SCHOOL OF MEDICINE & DENTISTRY
UNIVERSITY of ROCHESTER MEDICAL CENTER

2019 PREP Symposium

Auditorium K-207 (3-2408)

June 12, 2019

Please join us for refreshments immediately following the presentations in CEL Room 2-7520

Post-baccalaureate Research and Education Program

Program Directors:

Jacques Robert, PhD & Elaine M. Smolock, PhD

2:00pm-2:15pm		**Introduction**
2:15pm-2:30pm	**Samantha Edelen**	**Interaction of endopeptidase RipA and PBP PonA2 and implications for cell wall stability of Mycobacteria** ***Advisor: Martin Pavelka, PhD***
2:30pm-2:45pm	**Vania Lopez Ruiz**	**The role of endothelial cell metabolism in the context of acute lung injury** ***Advisor: Fabeha Fazal, PhD***
2:45pm-3:00pm	**Seble Negatu**	**Understanding T cell activation at sites of inflammation: myeloid cells nucleate T_h1 cells by antigen presentation and chemokine production** ***Advisor: Deborah Fowell, PhD***
3:00pm-3:15pm	**Christie Gilbert**	**Elucidating mechanisms of regulation for the LFR islet in *Staphylococcus aureus* clonal complex 30** ***Advisor: Steven Gill, PhD***

Thank you to everyone involved in PREP!

Mentors, Bench Mentors, & Committee Members

PREP Council Leader:

Dr. Fatima Rivera-Escalera

Skills Workshop Leaders:

Nicholas Battaglia, Monique Mendes, Emily Weber, & Alexis Zavez

Administrative Assistance:

Jennifer Brennan, Daisy Bird Geer, & Benjamin Lovell

Life Sciences Learning Center:

Danielle Alcena, Ph.D. & Dina Markowitz, Ph.D.

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Examining Indoleamine 2,3-dioxygenase Expression in the Human Placenta

Aria Alexander[1], Sarah Latchney[2], Martha Susiarjo[2], and Shawn Murphy [3]

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Successful pregnancies require the maternal immune system to establish fetal tolerance and to defend against infection, but the exact immunological mechanisms used to achieve this balance are still unknown. A pair of homologous enzymes, indoleamine 2,3-dioxygenase (IDO) 1 and 2 have been suggested to be potential modulators of the maternal immune system during pregnancy. Rate-limiting enzymes in tryptophan catabolism, IDO1 and IDO2 are expressed in the placenta, the major organ at the maternal-fetal interface. By catabolizing tryptophan, they are able to starve pathogens and contribute to noninfectious periods. Furthermore, once IDO1 and IDO2 degrade tryptophan, they produce downstream metabolites that promote regulatory T cell expansion and suppression of effector immune cells. While it is known that both enzymes are expressed in the human placenta upon delivery, their expression patterns in the human placenta throughout gestation still have not been thoroughly described. Through collection of placental tissue at University of Rochester Medical Center, we saw different patterns of expression of IDO1 and IDO2 during gestation. These differences suggest they may have distinct roles in the placenta during gestation.

Interaction of endopeptidase RipA and PBP PonA2 and implications for cell wall stability of Mycobacteria

Samantha L. Edelen, Daniel J. Martinelli, Maria-Magdalena Patru, and Martin S. Pavelka

Tuberculosis (TB) remains a top killer worldwide and continues to exist within an estimated 1 in 3 people as a latent infection by *Mycobacterium tuberculosis (Mtb).* Particularly, transition from latent to active infection is a common problem for HIV patients and those taking immunosuppressive medications. Research into TB pathogenesis and resuscitation from latency is of critical importance as a growing number of these infections become multi-drug resistant. This study aims to further our understanding of how two peptidoglycan maintenance proteins interact to balance the need for wall synthesis and degradation throughout growth and latent stages of the bacterium. PonA2 is a penicillin binding protein that polymerizes peptidoglycan monomers and was shown to be important during non-replicative conditions, similar to those faced during a latent human infection. RipA is an endopeptidase that both cleaves cross links during cell wall remodeling and synergizes with resuscitation promoting factors when returning to an active metabolic state. We show in model organism *Mycobacterium smegmatis* that deletion of *ripA* in a Δ*ponA2* mutant rescues this mutant from decreased survival after anaerobic stress. Interestingly, when observing mutants with deletion of these proteins under starvation stress, we are unable to rescue phenotypes shown by the Δ*ponA2* mutant with deletion of *ripA.* We hope to next observe the potential physical interaction of these two proteins through coimmunoprecipitation. A clearer picture of how mycobacterial cells regulate resuscitation from dormancy and survival under stress conditions faced during infection will provide insight into potential areas for treatment of TB or prevention of active disease.

Elucidating mechanisms of regulation for the LFR islet in *Staphylococcus aureus* clonal complex 30

Christie Gilbert[1], Kelly Vore[2], and Steven Gill[1]
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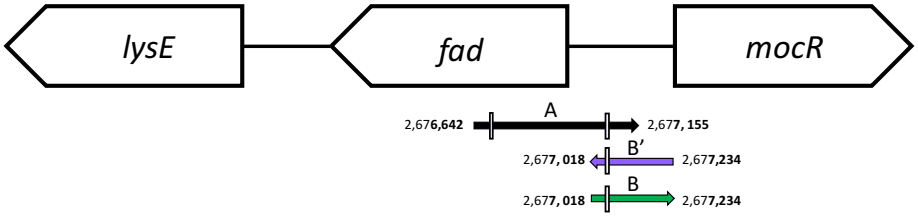
Background

Staphylococcus aureus is an opportunistic pathogen with the cause of infections ranging from asymptomatic skin colonization to highly severe infections leading to osteomyelitis, endocarditis and death. It is a commensal of the nares and skin, with a majority of individuals persistently colonized being susceptible to *S. aureus* infections. The genome of *S. aureus* is highly variable, resulting in specific lineages or clonal complexes (CC) with distinct clinical outcomes. One such lineage is Clonal Complex 30 (CC30), a lineage associated with persistent and complicated osteoarticular infections due to the metabolic strategies it has acquired, potentially enabling survival in nutrient limiting environments. Complete genome assessment of CC30 strains revealed 16 islets unique to *S. aureus* and CC30 that are predicted to contribute to metabolic flexibility, virulence, and niche adaptation. Among these islets is LFR, which encodes a newly discovered global regulator, *moc**R***. Additionally, the LFR islet contains ***l****ysE* and ***f****ad*, which both play essential functions in the cell allowing for stability and survival under stressful conditions. Our work aims to understand the mechanisms that control *mocR* expression.



Methods

mocR promoter activity was assessed using a *lacz* vector, pJB185, which produces β-Galactosidase relative to promoter activity. The activity of β-Galactosidase was normalized by protein concentration. First, a 500bp region upstream of *mocR* was inserted into pJB185 and assessed for β-Galactosidase activity (Region A). A smaller 200bp region was then used in pJB185 in the *fad* and *mocR* direction, and assessed for β-Galactosidase activity (Region B' and B, respectively). Constructs were transduced into wildtype UAMS-1 and UAMS-1 Δ*mocR*, UAMS-1 Δ*codY* to assess the autoregulation potential of MocR and CodY.

Results

Using Region A, both wildtype and Δ*mocR* strains had increasing activity in the stationary phase, this matched preliminary QRT-PCR data of gene expression of the LFR islet. *mocR* promoter activity was significantly higher in wildtype UAMS-1 vs. Δ*mocR*. Taken together, this data suggests positive autoregulation of this region by MocR, in addition to unknown global regulator(s) that allow the activity seen in Δ*mocR* UAMS-1. In UAMS-1 wild-type, activity levels of region B increased into the stationary phase (OD_{600} 1.2). Activity levels were significantly decreased in Δ*mocR*, further confirming MocR acts to upregulate of *fad*. To gain a better understanding of potential global regulators compensating for activity in UAMS-1 Δ*mocR*, Regions A-B' were transduced into UAMS-1 *ΔcodY.* Each of these regions had significantly higher activity levels in UAMS-1 *ΔcodY,* indicating that CodY binds to these regions and acts to repress *fad* and *mocR.*

The role of endothelial cell metabolism in the context of acute lung injury

Vania Lopez, Antony Leonard, Arshad Rahman, and Fabeha Fazal

Acute lung injury (ALI) is a common cause of respiratory failure in critically ill patients with a mortality rate of 38.5%. ALI can be precipitated by either direct insults such as pneumonia, aspiration or via indirect insults such as sepsis and multiple trauma, to the lungs. The vascular endothelium forming the innermost lining of all pulmonary blood vessels is the major barrier that protects air spaces against vascular fluid entry. Because of their localization, endothelial cells (EC) are constantly exposed to oxygen and nutrients that fuel cell metabolism. However, very little is known about the role of EC metabolism in the regulation of EC inflammation and permeability, the two major pathogenic features of ALI. Recent studies have highlighted that fatty acid oxidation (FAO) is central to the tight regulation of EC permeability required to maintain vessel functionality. Our lab has previously shown that BiP/GRP78, an endoplasmic reticulum (ER) chaperone and signaling regulator, is a critical determinant of EC inflammation and permeability associated with ALI. The purpose of the present study is to understand the role of BiP/GRP78 in mediating EC metabolic pathways and whether there is a linkage between EC metabolism, inflammation and permeability in the context of ALI. Human Pulmonary Artery Endothelial cells (HPAEC) were stimulated with proinflammatory mediator thrombin in the presence and absence of Etomoxir, an inhibitor of FAO or dichloroacetate (DCA), a promoter of glucose oxidation. Our results indicate that both inhibition of FAO by Etomoxir and the promotion of glucose oxidation by DCA, inhibited thrombin-induced inflammatory responses as observed by decrease in the synthesis and secretion and cytokines IL-6 and IL-8. Next, thrombin-induced disruption in endothelial barrier integrity was measured using Trans-endothelial resistance (TER), which is a real time measurement of EC permeability. Our data indicate that Etomoxir had no effect on thrombin-induced decrease in TER; however, it delayed the recovery 1 hour after thrombin challenge. Interestingly, DCA had no effect on thrombin-induced decrease in TER and subsequent recovery. Together our data suggest that metabolic pathways are critical to EC inflammation and permeability induced by thrombin.

Understanding T cell activation at sites of inflammation: myeloid cells nucleate T_h1 cells by antigen presentation and chemokine production

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T cell-mediated adaptive immune responses are critical for intracellular pathogen clearance. How T_h1 cells are recruited to the initial site of infection and activated through interactions with antigen presenting cells (APCs) to elucidate appropriate immune responses is poorly understood. Preliminary data using intravital microscopy and the REX3 transgenic mouse, in which chemokines CXCL9/10 producing cells express RFP and BFP, respectively, suggest that chemokine-producing cells form dense perivascular clusters that attract and position $CXCR3^+$ T_h1 cells upon immunization with CFA/OVA. We hypothesize that these CXCL10-producing cell clusters are hubs for antigen presentation and are essential for T_h1 activation and interferon-γ (IFN-γ) production. Our data show that myeloid cells are the primary chemokine producers in the inflamed ear. Specifically, monocyte-derived dendritic cells (MoDCs) are enriched for CXCL10 and MHC-II co-expression, suggesting MoDCs have the capability to both attract and activate T_h1 cells. To further understand the immune subsets and effect of the chemokine-rich clusters on T_h1 activation, we crossed the REX3 transgenic to a Photoactivatable-GFP (PA-GFP) mouse which enables us to label clustered cells *in-vivo* for *ex-vivo* analysis. We have successfully photoactivated clustered cells using precise delivery 830nm light on multiphoton microscopy (MPM). Using the PA-GFPxREX3 mouse, we plan to further determine if these cell clusters enhance T_h1 function by sorting photoactivated (in clusters) and non-photoactivated (outside clusters) T_h1 cells from the inflamed ear and analyzing IFN-γ expression. Understanding how T_h1 cells are positioned and activated at sites of inflammation will allow the field to boost responses to infectious agents and therapeutically attenuate T_h1 responses in autoimmunity.