INTERNATIONAL CONFERENCE ON GRAM-POSITIVE PATHOGENS

5th Meeting + October 12-15 2014 + Omaha, NE

WELCOME to the International Conference on Gram-Positive Pathogens (ICG$^+$P)! We are very pleased you have travelled to Omaha to join us and we hope that you have a relaxing, yet intellectually stimulating meeting. Infections caused by gram-positive pathogens, including *Staphylococcus aureus, Streptococcus pyogenes, Clostridium difficile,* and *Enterococcus faecium,* among others, are a burden on our society causing significant morbidity and mortality. This conference seeks to better understand these bacteria through fostering interactions between investigators studying multiple aspects of gram-positive pathogenesis, biology, and host defense. Another important aspect of the ICG$^+$P is the active support of pre- and post-doctoral trainees; most all oral presentations are awarded to trainees or junior faculty. Ultimately, the goal of this conference is to broaden our understanding of gram-positive pathogenesis and biology through the generation of new collaborations and to gain new insights through the study of similar systems in these related pathogens.

Finally, we are very excited to have the following four keynote presentations:

Sunday, October 12th
7:15-8:15 pm
Dr. Jean Lee**,** Associate Professor of Medicine at Harvard Medical School and an Associate Microbiologist at Brigham and Women's Hospital.
"Prevention of staphylococcal infections by glycoprotein vaccines synthesized in *E. coli*."

Monday October 13th
8:00-9:00 am
Dr. Andrew Camilli**,** Professor and Howard Hughes Medical Institute Investigator in the Department of Molecular Biology and Microbiology at Tufts University in Boston, MA.
"High throughput genetic analysis of *Streptococcus pneumoniae* virulence and desiccation tolerance."

4:20-5:20 pm
Dr. Bruno Dupuy, Associate Professor and Laboratory Head of the Bacterial Anaerobes Section at the Institut Pasteur in Paris, France.
"Control of *Clostridium difficile* virulence factors by metabolism."

Wednesday October 15th
8:30-9:30 am
Dr. Kelly Doran, Associate Professor in the Department of Biology at San Diego State University
"Microbial Warfare: Domestic and Foreign Policy."

SPONSORS

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ICG+P CONFERENCE SCHEDULE

Sunday, October 12th

5:00 p.m.-6:00 p.m. **Conference Check-in**
Conf. Registration Desk
Embassy Suites Hotel

6:00 p.m.-7:00 p.m. **Buffet Dinner**
Riverfront Ballroom
Embassy Suites Hotel

7:00 p.m.-7:15 p.m. **Opening Remarks**

Co-Chairs: Alexander Horswill and Nancy Freitag

7:15 p.m.-8:15 p.m. **KEYNOTE SPEAKER 1**
Riverfront Ballroom

'Prevention of staphylococcal infections by glycoprotein vaccines synthesized in *E. coli*.'

Dr. Jean Lee, Associate Professor of Medicine at Harvard Medical School and an Associate Microbiologist at Brigham and Women's Hospital.

Monday, October 13th

8:00 a.m.–9:00 a.m. **KEYNOTE SPEAKER 2**
Riverfront Ballroom

'High throughput genetic analysis of *Streptococcus pneumoniae* virulence and desiccation tolerance.'

Dr. Andrew Camilli, Professor and Howard Hughes Medical Institute Investigator in the Department of Molecular Biology and Microbiology at Tufts University in Boston, MA.

Session 1: Microbial Heartaches (aka Cardiac colonization)
Moderators: Marat Sadykov and Shaun Brinsmade

9:00 a.m.- 9:20 a.m.
Wilmara Salgado-Pabón, University of Iowa
'Tissue-specific effects of superantigens and their contribution to *Staphylococcus aureus* infective endocarditis.'

9:20 a.m.– 9:40 a.m.
P. David McMullen, University of Illinois at Chicago, "Establishing a new niche for bacterial replication: the surface invasin InlB confers cardiotropism to subpopulations of *Listeria monocytogenes*.'

9:40 a.m.– 10:00 a.m.
Armand Brown, University of Texas Health Science Center at San Antonio
'Streptococcus pneumoniae translocates into the myocardium and forms unique microlesions that disrupt cardiac function.'

10:00 a.m.-10:20 a.m. **Break**
Outside Riverfront Ballroom

Session 2: Hide and Seek (aka Host binding/host immune evasion)
Moderators: Sriram Varahan and Michael Watson

10:20 a.m.-10:40 a.m.
Alejandro Aviles Reyes, University of Rochester
'The collagen binding protein Cnm is modified by a novel glycosylation pathway in *Streptococcus mutans.'*

10:40 a.m.-11:00 a.m.
Beth Bachert, West Virginia University
'Unique *scl* loci are associated with invasive M3-type group A Streptococcus.'

11:00 a.m.-11:20 a.m.
Adnan Syed, University of Michigan
'Triclosan Promotes *Staphylococcus aureus* Nasal Colonization.'

11:20 a.m.-11:40 a.m.
Nina Gratz, St. Jude Children's Research Hospital
'A novel role for the pneumococcal neuraminidase NanA during pneumococcal meningitis.'

11:40 a.m.-12:00 p.m.
Tyler Scherr, University of Nebraska Medical Center
'Importance of carbamoyl phosphate synthetase (CPS) in *Staphylococcus aureus* biofilm immune evasion.'

12:00 p.m.-12:20 p.m.
Colin Kietzman, St. Jude Children's Research Hospital
'Dynamic capsule restructuring in Pneumococci caused by anti-microbial peptides.'

12:20 p.m.-1:10 p.m. **Lunch break**
Outside Riverfront Ballroom

ICG+P CONFERENCE SCHEDULE

Session 3: You are what you eat (aka Metabolism -controlled virulence)
Moderators: Tony Richardson and Jeff Boyd

1:10 p.m.-1:30 p.m.
Shaun Brinsmade, Georgetown University
'Graded CodY activity coordinates metabolism and virulence gene expression in *Staphylococcus aureus.'*

1:30 p.m.-1:50 p.m.
Elyse Paluscio, Washington University School of Medicine
'Streptococcus pyogenes Malate Degradation Pathway Links pH Regulation and Virulence.'

1:50 p.m.-2:10 p.m.
Nicole Spahich, University of North Carolina Chapel Hill
'The Evolution of Lactate Catabolism and Nitric Oxide Resistance in Staphylococci.'

2:10 p.m.-2:30 p.m.
Jeffrey Bose, The University of Kansas Medical Center
'The Fatty Acid Kinase of *Staphylococcus aureus* controls virulence.'

2:30 p.m.-2:50 p.m.
Nicholas Vitko, University of North Carolina
'Glycolysis-Dependent Resistance of *Staphylococcus aureus* to High Level Nitric Oxide Stress.'

2:50 p.m.-3:20 p.m. **Break**
Outside Riverfront Ballroom

Session 4: Fighting Back (aka Therapeutics)
Moderators: Cassandra Quave and Ronan Carroll

3:20 p.m.-3:40 p.m.
Nabil Abraham, Yale University School of Medicine
'Protein moonlighting: The anti-virulence function of an anti-freeze protein.'

3:40 p.m.-4:00 p.m.
Kathryn Patras, San Diego State University,
'The probiotic microbe *Streptococcus salivarius* K12 limits Group B Streptococcal vaginal colonization.'

4:00 p.m.-4:20 p.m.
Jeffrey Freiberg, University of Maryland
'Evaluation of Potential Group A Streptococcus Vaccine Candidates Identified by Immunoproteomics.'

4:20 p.m.-5:20 p.m. **KEYNOTE SPEAKER 3**
Riverfront Ballroom

'Control of *Clostridium difficile* virulence factors by metabolism.'

Dr. Bruno Dupuy, Associate Professor and Laboratory Head of the Bacterial Anaerobes Section at the Institut Pasteur in Paris, France.

7:30 p.m.-9:30 p.m.
Poster Session A and Networking Session
Riverfront Ballroom with Cash Bar

Tuesday, October 14th

Session 5: High Anxiety (aka Stress induced virulence)
Moderators: Michael Olson and Rita Tamayo

8:30 a.m.–9:00 a.m. **NIH UPDATE**
Riverfront Ballroom
Clayton Huntley National Institutes of Health

9:00 a.m.–9:20 a.m.
Kelly Rice, University of Florida
'Examination of the *Staphylococcus aureus* Nitric Oxide Reductase (saNOR) Reveals its Contribution to Modulating Intracellular NO Levels and Cellular Respiration.'

9:20 a.m.–9:40 a.m.
Anthony Gaca, University of Rochester
'The small alarmone synthase RelQ produces pGpp: a new member of the (pp)pGpp family of nucleotides.'

9:40 a.m.–10:10 a.m. **Break**
Outside Riverfront Ballroom

10:10 a.m.–10:30 a.m.
Bettina Buttaro, Temple University School of Medicine
'Transfer of antibiotic resistance gene *tetM* from *Enterococcus faecalis* pCF10 to *Staphylococcus aureus* and to *S. Typhimurium* in mixed species biofilms.'

10:30 a.m.–10:50 a.m.
Gary Port, Washington University School of Medicine
'Analysis of ClpX and SpxA mutants reveal a lack of correlation between stress resistance and virulence in *Streptococcus pyogenes*.'

ICG+P CONFERENCE SCHEDULE

10:50 a.m.–11:10 a.m.
Vinai Thomas, University of Nebraska Medical Center
'The Staphylococcus aureus CidAB proteins affect bacterial programmed cell death by modulating overflow metabolism.'

Session 6: Stealth Warfare (aka Small molecule inhibitors)
Moderators: Michael Federle and Gary Port

11:010a.m.–11:30a.m.
Pamela Hall, University of New Mexico
'ω-Hydroxymodin Limits *Staphylococcus aureus* agr-Dependent Pathogenesis and Inflammation.'

11:30 a.m.–11:50 a.m.
Cassandra Quave, Emory University School of Medicine
'Natural product quorum quenchers block MRSA virulence.'

11:50 a.m.-12:30 p.m. **Lunch Break**
Outside Riverfront Ballroom

12:30 p.m.-2:30 p.m. **Poster Session B**
Riverfront Ballroom

Session 7: Can't fight the feeling (aka Quorum/ environmental sensing)
Moderators: Kelly Rice and Megan Kiedrowski

2:30 p.m.-2:50 p.m.
'Laura Cook, University of Illinois at Chicago
'The role of quorum sensing in vaginal colonization by pathogenic streptococci.'

2:50 p.m.-3:10 p.m.
Jeff Boyd, Rutgers University
'Cellular Respiration as a Trigger for Multicellular Behavior in *Staphylococcus aureus*.'

3:10 p.m.-3:30 p.m.
Bobbi Xayarath, University of Illinois at Chicago
'Listeria monocytogenes secretes a peptide-pheromone that enhances vacuolar escape from host cells.'

3:30 p.m.-3:50 p.m.
Jessica Hastie, University of Iowa
'The dual role of anti-σ factor RsiV: lysozyme inhibitor and receptor.'

3:50 p.m.-4:20 p.m. **Break**
Outside Riverfront Ballroom

Session 8: Microbial manipulation (Pathogen immune interference)
Moderators: Wilmara Salgado-Pabon and Pam Hall

4:20 p.m.-4:40 p.m.
Cortney Heim, University of Nebraska Medical Center
'IL-10 is critical for myeloid-derived suppressor cell (MDSC) recruitment and bacterial persistence during *Staphylococcus aureus* orthopedic biofilm infection.'

4:40 p.m.-5:00 p.m.
Michael Watson, University of Michigan
'Interleukin-17A and Host Responses in Asymptomatic and Inflammatory Models of *Streptococcus pyogenes* Infection.'

5:00 p.m.-5:20 p.m.
Lance Thurlow, University of North Carolina
'Immune suppression in diabetics contributes to increased severity of *Staphylococcus aureus* skin and soft tissue infections.'

5:20 p.m.-5:40 p.m.
Salai Madhumathi Parkunan, University of Oklahoma Health Sciences Center
'Role Of Tlr4 In A Gram-Positive Ocular Infection Model.'

5:40 p.m.-6:00 p.m.
Ejiofor Ezekwe Jr, University of North Carolina at Chapel Hill
'Cellular Mechanisms of α-Hemolysin-mediated Activation of the NLRP3 Inflammasome.'

6:30 p.m.-9:30 p.m. **Conference Banquet**
Durham Western Heritage Museum

ICG+P CONFERENCE SCHEDULE

Wednesday, October 15th

8:30 a.m.–9:30 a.m. **KEYNOTE SPEAKER 5**
Riverfront Ballroom

'Microbial Warfare: Domestic and Foreign Policy.'

Dr. Kelly Doran, Associate Professor in the Department of Biology at San Diego State University

9:30 a.m.–10:00 a.m. **Break**
Outside Riverfront Ballroom

Session 9: Who's in Charge? (aka Regulatory mechanisms)
Moderators: Jeff Bose and Craig Ellermeier

10:00 a.m.–10:20 a.m.
Ravi Gupta, University of Arkansas for Medical Sciences
'Staphylococcus aureus RNAIII of the agr quorum sensing system stabilizes mgrA mRNA.'

10:20 a.m.-10:40 a.m.
Danielle Garsin, The University of Texas Health Science Center at Houston
'Regulation of Ethanolamine Utilization in *Enterococcus faecalis.'*

10:40 a.m.-11:00 a.m.
Catherine Vrentas, NIAID, US National Institutes of Health
'Expression, Purification, and Characterization of Hfqs in *Bacillus anthracis.'*

11:00 a.m.-11:20 a.m.
Heidi Crosby, University of Iowa
'Regulation of agglutination in *Staphylococcus aureus*.'

11:20 a.m.-11:40 a.m.
Scott Nguyen, University of Oklahoma Health Sciences Center
'Streptococcal Phage-like Chromosomal Islands and Impact on Host Phenotype.'

11:40 a.m.-12:00 p.m.
Revathi Govind, Kansas State University
'Alternative translational starts at tcdE in *Clostridium difficile.'*

12:00 p.m.-12:20 p.m.
Derek Moormeier, University of Nebraska Medical Center
'Temporal and Stochastic Control of *Staphylococcus aureus* Biofilm Development.'

12:20 p.m. Closing remarks
Riverfront Ballroom

Co-Chairs: Alexander Horswill and Nancy Freitag

12:30 Lunch Break
Outside Riverfront Ballroom

ICG+P ORAL ABSTRACTS

SESSIONS AND PRESENTERS

Session 1: Microbial Heartaches (aka Cardiac colonization)

Wilmara Salgado-Pabón
David McMullen
Armand Brown

Session 2: Hide and Seek (aka Host binding/host immune evasion)

Alejandro Aviles Reyes
Beth Bachert
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Derek Moormeier

ORAL ABSTRACTS

Session 1: Microbial Heartaches (aka Cardiac colonization)

9:00 a.m.-9:20 a.m.

Tissue-specific effects of superantigens and their contribution to *Staphylococcus aureus* infective endocarditis

Christopher S. Stach, Jessica M. King, Katarina Kulhankova, Patrick M. Schlievert, and **Wilmara Salgado Pabón**

University of Iowa, Carver College of Medicine, Iowa City, IA, USA

Staphylococcus aureus is the leading cause of infective endocarditis (IE). Valve colonizationl eads to the rapid formation of large vegetations associated with a high risk for congestive heart failure, strokes, and toxic shock syndrome (TSS). The *S. aureus* USA100, 200, and 600 clonal groups represent up to 60% of IE isolates. Strains within these groups encode for superantigens (SAgs) of the enterotoxin gene cluster or *egc* (a group of six largely uncharacterized SAgs), staphylococcal enterotoxin C (SEC), and/or TSS toxin-1 (TSST-1). The great majority of IE isolates also encode for these SAgs. USA300 strains represent <9% and lack these SAgs. Consistent with this, select USA100, 200, 400, and 600 strains caused IE in rabbits, while USA300 were deficient (LAC was found not to cause IE). SAgs are known for causing massive immune system activation leading to TSS. However, SAgs differ in their capacity to induce systemic effects. For many, their production is not high enough to reach the systemic circulation or are not efficient in membrane crossing causing them to remain localized within tissues where they exert their effects. We used MN8 (USA200), which encodes for TSST-1 and the *egc* Sags to determine their contribution to IE severity and progression. We provide evidence that the *egc* SAgs and TSST-1 significantly contribute to development of large septic vegetations with concomitant lethality in a highly sensitive rabbit model. Our previous studies demonstrated the critical role of SEC in *S. aureus* IE and sepsis. Altogether, we conclude that the *egc* SAgs, TSST-1, and SEC are critical contributors to the etiology and fatal outcomes that are characteristic of *S. aureus* IE.

9:20 a.m.-9:40 a.m.

Establishing a new niche for bacterial replication: the surface invasin InlB confers cardiotropism to subpopulations of *Listeria monocytogenes*

P. David McMullen and Nancy E. Freitag

Department of Immunology and Microbiology, University of Illinois at Chicago, Chicago, IL

The intracellular bacterial pathogen *Listeria monocytogenes* causes serious invasive infections and it has been recently determined that subpopulations of this bacterium have the capacity to target the heart. *L. monocytogenes* 07PF0776, isolated from a fatal human myocardial abscess, not only demonstrates enhanced cardiac colonization in mice but also invades cardiac myocytes with high efficiency. Two major bacterial surface proteins known as internalin A and B (encoded by *inlA* and *inlB*) facilitate *L. monocytogenes* invasion of many cell types, and we hypothesized that one or both of these invasins may contribute to cardiotropism. Deletion of *inlA* or *inlB* in the non-cardiotropic strain 10403S and in the cardiotropic strain 07PF0776 indicated that cardiac myocyte invasion was *inlB* dependent. The expression of the 07PF0776 derived *inlB* allele increased cardiac cell invasion of *L. monocytogenes* strains by 3 to 5 fold in comparison to 10403S derived *inlB*. Multiple amino acid substitutions present within the cardioinvasive InlB C-terminal region resulted in a lesspositively charged form of InlB that migrated more readily from the bacterial surface in response to host cell heparan sulfated proteoglycans (HSPGs) leading to enhanced host cell invasion via activation of the growth factor receptor Met. Overall, our data indicate that amino acid variations within an existing virulence factor can enable *L. monocytogenes* to access and exploit novel host replication niches.

9:40 a.m.-10:00 a.m.

Streptococcus pneumoniae translocates into the myocardium and forms unique microlesions that disrupt cardiac function

Armand O. Brown1, Beth Mann2, Geli Gao2, Jane S. Hankins3, Jessica Humann2, Jonathan Giardina2, Paola Faverio4, Marcos I. Restrepo5, Ganesh V. Halade6, Eric M. Mortensen7, Merry L. Lindsey8, Martha Hanes9, Kyle I. Happel10, Steve Nelson10, Gregory J. Bagby10, Jose A. Lorent11, Pablo Cardinal11, Rosario Granados11, Andres Esteban11, Claude J. LeSaux12, Elaine I. Tuomanen2, Carlos J. Orihuela1

1Dept. of Microbiology and Immunology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA. 2Dept. of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN, USA. 3Dept.of Hematology, St. Jude Children's Research Hospital, Memphis, TN, USA. 4University of Milan Bicocca and Dept. of Respiratory Medicine, San Gerardo Hospital, 33 Monza, Italy. 5Dept. Of Medicine, South Texas Veterans Health Care System and University of Texas Health Science Center at San Antonio, TX, USA. 6Division of Cardiovascular Disease, Dept. of Medicine, The University of Alabama at Birmingham, AL, USA. 7Medical Service, VA North Texas Health Care System and Dept. of Internal Medicine and Clinical Sciences, University of Texas Southwestern Medical Center, Dallas, TX, USA. 8Dept. of Physiology and Biophysics University of Mississippi Medical Center, Jackson, MS, USA. 9Dept. of Laboratory Animal Resources. University of Texas Health Science Center at San Antonio, San Antonio, TX, USA. 10Dept. of Physiology and Section of Pulmonary/Critical Care Medicine. Louisiana State University Health Sciences Center, New Orleans, LA, USA. 11CIBER de Enfermedades Respiratorias, Hospital Universitario de Getafe, Madrid, Spain. 12Division of Cardiology, Dept. of Medicine, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA.

Hospitalization of the elderly for invasive pneumococcal disease is frequently accompanied by the occurrence of an adverse cardiac event; these are primarily new or worsened heart failure and cardiac arrhythmia. Herein, we describe previously unrecognized microscopic lesions (microlesions) formed within the myocardium of mice, rhesus macaques, and humans during bacteremic *Streptococcus pneumoniae* infection. In mice, invasive pneumococcal disease (IPD) severity correlated with levels of serum troponin, a marker for cardiac damage, the development of aberrant cardiac electrophysiology, and the number and size of cardiac microlesions. Microlesions were prominent in the ventricles, vacuolar in appearance with extracellular pneumococci, and remarkable due to the absence of infiltrating immune cells. The pore-forming toxin pneumolysin was required for microlesion formation but Interleukin-1β was not detected at the microlesion site ruling out pneumolysin-mediated pyroptosis as a cause of cell death. Antibiotic treatment resulted in maturing of the lesions over one week with robust immune cell infiltration and collagen deposition suggestive of long-term cardiac scarring. Bacterial translocation into the heart tissue required the pneumococcal adhesin CbpA and the host ligands Laminin receptor (LR) and Platelet activating factor receptor. Immunization of mice with a fusion construct of CbpA or the LR binding domain of CbpA with the pneumolysin toxoid L460D protected against microlesion formation. We conclude that microlesion formation may contribute to the acute and long-term adverse cardiac events seen in humans with IPD.

Session 2: Hide and Seek (aka Host binding/host immune evasion)

10:20 a.m.-10:40 a.m.

ORAL ABSTRACTS

The collagen binding protein Cnm is modified by a novel glycosylation pathway in *Streptococcus mutans*

Alejandro Avilés Reyes, Jacqueline Abranches & José A. Lemos

Center for Oral Biology and Department of Microbiology and Immunology, University of Rochester, Rochester, NY

Cnm is a collagen binding surface protein of *Streptococus mutans* present in 10-15% of clinical isolates worldwide and has been regarded as an important virulence factor in extra-oral infections. Specifically, the expression of Cnm was shown to mediate binding to both collagen and laminin, enhance the ability to invade human coronary artery endothelial cells (HCAEC), and was associated with increased virulence in the *Galleria mellonella* model. Recently, we described the glycosylated nature of Cnm and the requirement of the glycosyltransferase PgfS for Cnm glycosylation. In the current study, we identified three new genes, named *pgfM1*, *pgfE* and *pgfM2*, and show that they are involved in the modification of Cnm. Both *pgfM1* and *pgfM2* are predicted to encode integral membrane proteins while *pgfE* encodes for a putative UDP-4-glucose epimerase. Transcriptional analysis revealed that *pgfM1* and *pgfE* are co-transcribed with *pgfS* whereas *pgfM2*, located downstream *pgfE*, is not. As seen with the □*pgfS* strain, single mutations in *pgfM1, pgfE* or *pgfM2* affected (i) Cnm mobility on SDS-PAGE, (ii) proteolytic stability of Cnm, (iii) interaction of Cnm to wheat germ agglutinin, (iv) binding to collagen, and (v) invasion of HCAEC. Thus, the phenotypes observed in all □*pgf* strains are likely associated to a defect in Cnm glycosylation that interferes with protein function, stability or both. Current efforts are under way to define the roles of each Pgf protein in Cnm modification and to identify the glycoconjugates associated with Cnm.

10:40 a.m.-11:00 a.m.

Unique *scl* loci are associated with invasive M3-type group A *Streptococcus*

Bachert, Beth A.1, Choi, Soo J.1, Durney, Brandon C.2, Holland, Lisa A.2, and Lukomski, Slawomir1.

1Department of Microbiology, Immunology, and Cell Biology; 2Department of Chemistry; West Virginia University, Morgantown, WV

Streptococcal collagen-like proteins 1 and 2, Scl1 and Scl2, are ubiquitous surface proteins and established virulence factors of group A *Streptococcus* (GAS). Scl1 transcription is Mga-dependent, while Scl2 expression is regulated at the level of translation. Several Scl variants were shown to bind extracellular matrix components, cellular fibronectin and laminin, facilitating biofilm formation and promoting stable tissue colonization, while several other Scl variants were shown to bind human plasma proteins. Here, we studied unique features of the *scl1.3 and scl2.3* loci, found in invasive M3-type GAS, with respect to their potential role in pathogenesis. First, the *scl1.3* locus harbors two distinct polymorphisms found in M3 strains but not in other M types: (i) the IS1548 element directly upstream of Mga binding site and (ii) a conserved null mutation within the *scl1.3* coding sequence. We demonstrate the null mutation results in a truncated form of the Scl1.3 protein which is secreted instead of being cell attached. Second, the Scl2.3 protein is detected in the majority of invasive M3 isolates, indicating selective pressure for Scl2 expression during invasive infection; this is in contrast to invasive M1 strains that do not express Scl2.1 protein. The six-helix bundle structure of the Scl2 non-collagenous domain has been solved and is conserved among all Scls. The collagen-like domain shows significant length polymorphism among M3 strains, as assessed by capillary gel electrophoresis. Ongoing structure-function studies in our laboratory investigate the role of Scl1.3 and Scl2.3 proteins in invasive GAS disease caused by M3-type GAS.

11:00 a.m.-11:20 a.m.

Triclosan Promotes *Staphylococcus aureus* Nasal Colonization

Adnan K. Syed1, Sudeshna Ghosh2, Nancy G. Love2, Blaise R. Boles3

1University of Michigan Department of Molecular Cellular and Developmental Biology Ann Arbor Michigan, 48109 2University of Michigan Department of Civil and Environmental Engineering Ann Arbor Michigan, 48109 3University of Iowa Carver College of Medicine Iowa City Iowa, 52242

The biocide triclosan is used in many personal care products including toothpastes, soaps, clothing, and medical equipment. Consequently it is present as a contaminant in the environment and has been detected in some human fluids including serum, urine, and milk. *Staphylococcus aureus* is an opportunistic pathogen that colonizes the nose and throat of approximately 30% of the population. Colonization with *S. aureus* is known to be a risk factor for several types of infection. Here we demonstrate that triclosan is commonly found in the nasal secretions of healthy adults and the presence of triclosan trends positively with nasal colonization by *S. aureus*. We demonstrate that triclosan can promote the binding of *S. aureus* to host proteins such as collagen, fibronectin, and keratin as well as inanimate surfaces such as plastic and glass. Lastly, triclosan exposed rats are more susceptible to nasal colonization with *S. aureus*. These data reveal a novel factor that influences the ability of *S. aureus* to bind surfaces and alters *S. aureus* nasal colonization.

11:20 a.m.-11:40 a.m.

A novel role for the pneumococcal neuraminidase NanA during pneumococcal meningitis

Nina Gratz, Lip Nam Loh, Beth Mann, Geli Gao, Elaine I. Tuomanen

Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN, 38105, USA

S. pneumoniae is the most common cause of bacterial meningitis in infants, young children and adults. If left untreated, this infection of the brain can result in serious complications or death. However, the molecular mechanisms underlying the ability of this bacterium to effectively cause meningitis are still not entirely understood. Pneumococcal neuraminidases are known to be important virulence factors involved during several stages of infection. Here we show that neuraminidase A (NanA) from the TIGR4 strain, which is believed to be non-functional due to a frame-shift mutation, is enzymatically active and, in contrast to NanA from the D39 strain, secreted. Our results demonstrate that in a mouse model of meningitis, the secreted version of the NanA protein is crucial for the breaching of the blood brain barrier (BBB), which is the firststep in the development of meningitis. We observed that NanA is involved in the downregulation of tight junction genes in human brain endothelial cells thereby making the BBB more permissive to infection. We propose a novel mechanism employed by *S. pneumoniae* to enhance the permeability of blood brain barrier, an important hallmark for the development of meningitis.

11:40 a.m.-12:00 p.m.

Importance of carbamoyl phosphate synthetase (CPS) in *Staphylococcus aureus* biofilm immune evasion

Tyler D. Scherr, Mark L. Hanke, and Tammy Kielian

Dept. of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE USA

S. aureus is a gram-positive, opportunistic pathogen and a leading cause of biofilm infections. From a clinical perspective, biofilm infections are particularly problematic because of their potential for dissemination and recalcitrance to antibiotic therapy, emphasizing

the need to better understand mechanisms that facilitate *S. aureus* biofilm maturation and persistence in an immune-competent host. Previously, our laboratory has shown that community-acquired methicillin-resistant *S. aureus* biofilms can inhibit macrophage phagocytosis and pro-inflammatory activation profiles. We utilized these biofilm-mediated immune defects to screen the *S. aureus* Nebraska Transposon Mutant Library, which identified carbamoyl phosphate synthetase (CPS) as a potential gene responsible for macrophage anti-inflammatory activity during biofilm formation. The purpose of this study was to assess the importance of CPS for *S. aureus* immune evasion by examining its role in macrophage phagocytosis and cell death *in vitro* and in modulation of the host inflammatory response *in vivo*. *In vitro*, *carA* mutant biofilms showed significantly increased macrophage invasion and phagocytosis concomitant with significantly decreased macrophage cell death by confocal microscopy. In a mouse orthopedic implant-associated biofilm model, infection with a *carA* mutant resulted in enhanced leukocyte accumulation and significantly decreased bacterial burdens compared to its isogenic wild type strain. Collectively, these findings confirm a key role for CPS in *S. aureus* biofilm immune evasion. Future studies will aim to shed light on the specific contribution of CPS to *S. aureus* biofilm pathogenesis. This work was supported by the NIH National Institute of Allergy and Infectious Diseases (NIAID; 2P01AI083211 Project 4) to T.K.

12:00 p.m.-12:20 p.m.

Dynamic capsule restructuring in Pneumococci caused by anti-microbial peptides.

Colin C Kietzman, Beth R Mann, Geli Gao and Elaine I Tuomanen

Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN, USA

The pneumococcus is a highly encapsulated bacterial member of the upper respiratory tract microflora in up to half of healthy individuals. Pneumococcal capsule is both necessary for colonization and virulence however it has long been apparent that within an infected host the degree of encapsulation is dependent on the tissue from which the bacteria is isolated. Little is currently known about how surface attached capsule is removed or what pathways are involved in determining the degree of encapsulation within the host. Our results show that exposure to human antimicrobial peptides drives a process where pneumococcal capsule is quickly removed from the bacterial cell surface. Using genetic inactivation it is apparent that several candidate pneumococcal genes influence this process and fall into two categories, genes that are required for the loss of capsule in response to antimicrobial peptides and genes that prevent the unregulated loss of capsule in the absence of antimicrobial peptides; these genes function similarly in a serotype independent manner. Growth in media containing antimicrobial peptides leads to steady state differences in encapsulation, which is also partially influenced by the genes controlling the short term removal of capsule from the cell surface. Furthermore the loss of capsule in response to antimicrobial peptides has a significant effect on the interaction of pneumococci with epithelial cells leading to increased invasiveness. Taken together these data suggest a novel pathway controlling capsule removal in response to a host derived molecule that will likely influence multiple aspects of pneumococcal virulence and physiology.

Session 3: You are what you eat (aka Metabolism -controlled virulence)

1:10 p.m.-1:30 p.m.

Graded CodY activity coordinates metabolism and virulence gene expression in *Staphylococcus aureus*

Deepak Sharma and **Shaun R. Brinsmade**

Department of Biology, Georgetown University, Washington, DC, USA

Staphylococcus aureus is a metabolically versatile pathogen responsible for hospital- and community-acquired infections. Much of this versatility stems from the bacterium's ability to express the appropriate genes in the host. CodY is a Firmicute-specific global regulator that adjusts metabolism and virulence gene expression based on the availability of the branched-chain amino acids (i.e., isoleucine, leucine and valine [ILV]) and GTP. Although the threshold DNA-binding activities of CodY required to regulate genes has been determined in *Bacillus subtilis*, no such details have been elucidated in a pathogen. Exploiting strains producing CodY variant proteins with residual activity to mimic nutrient limitation, we report the positions of CodY-controlled virulence genes within the gene expression hierarchy of CodY in *S. aureus*. Interrogating the abundance of both negatively regulated and positively regulated transcripts in these strains (e.g., *ilvD* required for ILV synthesis; *fnbA* for host colonization), we reveal a variety of responses to nutrient limitation. Consistent with previous results demonstrating strong negative and direct regulation of *ilvD* (~200-fold), ILV biosynthesis is relatively insensitive to changes in CodY activity. In contrast, positively regulated *fnbA* (~3-fold regulation; its gene product directs adhesion and biofilm formation in some strains) is sensitive to changes in CodY activity and exhibits a complex expression profile. We are using RNA-Seq to deduce the strategy by which *S. aureus* connects the need to eat with the production of virulence factors, proposing that during infection, CodY likely samples its activity landscape based on tissue availability of key metabolites to integrate metabolism and pathogenesis.

1:30 p.m.-1:50 p.m.

***Streptococcus pyogenes* Malate Degradation Pathway Links pH Regulation and Virulence**

Elyse Paluscio1 and Michael G. Caparon1

1Department of Molecular Microbiology Washington University School of Medicine St Louis, MO

The ability of *Streptococcus pyogenes* to infect different niches within its human host most likely relies on its ability to utilize alternative carbon sources. In examining this question, we noticed that all sequenced *S. pyogenes* strains possess the genes for the malic enzyme (ME) pathway, which allows malate to be used as a supplemental carbon source for growth. ME consists of four genes in two adjacent operons, with the regulatory two-component MaeKR required for expression of genes encoding a malate permease (*maeP*) and malic enzyme (*maeE*). Analysis of transcription indicated that expression of *maeP* and *maeE* are induced by both malate and low pH and induction in response to both cues is dependent on the MaeK sensor-kinase. Furthermore, both *maePE* and *maeKR* are repressed by glucose and this occurs via a CcpA-independent mechanism. Malate utilization requires the PTS transporter E1 enzyme (PtsI), as a PtsImutant fails to express the ME genes and is deficient in growth. Virulence of selected ME mutants was assessed in a murine model of soft tissue infection. A MaeP- mutant was attenuated for virulence, whereas a MaeE- mutant was hypervirulent as compared to wild type. Taken together, these data show that ME contributes to *S. pyogenes'* carbon source repertory, that malate utilization is a highly regulated process, that a single regulator controls ME expression in response to diverse signals, that malate uptake and utilization may be part of the adaptive pH response and that ME can influence the outcome of infection.

ORAL ABSTRACTS

1:50 p.m.-2:10 p.m.

The Evolution of Lactate Catabolism and Nitric Oxide Resistance in Staphylococci

Nicole A. Spahich

University of North Carolina - Chapel Hill

Staphylococcus aureus is a Gram-positive pathogen that resists many facets of innate immunity including nitric oxide (NO·). *S. aureus* NO·-resistance stems from its ability to evoke a metabolic state that circumvents the negative effects of reactive nitrogen species. The combination of L-lactate and peptides promotes *S. aureus* growth at moderate NO·- levels, however neither nutrient source alone allows for NO·-resistance. Here we investigate the staphylococcal family of malate:quinone and L-lactate:quinone oxidoreductases (Mqo and Lqo), both of which are critical during NO·-stress for the combined utilization of peptides and L-lactate. We address the specific contributions of Lqo-mediated L-lactate utilization and Mqo-dependent amino acid consumption during NO·-stress. We show that Lqo conversion of L-lactate to pyruvate results ultimately in the formation of acetate and ATP, an essential energy source for peptide utilization. Thus, both Lqo and Mqo are essential for growth under these conditions making them attractive candidates for targeted therapeutics. Accordingly, we exploited a modeled Mqo/Lqo structure to define the catalytic amino acids as well as key substrate-binding residues. We also compare the *S. aureus* Mqo/Lqo enzymes to their close relatives throughout the staphylococci and explore the substrate specificities of this enzyme family. Furthermore, *gfp*-promoter fusions of Mqo/Lqo orthologs suggest that there is differential regulation of this enzyme family between species. This study, therefore, provides the initial characterization of the mechanism of action and the immunometabolic roles for a newly defined staphylococcal enzyme family.

2:10 p.m.-2:30 p.m.

The Fatty Acid Kinase of *Staphylococcus aureus* controls virulence

Jeffrey L. Bose

Department of Microbiology, Molecular Genetics and Immunology, The University of Kansas Medical Center, Kansas City, KS, USA

During a screen of the Nebraska Transposon Mutant Library, we identified mutants in an operon encoding two hypothetical proteins that had a dramatic decrease in α-hemolysin production. Further analysis using non-polar mutations revealed that the second protein (termed VfrB) encoded in the operon was the primary contributor to conditionally-controlled α-hemolysin regulation as well as the expression of proteases and other secreted virulence factors. Bioinformatics suggested that VfrB may be a kinase, and it was found that this protein was essential for uptake of exogenous fatty acids. Furthermore, it was discovered that VfrB is a fatty acid kinase which works in cooperation with two additional previously hypothetical proteins (termed FakB1 and FakB2). Importantly, while biochemical evidence suggested the existence of such an enzyme, no protein performing this function had been described. While VfrB serves as a fatty acid kinase, FakB1 and FakB2 are fatty acid-binding proteins with differing specificity. In vivo studies using a murine model of dermonecrosis revealed that the *vfrB* mutant was hyper-virulent as observed by increased tissue damage, despite similar numbers of bacteria present. Together, these studies identify a previously undescribed fatty acid kinase complex found in gram-positive bacteria, and reveals the connection between fatty acid metabolism and virulence factor regulation in *Staphylococcus aureus*.

2:30 p.m.-2:50 p.m.

Glycolysis-Dependent Resistance of *Staphylococcus aureus* to High Level Nitric Oxide Stress

Nicholas P. Vitko, Anthony R. Richardson

Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, North Carolina, USA

Staphylococcus aureus is uniquely resistant to the innate immune effector nitric oxide (NO·). We have previously demonstrated that aerobic hetero-lactic acid fermentation is required for this resistance and that unlike other closely related *Staphylococci, S. aureus* possesses a second Rex-regulated lactate dehydrogenase (*ldh1*). However, it was unclear what environmental conditions would support this fermentative activity. Thus we examined that ability of various carbon sources to support growth of *S. aureus* in the presence of high NO· and found that only a subset of glycolytic carbon sources (hexoses) were capable of supporting *S. aureus* NO· resistance. Subsequent experimentation demonstrated that this is largely due to the respiration-dependent catabolism of most carbon sources, which results from either the use of quinonedependent enzymes or the production of a robust redox imbalance that cannot be overcome by fermentation. In support of these *in vitro* observations we were able to show that *S. aureus* survival during phagocytosis by macrophages is glycolysisdependent, as a result of iNOS activity, and that elevated levels of extracellular glucose support greater survival of *S. aureus* in an iNOS-dependent manner. Additionally, glycolytic-deficient mutants of *S. aureus* are severely attenuated at early time points in several animal models of infection. Together, these data implicate *S. aureus* glycolysis as a critical factor in innate immune resistance and suggest that host hyperglycemia may contribute to greater pathogenicity by supporting this resistance.

Session 4: Fighting Back (aka Therapeutics)

3:20 p.m.-3:40 p.m.

Protein moonlighting: The anti-virulence function of an anti-freeze protein

Nabil M. Abraham 1, 4, Martin Heisig 1, Lei Liu 1, Girish Neelakanta 6, Sarah Mattessich 1, Hameeda Sultana 6, Zhengling Shang 1, Juliana Mastronunzio 1, Charlotte Killiam 1, Wendy Walker 7, Lynn Cooley 2, Richard A. Flavell 3, 4, Herve Agaisse 5, and Erol Fikrig 1, 4

1 Department of Internal Medicine, Section of Infectious Diseases, Yale University School of Medicine, New Haven, CT, USA; 2 Department of Genetics, Yale University School of Medicine, New Haven, CT, USA; 3 Department of Immunobiology, Yale University School of Medicine, New Haven, CT, USA; 4 Howard Hughes Medical Institute, Chevy Chase, MD, USA; 5 Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, CT, USA; 6 Department of Biological Sciences, Old Dominion University, Norfolk, VA, USA; 7 University Health Sciences Center, Texas Tech University, El Paso, TX

Infectious diseases are a major cause of morbidity and mortality worldwide and are responsible for more than 4 million hospitalizations annually in the US alone. Moreover, with the systematic emergence of antibiotic resistance among bacteria there is a critical need for new classes of compounds to combat infectious diseases. Recently identified *Ixodes scapularis* tick antifreeze glycoprotein, IAFGP, which was found to protect the tick against cold damage was further identified to promote a secondary anti-virulence function against bacterial pathogens including MRSA. Recombinant IAFGP and a peptide, P1, derived from this protein bound a myriad of different microbes. Using *Staphylococcus aureus* as a model pathogen, bacterial binding of IAFGP or peptide P1 correlated with a severe depreciation in biofilm formation. Transgenic *iafgp*-expressing flies and mice challenged with bacteria were resistant to infection and septic shock. To further investigate biofilm formation *in vivo*, peptide coated catheters implanted subcutaneously in mice demonstrated a 40-fold reduction in the number of attached bacteria compared to mock-treated group. These studies highlight the practical potential of this peptide, and suggest a new therapeutic strategy to prevent or control bacterial disease in humans, including

infections associated with implanted biomaterials, either directly or in synergy with traditional antibiotics.

3:40 p.m.-4:00 p.m.

The probiotic microbe *Streptococcus salivarius* K12 limits Group B Streptococcal vaginal colonization

Kathryn A. Patras1, Philip Wescombe2, Berenice Rösler1, John Hale2, John Tagg2 and Kelly S. Doran1.

1San Diego State University, San Diego, California, USA. 2BLIS Technologies Ltd., Center for Innovation, Dunedin, New Zealand.

Streptococcus agalactiae (Group B Streptococcus, GBS) colonizes the rectovaginal tract in 20-30% of women; however, during pregnancy GBS can be transmitted to the newborn, causing severe invasive disease. Routine screening and antibiotic prophylaxis have failed to completely prevent GBS transmission and GBS remains a leading cause of neonatal pathogenesis. We have investigated ability of the probiotic bacterium, *Streptococcus salivarius*, a predominant member of the native oral microbiota, to control GBS colonization. We examined antibacterial activity of multiple *S. salivarius* strains against GBS using a deferred antagonism test and found that *S. salivarius* K12 (K12) exhibited the broadest spectrum of activity. K12 effectively inhibited all GBS strains tested including disease isolates from newborns, and from the vaginal tract of pregnant women. Inhibition was dependent on production of two bacteriocins, salivaricin A and salivaricin B, encoded on the megaplasmid pSsal-K12. We also determined K12 adhered to human vaginal epithelial cells at comparable levels to GBS. To examine *in vivo* activity of K12, we used a mouse model of GBS vaginal colonization developed in our laboratory. Mice were first colonized with GBS then treated with daily vaginal administration of K12. Excitingly, K12 treatment significantly reduced GBS vaginal colonization compared to non-treated controls. Future work seeks to establish the mechanism of *in vivo* GBS reduction, as well as K12 dosage efficacy in multiple murine backgrounds and GBS strains. We conclude that K12 may have potential as a preventative therapy to control GBS vaginal colonization during pregnancy to effectively prevent neonatal transmission.

4:00 p.m.-4:20 p.m.

Evaluation of Potential Group A *Streptococcus* Vaccine Candidates Identified by Immunoproteomics

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1Graduate Program in Life Sciences, University of Maryland, Baltimore, MD, USA

2Department of Cell Biology and Molecular Genetics, Maryland Pathogen Research Institute, University of Maryland, College Park (UMCP), College Park, MD, USA

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4Department of Microbiology and Immunology, School of Medicine, University of Maryland, Baltimore, MD, USA

Group A *Streptococcus* (GAS) is an important human pathogen that causes a number of diseases with a wide range of severity. While the ability to form *in vitro* biofilms in the laboratory has been shown, there is a lack of understanding of the role of GAS biofilms during an infection. Therefore, we developed an animal model of a tibial foreign body infection in order to test the hypothesis that GAS chronic infections are in a biofilm mode of growth *in vivo*. Histological samples of the infected tibia were prepared and the formation of a biofilm *in vivo* was visualized using peptide nucleic acid fluorescent in situ hybridization (PNA-FISH) and confocal microscopy. GAS formed localized microbial biofilm communities on the surface of the indwelling device and within the host bone in infected animals. Convalescent sera samples were collected and used to detect cell wall proteins expressed *in vitro* under biofilm and planktonic growth conditions. Immunogenic proteins were then identified using matrix-assisted laser desorption ionization–time of flight tandem mass spectrometry (MALDI-TOF/TOF MS). The genes for several of these immunogenic proteins were then evaluated for their potential to serve as future vaccine candidates. Genes were cloned into expression vectors in order to produce recombinant proteins that were subsequently recognized by sera from multiple animal models of GAS infection. Sera raised against the recombinant proteins demonstrated enhanced bactericidal activity in an *in vitro* assay. Based on these results, we believe these proteins may contribute to a future multivalent Group A *Streptococcus* multi-subunit vaccine.

Session 5: High Anxiety (aka Stress induced virulence)

8:10 a.m.–8:30 a.m.

Examination of the *Staphylococcus aureus* Nitric Oxide Reductase (saNOR) Reveals its Contribution to Modulating Intracellular NO Levels and Cellular Respiration

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Staphylococcus aureus nitrosative stress resistance is due in part to the nitric oxide (NO)- oxidizing flavohemoprotein (Hmp). Although *hmp* is present in all sequenced genomes, 35% of strains also contain *nor*, encoding a predicted quinol-type NO reductase (saNOR). DAF-FM diacetate staining of NO-challenged wild-type, *nor*, *hmp*, and *nor hmp* mutant biofilms suggested that Hmp may have a greater contribution to intracellular NO detoxification relative to saNOR. However, saNOR still has a significant impact on intracellular NO levels, and can complement NO detoxification in an *hmp nor* double mutant. As expected, an *hmp* mutant displayed an impaired ability to initiate growth under nitrosative stress conditions. However, *nor* mutant growth was comparable to the wild-type strain. Membrane potential and respiratory activity assays indicated that saNOR contributes to membrane potential and respiration under low-oxygen nitrosative stress conditions. Expression of *nor* was upregulated under low-oxygen growth conditions and dependent on SrrAB, a two-component system that regulates expression of respiration and nitrosative stress resistance genes. High-level *nor* promoter activity was also detectable in only a subpopulation of cells near the biofilm substratum. These results suggest that saNOR contributes to energy generation via respiration of NO during nitrosative stress, possibly conferring an advantage to *nor*+ strains *in vivo*.

8:30 a.m.–8:50 a.m.

The small alarmone synthase RelQ produces pGpp: a new member of the (pp)pGpp family of nucleotides

Anthony O. Gaca1, Brent Anderson2, Kuanqing Liu2, Jade D. Wang2, and José Lemos1

1Center for Oral Biology, University of Rochester, Rochester, NY, USA 2Department of Bacteriology, University of Wisconsin-Madison, Madison, WI, USA

The bacterial stringent response (SR) is a conserved stress tolerance mechanism that orchestrates broad physiological alterations designed to enhance survival under adverse conditions. This response is mediated by the intracellular accumulation of the alarmones guanosine 5'-diphosphate, 3'-diphosphate (ppGpp) and guanosine 5'-triphosphate, 3'-

diphosphate (pppGpp), collectively termed (p)ppGpp. In *Enterococcus faecalis*, metabolism of (p)ppGpp is carried out by the bifunctional synthase/hydrolase Rel and the small alarmone synthase (SAS) RelQ. RelEf is responsible for rapid accumulation of (p)ppGpp during SR induction, while RelQEf appears to maintain basal (p)ppGpp levels in the absence of stress. Although Rel is the main enzyme responsible for SR activation in Firmicutes, there is emerging evidence that SAS, such as RelQ, can make important contributions to cellular homeostasis. The goal of this work was to initiate the biochemical characterization of RelQ. We found that RelQEf preferentially synthesizes ppGpp (from GDP) over pppGpp (from GTP), and surprisingly, also utilized GMP to form guanosine 5'-monophosphate, 3'-diphosphate (pGpp) with a substrate preference: GDP>GMP>GTP. *In vitro* assays revealed that RelEf hydrolyzes pGpp, ppGpp, and pppGpp with seemingly equal efficiency. In *E. faecalis* and *Bacillus subtilis*, (p)ppGpp was shown to be a strong repressor of GTP biosynthesis through direct inhibition of HprT and Gmk that convert guanine to GMP and GMP to GDP, respectively. Using purified Gmk and HprT from *E. faecalis*, we show that pGpp more potently inhibits the GTP biosynthesis pathway than (p)ppGpp, indicating that pGpp constitutes a new member of the (pp)pGpp family of regulatory nucleotides.

8:50 a.m.-9:10 a.m.

Transfer of antibiotic resistance gene *tetM* from *Enterococcus faecalis* pCF10 to *Staphylococcus aureus* and to *S.* Typhimurium in mixed species biofilms.

Amanda Platt, Ella Massie-Schuh, Dhara Patel, Vasant Chary and **Bettina Buttaro**

Temple University School of Medicine, Philadelphia, PA, USA *E. faecalis* has been implicated in the spread of antibiotic resistance determinants to other species. The pheromone-inducible conjugative plasmid pCF10 carries a tetracycline resistance gene, *tetM*, on the conjugative transposon *Tn*925. pCF10 transfer between enterococci is efficient (10-1) and *Tn*925 is also capable of transferring at lower rates (10-5 to 10-8) independent of the plasmid. Using static mixed species biofilms, we have observed transfer of *Tn*925 and *tetM* from *E. faecalis* to *S. aureus* at a rate of 10-8. The transfer of *Tn*925 to *S. aureus* depended on pheromone induction of plasmid conjugation, suggesting it was dependent on pCF10. We have also demonstrated transfer to *S. enterica* serovar Typhimurium using a conical tube biofilm model. *Tn*925 can conjugatively transfer to *Escherichia coli*, however, we observe approximately 10 to 100-fold higher transfer rates than those reported for *Tn*925 in the chromosome. Taken together, these data lead to the hypothesis that residence of the conjugative transposon on a conjugative plasmid increases the rate of transposon transfer to other genera. Since the *Tn*925 transfer experiments were not done using mixed species biofilms, we are comparing the transfer rates of *Tn*925 located in the chromosome with those obtained with pCF10 in our model system. In addition, we have recently found that H2O2 oxidative stress increases the copy number of pCF10. This change in copy number is dependent on three conserved cysteines in the replication initiation protein PrgW. Experiments examining the transfer rates in mixed species biofilms under oxidative stress are currently being conducted.

9:10 a.m.-9:30 a.m.

Analysis of ClpX and SpxA mutants reveal a lack of correlation between stressresistance and virulence in *Streptococcus pyogenes*

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Department of Molecular Microbiology, Washington University School of Medicine, St Louis, MO, USA.

For *Streptococcus pyogenes* and other pathogens, it is generally assumed that resistance to thermal and oxidative stresses is essential for pathogenesis. In investigating this question, we recently identified the multi-stress regulator ClpX as a regulator of toxin expression, particularly for the secreted SpeB cysteine protease. Although much is known about the conditions that stimulate SpeB expression, little is known regarding the genetic circuitry that integrates these various signals and its intersection with stress resistance. For many grampositive bacteria, ClpX regulates stress resistance via interactions with two orthologs of the Spx transcriptional regulator, SpxA1 and SpxA2. Analysis of Spx mutants revealed that while ΔSpxA1 had a defect only for growth under aerobic conditions, ΔSpxA2 was severely attenuated for multiple stresses, including thermal and oxidative stress. When examined in a murine model of soft-tissue infection, ΔClpX and ΔSpxA1 were highly attenuated. In contrast, the highly stresssensitive ΔSpxA2 was hyper-virulent with higher tissue damage and bacterial burdens than the wild type strain. Furthermore, while the attenuation of ΔClpX and ΔSpxA1 was associated with reduced SpeB expression, ΔSpxA2 hyper-virulence was associated with SpeB hyper-expression and ΔSpxA2 virulence could be restored to wild-type levels by deletion of *speB*. Taken together, these data demonstrate: 1. that *S. pyogenes'* ability to damage tissue is combinatorial, involving fine-tuning expression of multiple toxins and 2. that there is no direct correlation between ClpXmediated multi-stress resistance and virulence. Further examination of ClpX's influence on virulence will provide insight into the genetic circuits that control the fine-tuning of toxin expression.

9:30 a.m.-9:50 a.m.

The *Staphylococcus aureus* CidAB proteins affect bacterial programmed cell death by modulating overflow metabolism

Vinai C. Thomasa, Sujata S. Chaudharia, Jeffery Boseb, Marat R. Sadykova and Kenneth W. Baylesa

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Although hypothesized to play a role in bacterial programmed cell death (PCD), the exact function of the holin-like CidA and co-expressed CidB membrane proteins has evaded characterization. In the present study, we monitored the fate of the CidABC expressing population by flow cytometry and show that both CidA and CidB antithetically regulate the survival of a subpopulation of cells under acidic stress by altering carbon partitioning at the pyruvate node into overflow metabolic pathways. While, mutation of *cidA* favored a partial redirection of carbon flux through the CidC pathway resulting in increased acetate excretion and higher rates of cell death relative to the wild-type, inactivation of *cidB* resulted in increased excretion of the neutral metabolite, acetoin through the AlsSD pathway and increased rates of survival. Additionally, evidence is presented to indicate that the observed metabolic changes in the *cidA* and *cidB* mutants do not result from differential transcription of either *cidC* or *alsSD* genes but possibly involves alterations in the translocation of the CidC protein to the lipid membrane where it attains optimal activity. These observations underscore a role for CidA and CidB in the metabolic modulation of bacterial PCD.

Session 6: Stealth Warfare (aka Small molecule inhibitors)

10:20 a.m.–10:40 a.m.

ω-Hydroxymodin Limits *Staphylococcus aureus agr*-Dependent Pathogenesis and Inflammation

Seth M. Daly,a Bradley O. Elmore,a Jeffrey S. Kavanaugh,b Kathleen T. Triplett,a Nadja B. Cech,c Alexander R. Horswill,b Nicholas H. Oberlies,c **Pamela R. Halla**

ORAL ABSTRACTS

Department of Pharmaceutical Sciences, College of Pharmacy, University of New Mexico, Albuquerque, New Mexico, USAa; Department of Microbiology, Carver College of Medicine, University of Iowa, Iowa City, Iowa, USAb; Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, North Carolina USAc

Antibiotic resistant pathogens are a global health threat. Small molecules that inhibit bacterial virulence and therefore pathogenesis are potential alternatives to conventional antibiotics as they may increase bacterial susceptibility to host killing while limiting selection for resistance. *Staphylococcus aureus* is a major cause of invasive skin and soft tissue infections (SSTI) in both the hospital and community, and has become increasingly antibiotic resistant. Quorum sensing (QS) via the *S. aureus* accessory gene regulator (*agr*) operon regulates virulence factor expression contributing to invasive SSTI. We recently identified ω-Hydroxymodin (OHM), a polyhydroxyanthroquinone isolated from solid-phase cultures of *Penicillium restrictum*, as an inhibitor of *S. aureus* QS and sought to further characterize it as an *agr* antagonist. At concentrations non-toxic to eukaryotic cells and which did not inhibit bacterial growth, OHM significantly inhibited *agr* signaling by all four *S. aureus agr* alleles. Mechanistically, OHM inhibited binding of the *agr* response regulator AgrA to cognate promoters as assessed by electrophoretic mobility shift assay and flow cytometry. Importantly, OHM was efficacious in a mouse model of SSTI. OHM treatment was associated with decreased dermonecrosis, enhanced bacterial clearance and significant reductions in inflammatory cytokine transcription and expression at the site of infection. These data suggest that bacterial disarmament through suppression of *S. aureus agr*-signaling may bolster the host innate immune response and limit inflammation.

10:40 a.m.–11:00 a.m.

Natural product quorum quenchers block MRSA virulence

Cassandra L. Quave, Department of Dermatology and Center for the Study of Human Health, Emory University School of Medicine, Atlanta, GA James T. Lyles, Center for the Study of Human Health, Emory University, Atlanta, GA Jeffery S. Kavanaugh, Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA Heidi A. Crosby, Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA Kate Neslon, Center for the Study of Human Health, Emory University, Atlanta, GA Alexander R. Horswill, Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA

Through the quorum sensing regulated accessory gene regulator (*agr*) cascade, *Staphylococcus aureus* is able to produce an impressive array of toxins, hemolysins, and enzymes with the capacity to inflict life threatening damage to the host. Small molecule inhibitors of the *agr* system hold the potential for mitigating the damage caused by this pathogen. Here, we discuss our work on quorum quenching natural products extracted from a medicinal plant used in the traditional treatment of skin and soft tissue infections (SSTI). Bioassay-guided fractionation techniques were used in the isolation of a refined fraction (224CF2) containing several bioactive natural products. Quorum quenching was detected through use of *agr::*P3 reporters and confirmed in each of the four *agr* types. Our findings include evidence of anti-virulence activity in the absence of growth inhibition as documented by HPLC quantification of delta-hemolysin, MIC testing, red blood cell lysis testing and assessment of supernatant damage to human keratinocyte (HaCat) cells. This work not only offers compelling evidence in support of the traditional use of this species to treat SSTIs, but also provides us with a new set of small molecule *agr* inhibitors for further study.

Session 7: Can't fight the feeling (aka Quorum/ environmental sensing)

2:30 p.m.-2:50 p.m.

The role of quorum sensing in vaginal colonization by pathogenic streptococci

Laura C.C. Cook1*, Jennifer C. Chang1, Reid V. Wilkening1, and Michael J. Federle1

1University of Illinois at Chicago, College of Pharmacy

In *Streptococcus pyogenes* (Group A Strep, GAS), an important human pathogen, quorum sensing signaling peptides Shp2 and Shp3 enhance biofilm formation. Shp peptides bind directly to the Rgg2 and Rgg3 transcription factors, controlling their ability to regulate gene expression. *Streptococcus agalactiae* (Group B Strep, GBS), a known colonizer of the female vaginal tract, can cause severe neonatal sepsis if passed to infants during vaginal birth. Nearly identical orthologs of *rgg2* and *shp2* are present in GBS, and have been annotated *rovS* and *shp1520*. RovS was found previously to regulate several virulence genes in GBS. Experiments conducted in our lab showed that production of Shp1520 by GBS can activate Shp2/3-regulated genes in GAS, signifying interspecies communication between GAS and GBS via this QS system. The previously observed role of these QS systems in biofilm formation and virulence, as well as their ability to mediate cross-species signaling, clearly demonstrates the importance of understanding their mechanism of action. To examine the role of this cross-species signaling in a host, we used a mouse model of vaginal colonization to determine whether mutants in the QS pathways affect carriage of streptococci. Preliminary data suggest that both the peptides and response regulators affect the ability of both species of streptococci to colonize the vaginal tract. Taken together, these studies provide great insight into the ability of two important human pathogens to communicate with each other in clinically relevant settings.

2:50 p.m.-3:10 p.m.

Cellular Respiration as a Trigger for Multicellular Behavior in *Staphylococcus aureus.*

Ameya Mashruwala and **Jeffrey M. Boyd**

Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ USA

Staphylococcus aureus is a health concern worldwide and a leading cause of biofilm related infection. The bioavailability of oxygen in the human body and infected tissues varies and we hypothesized that *S. aureus* would sense and respond to the presence of oxygen by altering its multicellular behavior. We find that multiple *S. aureus* clinical isolates form robust biofilms when cultured in the absence of oxygen. Although oxygen is a cell diffusible signal our data suggest that the effect of oxygen on biofilm formation is a result of its role as a terminal electron acceptor (TEA). We find that decreased concentrations of TEA result in an impaired ability of *S. aureus* to respire and this causes the bacterium to switch to a multicellular lifestyle mode. Consistent with this hypothesis we find that supplementation of anaerobic biofilms with nitrate, an alternative TEA, results in reduced biofilm formation. Biofilm formation can be the result of either stochastic or deterministic cellular processes. We find that biofilm formation under conditions of impaired respiration is a deterministic process and the lack of a TEA is sensed by a membrane spanning two-component regulatory system called SrrAB. Our data suggest that respiratory flux through electron transport chains alters the redox status of the cellular quinone pool thereby altering SrrAB activity. Finally, we show that upon sensing the cellular respiratory status SrrAB sets in motion a programmed cell death (PCD) response, which results in the release of extracellular DNA and increased biofilm formation.

3:10 p.m.-3:30 p.m.

ORAL ABSTRACTS

***Listeria monocytogenes* secretes a peptide-pheromone that enhances vacuolar escape from host cells**

Bobbi Xayarath, Francis Alonzo, III, and Nancy E. Freitag

University of Illinois at Chicago, Department of Microbiology & Immunology, Chicago, IL U.S.A.

Small peptide-based signaling systems serve to coordinate many diverse cellular functions and physiological processes in a number of Gram-positive bacteria. The bacterium, *Listeria monocytogenes (Lm)*, transitions from living as an environmental bacterium to surviving as a facultative intracellular pathogen in the mammalian host cell. Gene products required for intracellular survival are regulated by a master transcriptional activator known as PrfA that becomes activated following *Lm* entry into host cells. The specific cues that enable *Lm* to sense its intracellular environment are not well defined, nor is the signal which stimulates PrfA activity known. Here we demonstrate that *Lm* secretes a small peptide pheromone, pPplA, that enhances escape of *Lm* from host cell vacuoles and may also contribute to PrfA activation. The pPplA pheromone is processed from the PplA lipoprotein secretion signal peptide. *Lm* lacking the pPplA pheromone, but not the lipoprotein, were significantly attenuated for virulence in mice and were delayed in escape from the vacuoles of nonprofessional phagocytic cells, but not in either a macrophage-like cell line or in bone-marrow derived macrophages. The vacuolar escape defect was consistent with reduced polymerization of host cell actin and co-localization of *Lm* with the late endosomal marker Rab7. Loss of the pPplA pheromone did not impair perforation of the vacuole, only escape. Interestingly, the introduction of a mutationally activated *prfA** allele restored any virulence defects associated with loss of pPplA and eliminated the need for pPplA-dependent signaling. These results suggests that the pPplA peptide may help *Lm* sense its presence within the confines of the host cell vacuole, stimulating the expression of gene products that contribute to vacuole escape as well as PrfA activation to promote bacterial growth within the cytosol.

3:30 p.m.-3:50 p.m.

The dual role of anti-σ factor RsiV: lysozyme inhibitor and receptor

Jessica L. Hastie*1, Kyle B. Williams1, Carolina Sepúlveda2, Lindsey L. Bohr3, Jon C. Houtman1, Katrina T. Forest2, and Craig D. Ellermeier1

1University of Iowa, Iowa City, Iowa, USA 2University of Wisconsin Madison, Madison, Wisconsin, USA 3Luther College, Decorah, Iowa, USA

Extra-Cytoplasmic Function (ECF) sigma factors in complex with RNA polymerase are a subset of sigma factors many organisms use to transcribe specific genes in response to environmental cues. ECF sigma factor σV is induced in response to lysozyme, an important component of the innate immune system. In the absence of lysozyme, the activity of σV is inhibited by the anti-sigma factor RsiV, a single pass transmembrane protein. We have shown that in the presence of lysozyme, RsiV is degraded by a step wise proteolytic cascade known as Regulated Intramembrane Proteolysis (RIP). Our data indicates initiation of σV activation is controlled by site-1 cleavage of RsiV. We demonstrate that the signal peptidase SipS is able to cleave RsiV at site-1 *in vitro* only in the presence of lysozyme. Here we propose a RIP dependent mechanism of σV activation that is contingent upon the anti-sigma (RsiV) directly binding the inducing signal, lysozyme. We cocrystalized the extracellular domain of RsiV and lysozyme, and identified several residues important for σV activation and RsiV degradation. We hypothesize lysozyme binding to RsiV reveals the site-1 cleavage site, which induces the proteolytic cascade leading to the destruction of RsiV, and activation of σV. ECF sigma factor σV is present in *Bacillus subtilis* and the nosocomial pathogens *Enterococcus faecalis*, and *Clostridium difficile*. σV is thought to promote bacterial survival within a host through increased lysozyme resistance by activating genes to modify the peptidoglycan, and the direct interaction of RsiV and lysozyme, which inhibits lysozyme activity.

Session 8: Microbial manipulation (Pathogen immune interference)

4:20 p.m.-4:40 p.m.

IL-10 is critical for myeloid-derived suppressor cell (MDSC) recruitment and bacterial persistence during *Staphylococcus aureus* orthopedic biofilm infection

Cortney E. Heim, Debbie Vidlak and Tammy Kielian Department of Pathology and Microbiology University of Nebraska Medical Center, Omaha, NE

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature monocytes and granulocytes that are potent inhibitors of T cell activation. Although MDSCs are well-recognized for their role in tumor immunosuppression, their immunologic functions during bacterial infection have only recently emerged, and little is known about MDSC function during *Staphylococcus aureus* (*S. aureus*) infection. *S. aureus* biofilms can subvert immune-mediated clearance, and a recent report from our laboratory identified a population of Ly6GhighLy6C+ MDSCs as the main cellular infiltrate during *S. aureus* orthopedic biofilm infection. Biofilm-associated MDSCs inhibited T cell proliferation and cytokine production, correlating with a paucity of T cell infiltrates. Analysis of FACS-purified MDSCs recovered from *S. aureus* biofilms revealed increased IL-10 expression, which was confirmed using IL-10-GFP mice where Ly6GhighLy6C+ MDSCs represented the main source of IL-10 during infection. IL-10 secretion by MDSCs has been implicated in inhibiting T cell activation and polarizing macrophages (MΦs) toward an alternatively activated M2 phenotype. To determine the functional importance of IL-10 in shaping the inflammatory milieu during *S. aureus* infection, IL-10 knockout (KO) mice were utilized. MDSC influx into implant-associated tissues was significantly reduced in IL-10 KO mice at day 14 post-infection concomitant with enhanced monocyte and macrophage infiltrates. The reduction in MDSC recruitment facilitated bacterial clearance as revealed by significant decreases in *S. aureus* burdens in the tissue, knee joint and femur of IL-10 KO mice. These results demonstrate that IL-10 produced by MDSCs contributes to the persistence of *S. aureus* biofilm infections by limiting monocyte/macrophage activity.

4:40 p.m.-5:00 p.m.

Interleukin-17A and Host Responses in Asymptomatic and Inflammatory

Models of *Streptococcus pyogenes* Infection

Michael E. Watson1, Irina Laczkovich1, Jason B. Weinberg1, Michael G. Caparon2, and Suzanne R. Dawid1

1Division of Pediatric Infectious Diseases, University of Michigan, Ann Arbor, MI, USA 2Department of Molecular Microbiology, Washington University, St. Louis, MO, USA

Streptococcus pyogenes causes significant disease at multiple sites, including skin and mucosal tissues. In response to infection the adaptive immune response via Th17 helper T lymphocytes promotes recruitment of innate immunity cells, largely via interleukin-17A (IL-17A) signaling. The role of IL-17A with regards to clearance of *S. pyogenes* remains poorly characterized. Our goal was to assess the role of IL-17A in promoting clearance of *S.*

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pyogenes from two distinct murine models of asymptomatic vaginal colonization and an inflammatory subcutaneous infection. C57BL/6 wild-type (WT) mice and IL-17A-/- mice were utilized. Inoculation of *S. pyogenes* into vaginal or subcutaneous tissue in WT mice produced significant elevation of IL-17A, in addition to IL-1 and IL-23, compared with non-inoculated tissues. IL-17A-/- mice exhibited prolonged vaginal carriage compared to WT mice, with greater quantities of *S. pyogenes* recovered from vaginal washes. Examination of neutrophil recruitment into vaginal fluid demonstrated an attenuated neutrophil influx in colonized IL-17A-/- mice compared to WT. In the subcutaneous infection model, IL-17A-/- mice exhibited a 48-72 h delay in the development of maximal skin ulcer size compared to WT mice, with a lag in neutrophil recruitment in IL-17A-/- mice to the site of infection. However, the maximal skin ulcer size was ultimately larger in IL-17A-/- mice compared to WT mice. IL-17A plays a critical role in host responses against *S. pyogenes* in these two distinct models of infection. The delay of neutrophil recruitment in IL-17A-/- mice suggests that neutrophils are an important component of IL-17A-mediated activity promoting clearance of *S. pyogenes*.

5:00 p.m.-5:20 p.m.

Immune suppression in diabetics contributes to increased severity of *Staphylococcus aureus* skin and soft tissue infections

Lance R. Thurlow and Anthony R. Richardson

University of North Carolina Chapel Hill School of Medicine Chapel Hill, NC, USA

Infections caused by community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) have dramatically increased in frequency in the past decade. One particular population that has increased CA-MRSA disease incidence and severity are diabetics. Diabetics have defects in several aspects of immunity that result in increased infection rates; however, the underlying mechanisms of immune dysfunction are not well defined. Previous studies in this area concluded that infections in diabetics are hyper-inflamed as indicated by increased cytokine production. Conversely, despite high levels of proinflammatory cytokines, our research shows that innate immune cells from diabetics cannot produce inflammatory effectors such as reactive oxygen and nitrogen species (ROS/RNS). ROS/RNS generation by immune cells is essential for killing CA-MRSA and generation of these radicals requires cells to undergo a dramatic metabolic shift that is reminiscent of Warburg metabolism. One effect of this shift is increased NADPH production which is the sole reductant needed for ROS/RNS generation. Using murine infection models and cell culture, we show that Toll-like receptor signaling induces Warburg metabolism through activation of the mammalian target of rapamycin (mTOR). Moreover, we show that mTOR signaling is suppressed in diabetics and results in decreased ROS/RNS generation and worse infection outcomes. Finally, we demonstrate that mTOR inhibition in diabetes is caused by the activation of the AMP-activated protein kinase as a result of energy stress caused by deficient insulin signaling. Thus, diabetics are more prone to CA-MRSA infections because of immune dysfunction caused by aberrant insulin signaling that results in mTOR suppression and diminished antimicrobial effector production.

5:20 p.m.-5:40 p.m.

Role of TLR4 in a Gram-positive Ocular Infection Model

S. Madhumathi Parkunan1, Phillip S. Coburn2, Roger Astley2, Rachel L. Staats3, and Michelle C. Callegan1,2

Department of Microbiology and Immunology1, Department of Ophthalmology, University of Oklahoma Health Sciences Center, Oklahoma City, OK2 and Department of Animal Science, Oklahoma State University, Stillwater, OK3

Bacillus cereus causes an intraocular inflammatory condition of the eye called endophthalmitis. Severe forms of *B. cereus* endophthalmitis lead to vision loss within one day. We previously investigated the mechanisms by which *B. cereus* causes significant intraocular inflammation by analyzing the roles of TLR adaptors (MyD88 and TRIF) in experimental *B. cereus* endophthalmitis. MyD88-/- and TRIF-/- mice had significantly less intraocular inflammation with preserved retinal architecture and greater retinal function compared to C57BL/6J controls. Both MyD88 and TRIF signal for TLR4, a TLR shown to be important in inflammation in Gram-negative infections. TLR4-/- eyes and C57BL/6J control eyes were intravitreally infected with 100 CFU *B. cereus*. Eyes were analyzed for bacterial growth, retinal function by electroretinography, and inflammation by histology and myeloperoxidase and cytokine/chemokine ELISAs. TLR4-/- and C57BL/6J neutrophils were also tested for their ability to phagocytize *B. cereus*. Bacterial loads in TLR4-/- and C57BL/6J eyes were similar ($P \geq 0.05$). Significantly greater retinal function was retained in TLR4-/- eyes compared to controls ($P \leq 0.002$). TLR4-/- eyes had significantly less neutrophil influx ($P \leq 0.005$) and proinflammatory cytokines/chemokines ($P \leq 0.05$) than C57BL/6J eyes at 8 and 12 h postinfection. The ability of TLR4-/- neutrophils to recognize and phagocytize *B. cereus* was similar to that of C57BL/6J neutrophils. Taken together, our results suggest that TLR4 is important in the pathogenesis of *B. cereus* endophthalmitis. Future studies will determine whether *B. cereus* carries a specific ligand for TLR4 or whether an endogenous TLR4 ligand is associated with *B. cereus* intraocular infection.

5:40 p.m.-6:00 p.m.

Cellular Mechanisms of α-Hemolysin-mediated Activation of the NLRP3 Inflammasome

Ejiofor A.D. Ezekwe Jr1, Joseph A. Duncan1,2

Department of Pharmacology1, Department of Medicine, Division of Infectious Diseases2, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Staphylococcus aureus toxin, α-hemolysin, is an important and well-studied virulence factor in staphylococcal infection. It is a soluble monomeric protein that once secreted by the bacterium forms a heptameric pore in the membrane of a broad range of host cell types. Hemolysin was recently discovered to bind and activate a disintergin and metalloprotease 10 (ADAM10). In epithelial and endothelial cells, ADAM10 activation is required for the toxin's activity against these cells. In host monocytic cells, α-hemolysin activates the nucleotide-binding domain and leucine-rich repeat containing gene family, pyrin domain containing 3 (NLRP3) inflammasome leading to production of pro-inflammatory cytokines and cell death. We now show that ADAM10 is critical for α-hemolysin-mediated activation of the NLRP3 inflammasome as siRNA knockdown or chemical blockade of ADAM10-α-hemolysin interaction leads to diminished inflammasome activation and cell death. Unlike epithelial cell and endothelial cell damage, which requires α-hemolysin induced ADAM10 activation, ADAM10 protease activity was not required for NLRP3 inflammasome activation. This work confirms the importance of ADAM10 in immune activation by α-hemolysin but indicates that host cell signal induction by the toxin is different between host cell types.

Session 9: Who's in Charge? (aka Regulatory mechanisms)

10:00 a.m.–10:20 a.m.

ORAL ABSTRACTS

Staphylococcus aureus RNAIII of the agr quorum sensing system stabilizes mgrA mRNA

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Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA 72205

Emerging discoveries and characterization of small, regulatory RNAs in bacteria discloses a new age of gene regulation. Although most bacterial small RNAs act directly to repress the target mRNAs by antisense mechanism, few also stabilize the mRNA to activate the virulence gene expression. *Staphylococcus aureus* MgrA is a global transcriptional regulator which plays a key role in the regulation and expression of many virulence factors. The mgrA gene is transcribed from two promoters, P1 and P2, to produce two mRNA species both with a long 5' untranslated region (UTR) with 123 nt and 301 nt in length, respectively. By deletion analysis, we found the 5' distal region of the mgrA mRNA transcribed from the P2 promoter contributed to the mRNA stability. Genomic search indicated that this region could form a stable complex with RNAIII, the effector of the agr regulatory system, which was subsequently confirmed by genetic analysis, RNA electrophoretic mobility shift assay in vitro and RNA-RNA cross-linking in vivo. The interaction was further corroborated with SHAPE-directed RNA secondary structure prediction, which suggest that it did not involve loop-loop interaction typically associated with RNAIII regulation. We further demonstrated that autolysis and expression of abcA could be regulated by RNAIII mediated through MgrA. Thus, we conclude that MgrA can serve as an intermediary regulator through which agr exerts its regulatory function.

10:20 a.m.-10:40 a.m.

Regulation of Ethanolamine Utilization in *Enterococcus faecalis*

Sruti DebRoy1, Margo Gebbie2, Arati Ramesh3, Jonathan R. Goodson2, Melissa R. Cruz1, Ambro van Hoof1, Wade C. Winkler2, **Danielle A. Garsin1**

1Department of Microbiology and Molecular Genetics, The University of Texas Health Science Center at Houston, Texas 77030, 2Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, Maryland 20742, 3Department of Biochemistry, The University of Texas Southwestern Medical Center, Dallas, Texas 75390

The ability to breakdown ethanolamine, a source carbon and nitrogen, can affect the infectivity of certain human bacterial pathogens including *Enterococcus faecalis.* Adenosyl cobalamine (AdoCbl) is required for the metabolism of ethanolamine (EA) in *E. faecalis,* both as an enzyme co-factor and for the induction of the EA utilization (*eut*) genes. It acts through an AdoCblbinding riboswitch to induce the *eut* genes, but the mechanism of control is incompletely understood. Gene expression also requires EA to activate a two-component system composed of the sensor kinase, EutW, and its cognate response regulator, EutV, by phosphorylation. Active EutV is an antiterminator that binds nascent transcripts by recognizing a dual-hairpin ligand and preventing terminator formation. We found that the AdoCbl-binding riboswitch is part of a small, *trans*-acting RNA. In the absence of AdoCbl, the sRNA imprisons EutV in an inactive complex. When AdoCbl is present, its binding to the riboswitch prevents complex formation between EutV and the sRNA, and EutV is free to induce gene expression. Therefore, riboswitch-mediated control of protein sequestration is described as a mechanism to coordinately regulate gene expression for the first time.

10:40 a.m.-11:00 a.m.

Expression, Purification, and Characterization of Hfqs in *Bacillus anthracis*

Catherine E. Vrentas1, Zonglin Hu1, Rodolfo Ghirlando2, Stephen F. Porcella3, Susan Gottesman4, and Stephen H. Leppla1

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2 Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, Maryland, 20892, USA

3 Genomics Unit, Research Technologies Section, Rocky Mountain Laboratories; NIAID/NIH, Hamilton, MT, 59840, USA.

4 Laboratory of Molecular Biology, National Cancer Institute, NIH, Bethesda, Maryland, 20892, USA

Homohexameric bacterial Hfq proteins regulate gene expression via binding of small RNAs (sRNAs) and/or mRNAs. While the role of Hfq in virulence has been characterized in Gram-negative pathogens, its role in Gram-positive pathogens is less clear. *Bacillus anthracis* is one of only a few species with multiple copies of Hfq expressed from the genome: two chromosomal copies (Hfq1, 2) and one on the pXO1 virulence plasmid (Hfq3). These unusual multiple homologs, combined with our previous discovery of two sRNAs (sRNAs-I and II) inducibly expressed from pXO1 under virulence-inducing conditions, prompted our investigation of the role of Hfqs in *B. anthracis*. Each of the Hfqs was successfully expressed and purified from *E. coli*. Consistent with its sequence conservation with Hfqs from related species, Hfq2 is hexameric; unexpectedly, both Hfq1 and Hfq3 were monomeric. Reversion of sequence variations in Hfq1 did not restore hexameric structure, and analysis of truncated and chimeric Hfq constructs provided additional information about determinants of hexamer formation. Expression of Hfq1 in *E. coli* disrupts Hfq-dependent regulation, consistent with the hypothesis that Hfq1 plays an anti-Hfq role *in vivo*, whereas expression of Hfq3 in Δhfq *E. coli* allowed for partial complementation of the hfq phenotype. Next, the roles of Hfq, sRNA-I, and sRNA-II were examined by RNA-Seq, identifying sRNAs and mRNAs which changed in abundance upon deletion of each gene in *B. anthracis*. Preliminary results of this analysis will be presented to identify potential physiological roles of each gene and delineate the pool of Hfq-regulated sRNAs in *B. anthracis*.

11:00 a.m.-11:20 a.m.

Regulation of agglutination in *Staphylococcus aureus*

Heidi A. Crosby1 and Alexander R. Horswill1

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One of the hallmarks of *S. aureus* is its ability to agglutinate (form clumps) by adhering to fibrinogen, a component of human plasma. This clumping allows *S. aureus* to build vegetations on heart valves, leading to endocarditis. Binding to fibrinogen is mediated by clumping factors A and B (ClfA and ClfB), which are surface proteins covalently attached to the *S. aureus* cell wall. Our laboratory recently demonstrated that clumping of *S. aureus* is regulated by the two-component system ArlRS. An *arlRS* mutant fails to form clumps in the presence of human plasma or fibrinogen, and the mutant is attenuated in a rabbit model of infective endocarditis. Surprisingly, ArlRS does not appear to regulate the expression of *clfA* or *clfB*. Instead, ArlRS represses the expression of *ebh*, which encodes a giant ~1.1 MDa membrane-anchored surface protein. The function of Ebh is unknown, but it appears to block clumping, as deleting *ebh* in an

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arlRS mutant partially restores its ability to clump. Preliminary evidence suggests that ArlRS regulates *ebh* via the global regulator MgrA. ArlRS activates expression of *mgrA*, and our findings indicate that MgrA is a direct repressor of Ebh expression. Similar to an *arlRS* deletion strain, an *mgrA* mutant fails to clump in the presence of human plasma, and clumping can be restored in either strain by overexpressing MgrA. Taken together, these results suggest that ArlR and MgrA both regulate clumping in *S. aureus*, and this regulation is mediated in part by repression of the giant surface protein Ebh.

11:20 a.m.-11:40 a.m.

Streptococcal Phage-like Chromosomal Islands and Impact on Host Phenotype

Scott V. Nguyen, Catherine J. King, Kimberly A. McCullor, Maliha Rahman, and W. Michael McShan

University of Oklahoma Health Sciences Center, Department of Pharmaceutical Sciences, Oklahoma City, OK, USA

Phage-like chromosomal islands have been reported in many Gram-Positive pathogens including the well-known *Staphylococcus aureus* pathogenicity islands (SaPIs). Our laboratory has characterized a *Streptococcus pyogenes* chromosomal island in the M1 genome strain (SpyCIM1) and identified related chromosomal islands in other streptococcal species. Through dynamic excision and integration into the 5' end of *mutL*, SpyCIM1 regulates host gene expression by interruption of a DNA mismatch repair operon. During stationary phase, integration of SpyCIM1 disrupts the operon, separating the upstream promoter from downstream genes *mutL* (mismatch repair), *lmrP* (multidrug efflux pump), *ruvA* (Holliday-junction helicase), and *tag* (base-excision repair). SpyCIM1 excises from the genome during early logarithmic growth, relieving the complex mutator phenotype. Interfering with the SpyCI's ability to excise results in hypermutator cells and poor long-term viability. Although SpyCIs do not carry any known toxins in their genomes such as the toxic shock syndrome toxin-1 in SaPIs, RNASeq analysis and *Galleria mellonella* (common waxworms) *in vivo* experiments show that SpyCI enhances virulence in *S. pyogenes*. Related chromosomal islands integrated in the 5' end of *mutL* in other streptococcal species suggest the dynamic control of the mutator phenotype is a common strategy in these pathogens. This study provides additional insight into the role of the mutator phenotype in an era of emerging antibiotic resistance.

11:40 a.m.-12:00 p.m.

Alternative translational starts at *tcdE* in *Clostridium difficile*

Rebekah Nicols, Leah Fitzwater and **Revathi Govind.**

Kansas State University, Manhattan, KS, USA

Clostridium difficile is the causative agent of antibiotic associated diarrhea and pseudomembranous colitis. The toxigenic *C. difficile* strains that cause disease secrete, toxins A and/or toxin B, which cause colonic injury and inflammation. *C. difficile* toxins A and B have no export signature and are secreted by an unusual mechanism, that involves TcdE, a holin like protein. We have isolated *tcdE* mutant in the epidemic R20291 strain and found that in the absence of TcdE, the toxin secretion was affected. Complementation with the functional TcdE restored toxin secretion in the mutant. In TcdE ORF, we could identify three possible translational starts and each translated form may have a role in TcdE pore formation for controlled toxin release. We have created plasmid constructs that exclusively express only one of these three different TcdE forms and complemented the *tcdE* mutant with these constructs. Western blot analysis of the complemented

Poster # A01

The use of complement-deficient A/J mice to assess vaccine efficacy in an inhalation anthrax model of infection

Jongsam Ahn1, Todd J. Widhelm1, Anthony R. Sambol1, Gregory E. Rutkowski2 and Kenneth W. Bayles1

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Bacillus anthracis is the etiological agent of anthrax and in humans, inhalational anthrax is the deadliest form of the disease, causing death in nearly all untreated individuals. For example, the 2001 anthrax mail attacks led to eleven cases of inhalational anthrax, resulting in five deaths. The FDA licensed anthrax vaccine AVA (anthrax vaccine adsorbed) has proven to be effective and well tolerated in both humans and animals. AVA is produced from a sterile culture filtrate composed primarily of protective antigen (PA; an ~83kDa protein common to both the anthrax lethal toxin and edema toxin) which is adsorbed to a standard aluminum adjuvant. PA when used as a vaccine can elicit broad toxin neutralizing antibody. In addition to PA, AVA also contains small amounts of other *B. anthracis* proteins whose contribution to vaccine efficacy is unknown. The main goals of our research are to characterize the essential molecular components of AVA by generating mutant derivatives of the *B. anthracis* vaccine strain, V770-NP1-R, that are defective in key virulence determinants, generate vaccine preparations, and then test their abilities to elicit a protective immune response in an inhalation anthrax model of infection. To conduct these experiments, we utilized complement-deficient A/J mice, which are susceptible to the avirulent V770-NP1-R strain. To establish this model, we determined 1) the spore dosages needed to deliver known quantities of spores to the mouse lung, 2) the lethal dose needed to kill 50% of infected animals (LD50), and 3) the mean time to death of infected animals. These results suggest that this animal model will be a useful tool for studying the essential components of AVA.

Poster # A02

I*Staphylococcus aureus* susceptibility to polyamines and the mechanism of polyamine transport

Rebecca E. Anthouard, Gauri S. Joshi, Justin R. Clark and Anthony R. Richardson

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major public health concern, resulting in ~20,000 deaths and over $30 billion in health care costs annually. The diminishing efficacy of antibiotics in treating MRSA infections has prompted the search of alternative strategies to kill this highly virulent pathogen. We have previously shown that *S. aureus* is uniquely susceptible to host polyamines and unable to synthesize them *de novo*. Polyamines (e.g. spermine (spm), spermidine (spd), and putrescine (put)) are cationic aliphatic amines produced host cells during the post-inflammatory phase of wound healing after a soft skin tissue infection. We are currently studying 1) polyamine transport in *S. aureus*, and 2) the mechanism(s) of polyamine toxicity in *S. aureus*. We have found that, contrary to other organisms known to import polyamines, *S. aureus* does not use ATPase-dependent transporters to take up polyamines. Cells treated with azide, a pan -ATPase inhibitor, were fully susceptible to Spm toxicity. In contrast, *S. aureus* was fully protected from Spm toxicity when treated with CCCP, which diminishes proton-motive force (PMF). Our preliminary findings suggest that *S. aureus* does not import polyamines through the PotABCD system, but rather Spm enters the cell in a PMF-dependent fashion. We are interested in exploiting the polyamine susceptibility of *S. aureus* to develop novel treatment options for use in combination with antibiotics.

Poster # A03

Identification of a two component system that regulates the *Listeria monocytogenes* secretion chaperones PrsA1 and PrsA2

Laty A. Cahoon*, Francis Alonzo III, and Nancy E. Freitag

strains demonstrated that TcdE to translate mostly from the start codon at 25th and 27th position in the predicted ORF, producing proteins with 142(TcdE142) and 140 (TcdE140) amino acids long, respectively. Among these two forms, TcdE141 over expression lead to cell lysis and not the TcdE140 over expression. Toxin secretion was more efficient in the *tcdE* mutant strain that was complemented with *tcdEWT* (that possibly translates both TcdE isoforms) than the strains that were complemented with either *tcdE140* or *tcdE142* alone.

12:00 p.m.-12:20 p.m.

Temporal and Stochastic Control of *Staphylococcus aureus* Biofilm Development

1Derek E. Moormeier, 1Jeffrey L. Bose, 2Alexander R. Horswill, and 1Kenneth W. Bayles

1Center for Staphylococcal Research, Department of Pathology & Microbiology, University of Nebraska Medical Center, Omaha, Nebraska; 2Department of Microbiology, University of Iowa, Iowa City, Iowa

Biofilm communities contain distinct microniches that result in metabolic heterogeneity and variability in gene expression. Previously, these niches were visualized within *Staphylococcus aureus* biofilms by observing differential expression of the *cid* and *lrg* operons during tower formation. In the current study, we examined early biofilm development and identified two new stages (designated multiplication and exodus) that were associated with changes in matrix composition and a distinct reorganization of the cells as the biofilm matured. The initial attachment and multiplication stages were shown to be protease sensitive but independent of most cell surface-associated proteins. Interestingly, after six hours of growth, an exodus of the biofilm population that followed the transition of the biofilm to DNase I sensitivity was demonstrated. Furthermore, disruption of the gene encoding staphylococcal nuclease (*nuc*) abrogated this exodus event, causing hyper-proliferation of the biofilm and disrupting normal tower development. Immediately prior to the exodus event, *S. aureus* cells carrying a P*nuc*::*gfp* promoter fusion demonstrated Sae-dependent expression, but only in an apparently random subpopulation of cells. In contrast to the existing model for tower development, the results of this study suggest the presence of a Sae-controlled nuclease-mediated exodus of a biofilm subpopulation that is required for the development of tower structures. Furthermore, these studies indicate that the differential expression of *nuc* during biofilm development is subject to stochastic regulatory mechanisms apparently independent of the formation of metabolic microniches.

ICG+P POSTER SESSION "A" ABSTRACTS

MONDAY, OCTOBER 13, 2014
7:30 P.M.-9:30 P.M.

POSTER NUMBERS AND PRESENTERS

A01 Jongsam Ahn
A02 Rebecca Anthouard
A03 Laty Cahoon
A04 Marie-Eve Charbonneau
A05 Michael Federle
A06 Radha Golla
A07 Bryan Hancock
A08 Timothy Hermanas
A09 Hsinyeh Hsieh
A10 Carolyn Ibberson
A11 Megan Kiedrowski
A12 Christina Krute
A13 McKenzie Lehman
A14 Jessica Lister
A15 Ameya Mashruwala
A16 Austin Mogen
A17 Salvatore Nocadello
A18 Eric Ransom
A19 Jason Rosch
A20 Marat Sadykov
A21 Gwenn Skar
A22 Ganesh Sundar
A23 Michelle Swick
A24 Sriram Varahan
A25 Debbie Vidlak
A26 Reid Wilkening
A27 Vijay Yajjala
A28 Lin Zeng

Department of Microbiology and Immunology, University of IL at Chicago

Listeria monocytogenes (*Lm*) is a facultative intracellular pathogen that relies on the regulated secretion and activity of a variety of gene products to promote life within diverse host environments. Our lab recently identified a secreted chaperone, PrsA2, which is dispensable for bacterial growth in broth culture but essential for *Lm* virulence. We hypothesize that during host infection PrsA2 regulates the proper folding, stabilization, and activity of secreted proteins that contribute to bacterial virulence and viability within the host cytosol. PrsA homologs in other species appear to be required for the folding and stability of secreted protein factors at the membrane-cell wall interface and *Lm* has two *prsA* alleles: *prsA1* and *prsA2*. Given that PrsA2 plays a major role in virulence, we characterized a PrsA2 second site suppressor mutation that compensates for some but not all PrsA2 associated phenotypes. Complete genome sequencing of the suppressor mutant revealed an E81V mutation in *lmo1507*, the response regulator of a two component regulatory system for which *lmo1508* is the predicted sensor kinase. DNA microarray analysis and qRT-PCR validation revealed the *lmo1507-1508* regulon which consists of five genes: *lmo442*, *lmo881*, *lmo1506*, *prsA1* and *prsA2*. This is the first established genetic linkage between *prsA1* and *prsA2*. Further, *in silico* analysis has revealed potential Lmo1507 binding sites in the upstream regions of target promoters. We have defined the role of PrsA2 and PrsA1 in *Lm* physiology and pathogenesis and plan to further define the molecular mechanisms that regulate *prsA1* and *prsA2* expression.

Poster # A04

Mechanism of action of a small molecule deubiquitinase inhibitor that promotes macrophage anti-infective capacity

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With increased emergence of antibiotic resistance, alternative strategies to fight infections are urgently required. Recently, the small molecule deubiquitinase (DUB) inhibitor WP1130 was reported as a potential host-targeted anti-infective drug against important human food-borne pathogens, notably the Gram-positive bacterium *Listeria monocytogenes*. Utilization of WP1130 itself is limited by poor solubility, but given the potential of this compound, we initiated an iterative rational design approach to synthesize new derivatives with increased solubility. Our studies identify a promising candidate, compound 9, which reduced intracellular growth of *L. monocytogenes* in macrophages and also showed anti-infective activity against murine norovirus at concentrations that caused minimal cellular toxicity. To define the mechanism of action of this drug, we aimed to determine the target DUBs of compound 9 that mediated the anti-infective activity. We performed a quantitative analysis of ubiquitinated proteins by mass spectrometry in macrophages infected with *L. monocytogenes* to define the pattern of host-proteins ubquitination after treatment with compound 9. We also used a siRNA library targeting DUB enzymes to identify potential deubiquitinases that affect *Listeria* intracellular growth. Potential pathways highlighted by these approaches include the ubiquitin proteasome and the ER -associated degradation pathways. Consistent with this finding, treatment of macrophages with a proteasome inhibitor or ER stress inducer reduced *Listeria* intracellular growth. This small molecule inhibitor may provide a chemical platform for further development of therapeutic deubiquitinase inhibitors with broad-spectrum anti-infective activity and may also be a useful probe to increase our knowledge of the functions of cellular deubiquitinases in cell biology and immunity.

Poster # A05

Disrupting Streptococcal Quorum Sensing Circuits

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Streptococcus pyogenes, a human pathogen, causes a variety of diseases including, but not limited to, pharyngitis, rheumatic fever and necrotizing fasciitis, and it accounts for substantial mortality worldwide. Recent studies indicate that streptococci produce and respond to several secreted peptide signaling molecules (pheromones), including those known as SHPs (short hydrophobic peptides). Upon transport into the bacterial cell, the pheromones bind to and modulate activity of receptor proteins belonging to the Rgg family of transcription factors. In *S. pyogenes*, four Rgg paralogs exist, each serving as transcriptional regulators of genes associated with pathogenesis (RopB), biofilm development (Rgg2 and Rgg3), or a cryptic competence regulon (ComR). Our ongoing aims are to elucidate the mechanisms by which communication is propagated and regulated during the course of bacterial colonization and infection and to identify small molecules that disrupt signaling as a therapeutic strategy to treat or prevent disease. We have conducted genetic, biochemical and mass spectrometry analyses to identify several components of Rgg signaling pathways that includes defining the mature pheromones. We have also begun identifying environmental signals and bacterial enzymes that control the expression and activity of signaling pathways. Finally, we have identified inhibitory compounds that specifically block individual Rgg pathways across several species.

Poster # A06

Screening of the Nebraska Transposon Mutant Library of *Staphylococcus aureus* USA300 for Genes Involved in Resistance to the Action of a Newly Engineered LL-37 Peptide

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The human immune system has evolved to fight microbial infections, while microbial pathogens have co-evolved to evade this system. With increasing resistance to traditional antibiotics, there is an urgent need for new class of antimicrobials. Antimicrobial peptides (AMP) are key defense molecules in humans. At present, over 2400 AMPs, primarily from natural sources, have been registered into the antimicrobial peptide database (*http://aps.unmc.edu/AP*). These peptides are usually cationic and contain less than 50 amino acids. They can attack pathogens rapidly, faster than the replication rate of bacteria. In addition, they typically disrupt bacterial membranes, rendering it difficult for bacteria to develop resistance. Thus, AMPs are promising templates for engineering new antimicrobials. Recently, we have succeeded in engineering human cathelicidin LL-37 into a cell selective, stable, and potent peptide 17BIPHE2. Here we study the effect of this novel peptide on *S. aureus* USA300 at the genetic level. The Nebraska Transposon Mutant Library was

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screened for genes involved in resistance to the action of 17BIPHE2. The transposon library consists of 1,952 strains with each strain containing a single insert within a non-essential gene of *S aureus* USA300. At a sublethal level of the peptide, we have identified 19 mutants whose growth can be inhibited below the minimal inhibitory concentration (MIC) needed to inhibit the wild type strain. The identification of these susceptible genes may help understand the mechanism of bacterial responses and lead to the discovery of more potent antimicrobials.

Poster # A07

Development of Zebrafish Models of Streptococcal Infection to Examine Host Pathogen Interactions and Disease Progression

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Streptococcus agalactiae (Group B *Streptococcus*, GBS) is a Gram-positive pathogen that can causes a variety of disease states including bacteremia, pneumonia, sepsis, and meningitis. We have developed adult and larval zebrafish (*Danio rerio*) models of GBS infection using the highly encapsulated hypervirulent serotype III, ST-17 strain. We observed that GBS infection of adult zebrafish resulted in significant mortality, >90%, as well as cerebral hemorrhage and edema. GBS were isolated from brain tissue suggesting that, as in humans and in murine infection models, GBS may penetrate the blood-brain barrier (BBB) to cause brain infection. Additionally we have developed a zebrafish larvae infection model that allows for the live imaging and tracking of disease progression in real time. GBS infection resulted in larval death in a dose dependent fashion. Virulence was dependent on the presence of GBS capsule, properly anchored LTA and toxin production as infection with GBS mutants lacking these factors resulted in no, or significantly less, mortality. Additionally, following GBS infection of larval zebrafish, proinflammatory cytokines and chemokines including IL1β and CXCL8 were significantly induced compared to uninfected controls. We further infected transgenic zebrafish expressing endothelial specific mCherry with GFP-GBS and subjected fish to live confocal imaging. After 24 hours GBS was observed outside the brain vasculature suggesting GBS penetration of the BBB during the course of infection. By modeling GBS infections in adult and larval zebrafish we hope to identify additional host and bacterial factors that contribute to disease pathogenesis in both fish and humans.

Poster # A08

Presence of a Cyclic-di-GMP Signaling System in Spores of *Bacillus anthracis*

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Bacillus anthracis is a gram-positive spore-forming organism that causes fatal disease in humans, cattle, sheep and goats. *B. anthracis* spore stability, combined with ability to inflict lethal disease, has resulted in its use as a biowarfare agent. The 2001 Amerithrax attacks, utilizing spores concealed in letters, resulted in five deaths and over $320 million dollars spent decontaminating areas of release. The *B. anthracis* spore structure and physiology, especially involving the outermost exosporium layer, are incompletely understood. The exosporium plays a role in spore uptake and intracellular survival in the host. Utilizing transposon mutagenesis, we identified a gene in the Sterne strain, *bas3594*, as important for the proper assembly of the exosporium. The *bas3594* mutant spores exhibited decreased BclA deposition and microarray data revealed an expression profile similar to that of *bclA*, the gene encoding the major surface glycoprotein of the spore. BAS3594 is a GGDEF/EAL-domain-containing protein. GGDEF/EAL domains are typically found in proteins that regulate intracellular levels of the messenger c-di-GMP, which modulates gene function and host immune responses. The *B. anthracis* genome also encodes nine other proteins that contain GGDEF/EAL domains. Expression of mCherry- GGDEF/EAL-protein fusions revealed these putative cyclic di-GMP regulatory proteins are expressed during specific phases of *B. anthracis* growth and sporulation. A subset of these proteins is contained within mature spores and incubation of *B. anthracis* spores with GTP resulted in the generation of c-di-GMP showing evidence diguanylate cyclase activity in mature spores.

Poster # A09

Role of bacteriophage in Diterpenoid A resistant in *Staphylococcus aureus*

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Diterpenoid A is a meroterphene that can be isolated in Eastern Redcedar and other plant species, which exhibited strong antimicrobial activity against Gram positive bacteria in our bioassay study. Moreover, it exhibits strong inhibitory activity against methicillin-resistant *Staphylococcus aureus*, (MRSA). MRSA is a serious worldwide public health problem, especially in health care facilities. Treatment of actively growing *S. aureus* or other Gram-positive bacteria with Diterpenoid A resulted in lysis of the bacterial cells. The cells could be stabilized in hyperosmotic media, suggesting that cell killing resulted from cell wall defects. Previously, two Diterpenoid-A resistant *S. aureus* strains were created by EMS-mutagenesis for studying the mode of action of Diterpenoid A selection in our lab. A bioinformatics analysis on genomic sequences of the mutant strains and the parent strain was performed using BWA alignment and SAMtools following by snpEff to determine the SNPs that might be responsible for resistance to Diterpenoid A. Approximately 40 allele-candidates were identified. Most of them are located in Staphylococcal temperate phage genes, which indicates the bacteriophage-encoded proteins might be responsible for the resistance to Diterpenoid A. One of the phage genes, a putative autolysin, was called among the SNPs located. An autolysis assay was performed with the diterpenoid A-sensitive parent strain and two resistant mutant strains to determine if the mutation in the gene would affect the autolysis of actively growing *S. aureus* strains. Studies are underway to verify the involvement of phage determinants in the resistance process and to characterize the mechanisms involved.

Poster # A10

Examining the Role of Hyaluronidase in MRSA Biofilms

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Staphylococcus aureus is a leading cause of chronic infections such as osteomyelitis, endocarditis, and implant-associated

infections, which often manifest in a biofilm-like state. Bacterial biofilms are communities of cells that are encased in an

Poster # A11

Genetic instability of an *Enterococcus faecalis* σ54 (*rpoN*) mutant during biofilm growth

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Enterococcus faecalis is a leading cause of nosocomial infections and is linked to several chronic, biofilm-mediated diseases. *E. faecalis* is a commensal in the gut, where it remains non-pathogenic unless it translocates into the bloodstream. The gene *rpoN*, encoding alternative sigma factor σ54, was found in a RIVET screen to be highly expressed in the *Manduca sexta* gut. An OG1RF Δ*rpoN* strain was defective in pathogenesis and had increased biofilm formation compared to wild type. Using a colony biofilm assay adapted for *E. faecalis*, the Δ*rpoN* strain displayed a distinctive phenotype where spotted regions and sectors developed. Confocal analysis showed Δ*rpoN* colony biofilms had increased levels of extracellular proteins and altered surface characteristics compared to wild-type biofilms, and Δ*rpoN* colony sectors were structurally distinct. The Δ*rpoN* biofilm phenotype was lost when grown on TSB lacking glucose, while addition of glucose led to increased biofilm growth and altered colony biofilm phenotypes for both wild-type and the Δ*rpoN* strain. Culturing from spots or sectors of Δ*rpoN* biofilms enriched sub-populations that displayed biofilm phenotypes distinct from the Δ*rpoN* parent, suggesting a mutant population is arising in the Δ*rpoN* background during biofilm growth. Subsequent generations of enriched mutant populations showed a decreased ability to form biofilms in microtiter plate assays. Whole-genome sequencing of enriched mutant populations is currently in progress. Determining the genotype of mutants arising in Δ*rpoN* biofilms will provide insight into factors required for fitness of *E. faecalis* in the biofilm environment.

Poster # A12

The Disruption of Prenylation Leads to Pleiotropic Rearrangements in Cellular Behavior and Virulence in *Staphylococcus aureus*

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Prenylation is the addition of prenyl groups to peptide chains or metabolites via the condensation of geranyl- or isopentenyl-diphosphate moieties by geranyltranstransferases. Although this process is extensively studied in eukaryotes, little is known about the influence of prenylation in prokaryotic species. To explore the role of this modification in bacteria, we generated a mutation in the geranyltranstransferase (IspA) of *S. aureus*. Quite strikingly, the *ispA* mutant completely lacked pigment, and exhibited a previously undescribed small colony variant-like phenotype. Further pleiotropic defects in cellular behavior were noted, included impaired growth, decreased ATP production, increased sensitivity to oxidative stress, increased resistance to aminoglycosides and cationic antimicrobial peptides (AMPs), and decreased resistance to cell wall-targeting antibiotics. These latter effects appear to result from differences in envelope composition, as *ispA* mutants have highly defuse cell walls (particularly at the septum), marked alterations in fatty acid composition, and increased membrane fluidity. Importantly, the absence of prenylation also leads to decreased virulence using murine models of systemic infection. Taken together, these data present characterization of prokaryotic prenylation, and demonstrate that this process is central to a wealth of pathways involved in mediating both cellular homeostasis and virulence in *S. aureus*.

Poster # A13

Identification of the Amino Acids Essential for Signal Transduction of the *Staphylococcus aureus* LytSR Two-Component System

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Recent studies have demonstrated that expression of the *Staphylococcus aureus lrgAB* operon is localized within tower structures during biofilm development. To gain a better understanding of the mechanisms underlying this spatial control of *lrgAB* expression, we carried out a detailed analysis of the LytSR two-component system. Specifically, a highly conserved aspartic acid (Asp53) of the LytR response regulator was shown to be the target of phosphorylation, which resulted in enhanced binding to the *lrgAB* promoter and activation of transcription. In addition, we identified His390 of the LytS histidine kinase as the site of autophosphorylation and Asn394 as a critical amino acid involved in phosphatase activity. Interestingly, LytS-independent activation of LytR was observed during planktonic growth, but minimally during biofilm growth where *lrgAB* expression was dependent on autophosphorylation of LytS to trigger the signal transduction cascade. In addition, over activation of LytR (resulting from *lytSN394A* and *lytRD53E* mutations) led to increased *lrgAB* promoter activity during planktonic and biofilm growth. Overall, the results of this study are the first to define the LytSR signal transduction pathway, as well as the metabolic context within biofilm tower structures that trigger these signaling events.

Poster # A14

Identification of eDNA-binding proteins in the *Staphylococcus aureus* biofilm matrix

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Staphylococcus aureus is a commensal organism capable of causing a diverse array of acute and chronic infections. Biofilm formation plays an important role in pathogenesis for many chronic infections by promoting antibiotic tolerance and immune evasion. Both protein and eDNA have previously been identified as major components of the biofilm matrix. However, little research has been conducted on the specific interactions between these factors, despite reports suggesting that DNA-binding proteins may play a role in biofilm formation. To address this, we screened for potential eDNA-binding proteins using Southwestern blotting to detect non-specific DNA-binding activity of secreted and cell-surface associated proteins. Protein bands displaying high DNA-binding activity were analyzed by mass spectrometry. From this screen, we selected Eap and IsaB as candidates for further study. We observed decreased *in vitro* biofilm formation in an *isaB* mutant in some strain backgrounds, but not in an *eap* mutant. A

plausible explanation for this result is that functional redundancy may require the deletion of multiple proteins. However, we observed a clear reduction in matrix-associated eDNA levels in an *eap isaB* double mutant using a modified eDNA isolation protocol. These results suggest that eDNA-binding proteins may play a role in protecting eDNA *in vivo*, thus addressing a concern which has previously been raised regarding the role of eDNA during infection.

Poster # A15

Cellular Respiration as a Trigger for Multicellular Behavior in *Staphylococcus aureus.*

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Staphylococcus aureus is a health concern worldwide and a leading cause of biofilm related infection. The bioavailability of oxygen in the human body and infected tissues varies and we hypothesized that *S. aureus* would sense and respond to the presence of oxygen by altering its multicellular behavior. We find that multiple *S. aureus* clinical isolates form robust biofilms when cultured in the absence of oxygen. Although oxygen is a cell diffusible signal our data suggest that the effect of oxygen on biofilm formation is a result of its role as a terminal electron acceptor (TEA). We find that decreased concentrations of TEA result in an impaired ability of *S. aureus* to respire and this causes the bacterium to switch to a multicellular lifestyle mode. Consistent with this hypothesis we find that supplementation of anaerobic biofilms with nitrate, an alternative TEA, results in reduced biofilm formation. Biofilm formation can be the result of either stochastic or deterministic cellular processes. We find that biofilm formation under conditions of impaired respiration is a deterministic process and the lack of a TEA is sensed by a membrane spanning two-component regulatory system called SrrAB. Our data suggest that respiratory flux through electron transport chains alters the redox status of the cellular quinone pool thereby altering SrrAB activity. Finally, we show that upon sensing the cellular respiratory status SrrAB sets in motion a programmed cell death (PCD) response, which results in the release of extracellular DNA and increased biofilm formation.

Poster # A16

VBacterial Nitric Oxide Synthase and its Contribution to *Staphylococcus aureus* Physiology

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Staphylococcus aureus is a devastating pathogen which can infect most tissue and organ systems. Bacterial nitric oxide synthase (NOS) enzymes produce nitric oxide (NO) and have been implicated in survival during *in vivo* infection as well as resistance to exogenous oxidative stress in several pathogens, including *S. aureus*. However, the contribution of *S. aureus* NOS (saNOS) to cell physiology has not been previously investigated. Growth curves in TSB containing glucose did not reveal differences in optical density (OD600) or CFU/ml between the clinical wild-type strain UAMS-1 and its isogenic *nos*::*erm* mutant. However, the *nos* mutant presented a lower OD phenotype and altered cell morphology when grown in TSB without glucose. Furthermore, UAMS-1 was able to out-compete the *nos* mutant when these two stains were co-cultured in planktonic TSB growth conditions. As observed in previously published studies, our UAMS-1 *nos* mutant displayed increased sensitivity to exogenous H2O2. Additionally, it appears that increased endogenous ROS accumulation occurs in the *nos* mutant compared to wild-type and complement strains. These results suggest that saNOS contributes to *S. aureus* growth by a currently unknown mechanism. On-going and future research efforts are aimed at elucidating the precise role that saNOS plays in *S. aureus* physiology, which could lead to new therapeutic techniques aimed towards combating *S. aureus* infection.

Poster # A17

Structural genomics for surface proteins of Gram-positive pathogen.

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The cell wall of gram-positive bacteria is a fundamental and unique subcellular organelle. Although the primary function of the cell wall is to provide a rigid exoskeleton to resist against mechanical and osmotic stress, it also serves to host proteins that can have a wide range of functions. They include binding of host tissues, binding to specific immune system components, protein processing, nutrient acquisition and inter-bacterial aggregation for the conjugal transfer of DNA. All these functions require the proteins are properly localized on the surface.

Previously, *in silico* analysis was performed in our lab using genomics data. It identified several surface proteins from different gram-positive pathogens such as *Staphylococcus aureus*, *Streptococcus pneumonia*, *Streptococcus agalatie, Listeria monocytogenes*, *Bacillus anthracis* and *Clostridium difficile*. Surface localization of many of those targets has been validated by experimental studies, i.e. surfome analysis, by scientific community in published papers or by direct communication.

To date, 272 structures from Gram-positive bacteria have been solved at Center of Structural Genomics of Infectious Disease (CSGID). 103 of them are from a specific focus area that include protein that are secreted, localized in the outer surface, involved in toxin production or in the cell envelope metabolism. These results represent a huge quantity of data and a starting point for further research and structure aided drug and antigens discovery.

In collaboration with request investigators from scientific community, 3D-structures are under evaluation in order to support and complement the functional characterization of specific protein.

Poster # A18

Use of oxygen-dependent fluorescent proteins in *Clostridium difficile* to determine subcellular protein localization and gene expression

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The Gram-positive bacterium *Clostridium difficile* is a major burden on healthcare systems in developed countries. *C. difficile* is an especially oxygen-sensitive anaerobe, which has discouraged the use of established oxygen-dependent reporters such as GFP. However in a recent publication, we utilized an oxygen-dependent fluorescent protein (cyan fluorescent protein) in *C. difficile* by fixing the cells anaerobically and then exposing the fixed cells to oxygen. Here, we expand upon our initial findings with a newly synthesized red fluorescent protein that was codon-optimized for AT-rich bacteria. Using this variant, gene expression and subcellular protein localization can be determined in *C. difficile*. For example, we found three *C. difficile*-specific proteins (MldABC) of unknown function localized to the mid-cell, suggesting a role in cell division. Mutants of *mldA* or *mldB* exhibit morphological defects, including loss of rod shape (a curved cell phenotype) and inefficient separation of daughter cells (a chaining

phenotype). Furthermore, mutants lacking these proteins were attenuated in the Syrian hamster model of *C. difficile* pathogenesis. Since these proteins are unique to *C. difficile* and play a role in pathogenicity, these newly identified cell division proteins could serve as therapeutic targets. In sum, we believe studying subcellular protein localization and gene expression with oxygen-dependent fluorescent proteins will prove invaluable for research in strict anaerobes such as *C. difficile*.

Poster # A19

Metal Export in Pneumococcus: Roles in Virulence and Antimicrobial Development

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For bacterial pathogens whose sole environmental reservoir is the human host, the acquisition of essential nutrients, particularly transition metals, is a critical aspect of survival due to tight sequestration and limitation strategies deployed to curtail pathogen outgrowth. To oppose the spread of infection, the human host has evolved multiple mechanisms to counter bacterial invasion, including sequestering essential metals away from bacteria and exposing bacteria to lethal concentrations of metals. We have identified a number of metal efflux systems that are vital for pneumococcal infection, including specific transporters for calcium, manganese, and copper. We demonstrate that the copper efflux system is essential during pulmonary infection and the primary role for this transporter is evasion a macrophage-mediated clearance. We further demonstrate a novel mechanism of copper-mediated bactericidal activity, inhibition of aerobic dNTP biosynthesis. Due to the role of such transporters during infection, a high throughput screen was initiated to identify inhibitors of these pathways. Our screening results identified a class of compounds that potentially target these efflux pathways. These data indicate that metal efflux is a critical facet of how the pneumococcus adapts to the host environment and can be targeted as a novel antimicrobial strategy.

Poster # A20

Inactivation of the Pta-AckA pathway in *S. aureus* impairs growth through membrane hyperpolarization

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Staphylococcus aureus is a highly adaptable human pathogen responsible for a variety of infections that range from folliculitis to life-threatening diseases such as severe sepsis, endocarditis and bacteremia. *S. aureus* infections represent an enormous challenge to physicians due to its ability to survive in diverse host environments and the emergence of multidrug-resistant strains. The versatility of this bacterium depends on the proficiency to adjust its metabolism to rapid environmental changes via modulation of expression of genes involved in different metabolic pathways. Our studies demonstrate the indispensable role of the phosphotransacetylase-acetate kinase pathway for *S. aureus* growth and fitness. We showed that inactivation of this major overflow metabolic pathway had a drastic inhibitory effect on growth and caused accumulation of dead cells. Interestingly, inactivation of the Pta-AckA pathway did not cause a decrease in the energy status of bacteria, but it led to a metabolic block at the pyruvate node that enhanced respiration and globally altered the intracellular metabolic status of the bacteria. Investigation of the molecular mechanisms that impair growth and potentiate cell death in the *pta* and *ackA* mutants revealed that the detrimental effects on growth and viability in these mutants were caused by redirection of carbon flux into the TCA cycle that lead to protonation of cytoplasm and causes membrane hyperpolarization. Overall, the results of this study illustrate the intimate relationship between the fundamental aspects of staphylococcal metabolism and the molecular control of cell death and suggest new therapeutic options to combat this important pathogen.

Poster # A21

Immune maturity plays a significant role in the immune response to CNS catheter infection with *S. epidermidis*

Gwenn Skar*, Yenis Gutierrez-Murgas, Matthew Beaver, Jessica Snowden *Presenting

Infants are known to be higher risk of ventriculoperitoneal shunt infection, particularly with *S.epidermidis*. As *S. epidermidis* has relatively limited virulence, we hypothesized that this increased infection risk is due to alterations in the immature response that allow for infection. To evaluate this hypothesis, we adapted our previously published CNS catheter infection model to generate infection with *S. epidermidis* in 14 day old C57BL/6 mouse pups (equating human infants). The pups tolerate the procedure well, with no observed mortality. However, infected pups do experience greater weight loss and worsened clinical illness scores than observed in pups implanted with sterile catheter. In comparison with previously infected adults, the pups have a significantly longer time period of weight loss before they return to normal weight gain patterns. Interestingly, in the immature pups, the infection was more likely to spread from the catheter to the brain parenchyma than we have observed in older mice. Additionally, the younger pups had significantly lower levels of pro-inflammatory mediators CXCL1, CXCL2 and IL6, all of which play a role in recruiting immune cells to the site of infection. As expected, we found a decrease in the number of macrophages and neutrophils recruited to the brain tissue surrounding the catheter in pups as compared to adult mice by flow cytometric analysis. This novel finding could have clinical significance, as the presence of neutrophils is frequently used in children as a marker to determine the presence or absence of infection. There was also a small increase in anti-inflammatory cytokine IL10 noted in the pups implanted with infected catheters on multiplex cytokine analysis and qRT-PCR. Collectively, these findings demonstrate an attenuated inflammatory response in the pups, distinct from that observed in older mice, which may explain the increased parenchymal spread of infection. On-going studies include behavioral studies in the mice to determine long term outcomes of infection on cognitive and motor skills, as these infections in human beings have been associated with IQ loss and other neurologic sequelae. Mice evaluated 2 weeks post-infection, when evidence of illness has resolved and bacterial infection has cleared, have been noted to have abnormal nest building behavior but normal Rotarod performance, suggesting a cognitive but not motor defect that persists following resolution of infection. Better understanding of the immune mechanisms that place infants at higher risk of these infections can be used to guide future screening and adjunctive therapies.

Poster # A22

Impact of a Putative PTS Fructose Transporter on the Physiology and Virulence of the Group A Streptococcus

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POSTER SESSION "A" ABSTRACTS

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Streptococcus pyogenes (Group A Streptococcus, GAS) leads to benign superficial and severe invasive infections in humans. GAS can infect numerous tissue sites, integrating environmental cues to coordinate its virulence program. GAS uses the phosphoenolpyruvate-dependent phosphotransferase system (PTS) to transport carbohydrates for metabolism. This system encompasses the general proteins EI and HPr, and sugar-specific EIIs required for their transport. We previously showed that an M1T1 GAS devoid of the PTS exhibited altered Streptolysin S activity, suggesting that transport of specific carbohydrates might play a significant role in the expression of GAS virulence factors. A transposon mutagenesis screen carried out by our group recently revealed that a putative fructose-specific EII (*fru*A) is essential for GAS survival in whole human blood. Here, we characterized the fructose metabolic operon (*fru*RBA) in the invasive serotype M1T1 GAS strain 5448. The EII *fru*A gene was shown to be transcribed as part of an operon encompassing *fru*B, a 1-phosphofructokinase, and *fru*R, the operon repressor. Growth of a non-polar Δ*fru*A mutant in CDM with fructose but not other PTS sugars as the sole carbon source is severely impaired compared to the parental and complemented strains, validating it as fructose-specific. Using RNA-seq, expression of *fruRBA* was induced during CDM-fructose growth compared to CDMglucose, further supporting this conclusion. Ongoing studies using mutants in *fruR, fruB,* and *fruA* are asssessing their individual roles for fitness and virulence in *ex vivo* and *in vivo* assays that will help to determine the broader impact of this PTS fructose transport system on GAS pathogenesis.

Poster # A23

Bacillus anthracis Camelysin Secretion and Function

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Apart from the anthrax toxins and capsule, *Bacillus anthracis* produces other proteins that are known or postulated to affect host -pathogen interactions. Here we focus on camelysin, an apparent homologue of a *B. cereus* protein reported to have proteolytic activity. The *B. anthracis* camelysin gene, *calY*, is controlled by the transition state regulator SinR, and is highly expressed in cultures during stationary phase. Our previous studies showed that culture supernates of a *calY*-null mutant had elevated levels of InhA1, a metalloprotease associated with *B. anthracis* virulence. We sought to characterize the activity of camelysin and its influence on InhA1 and virulence. Using camelysin-specific antibodies and a cell fractionation assay, we found that camelysin is secreted into the culture medium of *B. anthracis* cells. This result is in contrast to reports for *B. cereus*, where camelysin was found to be cell-associated. Recombinant camelysin, purified from *Bacillus anthracis*, did not cleave casein, gelatin, or purified InhA1. Our data suggest that *B. anthracis* camelysin differs from that produced by *B. cereus*. Despite the lack of activity *in vitro*, camelysin negatively affects both InhA1 and toxin protein abundance in culture supernates, as shown using a *calY*-null mutant. This influence on known virulence factors did not translate to hypervirulence in a mouse model for late-stage disease. *B. anthracis* camelysin may require additional cofactors for proteolytic activity or may control InhA1 levels indirectly. These studies will enhance our understanding of interactions between virulence factors during infection and increase our knowledge of differences between these two pathogens.

Poster # A24

Deletion of a hypothetical protein encoding gene results in hyper biofilm formation in _Enterococcus faecalis_

Sriram Varahan and Lynn E. Hancock

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Enterococcus faecalis is an opportunistic pathogen that has emerged in recent years as one of the leading causes of nosocomial infections. Biofilm formation is considered to be one of the hallmarks of enterococcal biology and allows *E. faecalis* to cause persistent infections in the host such as catheter associated urinary tract infection (CAUTI) and surgical site infections. Several genes have been shown to be important for biofilm development. Here we describe a previously uncharacterized hypothetical protein, EF0073. Deletion of *ef0073* resulted in hyper biofilm formation in comparison to the parental V583. When EF0073 was overexpressed using high copy plasmid system, this resulted in a severe biofilm defect suggesting that this protein might be a repressor of biofilm formation in *E. faecalis*. Scanning electron microscopy analysis revealed the presence of an abundant exo-polymer matrix in the mutant biofilms in comparison to the parental V583 strain. The biofilms formed by the *ef0073* deletion mutant was more resistant to proteinase K treatment compared to the wild-type which is indicative of an altered biofilm matrix composition. Bioinformatic analysis revealed that EF0073 shared weak homology with the RNPP family proteins that regulated gene expression in Gram positives via binding to peptide pheromones. Elucidating the exact mechanism by which EF0073 regulates biofilm formation in *E. faecalis* is the focus of ongoing studies in the laboratory.

Poster # A25

Interleukin-12 is critical for the establishment of _S. aureus_ orthopedic implant infection

Cortney E. Heim1, **Debbie L. Vidlak1**, Tyler D. Scherr1, Curtis W. Hartman2, and Tammy L. Kielian1

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Staphylococcus aureus (*S. aureus*) is a leading cause of prosthetic joint infections typified by biofilm formation. Recent work from our laboratory has identified critical roles for myeloid-derived suppressor cells (MDSCs) in biofilm persistence. Although seemingly counterintuitive based on the suppressive properties of MDSCs, pro-inflammatory signals have been reported to induce MDSC recruitment and activation. Here we report that numerous cytokines (IL-12p40, IL-1β, TNF-α, IL-6, IL-17, and G-CSF) were significantly elevated in a mouse *S. aureus* post-surgical joint infection model, which coincided with significantly increased Ly6GhighLy6C+ MDSC infiltrates concomitant with reduced inflammatory monocyte, macrophage, and neutrophil influx over the one month interval examined. Of particular interest was IL-12, based on its ability to elicit either pro- or anti-inflammatory effects, mediated through p35-p40 heterodimers (p70) or p40 homodimers, respectively. Therefore, we utilized IL-12p40 and IL-12p35 knockout (KO) mice to identify which molecule influences the non-productive immune response during *S. aureus* orthopedic biofilm infection. Ly6GhighLy6C+ MDSC recruitment and inflammatory cytokine/chemokine expression was significantly reduced in both p40 and p35 KO mice concomitant with increased monocyte and neutrophil infiltrates, which correlated with enhanced bacterial clearance. Additionally, adoptive transfer of MDSCs from wild type mice into infected p40 KO animals restored the local biofilm-permissive environment, as evidenced by increased *S. aureus* burdens. Taken together, these findings reveal a critical role for early IL-12p70 production in shaping the evolving anti-inflammatory biofilm milieu by targeting MDSCs.

Poster # A26

Group A Streptococcus quorum sensing and the CovRS system – Identifying new genetic links

POSTER SESSION "A" ABSTRACTS

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Group A Streptococcus (GAS, Streptococcus pyogenes) is a major cause of human disease, resulting in over 600 million cases of streptococcal pharyngitis per year. GAS has many phenotypic states, ranging from a cause of simple pharyngitis to invasive disease. It has been shown that GAS also exists in both an avirulent carrier state and a virulent disease causing state. The mechanism by which GAS maintains these lifestyles is unclear, but the ability to coordinate gene expression across bacterial populations plays an important role. Our lab has identified a novel GAS quorum-sensing (QS) pathway that appears to control a number of genes relating to competence and biofilm formation, as well as capable of sustained selfinduction. Short hydrophobic peptides (SHPs) are produced, secreted and processed. Upon reaching a critical concentration, SHPs are reimported into the cell where they bind their cognate Rgg receptors, which serve as transcription factors, resulting in downstream gene expression. Despite the advances we have made in understanding this QS system, we do not have a clear understanding of how QS in GAS impacts human health. Preliminary animal studies indicate that Rgg pathways play an important role in maintaining colonization, and genetic studies have identified a link to CovRS, a central controller of GAS virulence. We hypothesize that proteases under the control of CovRS are able to alter Rgg2/3 induction via degradation of the SHP pheromones, contributing to evidence that induction of Rgg2/3 may be important to avirulent carriage.

Poster # A27

Innate immune responses to Methicillin-resistant *Staphylococcus aureus* lung infection

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Methicillin-resistant *Staphylococcus aureus* (MRSA), a gram positive bacterial pathogen, has become one of the leading etiological agents for both hospital and community-acquired pneumonia. To determine the pathogenic mechanism, we assessed innate immune response, bacterial clearance and survival rate in C57BL/6 mice after respiratory infection with MRSA strains LACJE2, MW2 and BAA 1695. MRSA infection induced similar levels of neutrophil recruitment regardless of bacterial strains used. However, pro-inflammatory cytokine production, particularly TNF-α and IL-6, was significantly different among groups. Specifically, early (5hr) TNF-α production was enhanced in mice infected with BAA 1695, which was correlated with their significantly reduced bacterial burdens when compared to animals which received LACJE2 or MW2 infection. Conversely, IL-6 production was significantly increased in mice challenged with BAA 1695 or LACJE2, which was consistent with their increased mortality rates when compared to MW2–infected mice. Moreover, a similar association in cytokine response and the severity of MRSA lung infection was observed when LACJE2 WT and mutants were assessed in this mouse model. In future studies, we will determine the possible cause and effect relationship between early cytokine production and the severity of *Staphylococcus aureus* pneumonia. (This work was supported by NIH/NHLBI R01 HL118408)

Poster # A28

Gene regulation in metabolism of amino sugars by *Streptococcus mutans*

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Glucosamine (GlcN) and N-acetyl-D-glucosamine (GlcNAc) are among the most abundant naturally-occurring sugars on the planet, and are catabolized by many bacterial species as a source of both carbon and nitrogen. Recent studies have indicated that metabolism of these amino sugars by the main etiologic agent of dental caries, *Streptococcus mutans,* affects many traits needed for persistence and virulence, including biofilm formation and pH homeostasis. Preliminary analysis of regulation of the genes for amino sugar metabolism by *S. mutans* indicates significant deviation from mechanisms established for paradigm Gram-negative and Gram-positive bacteria. Specifically, a single transcription regulator, NagR, in *S. mutans* controls not only the expression of *glmS,* which encodes a GlcN-6-P synthase that converts fructose-6-P and glutamine into GlcN-6-P for cell wall biogenesis, but also *nagA* (GlcNAc-6-P deacetylase) and *nagB* (GlcN-6-P deaminase), which are required for catabolism of GlcNAc and GlcN. Two NagR-binding elements (*dre*) have each been predicted in the promoter regions of *nagB* and *glmS*. Using a promoter:reporter fusion strategy, we investigated the role of each *dre* in regulating *glmS* and *nagB* promoter activities in the presence of GlcNAc or GlcN. The results revealed a synergistic relationship between the two *dre* elements in *glmS* promoter that required proper spacing, but that was not the case for *nagB*. Furthermore, binding interactions of NagR and DNA fragments from both promoters were strongly influenced by particular sugar-phosphates. Collectively, these results reveal that different modes of interaction of NagR with target promoters is modulated by catabolic intermediates and the positioning of *dre* in relation to the gene promoters.

POSTER SESSION "A" ABSTRACTS

Poster # B01

Streptococcus agalactiae cell envelope regulation by capsular

polysaccharide A (CpsA)

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Streptococcus agalactiae, known as Group B Streptococcus (GBS), is the principal cause of newborn infections. Infants become infected by inhaling contaminated vaginal fluids during delivery or through an ascending infection of the placental membranes. To prevent infection, intravenous antibiotics are administered during delivery to GBS-colonized mothers, or to infants, increasing the risk of antibiotic resistance. The truncated protein GBSCpsAΔLytR is a promising candidate as a non-antibiotic treatment for GBS infections. Expression of GBSCpsAΔLytR in wild type GBS results in a repressive/dominant negative phenotype, with capsule levels severely reduced. The capsule of GBS is an essential virulence factor, allowing host immune system evasion. Previous work demonstrated that the CpsA protein binds the capsule operon promoter. We hypothesized the dominant negative effect may change transcription of the capsule operon. To test this hypothesis, we used an alkaline phosphatase reporter plasmid to measure transcription from the capsule operon promoter in the chromosomal GBSCpsAΔLytR strain compared to the wild type and *ΔcpsA* GBS strains. Preliminary results indicate there is no significant difference in capsule promoter activity between the wild type and *ΔcpsA* strains, while there is a significant difference between the wild type and the GBSCpsAΔLytR strains. This suggests that additional proteins are responsible for activation of transcription at the GBS capsule operon promoter. While a significant difference in capsule promoter activity between the wild type and chromosomal GBSCpsAΔLytR strains occurs, our data suggests the majority of the dominant negative effect on capsule production occurs post-transcriptionally, possibly due to changes in protein-protein interactions.

Poster # B02

Characterization of DNA methylation in _Staphylococcus aureus_

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Staphylococcus aureus is a common human pathogen capable of causing a variety of diseases ranging from minor skin infections to potentially fatal infections such as necrotizing pneumonia and endocarditis. In order to combat the continued evolution of drug resistance in this organism, a detailed understanding of the physiology of *S. aureus* is required. Epigenetic modification of DNA through methylation is utilized by a variety of organisms to provide an additional layer of regulatory information to the standard four nucleotide sequence. The nature and significance of epigenetic modification in *Staphylococcus aureus* remains unknown. Knowledge of DNA methylation in this organism is limited to research conducted on two type I restriction systems. Previous work in *S. aureus* generated predicted methylated motifs for these systems and evaluated methylation primarily through plasmid-based experimentation. In the present study, we examine genome-wide methylation patterns in *S. aureus* by performing single molecule real-time (SMRT) sequencing on a clinically-relevant USA300 strain. Furthermore, we examine the possibility of epigenetic regulation by comparing methylation patterns following exposure to human serum and in strains deficient in quorum sensing. Current evidence suggests little variance in methylation patterns between diverse conditions, indicating an unlikely use of methylation in epigenetic regulation. Thus, DNA methylation appears to function solely as a barrier to the uptake foreign DNA in *Staphylococcus aureus*. Future investigations are underway to evaluate the relevance of detected off-target adenine and cytosine methylation signatures in *S. aureus* genomic DNA.

Poster # B03

Global identification and genome annotation of sRNAs in three strains of _Staphylococcus aureus_

Ronan K. Carroll[1], Andy Weiss[2], Austin B. Morgen[3], Kelly C. Rice[3] and Lindsey N. Shaw[2]

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The use of next-generation DNA sequencing for transcriptomic studies has resulted in increased awareness of the role and importance of non-coding RNA in the bacterial cell. These molecules, commonly referred to as sRNAs (for small RNAs), have diverse functions in the cell, and many, including the agr effector RNAIII, have been shown to play a role in gene regulation. In *Staphylococcus aureus* gene regulation is complex with over 130 transcription factors encoded within the genome. Estimates now suggest that the number of sRNAs encoded within the *S. aureus* genome exceeds that of proteinaceous transcription regulators, underlying the importance of this class of regulators to the cell. Despite their apparent abundance, comprehensive global identification, annotation and analysis of sRNAs has lagged far behind that of protein regulators, in part due to difficulties in their identification and classification. To facilitate a better understanding of the abundance of sRNAs in the *S. aureus* genome we have generated updated genbank files for three commonly used strains (MRSA252, NCTC8325 and USA300 FPR), containing annotations for approx. 130 previously identified sRNAs. The position of each sRNA was determined based on previously published data and RNA-seq alignment files, generated for each of the three strains. The completed files provide a valuable reference for know sRNAs in *S. aureus* which we utilized to perform a global analysis of sRNA expression during growth of USA300 in human serum. This analysis also lead to the discovery of previously unidentified sRNAs that are strongly expressed in human serum.

Poster # B04

***S. aureus* SaeRS facilitates toxicity of a small molecule inhibitor of fermentation.**

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ICG+P POSTER SESSION "B" ABSTRACTS

TUESDAY, OCTOBER 14, 2014
12:30 P.M.-2:30 P.M.

POSTER NUMBERS AND PRESENTERS

B01 Ashley Anderson
B02 Matthew Brown
B03 Ronan Carroll
B04 Jacob Choby
B05 Manisha Diaz
B06 Michael Francis
B07 Melinda Grosser
B08 Theresa Ho
B09 Tramy Hoang
B10 Lauryn Keeler
B11 Brandon Kim
B12 Yoann Le Breton
B13 Ting Lei
B14 David Mains
B15 Biswajit Mishra
B16 Cara Mozola
B17 Alexandra Paharik
B18 Veronica Medrano Romero
B19 Berenice Rosler
B20 Gyan Sahukhal
B21 Joseph Sorg
B22 Eli Sward
B23 David Sychantha
B24 Luis Alberto Vega
B25 Andy Weiss
B26 Ian Windham
B27 Junshu Yang
B28 Sujata Chaudhari

POSTER SESSION "B" ABSTRACTS

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Staphylococcus aureus is responsible for extensive morbidity and mortality worldwide. *S. aureus* causes a variety of infections including soft tissue abscesses and osteomyelitis. At these foci of infection, *S. aureus* must respond to niche conditions, including oxygen tension. In the absence of oxygen or other terminal electron acceptors, *S. aureus* can produce energy by fermentation instead of aerobic respiration. The metabolic switch to fermentation also produces the clinically relevant small colony variant phenotype which is recalcitrant to antibiotic treatment and can be the etiological agent of persistent infections. Our previous work has shown that a small molecule ('882) identified in a high-throughput screen which stimulates endogenous heme biosynthesis, also diminishes fermentative activity and is bacteriostatic to fermenting *S. aureus*. In order to understand the mechanism of toxicity, *S. aureus* strain Newman was screened for spontaneously '882-resistant isolates; whole genome sequencing yielded seven isolates all with lesions in the *saePQRS* locus. SaeRS is a staphylococcal two-component system which regulates toxin production and other critical cellular processes. The Newman SaeS histidine kinase harbors a point mutation which results in constitutive kinase function and is necessary and sufficient for SaeRS-facilitated '882 toxicity. Adding to our understanding of the connection between SaeRS and toxicity, a reverse genetics approach has identified two genes that are SaeRS-regulated and required for '882 toxicity. Taken together, the complementary strategies of forward and reverse genetics have identified numerous genes which will reveal underlying principles of *S. aureus* metabolism and toxicity associated with perturbing fermentation using chemical biology.

Poster # B05

Regulation of AtlA activity in *Enterococcus faecalis*

Manisha R. Diaz and Lynn E. Hancock

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Enterococcus faecalis is one of the leading causes of endocarditis, urinary tract infections, bacteremia, and intra-abdominal infections. In several disease models, *E. faecalis* mediates pathological changes *via* secretion of two co-regulated extracellular proteases, gelatinase (GelE) and serine protease (SprE). *E. faecalis* also encodes a major autolysin (AtlA) with muramidase-2 activity. Autolysins function as regulators of cell death and biofilm development. Purified GelE and SprE exhibit differential proteolytic cleavage specificities for AtlA wherein, GelE cleaves at both the N- and C-terminus and SprE cleaves at only the N-terminus. Previous work from our laboratory has shown that regulation of AtlA by proteolytic cleavage is critical for autolysis and release of eDNA. The present work is an attempt to understand how GelE and SprE control autolysis. To address this, we mapped the N-terminal cleavage sites of the processed forms of AtlA by Edman degradation. A second part of the present work is an attempt to examine cell wall modifications that are thought to affect AtlA activity. In response to lysozyme, a cascade of proteolytic cleavage events leads to the release and activation of the sigma factor SigV from anti-sigma factor RsiV. Our laboratory has shown that an *rsiV* mutant is defective in daughter cell separation, similar to the phenotype of an *atlA* mutant, suggesting that cell wall modifications important in lysozyme resistance might also play a determining role in regulating AtlA activity. Here, we have used standard zymographic analyses to examine the effect of these cell wall modifications on the autolysin activity.

Poster # B06

Identifying the *Clostridium difficile* germinant receptor reveals a novel pathway for spore germination

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Clostridium difficile is an anaerobic, spore forming bacterium that infects antibiotic-treated hosts. Spores are metabolically dormant forms of bacteria that are resistant to harsh environmental conditions, including antibiotics. Though metabolically dormant, spores sense changes in environmental conditions and begin the transition from dormancy to vegetative growth (germination) upon sensing appropriate signals (germinants). Work in our lab has focused on understanding how spore germinants initiate the germination process. In *Bacillus subtilis*, one of the first measurable processes in germination is the release of dipicolinic acid (DPA) from the spore core. DPA release then activates enzymes found in the spore cortex, a thick layer of specialized peptidoglycan which surrounds the DNA-containing core. Cortex degradation must be completed so that a vegetative cell can grow from the germinated spore. Previous work in our lab revealed a novel *C. difficile* germinant receptor, CspC. We predict that this receptor is embedded within the spore cortex. All other known germinant receptors in spore-forming bacteria are localized to the spore's inner membrane; *C. difficile* does not encode the canonical germinant receptors found in other spore-forming bacteria. This implies that there may differences in how *C. difficile* spores germinate, when compared to other spore-forming bacteria (e.g. *B. subtilis*). We hypothesize that activation of the *C. difficile* germinant receptor initiates cortex hydrolysis and this action then allows DPA release from the *C. difficile* core. This proposed mechanism differs from the *B. subtilis* model and suggests a more simplified mechanism of germination for *C. difficile* spores.

Poster # B07

Regulation of nitric oxide resistance in *Staphylococcus aureus*

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Staphylococcus aureus is a leading cause of bacterial sepsis, skin and soft tissue infections, and infective endocarditis worldwide, which is facilitated by its ability to subvert host innate immunity. Unlike other bacteria, *S. aureus* is able to replicate efficiently in the presence of nitric oxide (NO·), an antimicrobial radical produced as part of the innate immune response. We sought to identify regulatory factors essential for the ability of *S. aureus* to respond to and resist NO·. We screened transposon mutants in 113 of 143 predicted transcriptional regulators in *S. aureus* for their ability to grow efficiently in the presence of NO· in broth culture. We identified five regulons essential for full NO· resistance: SrrAB, Fur, CodY, SarA, and Rot. Whereas SrrAB and Fur have previously been implicated as critical regulators during the *S. aureus* response to NO·, SarA, CodY and Rot were not formerly known to have a role in NO· resistance. Interestingly, all three of these proteins are also important virulence regulators in *S. aureus*. Therefore, we next investigated the detailed mechanisms of NO·-sensitivity in the *sarA, codY,* and *rot* mutants. Because the *sarA* mutant is the most NO·-sensitive of the three, we performed RNA-seq to further characterize the SarA regulon under NO·-stress. Altogether, our results provide key insights into the requirements for NO·-resistance in *S. aureus* and demonstrate a significant overlap between regulation of NO·-resistance and virulence.

Poster # B08

Ferric Uptake Regulator Fur control of Putative Iron Acquisition Systems in *Clostridium difficile*.

Theresa Ho

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POSTER SESSION "B" ABSTRACTS

Clostridium difficile is an anaerobic, Gram-positive, spore-forming opportunistic pathogen which causes diarrhea. Although iron acquisition in the host is important for survival of many bacterial pathogens, high levels of intracellular iron can increase oxidative damage. Therefore, expression of iron acquisition mechanisms is tightly controlled by transcriptional regulators such as the iron-responsive Fur repressor in many pathogens. Using targetron mutagenesis we generated a *fur* insertion mutant in *C. difficile*. To determine the genes regulated by *C. difficile* Fur, we used microarray analysis to compare transcriptional differences between the *fur* mutant and the wild type when grown in high iron medium. The *fur* mutant had increased expression of greater than 70 putative transcriptional operons. We have analyzed expression of several of the Fur-regulated genes and verified that they are both iron- and Fur-regulated in *C. difficile*. Included in those genes which were repressed by Fur were several putative cation transport systems, which are homologous to the ATP-Binding Cassette (ABC), Feo or other cation transporters. We also demonstrate that expression of several Fur-regulated putative iron acquisition systems were increased during *C. difficile* infection using the hamster model of infection. Interestingly, while the *C. difficile* genome encodes 3 orthologous FeoAB systems, 2 FeoAB systems were induced in the absence of iron or Fur. Using an iron -transport reporter fusion *E. coli* strain, we demonstrate that the Feo1 system was able to restore iron transport. Our data suggest that Fur controls iron transport in *C. difficile* and the Fur regulon is induced *in vivo* suggesting iron may be limiting during an infection.

Poster # B09

icaADBC is Negatively Regulated by TcaR in _Staphylococcus epidermidis_ 1457

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Staphylococcus epidermidis is a commensal organism commonly isolated from infected surgically implanted medical devices. This leads to persistent infections that are resistant to host immune responses and antimicrobial therapy. The major virulence factor is the ability to form biofilms, which are composed of polysaccharide intercellular adhesin (PIA) made by the products of the *icaADBC* operon. Previous studies in *S. aureus* suggest that *icaADBC* transcription is strongly repressed via IcaR and weakly repressed via TcaR. In contrast, studies in our laboratory found that *icaADBC* transcription and PIA production was not significantly increased in *S. epidermidis* 1457 *icaR::tetM* suggesting that TcaR may strongly repress *icaADBC* transcription in *S. epidermidis*. The purpose of this study was to determine the function of TcaR with regards to *icaADBC* transcription in *S. epidermidis*. *icaADBC* northern and PIA immunoblot analyses were performed with *S. epidermidis* 1457, 1457 *tcaR::dhfr,* 1457 *icaR::tetM* and 1457 *icaR::tetM tcaR::dhfr*. These experiments demonstrated *icaADBC* transcription, and subsequently PIA synthesis, was negatively regulated via TcaR and, to a lesser extent, IcaR. Accordingly, PIA immunoblot demonstrated that 1457 *tcaR::dhfr* and 1457 *icaR::tetM tcaR::dhfr* made significantly more PIA than WT 1457 or 1457 *icaR::tetM.* To study spatial and temporal expression of *icaADBC*, a microfluidic flow cell system was used to generate time-lapse epifluorescence images of biofilm formation. *icaADBC* expression was significantly enhanced in 1457 *tcaR::dhfr* and 1457 *icaR::tetM tcaR::dhfr*. Additionally, 1457 *tcaR::dhfr* and 1457 *icaR::tetM tcaR::dhfr* produced more biofilm in this system than 1457 or 1457 *icaR::tetM*. Our data demonstrate that, in *S. epidermidis* 1457, *icaADBC* transcription, and subsequent PIA synthesis and biofilm formation, is negatively regulated via TcaR instead of IcaR.

Poster # B10

Genomic Analysis and Characterization of Colonizing and Invasive Group B Streptococcal Clinical Isolates

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Many microbes exhibit dual roles within hosts existing both as colonizing normal flora and invasive pathogens. One such organism, *Streptococcus agalactiae* (Group B *Streptococcus*, GBS), is frequently isolated from the gastrointestinal and vaginal tract of 20-30% of healthy adults but is also a leading cause of morbidly and mortality in susceptible hosts including newborns, the elderly and those with underlying immune deficiencies. Currently, the molecular and genetic mechanisms controlling the switch from a colonizing to an invasive pathogen are not well understood. Thus far, the genomes of only three GBS strains isolated from humans and seven strains isolated from other animals have been fully sequenced and completely assembled. We obtained 45 vaginal colonizing isolates collected from pregnant women and five invasive strains isolated from the cerebral spinal fluid (CSF) of newborns. Following isolation of genomic DNA we conducted whole genome sequencing using the illumina Miseq platform. Multilocus sequence typing (MLST) and serotyping was performed using sequence data and PCR. The isolates clustered into 7 clonal complexes, with the majority of isolates being ST-22. Initial genome mapping against a reference strain revealed non-homologous regions that code for many proteins including those with phage, enzymatic, and hypothetical functions. In addition we examined the ability of all GBS isolates to adhere to human vaginal epithelial cells. Preliminary results suggest that increased adherent capability may correlate with the presence and/or absence of specific genes. Continued analysis of the genetic differences between colonizing and invasive isolates may provide insight into the pathogenesis of GBS disease.

Poster # B11

Regulation of Tight Junction Complexes in the Brain Endothelium by Bacterial Meningeal Pathogens

Brandon J. Kim1, Bryan M. Hancock1, Andres Bermudez1, Natasha Del Cid2, Efren A.

Reyes1,2, Brett Hilton1, Cameron Smurthwaite1, Roland Wolkowicz1, David Traver2, Kelly S. Doran1,3

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Bacterial meningitis is the most common serious infection of the central nervous system and a major cause of death and disability worldwide. To cause disease blood-borne bacteria must interact with, and penetrate the blood-brain barrier (BBB). The BBB is comprised of specialized brain microvascular endothelial cells that contain tight junctions, which promote barrier function and maintenance of brain homeostasis. We, and others, have shown that meningeal pathogens are able to disrupt BBB integrity, however little is known about the disruption mechanism. Using Group B Streptococcus (GBS) as a model meningeal pathogen, we found that transcript and protein levels of the major tight junction proteins in brain endothelium were significantly reduced during infection. Furthermore, the global tight junction repressor, Snail1, was upregulated in response to GBS. Using overexpression and knockdown methods, we demonstrated that Snail1 is necessary and sufficient to promote BBB tight junction disruption during infection. Snail1 induction was observed in brain tissue during infection *in vivo* in both mouse and zebrafish models of GBS meningitis. To examine the impact of Snail1 on BBB leakage *in vivo*, we have established an inducible dominant-

POSTER SESSION "B" ABSTRACTS

negative transgenic Snail1a zebrafish. Our results suggest a novel mechanism in which bacterial pathogens may modulate the host transcriptional repressor Snail1 to disrupt tight junctions, promoting BBB permeability and the development of meningitis. Future studies will seek to identify the bacterial factors and host signaling pathways that initiate this process during pathogen infection and disease progression.

Poster # B12

Saturation Mutagenesis and Identification of Core Essential Genes in the Genomes of Two Divergent Group A Streptococcal Isolates using Tn-seq

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Streptococcus pyogenes (Group A Streptococcus, GAS) is a human-restricted pathogen that produces a wide range of diseases, including superficial infections of the skin (impetigo) and throat (pharyngitis) as well as severe invasive infections of sterile sites (necrotizing fasciitis, toxic shock syndrome). Thus, GAS is a major public health concern worldwide and design of effective treatments is desirable. Characterization of all essential genetic determinants in a pathogen's genome is an extremely powerful approach to identify attractive new targets for therapeutic interventions. Although numerous GAS genome sequences are available, functional genome-wide analyses of fitness to better understand GAS pathogenesis have been limited due to available methodologies. In this work, we developed a *mariner*-based system (*Krmit*) to generate complex mutant libraries in two divergent GAS genetic backgrounds: M1T1 5448 (associated with throat and invasive infections) and M49 NZ131 (associated with skin and throat infections). We used transposon deep sequencing (Tn-seq) to precisely locate *Krmit* insertion sites within both GAS libraries. Alignment of *Krmit*-specific reads to the relevant reference genome revealed that the transposon inserted, on average, in one out of three potential insertion sites (TA) present in either GAS genome, resulting in over 50,000 independent mutations. The high level of transposon saturation allowed us to use a Bayesian statistical model to make rigorous predictions about the essentiality of individual genes (ORFs) in both GAS genomes. We identified a set of 298 and 284 genes predicted to be essential for the growth of GAS 5448 and NZ131, respectively, when grown in THY rich media *in vitro* for various lengths of time. Comparison of our datasets to the GAS core genome obtained after alignment of 20 GAS chromosome sequences revealed a total of 88 essential genes common to all these GAS strains. Taking our study as well as results from essentiality screens in *S. sanguinis* and *S. pneumoniae* revealed significant overlaps as well as interesting differences that we will discuss further. To validate our Tn-seq data, we have used conditional expression of identified essential genes to demonstrate their necessity for GAS survival *in vitro*. The new *Krmit* system is now being applied in both *ex vivo* and *in vivo* models of GAS disease to define the genetic determinants required for GAS pathogenesis.

Poster # B13

Global Effects on Transcriptome of the Essential Protein Gcp of *Staphylococcus aureus*

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The conserved protein Gcp is essential for bacteria growth; however, its essential mechanism remains unclear. In this study, we utilized the next generation RNA-seq technology to determine the effect of depletion of Gcp on global gene transcription in *Staphylococcus aureus* and identified many operons that were dramatically affected, providing fundamental information to elucidate essential function of Gcp.

Poster # B14

Identification of *Listeria monocytogenes* genes contributing to oxidative stress resistance

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The Gram-positive bacterium *Listeria monocytogenes* (*Lm*) is a facultative intracellular pathogen capable of subverting host phagocytosis to avoid destruction. Host phagocytes utilize several approaches to neutralize and destroy bacteria within the phagosome, including a respiratory burst that is characterized by the rapid production of reactive oxygen species (ROS) by NADPH oxidase within the maturing phagosome. The generation of ROS with the phagosome serves to neutralize bacterial products and contain bacterial infection. Activation of a transcriptional regulator known as PrfA plays a critical role in *Lm* intracellular survival as PrfA induces the expression of a number of gene products required for phagosome escape and cytosolic replication. Our lab has observed that mutant strains that encode constitutively-activated PrfA (PrfA* strains) are more resistant to hydrogen peroxide-mediated killing than the parent strain 10403S. Using transposon mutagenesis with the *Himar1* transposon introduced into the *prfA** background, we screened a library of over 7,500 *Lm* mutants for sensitivity to hydrogen peroxide *in vitro* and identified several uncharacterized genes encoding products that potentially contribute to oxidative stress resistance. These gene products include Lmo0779, a putative membrane protein with a C-terminal nitrite/sulfite reductase domain; Lmo0553, similar to a magnesium and cobalt efflux protein; and HemL (encoded by *lmo1553*), a protein highly similar to glutamate-1-semialdehyde 2,1-aminotransferase and thought to be necessary in the biosynthesis of hemin. We have confirmed that these mutants are more susceptible to hydrogen peroxide-mediated killing than their parent strain despite similar growth characteristics in medium. This study has thus identified several new *Lm* proteins that may play roles in metal ion homeostasis and in the mitigation of ROS within the host.

Poster # B15

Anti-biofilm and Immune Modulatory Activities of Human Cathelicidin LL-37 Engineered Compounds

Biswajit Mishra,†# Mark L. Hanke,‡ # Kyle Lau,† Tamara Lushnikova,† Cortney E. Heim,‡

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# Equal contribution

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Currently, *Staphylococcus aureus* infections cause a comparable number of deaths as human immunodeficiency virus type 1 (HIV-1). This problem is further worsened due to the formation of biofilms in vivo and emergence of multidrug resistant staphylococcal species. In this poster, we have evaluated the

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potential of newly engineered human LL-37 antimicrobial peptides against planktonic and biofilm forms of methicillin resistant *Staphylococcus aureus* USA300. The peptides were generated by combining peptide library screening with structure-based design. 17BIPHE2, the most potent peptide, was found to be stable to the action of a panel of host and pathogen proteases. The peptide not only possessed the ability to effectively kill MRSA but also other ESKAPE pathogens. However, it displayed no hemolytic activity at the concentration needed to kill MRSA USA300. *In vitro*, 17BIPHE2 was effective in inhibiting bacterial attachment, biofilm formation, and could destroy established biofilms. Importantly, the peptide also prevented biofilm formation in a mouse model of catheter-associated infection. In addition, it also enhanced the innate immune response by augmenting CCL2 (MCP-1) and CXCL10 (IP-10) chemokine expression. Having been endowed with all these desired properties we propose this molecule has immense potential for future drug development against infections caused by *S. aureus* USA300 or other ESKAPE pathogens.

Poster # B16

A Novel Cholesterol-Insensitive Mode of Membrane Binding by Streptolysin O Promotes Cytolysin-Mediated Translocation of the Co-Toxin SPN

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Cytolysin-mediated translocation (CMT), performed by *Streptococcus pyogenes,* utilizes the cholesterol-dependent cytolysin Streptolysin O (SLO) to translocate the NAD+-glycohydrolase SPN into the host cell during infection, causing cytotoxicity. SLO is required for CMT and can accomplish this activity without pore formation, but the details of SLO's interaction with the membrane preceding SPN translocation are unknown. Analysis of binding domain mutants of SLO and binding domain swaps between SLO and homologous cholesterol-dependent cytolysins revealed that membrane binding by SLO is necessary but not sufficient for CMT, demonstrating a specific requirement for SLO in this process. Despite being the only known receptor for SLO, this membrane interaction does not require cholesterol. Depletion of cholesterol from host membranes and mutation of SLO's cholesterol recognition motif abolished pore formation but did not inhibit membrane binding or CMT activity. Surprisingly, SLO requires the co-expression and membrane localization of SPN to achieve cholesterol-insensitive membrane binding; in the absence of SPN, SLO's binding is characteristically cholesterol-dependent. This SPN-dependent mode of binding can be distinguished from the canonical cholesterol-dependent mode by differential extraction with the detergent saponin, suggesting SPN mediates SLO's localization to a separate fraction of the plasma membrane or modulates its level of insertion into the membrane. SPN's membrane localization also requires SLO, and mutants of SLO that cannot perform CMT do not permit SPN-membrane binding. These data suggest that a co-dependent, cholesterol-independent mode of binding to an alternative receptor results in SPN translocation. This represents a novel mechanism of membrane binding by a cholesterol-dependent cytolysin.

Poster # B17

Role and Regulation of Extracellular Proteases in *Staphylococcus epidermidis* Biofilm Formation

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2. University of Nebraska Medical Center Department of Pathology and Microbiology Omaha, NE USA

Staphylococcus epidermidis is a human commensal organism that is responsible for a significant portion of healthcare-associated infections (HAI). It is the most frequent cause of central line-associated bloodstream infections and the second most frequent cause of surgical site infections. The major *S. epidermidis* virulence determinant is its ability to form a biofilm. Although the polysaccharide intercellular adhesin (PIA) has been thoroughly investigated as a mediator of *S. epidermidis* biofilm development, multiple studies have reported that 30-70% of clinical isolates lack the genes for PIA synthesis. In PIA-negative strains, a cell surface-bound adhesin called Aap (accumulation-associated protein) is required for biofilm formation. Proteolytic processing of Aap is required for this function, but the *S. epidermidis* mechanism to process Aap is not clear. Using a microtiter plate assay, we found that mutants of the proteases Ecp and SepA in PIAnegative *S. epidermidis* exhibit diminished biofilm formation ($p < 0.05$). Further, exogenous addition of proteases to *S. epidermidis* cultures results in dramatically increased biofilm formation in an Aap-dependent manner. We have demonstrated by microarray and activity assays that Ecp is positively regulated by the staphylococcal quorum-sensing system Agr. These findings indicate that secreted proteases are critical for *S. epidermidis* PIA-independent biofilm formation. Further, the results suggest that the Agr system is required for *S. epidermidis* biofilm maturation, a phenomenon that has not been observed in *S. aureus.* On the whole, this study identifies a novel role for the Agr system and secreted proteases in promoting staphylococcal biofilm development.

Poster # B18

Involvement of the transcription factor ComK in *Staphylococcus aureus* competence regulation

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Natural genetic transformation is one of the ways by which bacteria acquire exogenous DNA and competence is the specific state developed to undergo transformation. Transformation was first discovered in Gram-positive bacteria, (*Streptococcus pneumoniae)* and has also been described for Gram-negative bacteria and Archaea. The machinery for DNA-uptake is similar among bacteria but the competence regulatory circuits seem to be species-specific. Among Gram-positive bacteria, there are two different and evolutionary distinct central competence regulators, the alternative sigma factor *sigX* and the transcription factor *comK*. Some species, like *Bacillus subtilis, Staphylococcus aureus* and *Listeria monocytogenes,* have both regulators. However, only in *B. subtilis* their function has been clearly and extensively studied; SigH (an alternative sigma factor that is evolutionary related to *sigX)* and ComK are the main regulators that activate the development of sporulation and competence, respectively. Our group has firstly established that the competence state in *Staphylococcus aureus* is induced by the expression of *sigH,* but the function of ComK remains unknown. The aim of this study is to identify if ComK is involved at any step of the competence development pathway for natural transformation in *Staphylococus aureus.*

Poster # B19

Host immunity involved in modulation of Group B Streptococcal vaginal colonization

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POSTER SESSION "B" ABSTRACTS

Doran1.

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Streptococcus agalactiae (Group B *Streptococcus*, GBS) is a Gram-positive bacterium that colonizes the cervicovaginal tract in 20-30% of healthy women. Colonization is asymptomatic, however during pregnancy, GBS can be vertically transmitted to newborns peripartum, causing pneumonia, sepsis or meningitis. Current prophylaxis, consisting of late gestation screening and intrapartum antibiotics, has failed to completely prevent transmission, and GBS remains the leading cause of bacterial neonatal meningitis in the United States. Gaining insight into factors controlling vaginal colonization is essential for developing novel therapeutics to limit maternal GBS carriage and prevent transmission to the vulnerable newborn. Using human cervical and vaginal cell lines, and our established mouse model of GBS vaginal colonization, we have begun to characterize key host factors that impact vaginal colonization. *In vitro*, we have observed increased transcript and protein levels of cytokines involved in innate and adaptive immunity, such as IL-1β, IL-6 and IL-36γ, after challenge with different GBS serotypes. Our work identified that a specific GBS strain, CJB111, exhibits increased persistence in the murine vaginal tract. Interestingly, it also adheres and invades cervical cells more readily than other strains, suggesting that certain GBS strains could use cervical cells as a reservoir to establish long-term colonization. Prolonged persistence might allow for adaptive immune responses. Correspondingly, we have detected increased IL-17 production in mice colonized with CJB111 compared to other GBS strains. Further, addition of exogenous IL-17 accelerated clearance of CJB111. We conclude that both host immune responses and bacterial strain differences are crucial in modulating GBS vaginal colonization.

Poster # B20

Regulation of Biofilm Development by the *msaABCR* operon

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Community-acquired, methicillin-resistant *Staphylococcus aureus* strains are causing severe infections among healthy individuals. The complex regulatory networks used by these strains to control virulence and biofilm development are still poorly understood. We have defined a new operon, *msaABCR* that includes two non-coding RNAs (*msaA* and *msaC*) and a regulatory anti-sense RNA (*msaR*), which play an important role biofilm development and virulence in *S. aureus*. In this study we have investigated the regulatory role of the *msaABCR* operon in biofilm development in CA-MRSA strain USA300 LAC. Confocal Microscopy after live dead staining with syto-9 and toto-3 of 48 hr biofilm, and analysis using COMSTAT showed the presence of increased cell death, underdeveloped biofilm in the *msaABCR* operon deletion mutant. Production of increased extracellular DNA (eDNA) in the mutant further support the increased cell death within the biofilm. Further study showed that increased protease activity and increased processing of major autolysin (Atl) contribute to the biofilm defect. We have also studied the ability of *msaABCR* deletion mutant to form biofilm *in vivo* using a catheter based murein model with and without treatment with daptomycin. The *msaABCR* deletion mutant is attenuated by 91.53% compared to wild type, whereas the *sarA* mutant was attenuated only 54.7% which was used as control. The daptomycin treatment showed 94.6 % clearance in the deletion mutant compared to wild type, whereas the *sarA* mutant showed 97.8% clearance. In conclusion, this study shows that the *msaABCR* operon regulates biofilm formation *in vitro* and *in vivo* and may be exploited as a therapeutic target for the treatment of staphylococcal biofilm associated infection.

Poster # B21

Characterizing the impact of germinant receptor levels on *Clostridium difficile* spore germination

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Clostridium difficile has become a major nosocomial pathogen and a burden on the healthcare systems in many countries. The survival of this strict anaerobe outside a host is dependent on the ability to form a dormant spore. Metabolically dormant spores persist in the aerobic environment and are resistant to many harsh conditions, including antibiotics. In a host, spores must germinate to actively growing bacteria to cause disease (the toxins necessary for disease on not deposited in or on the spore form). Key to the germination of *C. difficile* spores are host-derived bile acids. Host bile acids are divided, generally, into two classes: cholic acid (CA) derivatives and chenodeoxycholic acid (CDCA) derivatives. CA is a germinant for *C. difficile* spores while CDCA inhibits CA-mediated germination. While many strains require bile acids to germinate, some have been recently described to germinate in rich medium alone, suggesting heterogeneity in germination responses among different *C. difficile* isolates. Previously, we identified the *C. difficile* bile acid germinant receptor, CspC, a subtilisin-like and catalytically-dead protease. To begin to understand the differences germination responses between *C. difficile* isolates, we determined the levels of CspC in several isolates and compared how these differences translated to rates of *C. difficile* spore germination and apparent affinities of the bile acids for the *C. difficile* spore.

Poster # B22

The SaeR/S Gene Regulatory System Regulates Pro-Inflammatory Cytokine TNF- α during *Staphylococcus aureus* Infection

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Staphylococcus aureus (*S. aureus*) is a Gram-positive bacterium that was once predominantly isolated from hospitalized patients but is now prevalent in communities. Once infected with *S. aureus* it can induce symptoms ranging from mild skin infections to fatal sepsis syndromes. Although our understating regarding the pathogenesis of this pathogen is advancing there is still a necessity to understand the relationship between *S. aureus* and the host immune system. This study primarily focuses on the production of tumor necrosis factor-alpha (TNF-α) in response to *S. aureus* infection. TNF-α is a pro-inflammatory cytokine produced by monocytes, T lymphocytes, and to a lesser extent neutrophils. Preliminary data suggests that there is an influential role for the SaeR/S two-component gene regulatory system of *S. aureus* in modulating TNF-α production. *In vitro* studies using isolated human monocytes synchronized with either wild-type (wt) or an isogenic *saeR/S* deletion mutant (Δ*saeR/S*) of S. aureus demonstrated a significant difference in TNF-α production, with Δ*saeR/S* having at least a 5-fold increase in TNF-α production by monocytes. Furthermore, the difference in TNF-α production between wt and Δ*saeR/S* was sustained for a pro-longed period of time; this suggests that the two-component SaeR/S gene regulatory system has an influential regulatory role in the production of TNF-α by monocytes. Other in vitro studies including: human whole blood assays, and isolated neutrophil assays, indicate that the regulation of TNF-α is an essential characteristic of *S. aureus* pathogenesis. These preliminary data suggest that recombinant TNF-α could be used as a therapeutic agent to help resolve the virulence of *S. aureus* in infected patients. Taken together, this study provides insights into specific mechanisms used by S. *aureus* during staphylococcal disease and characterizes a relationship between a bacterial global

regulator of virulence, SaeR/S, and the production of the pro-inflammatory cytokine TNF- α by the host immune system.

Poster # B23

Characterization of peptidoglycan *O*-acetyltransferase OatA from *Streptococcus pneumoniae*

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Variations in the chemical structure of peptidoglycan (PG) such as stem peptide branching and O-acetylation contribute to penicillin resistance in *Streptococcus pneumoniae*. Deletion of the PG O-acetyltransferase *oatA* (*adr*) which is involved in the C6 O-acetylation of *N*acetylmuramic (MurNAc) acid has been shown to decrease the inherent minimum inhibitory concentration of penicillin and increased sensitivity to lysozyme *in vitro.* OatA is a bi-modular protein that contains an N-terminal transmembrane domain and a C-terminal extracellular catalytic domain. We have cloned *oatA* coding for its C-terminal domain (ΔOatA, residues 423-602) in frame with an N-terminal His6-tag which was produced in a soluble form when expressed in *Escherchia coli.* To better understand its substrate specificity and mechanism of action, kinetic analyses were performed using the artificial acetyl-donor *p*-nitrophenyl acetate and polysaccharide acceptors of varying length. ΔOatA is able to use β-1,4-*N*acetylglucosamine polymers (GlcNAcn) as acetyl-acceptors and it exhibits a strong preference towards longer chains. ESI-MS2 analyses of ΔOatA-catalyzed reaction products show that when using GlcNAc4 as an acetyl-acceptor the enzyme prefers to modify the terminal non-reducing residues. Interestingly, OatA is unable to O-acetylate the synthetic muropeptide GlcNAc- MurNAc-L-Ala-D-isoGln suggesting a preference toward long nascent or mature glycan strands. The biochemical characterization presented shows that ΔOatA is an enzymatically active domain and may be exploited for the identification of novel inhibitors for targeting penicillin resistant pneumococci.

Poster # B24

Mapping Genetic Determinants Important to Group A Streptococcus Interaction with Phagocytes

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Dept. of Cell Biology and Molecular Genetics & Maryland Pathogen Research Institute, University of Maryland, College Park, MD 20742

Group A Streptococcus (GAS) is a significant human-restricted pathogen causing a wide array of acute and fatal diseases. In colonizing various tissue sites, GAS simultaneously adapts to nutrient availability in its environment and evades immune responses, including killing by host neutrophils, which appear to play a prominent role in the development of invasive streptococcal infections. Recent work in our lab revealed an important connection between the metabolic sensing pathway of the phosphoenolpyruvate phosphotransferase system (PTS) and the Mga regulatory network controlling virulence gene expression. Further characterization is necessary to elucidate whether PTS-signaling affects GAS interaction with host immune responses. Although multiple virulence factors have been identified as required for GAS to evade or survive within phagocytes, a comprehensive examination of their genome-wide genetic interactions has not been performed. We seek to map PTS-dependent virulence gene regulatory networks involved in host immune evasion in GAS by combining existing neutrophil killing assays with transposon deep sequencing (Tn-seq) of complex mutant libraries generated in divergent GAS genetic backgrounds using a *mariner*-based transposon system (*Krmit*). Thusly, we aim to identify factors essential for GAS to evade killing by host neutrophils and infer their genetic interactions. Preliminary data indicates a functional PTS is required by GAS for surviving neutrophil killing; mutants in an operon encoding a fructose-specific PTS transporter, identified as necessary for growth in blood, display aberrant neutrophil survival phenotypes. Ultimately, we hope to utilize this approach to further dissect mechanisms linking PTS signaling with virulence via Mga and other potential PTS-dependent virulence regulators.

Poster # B25

The δ subunit of RNA polymerase guides promoter selectivity and virulence in *Staphylococcus aureus*

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In Gram-positive bacteria, and particularly Firmicutes, the DNA-dependent RNA polymerase (RNAP) complex contains an additional subunit, termed the δ factor, or RpoE. This enigmatic protein has been studied for over 30 years in various organisms, but its function is still not well understood. In this study we investigate its role in the major human pathogen *Staphylococcus aureus*. We show conservation of important structural regions of RpoE in *S. aureus* and other species, and demonstrate binding to core-RNAP that is mediate by the β and/or β' subunits. To identify the impact of the δ subunit on transcription we performed RNA-seq analysis, and observed 191 differentially expressed genes in the *rpoE* mutant. Ontological analysis revealed, quite strikingly, that many of the downregulated genes were known virulence factors, whilst several mobile genetic elements (SaPI5 and prophage ΦSA3usa) were strongly upregulated. Phenotypically, the *rpoE* mutant had decreased accumulation and/or activity of a number of key virulence factors, including α-toxin, secreted proteases and PVL. We further observed significantly decreased survival of the mutant in whole human blood, increased phagocytosis by human leukocytes, and impaired virulence in a murine model of infection. Collectively, our results demonstrate that the δ subunit of RNAP is a critical component of the *S. aureus* transcription machinery and plays an important role during infection.

Poster # B26

Identification of the CidR binding site in the promoters of the *cidABC* and *alsSD* operons

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S. aureus is an important human pathogen, able to persist in humans with its ability to form biofilms. An important component of the biofilm matrix is DNA released by lysed bacterial cells. *S. aureus* biofilms release DNA in a controlled manner, through the lysis of a subpopulation of cells in the biofilm via programmed cell death (PCD). Involved in PCD are the *cidABC* and *alsSD* operons, which have been shown to be positive and negative regulators of cell death and lysis, respectively. The current study has focused on CidR-mediated control of *cidABC* and *alsSD* expression and the interactions of this regulator with the promoter regions of these operons. The strategy used involved the creation of transcriptional *lacZ* fusion plasmids to assess the effects of truncations and site-directed mutations in putative CidR-binding sites within the *cidABC* and *alsSD* promoter regions. Cells were grown under inducing conditions, harvested and assayed for β-galactasidase activity as a measure of promoter activity. The results demonstrate that important *cis*-acting regulatory elements controlling *cid* expression lie between -93 to -58 bp in the *cid*

promoter. This region overlaps a 12-bp element that is conserved in the *cidABC* and *alsSD* promoter regions. In support of the importance of these sites in the regulation of promoter activity, electrophoretic mobility-shift assays demonstrated a CidR-dependent shift in a 60-bp DNA fragment containing this element. Combined, the results of this study demonstrate the existence of a conserved cis-acting element that is the target for CidR-mediated control of *cidABC* and *alsSD* expression.

Poster # B27

The Novel Regulator SA1804 Mediates the Virulence of *Staphylococcus aureus*

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Staphylococcus aureus is major hospital- and community-associated pathogen and is responsible for thousands of invasive infections annually. The ability of this organism to cause infectious is due to its expression of virulence factors, which are tightly controlled by a variety of regulators, including an important two-component signal transduction regulatory system, SaeRS. We have undertaken a transcriptomics study for the identification of SaeRS regulon and found that inactivation of SaeRS dramatically enhances the transcription of a novel transcriptional regulator (SA1804). This led us to hypothesize that SaeRS mediates the expression of virulence factors, including toxins and adhesins through its regulation of *sa1804*. To test this hypothesis, we created *sa1804, saeRS, and sa1804/saeRS double* deletion mutants in a USA300 community-acquired MRSA strain, 923, and determined their impact on the pathogenicity. The deletion of *sa1804* dramatically increased the production of toxins and cytotoxicity, and enhanced the capacity of bacteria to adhere to and invade into the epithelial cells, whereas the deletion of *saeRS* eliminated the expression of toxins and cytotoxicity, and abolished the bacterial ability to adhere to and invade into the epithelial cells. Moreover, the double deletions of *sa1804* and *saeRS* appeared a similar phenotype with the *saeRS* null mutation. Furthermore, we determined the regulatory mechanism of SA1804 using qPCR, promoter-lux reporter and gel-shift approaches. Our data indicate that the novel virulence repressor SA1804 is dependent on SaeSR regulator. This study sheds light on the regulatory mechanism of virulence factors and allows for us further elucidate the molecular pathogenesis of *S. aureus*.

Poster # B28

The alternate sigma factor B promotes staphylococcal fitness by optimizing intracellular carbon economy

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The pleiotropy associated with the staphylococcal alternate sigma factor B (SigB) regulon imparts a significant challenge in understanding its native physiological role. However, in this study we advance a previously unappreciated role for this regulator in optimizing intracellular carbon economy. We show that during growth under conditions of excess glucose, inactivation of *sigB* leads to increased flux of intracellular carbon through overflow metabolic pathways. Consequently, a competitive fitness defect was observed in the Δ*sigB* mutant relative to wildtype under both planktonic and biofilm growth modes. As the fitness defect of the Δ*sigB* mutant was not evident during growth in the absence of glucose or following inactivation of *cidC* in the Δ*sigB* mutant background, a significant role for acetate in influencing fitness of the former mutant is surmised. Finally using a mouse osteomyelitis model, we show that the CidC-dependent fitness defect of the Δ*sigB* mutant is also evident during the chronic biofilm infection process. These observations not only have important implications for staphylococcal pathogenesis but may also help explain the often confounding attenuated *in vivo* phenotype of the *sigB* mutant in spite of its ability to produce excess virulence factors.

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Step outside the front doors of the Embassy Suites Omaha Downtown/Old Market, and you will be immersed in the Old Market neighborhood with its myriad of restaurants, pubs, shopping, art galleries and museums including the Bemis Center for Contemporary Arts and the Durham Museum. Most restaurants in the Old Market are causal dining and do not require reservations.

Downtown

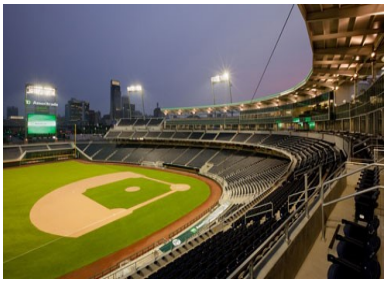
West of the Old Market, is the heart of downtown Omaha with its primary business towers: Woodman of the World Tower, First National Bank Tower, Union Pacific Center, and several other commercial and residential towers. The Orpheum Theater is a historic entertainment venue of Omaha that continues to bring in top productions from across the globe. The Omaha Children's Museum and the Rose Theater provide wonderful adventures for our young and young-at-heart visitors.

Just north of the hotel, Omaha opens up to several walking opportunities such as a quick stroll to the Gene Leahy Pedestrian Mall flanked by the Holland Center for Performing Arts. One can wander through sculptures of a wagon train or wild bison or geese at First National's Spirit of Nebraska's Wilderness and Pioneer Courage Park. Further west, but still is easy walking distance from downtown, is the Joslyn Art Museum and the Omaha Civic Auditorium.

A slightly longer venture leads east from the hotel to the Missouri riverfront which encompasses the Heartland of America Park and Fountain adjacent to the ConAgra campus; Lewis & Clark Landing; and the Bob Kerrey Pedestrian Bridge that connects Omaha with Council Bluffs, which is home to three major casinos.

North Downtown District

Omaha's North Downtown district hosts several key sporting venues, including TD Ameritrade Park, home of baseball's NCAA College World Series; Creighton University soccer at Morrison Stadium; and hockey at CenturyLink Center Omaha, the latter puts on major concerts and conventions for the city. This neighborhood also supports entertainment and shopping including Film Streams and Hot Shops Art Center.



South Omaha District and Midtown Crossing

A little further afield are several not-to-be-missed tourist destinations around Omaha. South of downtown Omaha is the world renowned Omaha's Henry Doorly Zoo, Lauritzen Gardens and Kenefick Park, El Museo Latino, and Sokol Auditorium. Westward, between downtown Omaha and the campus of the University of Nebraska Medical Center is Midtown Crossing, a newly regenerated neighborhood with outstanding dining, shopping, and entertainment opportunities.