermucoviscosity in Klebsiella pneumoniae

Logan Treat[1], Kimberly Walker[1], Virginia Miller[1,2]
[1]Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, [2]Department of Genetics, University of North Carolina at Chapel Hill

Hypervirulent Klebsiella pneumoniae (hvKp) causes community-acquired, invasive infections in immunocompetent hosts. Typically, hvKp possess the phenotype of hypermucoviscosity (HMV), which is dependent on the polysaccharide capsule and is characterized by stringy colonies and viscous liquid cultures. It has long been assumed that HMV is a consequence of hyper production of capsule. The genes rmpA and rmpC encode transcriptional regulators linked to hypervirulence. Investigation of these genes in connection with HMV and capsule led to the discovery of rmpD. The rmpD gene encodes a small protein that is necessary for HMV but does not alter capsule expression or production, contradicting the assumption that HMV resulted from capsule overproduction. In a mouse model of pneumonia, a rmpD mutant demonstrated that HMV is essential for virulence, and distinct roles for HMV and capsule were shown for protecting Kp from adherence to and phagocytosis by immune cells. RmpD contains a putative N-terminal transmembrane domain, and topology experiments indicate the highly basic C-terminal region localizes to the cytoplasm. As small proteins often function in conjunction with other proteins, we investigated potential interacting partners for RmpD. Preliminary data supports an interaction between RmpD and Wzc, a polysaccharide co-polymerase necessary for capsule production. Ongoing experiments are focused on characterizing this interaction to understand how it leads to HMV. As the frequency of convergence of hypervirulence traits with multi-drug resistant Kp increases, it is essential to understand the molecular mechanism underlying HMV to estimate the risk of emerging Kp strains.

Visualizing Interleukin-27 Production in a Murine Model of Neonatal Gram-Negative Bacterial Sepsis

Jordan Vance[1], Ashley Divens[1], Jessica Povroznik[1], Dr. Cory Robinson[1]
[1]*Department of Microbiology, Immunology, and Cell Biology; West Virginia University School of Medicine*

The neonatal immune system displays a unique immune profile consisting of Th2-biased responses, reduced toll-like receptor activity, and reduced pro-inflammatory activity. Taken together, these characteristics contribute to the increased susceptibility to infection observed in the neonatal population. In 2018 alone, 2.5 million infants faced mortality due to infection. In order to combat infant deaths from infection, we need to better understand the neonatal immune system. Our laboratory has previously reported increased levels of the immune suppressive cytokine interleukin (IL)-27 in the neonatal population. We believe that IL-27 plays a key role in the immune response, particularly during infection.

IL-27 is a pleiotropic cytokine with immunosuppressive activity comprised of the subunits p28 and EBI3. A variety of cell types produce IL-27, however, a comprehensive profile of these cells is unknown. We hypothesize that IL-27 compromises host control of infection in early life and IL-27 is produced by a heterogeneous population of cells that contributes to reduced bacterial control. To explore this hypothesis, our laboratory uses a murine model of neonatal bacterial sepsis to investigate the regulatory role of IL-27 in early life. This model recapitulates many clinical observations in humans including reduce weight gain, dehydration, and circulating bacteria in the blood and periphery. Here we employ an IL-27 reporter mouse with a transgene that contains eGFP controlled by the IL-27p28 regulatory region and a bioluminescent strain of K1-encapsulated Escherichia coli. This allows us to visualize fluorescent signal indicative of IL-27 production relative to the bacterial burden in real time by whole animal imaging as well as sort IL-27 producers for further analysis. This confirmed systemic infection and IL-27 production. Flow cytometric analysis of the spleen demonstrated a discreet population of GFP+ myeloid cells that was more robust in infected pups. Further single-cell analysis will elucidate the transcriptional and functional characteristics of these cells.

To fully understand the role and impact of elevated IL-27 levels in neonates, we must understand the dynamics of IL-27 production during bacterial infection, as well as the cellular phenotype and functionality of IL-27 producers. The work described here establishes the tools and approach required to achieve these objectives and enhance our understanding of IL-27 biology in the context of early life immunity.

Import of a unique host-derived sugar *N*-acetylglucosamine 1-P by rickettsiae for biosynthesis of cell envelppe glycoconjugates

Victoria I Verhoeve[1], Hyojik Yang[2], Timothy P. Driscoll[3], Alan S. Cross[1,4], Robert K. Ernst[1,2], Joseph J. Gillespie[1]
1. Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, Maryland, USA
2. Department of Microbial Pathogensis, University of Maryland School of Dentristy, Baltimore, Maryland, USA
3. Department of Biology, West Virginia University, Morgantown, West Virginia, USA
4. Institute for Global Health, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland, USA

The global impact of rickettsial diseases is highlighted by historical records, reemergence of fatal arthropod-borne spotted and typhus fever rickettsioses, and emergence of new pathogens. The intracellular lifestyle of *Rickettsia* spp. poses immense challenges to research; nevertheless, a transdisciplinary approach alleviates the near intractability of these bacteria to genetic manipulation. We employ such an approach, targeting one of the most understudied aspects of rickettsial virulence, host-dependent metabolic parasitism. Our recent reconstruction of the *Rickettsia* metabolic and transport network identified 51 host-acquired metabolites needed to compensate for degraded biosynthesis pathways. Without glycolysis and the pentose phosphate pathway, peptidoglycan (PGN) and lipopolysaccharide (LPS) must be synthesized using host sugars. *N*-acetylglucosamine 1-P (*N*AG-1-P), a precursor of both PGN and lipid A disaccharide backbones (as well as other LPS sugars), is usually synthesized by bacteria using the bifunctional enzyme GlmU: the C-terminal acetyltransferase domain generates *N*AG-1-P from glucosamine-1-P (GlcN-1-P), while the N-terminal uridyltransferase domain converts *N*AG-1-P to UDP-*N*AG. Curiously, *Rickettsia* spp. contain a truncated GlmU enzyme with only the uridyltransferase domain, indicating rickettsiae do not synthesize GlcN-1-P. Given the eukaryotic pathway (synthesizing sialic acid or chitin) generates *N*AG-1-P from *N*AG-6-P (not GlcN-1-P), we speculate that rickettsiae import host *N*AG-1-P and convert it to UDP-*N*AG using a streamlined enzyme (GlmU_N) tailored to eukaryotic metabolism. **Specifically, we hypothesize that rickettsiae uniquely utilize host-derived *N*AG-1-P for biosynthesis of cell envelope components and that such metabolite thievery from the host amino sugar biosynthesis pathway is essential for rickettsial intracellular replication and survival**. To test our hypothesis, we are characterizing rickettsial uptake and metabolism of host *N*AG-1-P by monitoring bioorthogonal sugars incorporated into LPS components using click chemistry. Preliminary data indicate that rickettsial growth is sensitive to limiting and excess *N*AG-1-P concentrations in host cells. We have successfully isolated and characterized PGN, lipid A and core and O-antigen polysaccharide (COPS) from rickettsiae to trace azidosugar incorporation into cell envelope glycoconjugates. Unexpectedly, our data revealed divergent lipid A structures for different *Rickettsia* pathogens. Furthermore, we have identified novel sugars within the Rickettsia COPS and potentially the S-layer. Collectively, our work will illuminate 1) a novel rickettsial trait (GlmU_N and a unique *N*AG-1-P transport system) that stands as a promising target for therapeutics aimed at combatting fatal rickettsioses, 2) the impact (pathogenesis) of *N*AG-1-P pilfering on host cell metabolism, and 3) the importance of *N*AG-1-P as a critical ingredient for developing a rickettsial axenic media required for future genetic manipulation.

The role of Rho factor in phase variation of flagella and toxins in Clostridioides difficile

Mercedes Warren[1], Dr Rita Tamayo[1]
[1]*University of North Carolina at Chapel Hill*

Clostridioides difficile causes antibiotic-associated intestinal disease. Toxins and flagella contribute to disease development, but these factors also stimulate an inflammatory immune response. Modulating toxin and flagellar gene expression is therefore critical for balancing disease onset with immune evasion. C. difficile exhibits heterogeneity in the production of flagella and toxins through phase variation, a bet-hedging strategy that occurs through ON/OFF modulation of a phenotype. Phase variation of flagellum and toxin gene expression is mediated through site-specific DNA recombination that reversibly inverts the flagellar switch, located upstream of the flgB operon. When the flagellar switch is in the ON orientation, bacteria are motile and toxigenic; in the OFF orientation, bacteria are non-motile and non-toxigenic. Our lab determined that Rho, a transcriptional termination factor, is involved in phase variation of flagella and toxins. We found that C. difficile Rho preferentially inhibits transcription of flg OFF mRNA over flg ON, but Rho from the model organism Bacillus subtilis does not. These results suggest C. difficile Rho possesses an activity absent in the B. subtilis orthologue. Notably, C. difficile Rho contains an N-terminal insertion domain (NID) absent from B. subtilis Rho. Only ~35% of Rho orthologues contain an NID, and these domains confer diverse functions. My research aims to address how Rho distinguishes between flg ON and OFF mRNAs and the function of the NID. Because the B. subtilis Rho is unable to terminate flg OFF mRNA, we hypothesized that the NID of C. difficile Rho is required for termination. Our results suggest that the NID is required for termination, as C. difficile RhoΔNID is unable to restore Rho function and the flg OFF phenotype in a background without full-length Rho. We do not yet know if the NID is important for RNA binding or transcription termination. We will use approaches including RNA binding assays and in vitro transcription to determine how Rho differentially affects transcription termination of flg ON and OFF mRNAs. To assess the importance of the NID domain in differentiating between flg ON and flg OFF mRNA, we will create chimeric Rho proteins combining domains from C. difficile and B. subtilis Rho and compare their ability to terminate transcription of flg ON and OFF mRNAs. This work will determine the mechanism by which Rho interacts with flg mRNA to mediate phase variation of flagella and toxins in C. difficile.

Characterization of acellular pertussis vaccine-induced memory responses and improvement of responses by use of novel adjuvant, SWE

Kelly Weaver[1]
[1]*West Virginia University*

Characterization of acellular pertussis vaccine-induced memory responses and improvement of responses by use of novel adjuvant, SWE

Kelly L. Weaver, Catherine B. Blackwood, Alex M. Horspool, Gage M. Pyles, Emel Sen-Kilic, Emily Grayson, Annalisa B. Huckaby, William T. Witt, Megan M. DeJong, M. Allison Wolf, F. Heath Damron, Mariette Barbier

Vaccine Development Center in the Department of Microbiology, Immunology and Cell Biology, School of Medicine, West Virginia University, Morgantown, WV

The Gram-negative pathogen Bordetella pertussis causes the respiratory disease whooping cough, which affects people of all ages, but is fatal primarily in infants. Despite vaccination, reemergence of pertussis is occurring at an alarming rate. According to the CDC, during a 2012 outbreak, there were 48,277 cases and 20 deaths, the highest incidence of pertussis in the last 50 years. Resurgence is hypothesized to be a result of pathogen evolution and limitation of vaccine-induced memory. In humans, protection from infection provided by acellular vaccines wanes over time, leaving populations vulnerable to disease. Whole cell pertussis vaccines were phased out due to safety concerns, however, they conferred long-term protection that did not wane. To address this issue and formulate the next generation of pertussis vaccines, we developed a model to study the vaccine-induced memory response. We compared the immune responses elicited by acellular and whole cell vaccination to inform development of an acellular vaccine in which protection does not wane over time. We monitored the murine immune response to vaccination and challenge over the course of 532 days, measuring changes in serum antibody titers, markers for germinal center formation, and antigen-specific memory B cell generation. Mice were vaccinated intramuscularly with whole cell, acellular, or mock vaccines on days 0 and 21. On days 20, 22, 35, 60, 90, 152, 365, and 532 mice were euthanized, and immunologically relevant samples were processed. We observed that whole cell and acellular vaccination were protective against challenge overtime. Serum antibody titers were similar between whole cell and acellular vaccinated mice and did not decline after one year. However, whole cell vaccinated mice had higher T follicular helper and antigen-specific memory B cell responses, and serum CXCL13 levels were significantly higher compared to mock and acellular vaccinated mice. These markers of longevity of vaccine-mediated protection against pertussis were then used to evaluate novel formulations of pertussis vaccines. We hypothesized that supplementing DTaP with an additional adjuvant will improve vaccine-induced memory responses. To test this, we supplemented DTaP with SWE, a squalene-in-water adjuvant, and observed improved antibody responses compared to both DTaP alone and mock-vaccinated animals. We will continue evaluating the vaccine-induced memory responses associated with the addition of SWE to DTaP in future studies. From this work, we identified potential markers of the longevity of the memory response to vaccination. These will be further investigated to inform the formulation of a next generation pertussis vaccine to protect vulnerable populations and prevent pertussis-related deaths.

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Investigation of Drug Resistant Acinetobacter baumannii Virulence Factors Implicated in Intracellular Survival Within Differentiated THP-1 Cells

Elias Wheibe[1], Kyleigh Connolly[1], Dr. Ryan Relich[2], Dr. Steven Fiester[1,3], Dr. Jennifer Grier[1]
[1]Department of Biomedical Sciences, USC School Of Medicine-Greenville, [2]Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, [3]Department of Pathology, Prisma Health

Purpose: Acinetobacter baumannii is a nosocomial opportunistic pathogen usually associated with mucosal infections such as pneumonia, urinary tract infections or sepsis and less commonly with more severe illnesses such as meningitis, wound infection, or necrotizing fasciitis. Of late, A. baumannii has been identified by the CDC as an urgent threat due to the multidrug- and pandrug-resistant properties it has acquired, making it a major concern within the public health community. Due to a sparsity of information on virulence factors and mechanisms of immune protection, it has been difficult to understand how this bacterium is able to gain a foothold and cause severe infections. We sought to study how necrotizing fasciitis-associated strains of A. baumannii interact with human macrophages to assess intracellular survival and potential virulence factors regarding evasion of the host immune response. Methods: To this end, we studied extensively drug resistant A. baumannii strains isolated at two timepoints (strains NFAb-1 and NFAb-2) from a patient over the course of a lethal necrotizing fasciitis infection that led to sepsis. The intracellular survival of these two strains was compared with each other, as well as to the type strains, ATCC 19606 and ATCC 17978, within a human macrophage-differentiated monocyte cell line, THP-1. Survival studies were complemented with quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) to investigate expression of potential bacterial virulence factors, catalase E (KatE), phospholipase C (PLC), outer membrane protein A (ompA), and pilus A (PilA), that could contribute to immune evasion. Results: The intracellular survival assay demonstrated enhanced survival and bacterial growth of NFAb-1 and NFAb-2 within macrophages compared to ATCC 19606 and 17978. Examining gene expression, there were no significant differences between bacterial strains regarding expression of KatE, PLC, or OmpA; however, PilA was found to be expressed at much higher levels in NFAb-1 and NFAb-2 compared to ATCC 19606 and 17978. Discussion: Taken together, this data establishes that these necrotizing fasciitis-causing A. baumannii strains are more likely to survive a macrophage-mediated immune response and expression of pilus-associated genes may play a role in these interactions. Our studies demonstrate a need to further investigate A. baumannii mechanisms of virulence to understand its ability to evade the immune response in hopes of understanding and combating infections.

Characterization of a Phase-Variable Two-Component Signal Transduction System in Clostridioides difficile

Caitlin Williams[1], Cynthia Thomas[1], Rita Tamayo[1]
[1]*University of North Carolina at Chapel Hill*

Clostridioides difficile is a Gram-positive, obligate anaerobe that causes diarrheal disease and is a leading cause of nosocomial infections worldwide. C. difficile must adapt to an ever-changing intestinal environment throughout the course of infection. One way that the pathogen may achieve this is through phase variation of key virulence factors. C. difficile was recently shown to phase vary expression of an atypical signal transduction system, CmrRST. This system includes a sensor histidine kinase, CmrS, and two response regulators, CmrR and CmrT, both of which have DNA-binding domains. Upstream of the cmrRST operon is an invertible sequence, named the cmr switch, that can be flipped by a site-specific recombinase. When the cmr switch is in the ON orientation, expression of cmrRST is increased, which has many associated phenotypes, including rough colony morphology, increased motility, and increased virulence. The goal of this research is to understand how CmrR and CmrT function separately and together to alter gene regulation and influence CmrRST-associated phenotypes. We hypothesize that CmrR and CmrT dimerize to bind unique DNA sequences and act as transcription factors. We are investigating both protein-protein and protein-DNA interactions to define the underlying mechanisms of regulation by CmrRST. Results of bacterial two-hybrid assays in C. difficile suggest that only CmrT readily homodimerizes and that heterodimers of CmrR with CmrT may also form, though at a lower frequency. Overexpression studies suggest that the two-hybrid tags inhibit CmrR function, therefore planned experiments include protein pull-down assays to detect dimers using smaller protein tags. To begin investigating protein-DNA interactions, we purified CmrR for use in electrophoretic mobility shift assays (EMSA). Our lab has previously identified four transcriptional start sites upstream of the cmrRST operon and shown that overexpression of CmrR results in increased expression of cmrRST. Preliminary EMSA results suggest that CmrR directly binds upstream of the cmrRST operon. We will use EMSA with additional probes to identify the precise binding location of CmrR and further define the autoregulatory mechanism. The intricate, layered control of expression and activity of CmrRST, coupled with its role in virulence, make it an interesting virulence factor of C. difficile. Furthering our understanding of the regulation and mechanisms of C. difficile pathogenesis will open doors for novel therapeutic options to combat this significant pathogen.

Multivalent mRNA vaccines provide protection from Bordetella pertussis infection in murine models

Allison Wolf[1,2], Post-doctoral fellow Dylan Boehm[1,2], Elisabeth Narayanan[3], Justin Bevere[1,2], Megan DeJong[1,2], Jesse Hall[1,2], Ting Wong[1,2], Samantha Falcone[3], Joanne O'Hara[3], Graham Bitzer[1,2], Emel Sen-Kilic[1,2], Obadiah Plante[3], Sunny Himansu[3], Mariette Barbier[1,2], Andrea Carfi[3], Associate Professor F. Heath Damron[1,2]
[1]West Virginia University, Department of Microbiology, Immunology, and Cell Biology, [2]Vaccine Development Center at West Virginia University Health Sciences Center, , [3]Modena, Inc

Bordetella pertussis is a highly contagious respiratory pathogen and is the causative agent of whooping cough. Reactogenic whole cell vaccines were replaced by acellular vaccines in many countries, but despite high vaccine coverage B. pertussis remains an issue among both children and adults. Acellular vaccines prevent symptomatic disease and infant mortality, but provide neither sustained nor complete protection against Bordetella pertussis. Messenger RNA (mRNA) vaccines provide a novel and adaptable immunization strategy to protect against pertussis infections. Using multiple murine models, we demonstrate that single antigen mRNA vaccines and multivalent mRNA vaccines can elicit an antigen specific immune response and provide protection against B. pertussis challenge. B. pertussis mRNA vaccines reduce the bacterial burden in the respiratory tract of infected animals and prevent leukocytosis and inflammation in the lungs. Collectively, this data supports further investigation into mRNA vaccines to protect against B. pertussis.

Challenges of genetic manipulation within diverse species in the genus Chlamydia

Katerina Wolf[1], Kenneth A. Fields
[1]*University Of Kentucky College Of Medicine*

Diverse chlamydial species share many aspects of the typical intracellular life style, such as the biphasic developmental cycle and the preference to primarily invade epithelial cells. However, each chlamydial strain also employs sophisticated species-specific strategies that significantly contribute to an extraordinary diversity in organ and/or tissue tropism and disease manifestation. In order to discover and understand underlaying mechanisms of how these pathogens infect particular hosts, utilize nutrients, respond to the eukaryotic intracellular environment, communicate with the host cell, and most importantly, cause specific diseases, it is imperative to utilize a genetic manipulation approach applicable to every chlamydial species. Chlamydial intracellular lifestyle posed a significant barrier to achieving of inactivation of specific genes within the chlamydial genome. Successful transformation of C. trachomatis (Ctr), serovar L2 with the shuttle vector pGFP::SW2, using CaCl2 treatment, was an essential first step leading to the development of tractable genetic system in this chlamydial species. Moreover, random mutagenesis approaches such as mutagenesis of Ctr genome using ethyl methanesulfonate (EMS) treatment and transposon mutagenesis of Ctr and C. muridarum (Cmuri) have been achieved. A mutagenesis of Ctr using the TargeTron or FRAEM system, producing mutants in specific target genes, were also developed. Whilst the TargeTron system necessitates the use of proprietary algorithms and limits integration to sites evaluated to be efficient, FRAEM method utilizes a large, often difficult to utilize pSUmC suicide vector derived from the entire native Ctr, L2 plasmid, pL2. Importantly, all currently developed approaches for targeted gene inactivation in Chlamydia are established exclusively for Ctr, L2. We present evidence that the four 22 bp tandem repeats, present in all chlamydial native plasmids, represent at least in part, the plasmid origin of replication. By introducing this ori into 5.45 kb pUC19, in a species-specific manner, we successfully generated a shuttle vector that can be transformed into and maintained by any chlamydia. pUC19 shuttle vector is rapidly lost upon removal of the selective antibiotic, thus providing an ideal strategy for an efficient generation of deletion mutants by allelic exchange via homologous recombination in any member of the genus Chlamydia. Indeed, we have successfully produced Δtarp in Cmuri using this pUC19 shuttle vector, which has been utilized in mouse infection model.

The Kingella kingae adhesins PilC1 and PilC2 have distinct functions in type IV pili-mediated processes

Taylor Yount[1], Dr. Alexandra Sacharok, Dr. Eric Porsch, Dr. Joseph St. Geme
[1]*University of Pennsylvania*

The gram-negative bacterium Kingella kingae is a leading cause of osteoarticular infections and septic arthritis in young children. K. kingae colonizes the oropharynx as a commensal organism, and it is capable of invading the bloodstream, disseminating to distant sites, and causing disease. The first step of K. kingae colonization involves adherence to the oropharyngeal epithelium, which is a process mediated by surface fibers called type IV pili (T4P). Our lab has identified two T4P-associated adhesins, PilC1 and PilC2, that directly mediate adherence to epithelial cells. These proteins have homologs in other T4P-expressing bacterial species and are members of the PilC family. Many proteins associated with the pilus machinery are well characterized; however, understanding of the function of PilC family members remains limited. Unlike other bacterial species with PilC homologs, K. kingae expresses two proteins, PilC1 and PilC2, with only 16% similarity, suggesting that PilC1 and PilC2 may have distinct roles or utilize different mechanisms to promote T4P-associated processes. Purified PilC1 protein binds with greater affinity to cultured epithelial cells when compared to purified PilC2 protein, suggesting that PilC1 and PilC2 bind to distinct host cell receptors. In further support of this hypothesis, our data demonstrate that there is a metal ion-dependent adhesion site (MIDAS) motif in the N-terminus of PilC1 but not PilC2, which is required for PilC1-mediated adherence to epithelial cells. This project aims to further characterize and identify the host cell receptor(s) for the K. kingae PilC1 and PilC2 adhesins. Understanding the molecular mechanisms that promote K. kingae adherence to the oropharyngeal epithelium may lead to the identification of novel targets for the disruption of K. kingae pathogenesis.

Two-component system YesMN responds to environmental zinc and contributes towards host-to-host transmission of Streptococcus pneumoniae.

M. Ammar Zafar[1], Alicia Costa-Terryl[1], Taylor Young[1]
[1]*Wake Forest School of Medicine*

Two-component systems allow bacteria to sense their environment and respond accordingly quickly. Streptococcus pneumoniae (Spn, pneumococcus), an obligate human pathogen, has 13 TCS that contributes towards its ability to colonize the upper respiratory tract (URT) and cause invasive disease. An in vivo genetic screen of random transposon mutants identified the TCS YesMN as a contributor towards Spn shedding without affecting its ability to colonize the URT. High shedding of Spn in nasal secretions is a prerequisite for host-to-host transmission. Using an infant mouse model of Spn colonization, shedding and transmission, we show that an isogenic mutant of yesMN (ΔyesMN) has reduced shedding that correlates with poor transmissibility without affecting colonization, validating the results from the genetic screen. As YesMN is not well characterized, we carried out an in vitro RNA-seq analysis to identify the regulon of YesMN. Top hits included genes involved in Zinc homeostasis, a critical metal for host and bacterial enzymatic activity that is tightly regulated at mucosal surfaces by the host. We determined through qRT-PCR that zinc homeostasis genes are under YesMN regulation, and it senses the availability of Zinc in the environment to modulate the expression of its regulon. Moreover, during coinfection with influenza A virus (IAV), we observed YesMN to be critical for Spn colonization of the upper respiratory tract. Thus, our coinfection data suggests that the TCS is advantageous in a highly inflamed URT environment created by IAV infection. Subsequently, in vitro human nasal fluid binding assays revealed that ΔyesMN bound more than the parental wild-type isolate. As there is an increase in nasal secretions during IAV infection, our results suggest that reduced colonization of Spn in the URT is because of increased clearance by the host. Taken together, our results provide a comprehensive understanding of one of the least characterized TCS of Spn and show that coinfection is not necessarily a synergistic environment for Spn.

Meeting Participants

Name	Organization	email
Haley Adcox	Virginia Commonwealth University	ADCOXHE@VCU.EDU
Herve Agaisse	University of Virginia	hfa5y@virginia.edu
Paige Allen	Virginia Commonwealth University	pallen7@lsu.edu
Sambuddha Banerjee	East Carolina University	banerjees17@ecu.edu
Srijon Banerjee	University of Pittsburgh	skb71@pitt.edu
Mariette Barbier	West Virginia University	mabarbier@hsc.wvu.edu
Andrew Barnes	Wake Forest School of Medicine	andbarne@wakehealth.edu
Ian Barton	East Carolina University Brody School of Medicine	bartoni19@ecu.edu
Shingo Bessho	Temple University	tul03801@temple.edu
Justin Bevere	West Virginia University Vaccine Development Center	jubevere@hsc.wvu.edu
Graham Bitzer	West Virginia University	gjb0014@mix.wvu.edu
Shelby Bradford	West Virginia University	sbradfo1@mix.wvu.edu
Amanda Brady	University of Louisville	a0brad06@louisville.edu
Kristen Brao	University of Maryland, Baltimore	Kristen.brao@som.umaryland.edu
Andrew Bray	Wake Forest School of Medicine	anbray@wakehealth.edu
Rezia Era Braza	Johns Hopkins University	rbraza1@jhu.edu
Shaun Brinsmade	Georgetown University	sb1344@georgetown.edu
Vincent Bruno	University of Maryland, School of Medicine	vbruno@som.umaryland.edu
Amaris Cardenas	University of Virginia	ajc5bq@virginia.edu
Greg Carey	University of Maryland School of Medicine	GCarey@som.umaryland.edu
Jason Carlyon	Virgina Commonwealth University	jason.carlyon@vcuhealth.org
Giovanna Castellanos	Walter Reed Army Institute of Research	giacas@umd.edu
Rhonda Caston	Uniformed Services University Of The Health Sciences	rhonda.caston.ctr@usuhs.edu
Clayton Caswell	Virginia Tech	caswellc@vt.edu
Mitchell Caudill	Virginia Tech	mtcau@vt.edu
Sarah Conlon	UNC Chapel Hill	seconlon@email.unc.edu
Melissa Cooper	West Virginia University	melissa.cooper1@hsc.wvu.edu
Maria Cortina	University of Virginia	Mec4nc@virginia.edu
Katherine Cotten	Johns Hopkins University	kcotten1@jhu.edu
Matthew Crawford	University of Virginia	mac5ee@virginia.edu
Connor Cribb	East Carolina University	cribbc20@students.ecu.edu
Alison Criss	University of Virginia	akc2r@virginia.edu
Casey Cunningham	West Virginia University	ccunni10@hsc.wvu.edu
Heath Damron	West Virginia University	fdamron@hsc.wvu.edu
Stephen Darnell	Uniformed Services University Of The Health Sciences	stephen.darnell.ctr@usuhs.edu

Name	Organization	email
Kim Davis	Johns Hopkins Bloomberg School of Public Health	kdavi140@jhu.edu
Megan DeJong	West Virginia University	madejong@mix.wvu.edu
Eric Devairakkam	University of Virginia	ead8jy@virginia.edu
Brittany Devasure	University Of Virginia	qsm9gp@virginia.edu
Aissata Diallo	Uniformed Services University	aissata.diallo.ctr@usuhs.edu
Lillian Dillard	University of Virginia	lrd3uu@virginia.edu
Ashley Divens	West Virginia University	amd0006@mix.wvu.edu
Samantha D'Spain	University Of Virginia	sd6eh@virginia.edu
Johanna Elfenbein	University of Wisconsin-Madison	jelfenbein@wisc.edu
Rachel Ende	University of Virginia	rje5tp@virginia.edu
Bob Ernst	University of Maryland, Baltimore	rkernst@umaryland.edu
Mariel Escatte	Walter Reed Army Institute of Research	marielescatte@gmail.com
Rory Eutsey	Carnegie Mellon University	reutsey@andrew.cmu.edu
Ken Fields	University Of Kentucky	ken.fields@uky.edu
Steven Fiester	University Of South Carolina School Of Medicine Greenville	fiester@greenvillemed.sc.edu
Timothy Fitzgerald	Walter Reed Army Institute Of Research	timothy.a.fitzgerald4.ctr@mail.mil
Matthew Flores	Virginia Tech	mjf1268@vt.edu
Taylor Franklin	University of North Carolina at Chapel Hill	taylor_franklin@med.unc.edu
Samantha Furr-Rogers	University of North Carolina at Charlotte	srfurr1@uncc.edu
Kathleen Furtado	University of North Carolina at Chapel Hill	kfurtado@unc.edu
Brandon Garcia	East Carolina University	garciabr18@ecu.edu
Jessica Gilibertie	VCOM	jgilbertie@vcom.vt.edu
Erin Gloag	The Ohio State Univerisity	erin.gloag@osumc.edu
Bill Goldman	University of North Carolina at Chapel Hill	goldman@med.unc.edu
Kaitlyn Grando	Temple University	kaitlyn.grando@temple.edu
Matthew Greenwald	University of North Carolina at Chapel Hill	mag617@unc.edu
Megan Grund	West Virginia University	meg0053@mix.wvu.edu
Maria de la Paz Gutierrez	West Virginia University	mariadelapaz.gutierrez@gmail.com
Ashley Haines	Norfolk State University	anhaines@nsu.edu
Chelsea Hall	University of Virginia	cph8ge@virginia.edu
Erin Harberts	Towson University	eharberts@towson.edu
Meaghan Hart	University of Maryland, College Park	mhart219@umd.edu

Name	Organization	email
Christopher Healy	Uniformed Services University Of The Health Sciences	christopher.healy.ctr@usuhs.edu
Kevin Hernandez	University of Pennsylvania	kahernan@pennmedicine.upenn.edu
Giovanna Hernandez	Wake Forest University	gihernan@wakehealth.edu
Matthew Higgs	University of North Carolina at Chapel Hill	hmatt1@email.unc.edu
Annie Hinson	Virginia Commonwealth University	hinsonan3@vcu.edu
Casey Hofstaedter	University of Maryland, Baltimore	casey.hofstaedter@som.umaryland.edu
Dariel Hopersberger	East Carolina University	hopersbergerd16@students.ecu.edu
Julia Hotinger	Virginia Commonwealth University	hotingerja@vcu.edu
Andrew Hudson	Wake Forest School of Medicine	awhudson@wakehealth.edu
Jason Hunt	VCU	jason.hunt@vcuhealth.org
Kelly Hurley	University of Pittsburgh	keh186@pitt.edu
Kelli Hutcheson	UNC Charlotte	khutche4@uncc.edu
Corine Jackman	Carnegie Mellon University	cjackman@andrew.cmu.edu
Ann Jerse	Uniformed Services University Of The Health Sciences	ann.jerse1@usuhs.edu
Brittany Johnson	University Of North Carolina At Chapel Hill	mjohn398@uncc.edu
Jason Kang	West Virginia University	jkang1@mix.wvu.edu
Jim Kaper	University of Maryland School of Medicine	jkaper@som.umaryland.edu
Melissa Kendall	University of Virginia	mmk5d@virginia.edu
Alexa Kerkan	East Carolina University	kerkana20@students@edu.edu
Jennifer Kerr	Notre Dame of Maryland University	jkerr@ndm.edu
Leigh Knodler	Washington State University	leigh.knodler@wsu.edu
Volkan Koseoglu	University of Virginia	vkk5b@virginia.edu
Evan Lamb	University of Virginia	el2emj@virginia.edu
Matthew Lawrenz	Univeristy of Louisville	matt.lawrenz@louisville.edu
Yoann Le Breton	Walter Reed Army Institute of Research	yoann.s.lebreton.ctr@mail.mil
Jess Lee	Cerillo	jess@cerillo.bio
Katherine Lee	West Virginia University	ksl0006@mix.WVU.edu
George Liechti	Uniformed Services University	george.liechti@usuhs.edu
Mary Clark Lind	Virginia Commonwealth University	lindmch@vcu.edu
Yiwei Liu	The Ohio State University	liu.6556@osu.edu
Ian Liyayi	University of Virginia	il3xb@virginia.edu
Lillian Lowrey	University of North Carolina at Chapel Hill	Lilowrey@live.unc.edu
Stephanie Majernik	Walter Reed Army Institute of Research	stephanie.n.majernik.ctr@mail.mil
Areej Malik	Old Dominion University	amali010@odu.edu

Name	Organization	email
Margalida Mateu-Borras	West Virginia University	mmateuborras@gmail.com
Eric Mayton	Cerillo	eric@cerillo.bio
Kevin McIver	University of Maryland, College Park	kmciver@umd.edu
Lily McKay	University of North Carolina at Chapel Hill	lsmckay@ad.unc.edu
Anchal Mehra	University of North Carolina at Chapel Hill	anchal@unc.edu
Stephen Melville	Virginia Tech	melville@vt.edu
Tod Merkel	FDA	tod.merkel@fda.hhs.gov
Nicole Messner	Virginia Tech	nicolem07@vt.edu
Jane Michalski	University of Maryland, Baltimore	Jmichalski@som.umaryland.edu
Virginia Miller	University of North Carolina at Chapel Hill	vlmiller@med.unc.edu
Daniel Miller	Virginia Commonwealth University	millerdp@vcu.edu
Amanda Miller	Temple University	Tug25971@temple.edu
Kevin Mlynek	US Army Medical Research and Materiel Command	kevin.d.mlynek.ctr@mail.mil
Nina Montoya	University of Pennsylvania	nmontoya@pennmedicine.upenn.edu
Daniel Morreale	Children's Hospital of Philadlephia	danmorr@pennmedicine.upenn.edu
Karina Mueller Brown	Carnegie Mellon University	karinam@andrew.cmu.edu
Zachary Nash	University of North Carolina at Chapel Hill	zachmnash@gmail.com
Adenrele Oludiran	Old Dominion University	aoludira@odu.edu
Humberto Ortega	Binghamton University	hortega1@binghamton.edu
Kristina Parman	Clemson University	kparman@g.clemson.edu
Krishna Patel	East Carolina University	patelkri21@ecu.edu
Augustus Pendleton	Georgetown University	ap1748@georgetown.edu
Aolani Perry	University of Maryland, College Park	aperry16@umd.edu
Everett Pesci	East Carolina University	pescie@ecu.edu
Eric Porsch	Children's Hospital of Philadelphia	porsche@chop.edu
Aimee Potter	University of Virginia	ap6ap@virginia.edu
Jessica Povroznik	West Virginia University	jessica.povroznik@hsc.wvu.edu
Deborah Powers	University Of Virginia	dap5mb@virginia.edu
Sarah Price	University of Louisville	sarah.price.2@louisville.edu
Chris Pritchett	East Tennessee State University	pritchettc@etsu.edu
Erin Purcell	Old Dominion University	epurcell@odu.edu
Gage Pyles	West Virginia University	Gp0024@mix.wvu.edu
Pranav Rana	The Ohio State University	rana.81@buckeyemail.osu.edu
David Rasko	University of Maryland School of Medicine	drasko@som.umaryland.edu

Name	Organization	email
Jocelyn Ray	University of Virginia	jray@virginia.edu
Curtis Read	Virginia Commonwealth University	readcb@vcu.edu
Anthony Richardson	University of Pittsburgh	anthony.richardson@pitt.edu
Cory Robinson	West Virginia University	cory.robinson1@hsc.wvu.edu
Cristian Roca	University of North Carolina at Chapel Hill	cristianroca@unc.edu
Miguel Rodriguez Anavitate	University of Pennsylvania	Miguel.rodriguez@pennmedicine.upenn.edu
Steven Rolland	University Of Virginia	yhj2jn@virginia.edu
Joseph Rom	University of Maryland	jrom@umd.edu
Andrew Roman	Uniformed Services University Of The Health Sciences	andrew.roman.ctr@usuhs.edu
Marty Roop	East Carolina University	roopr@ecu.edu
Stephen Salisbury	University of North Carolina at Chapel Hill	stephen.salisbury@unc.edu
Joshua Saliutama	Clemson University	jsaliut@g.clemson.edu
Savannah E. Sanchez	Virginia Commonwealth University School of Medicine	sanchez.s.elizabeth@gmail.com
Camila Saunders	University of North Carolina at Chapel Hill	camila_saunders@med.unc.edu
Alison Scott	University of Maryland, Baltimore	aliscott@umaryland.edu
Nathaniel Seals	East Carolina University Brody School of Medicine	sealsn17@students.ecu.edu
Victoria Sepulveda	University of North Carolina at Chapel Hill	vsepulve@email.unc.edu
Matthew Sherman	University of Maryland, Baltimore	matthew.sherman@som.umaryland.edu
Thomas Siff	Virginia Commonwealth University	siffte@vcu.edu
Sophie Sipprell	UNC Charlotte	ssipprel@uncc.edu
Jamison Smiley	University of Virginia	jas5ve@virginia.edu
Bailey Smith	Carnegie Mellon University	baileysm@andrew.cmu.edu
Emily Smith	University of Maryland, Baltimore	emily.smith@som.umaryland.edu
Richard Smith	University of Maryland, Baltimore	richard.smith@umaryland.edu
Alexandra Soare	University of Maryland, Baltimore	aysoare@gmail.com
Anthony Soc	Uniformed Services University Of The Health Sciences	anthony.soc.ctr@usuhs.edu
Amelia Stephens	University of Pittsburgh	acs208@pitt.edu
Tristan Stoyanof	Virginia Tech	tstoy@vt.edu
Margaret Stroud	Furman University	margaret.stroud@furman.edu
Kylie Sumner	University of Maryland, Baltimore	ksumner@som.umaryland.edu
Jessica Taddeo	Temple University LKSOM	jessica.taddeo@temple.edu
Rita Tamayo	University of North Carolina at Chapel Hill School of Medicine	rita_tamayo@med.unc.edu
Ashoka Taviti	Uniformed Services University	ashoka.taviti.ctr@usuhs.edu

Name	Organization	email
Holly Torsilieri	University of Virginia	hmt4nk@virginia.edu
Lam Thuy Vi Tran Ho	Uniformed Services University Of The Health Sciences	lam-thuy-vi.tran-ho.ctr@usuhs.edu
Logan Treat	University of North Carolina at Chapel Hill	ltreat95@email.unc.edu
Cagla Tukel	Temple University	ctukel@temple.edu
Jordan Vance	West Virginia University	jkv0003@mix.wvu.edu
Victoria Verhoeve	University Of Maryland Baltimore	vverhoeve@som.umaryland.edu
Kimberly Walker	University of North Carolina at Chapel Hill	kawalker@med.unc.edu
Mercedes Warren	University of North Carolina at Chapel Hill	warrenm1@live.unc.edu
Kelly Weaver	West Virginia University	Kyweaver@mix.wvu.edu
Erin Weddle	University of Virginia	eaw6g@virginia.edu
Cody Weidenthaler	Uniformed Services University Of The Health Sciences	cody.weidenthaler.ctr@usuhs.edu
Lacie Werner	University of Virginia	lmw6qx@virginia.edu
Elias Wheibe	USC School of Medicine Greenville	ewheibe@email.sc.edu
Martin Wiedmann	Cornell University	MW16@CORNELL.EDU
Caitlin Williams	University of North Carolina at Chapel Hill	clwilliams@unc.edu
Katerina Wolf	University Of Kentucky College Of Medicine	kwo227@uky.edu
Allison Wolf	West Virginia University	allison.wolf@hsc.wvu.edu
Matthew Wolfgang	University of North Carolina at Chapel Hill	matthew_wolfgang@med.unc.edu
Ting Wong	West Virginia University	twong4@mix.wvu.edu
Daniel Wozniak	Ohio State University	daniel.wozniak@osumc.edu
Taylor Yount	University of Pennsylvania	tyount@pennmedicine.upenn.edu
Muhammad Zafar	Wake Forest School of Medicine	mzafar@wakehealth.edu
Daniel Zurawski	Walter Reed Army Institute Of Research	daniel.v.zurawski.civ@mail.mil

Wintergreen RESORT & CONFERENCE CENTER

