UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 18, 2018 Mary Gates Hall

Online Proceedings

Poster Session 1

MGH 241, Easel 130

11:00 AM to 1:00 PM

Neutralization Breadth and Epitope Binding of Infantile Broadly Neutralizing Antibodies Against HIV

Noah Arthur James Cassidy, Senior, Microbiology
Mary Gates Scholar
Mentor: Julie Overbaugh, Microbiology
Mentor: Jeremy Roop

This abstract is no longer available.

Session 1E

From Viral Pathogenesis to Genetic Diseases to Building a Better Kidney

Session Moderator: Michael Lagunoff, Microbiology

MGH 231

12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Hypoxia-Induced Factors in Latent KSHV Infection of Endothelial Cells

Jie Yin, Senior, Biochemistry, Microbiology
Levinson Emerging Scholar, Mary Gates Scholar, UW Honors Program
Mentor: Michael Lagunoff, Microbiology
Mentor: Daniel Holmes, Microbiology

Kaposi's sarcoma-associated herpesvirus (KSHV) is the etiological agent of Kaposi's Sarcoma (KS), a highly vascularized tumor made up of cells of endothelial origin. KSHV establishes a predominantly latent infection in endothelial cells in culture and in the KS tumor. A previous study has shown that KSHV induction of the Warburg effect is required for the survival of latently infected endothelial cells. The Warburg effect, a common metabolic alteration in cancer cells, refers to an increase in glycolysis and a decrease in oxidative phosphorylation. The mechanism of Warburg induction by KSHV is currently unknown. I proposed to evaluate the role of endothelial cell specific hypoxia-induced factors (HIFs) on KSHV Warburg induction since HIFs have been implicated in Warburg induction in other types of cancer. I hypothesized that HIF2α mediates KSHV Warburg induction through expression of glycolytic genes. To test this, I constructed HIF2α knock-out cells using CRISPR/Cas9 gene editing. I then used RT-qPCR to measure glycolytic gene expression during KSHV infection of wild-type and HIF2α knock-out cells. I found that the transcript levels of certain glycolytic genes remained constant in KSHV-infected HIF2α knock-out cells as compared to KSHV-infected wild-type cells, showing that HIF2α is not responsible for glycolytic gene expression during KSHV infection. I am following up on the role of the KSHV latent gene KapA on the induction of glycolytic gene expression. KapA was previously found to interact with components of the Ras pathway. As the Ras pathway activates glycolytic genes through HIF1α, I hypothesize that exogenous expression of KapA will lead to increased glycolytic gene expression through increased expression of HIF1α. I will construct an endothelial cell line that overexpresses KapA using CRISPR/Cas9 system and then use RT-qPCR to evaluate glycolytic gene expression. These results will aid in the future efforts to develop antiviral drugs by targeting cellular metabolism.

Session 1J

Mechanisms of Cellular Regulation

Session Moderator: Hannele Ruohola-Baker, Biochemistry

MGH 251

12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Understanding Mechanisms of Antibiotic Resistance Development

Chris Hsu, Senior, Biochemistry
Mentor: Houra Merrikh, Microbiology

Antibiotic resistance is an intractable clinical challenge that disproportionally affects much of the world's most impoverished populations. A critical driver of resistance stems from DNA mutations. Therefore, insights into how mutations arise in the genome is critical to understanding the development of antibiotic resistance. Our lab is currently identifying factors and environmental conditions that promote mutagenesis and bacterial evolution. Utilizing mutation rate analysis, we

have identified factors and conditions that promote mutagenesis in divergent bacterial species. Additionally, using short-term evolution experiments, we have found that these factors promote the development of antibiotic resistance over time. This work provides novel mechanistic insights into mutagenesis and antibiotic resistance development in bacteria.

SESSION 1P

MCNAIR SESSION - SCIENCE AND TECHNOLOGY FROM CELLS TO OUTER SPACE

Session Moderator: Laura Pina, Human Centered Design and Engineering

MGH 295

12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Identifying Gene Inactivations that Produce Gallium Resistance in *Pseudomonas aeruginosa*

Lisa Khuu, Senior, Microbiology

McNair Scholar, UW Honors Program

Mentor: Pradeep Singh, Microbiology

Mentor: Richard Siehnel, Microbiology

Antibiotic resistance is a major public health problem. For example, in people with the genetic disease cystic fibrosis, *Pseudomonas aeruginosa* causes chronic lung infections that resist treatment to all existing antibiotics. Gallium is a promising new antimicrobial agent that works through a novel mechanism of action. Bacterial uptake systems are unable to distinguish gallium from iron due to their nearly identical ionic radii. Once inside, bacterial gallium is thought to disrupt iron-dependent processes because Ga^3+ cannot be reduced in physiological conditions, and iron's biological functions depend on its capacity for redox cycling. Here I sought to better understand gallium's mechanism of action by identifying gene mutations that decrease *P. aeruginosa* gallium sensitivity. To accomplish this, I used transposon (Tn) mutagenesis, which transfers a genetic element to random locations on the bacterial chromosome thereby inactivating genes. Of the 295,000 mutants generated, I selected for mutants capable of growing in the presence of increased gallium compared to the parental strain. Using a procedure known as rescue cloning, a restriction digest was performed on the chromosomal DNA of these mutants to produce individual fragments. One of these DNA fragments will contain the Tn insert and chromosomal DNA flanking that insert. The Tn insert encodes resistance to gentamycin and holds the capacity to replicate as a plasmid in *E. coli*. I ligated the isolated fragments and transformed *E. coli*, selecting for gentamycin resistance. I isolated this plasmid DNA from two mutants and their Tn insertion sites (and the genes inactivated by the Tn) were identified by sequencing the DNA surrounding the Tn inserts. These genes were found to be involved in iron transport. By completing these experiments, I hope to suggest hypotheses for the roles these genes play in gallium sensitivity and to provide data useful toward guiding its development as an antibacterial agent.

POSTER SESSION 2

Balcony, Easel 87

1:00 PM to 2:30 PM

Characterizing the Role of LCN2 during the Gastric Epithelial Cell Immune Response Initiated by *Helicobacter pylori*

Rohan Hassan, Senior, Biology (Molecular, Cellular & Developmental)

Mary Gates Scholar, Washington Research Foundation Fellow

Mentor: Nina Salama, Human Biology, Fred Hutchinson Cancer Research Center

Mentor: Tina Gall, Molecular and Cellular Biology, Fred Hutchinson Cancer Research Center

Helicobacter pylori is a gastric bacterial pathogen that infects about 50% of the world's population. Chronic infection with *H. pylori* causes inflammation and increases the risk for developing gastric cancer. *H. pylori* colonizes the human stomach where it uses a type IV secretion system to deliver bacterial factors to the gastric epithelial cells. Once infected, gastric epithelial cells initiate different immune responses, one of which is the release of a protein; lipocalin 2 (LCN2). Like nearly all pathogenic bacteria, *H. pylori* must acquire iron, which is important for colonization, persistence, and virulence. LCN2 behaves like a competitor for iron and limits bacterial growth by depleting intracellular iron stores. LCN2 is highly upregulated in gastric tissue during *H. pylori* infection, however it is still unclear if LCN2 plays a beneficial role for the host by controlling *H. pylori* growth. My project tests the hypothesis that if host LCN2 is sequestering iron, then *H. pylori* survival rates will be decreased. My first aim characterizes the kinetics of LCN2 expression determining when this gene is most highly expressed during *H. pylori* infection. My second aim uses novel and innovative CRISPR/Cas9 genome editing techniques to engineer LCN2 knockout gastric cells. If my hypothesis is correct, I expect that *H. pylori* survival rates will increase when co-cultured with LCN2 knockout cells since there will be no sequestering of iron by LCN2. Understanding how the host tries to defend itself through proteins such as LCN2 will provide better insight into the host immune response and may inform vaccine design and novel treatment strategies for populations with a higher predisposition for developing gastric cancer as a result of *H. pylori* infection.

SESSION 2B

ENHANCING IMMUNE RESPONSES TARGETING INFECTION, INJURY AND CANCER

Session Moderator: Kristin Anderson, Immunology
MGH 228
3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

ELISA Detection of Antibodies Against *Klebsiella pneumoniae* Capsule in Rabbit Immune Sera
Kathryn Shea Willebrand, Senior, Microbiology
Mary Gates Scholar
Mentor: Frank DeLeo, Laboratory of Bacteriology
Mentor: Scott Kobayashi, Laboratory of Bacteriology

Klebsiella pneumoniae is a gram-negative bacterium that asymptomatically colonizes the intestinal and upper respiratory tract of healthy humans. It is also an opportunistic pathogen that causes bacteremia, pneumonia, urinary tract infections, and other severe infections in individuals with significant comorbidities. *Klebsiella* infections are often healthcare-associated and antibiotic-resistance is a major problem for treatment. A *K. pneumoniae* lineage known as multilocus sequence type 258 (ST258) is resistant to virtually all beta-lactam antibiotics, including the carbapenems, and many clinical isolates are also resistant to fluoroquinolones and aminoglycosides. Additionally, the capsule of ST258 confers resistance to phagocytosis by neutrophils and killing by the complement system. However, it is readily killed if phagocytosed. One possible therapeutic approach is to develop an antibody-based immunotherapy that targets the capsular polysaccharide (CPS) of ST258. As a step toward developing the tools needed for such an approach, we developed an enzyme-linked immunosorbent assay (ELISA) that can be used to detect CPS and titer CPS-specific IgG antibodies. Polystyrene ELISA plates were coated with purified ST258 CPS and then incubated with rabbit antiserum containing antibodies specific for CPS. Anti-CPS IgG was then detected with goat anti-rabbit IgG antibodies and a detection reagent. We anticipate the ELISA will be an important quantitative tool in our efforts to develop an immunotherapy that targets the CPS of *K. pneumoniae.*

POSTER SESSION 4

MGH 241, Easel 136
4:00 PM to 6:00 PM

Internal Deletion Induced Interferon Response to Influenza A
Jacob Richard Kowalsky, Senior, Microbiology
Mary Gates Scholar, Washington Research Foundation Fellow
Mentor: Jesse Bloom, Division of Basic Sciences
Mentor: Alistair Russell, Basic Sciences, Fred Hutchinson Cancer Research Center

As an airborne virus, influenza A is a widespread threat to global economies and a consistent danger to public health. Through high reassortment and evolutionary rates, influenza is even able to infect those who have been previously vaccinated against the virus. The innate immune system serves as a key first line of defense against this pathogen, with the signaling components, called interferons, driving the production of a potent cellular antiviral response. Studies have indicated that viral populations replete in defective virus particles, virions with a deletion in a portion of their genome, are less efficient at blocking the antiviral response, as shown by increased interferon in the host. Our project seeks to explore this phenomenon of RNA deletions leading to increased interferon expression in host cells by testing the hypothesis that deletions in the three polymerase genes of influenza alone are sufficient to cause an increase in the interferon response. In addition, we are currently testing if mutational deactivation of one of the other genome segments, or absence of such segments, is capable of producing a more robust immune response when combined with polymerase gene deletions. In order to support this analysis, I began by creating pure populations of PA defective influenza particles grown on PA expressing host cells. Similar to results observed by my mentor Dr. Alistair Russell with PB1 and PB2 defective populations, it was found that these PA defective influenza particles were sufficient to induce the interferon response. Recently, I have assisted in the creation of multiple influenza protein expressing cell lines and influenza populations with simultaneous modifications to the HA, NS, and polymerase genes. It is hoped that immune stimulation data derived from these custom viruses, in combination with previous findings, will improve current antiviral therapies and models of the human immune response to influenza.

POSTER SESSION 4

MGH 241, Easel 123
4:00 PM to 6:00 PM

Binding and Inhibition of Heme-Regulated eIF2α Kinase by the Human Cytomegalovirus TRS1 Protein
Munif Nyem Chowdhury, Senior, Microbiology
Mentor: Adam Geballe, Allergy and Infectious Diseases

Human cytomegalovirus (HCMV) is a member of the Herpesvirus family. Most of the US population has been exposed to this virus, but it is only deadly in immunocompromised

individuals. When a human cell becomes infected, one of the earliest mechanisms of anti-viral defense is the activation of protein kinase R (PKR). Active PKR phosphorylates the translation initiation factor, eIF2α, shutting down most protein synthesis in the cell. HCMV circumvents cellular shutdown by expressing protein TRS1, which binds to PKR and inhibits it. Another cellular kinase, the heme-regulated inhibitor (HRI), also phosphorylates eIF2α upon activation by reactive oxygen species. Because HRI and PKR are similar in structure, I hypothesize that HRI activation during HCMV infection is inhibited by TRS1. I infected PKR-knockout human fibroblasts with HCMV and then activated HRI expression by inducing production of reactive oxygen species. These cells also have a luciferase gene, expression of which is induced upon eIF2α phosphorylation. I measured the abundance of phosphorylated eIF2α (eIF2α-P) and luciferase activity in the lysate of infected cells in which HRI has been activated. I expect that TRS1 will bind and inhibit HRI, therefore eIF2α-P levels and luciferase activity will be low compared to cells lacking TRS1. These results reveal that HCMV replication is sensitive to HRI activation in reactive oxygen-rich inflammatory tissues and provides insights into the evolutionary history and structural similarities between HRI and PKR.

POSTER SESSION 4

MGH 241, Easel 140

4:00 PM to 6:00 PM

Role of STING in KSHV Infection and Immune Response of Lymphatic Endothelial Cells

Alice Ponsawarn Ranjan, Junior, Microbiology, Biology (Molecular, Cellular & Developmental)

Mary Gates Scholar

Mentor: Michael Lagunoff, Microbiology

Mentor: Danny Vogt, Microbiology

Kaposi's sarcoma-associated herpesvirus (KSHV) is the causative agent of Kaposi's Sarcoma (KS), a highly vascularized tumor composed of cells of endothelial origin. While KSHV infects both blood (BECs) and lymphatic (LECs) endothelial cells, LECs are more susceptible to infection and express fewer antiviral genes during infection compared to BECs. Recent experiments have shown that LECs, but not BECs, have a defect in STING-mediated signaling, a critical antiviral pathway that is activated during herpesvirus infections. STING induces expression of the antiviral signaling molecule interferon and pro-inflammatory cytokines through the transcription factors IRF3 and NF-kB, respectively. While STING is unable to signal through IRF3 in LECs, it is unknown if NF-kB activation is similarly impaired. Moreover, it is unknown if the defect contributes to the increased susceptibility to KSHV infection in LECs. Accordingly, I hypothesize that in LECs, NF-kB signaling via STING will be impaired and that the STING defect increases susceptibility to infection relative to BECs. To determine if STING activates NF-kB, I will stimulate STING in BECs and LECs and measure expression of NF-kB-activated genes. I expect NF-kB-activated genes to be expressed in BECs but not in LECs, indicating that STING is unable to signal through NF-kB in LECs. To determine if the STING defect renders LECs more susceptible to KSHV infection, I will use CRISPR-Cas9 to knock out STING in BECs. I will then use KSHV to infect the knock-out BECs as well as wild-type BECs and LECs and compare infection rates. I expect the infection rate in the knockouts to be increased relative to wild-type BECs and similar to the infection rate in LECs. The results from these experiments will further elucidate how KSHV exploits defects in innate-immunity to infect and transform host cells.

POSTER SESSION 4

MGH 241, Easel 139

4:00 PM to 6:00 PM

The Role of the Pentose Phosphate Pathway in the Survival of Endothelial Cells Latently Infected with KSHV

Madeleine Hart, Recent Graduate, Biochemistry, Public Health, University of Washington

UW Post-Baccalaureate Research Education Program

Mentor: Michael Lagunoff, Microbiology

Mentor: Daniel Holmes, Microbiology

Viruses are nonliving entities and therefore lack their own metabolism, but they do have the ability to alter the host cell's metabolic pathways for their own gain. Kaposi's Sarcoma-associated herpesvirus (KSHV), a human herpesvirus, is the etiological agent of Kaposi's Sarcoma (KS), the most common tumor in AIDS patients worldwide. In KS tumors and cultured endothelial cells, KSHV is predominantly in the latent state, with only a small portion of viral genes and a microRNA cluster expressed, and no virion assembly. Our lab has shown that KSHV dramatically activates many host cell metabolic pathways during latent infection, including glycolysis. A global metabolomics screen suggested that glycolytic intermediates might be shuttled through the pentose phosphate pathway, (PPP) during KSHV infection. Our hypothesis is that the PPP is important for viral perseverance and the survival of human endothelial cells latently infected with KSHV. Infected endothelial cells were treated with the glucose-6-phosphate dehydrogenase (G6PD) inhibitor 6-aminonictotinamide (6-AN) at different concentrations and the amount of cell death was quantified. The inhibition of the PPP by 6-AN significantly increases cell death in KSHV infected TIME cells, but not in matched uninfected cells. KSHV microRNAs are expressed during latent infection and have been shown to be sufficient to induce glycolysis. Interestingly, a KSHV mutant, ???miR, which lacks the

microRNA cluster is less sensitive to inhibition of the PPP. This suggests that the microRNA cluster may be responsible for the reliance of KSHV infected endothelial cells on the PPP. The data suggest that inhibitors of the PPP provide novel therapeutic agents for KS tumors.