Undergraduate Research Symposium May 20, 2016 Mary Gates Hall

Online Proceedings

POSTER SESSION 2

MGH 241, Easel 149

1:00 PM to 2:30 PM

Mechanism of Horizontal Gene Transfer

Kathryn Shea Willebrand, Sophomore, Microbiology
Mentor: Beth Traxler, Microbiology

Our lab is investigating the process of horizontal transfer of mobile DNA molecules in microbes, focusing on plasmids of bacteria. A plasmid is a circular piece of DNA that replicates separately from the host chromosome and can be passed from one bacterial cell to another via conjugation, allowing advantageous traits to spread among diverse species. My work has focused on the pQil plasmid, which was originally found in pathogen *Klebsiella pneumonia*, but has been observed to transfer to other bacterial species in human hosts. The pQil plasmid encodes resistance to the carbapenem antibiotics, a class of antibiotics used to combat multi-drug resistant bacterial infections and important in the treatment of bacterial pneumonia. We are characterizing the mobility of the pQil plasmid to create a laboratory model of a multi-plasmid system. We paired pQil with the F plasmid in *E. coli* to measure the transfer of these plasmids in the laboratory. We mated a plasmid-free recipient with the *E. coli* donor strain containing pQil, F, or both. The recipient cells were then characterized for the presence of the plasmids. We determined that the frequency of transfer for pQil is increased in the presence of F'42 and that the transfer of F'42 is slightly inhibited by the presence of pQil. Continuing studies will analyze the mechanisms through which bacterial conjugation occurs. Ultimately our work will inform us on how complex microbial systems evolve in natural environments, such as humans infected with pathogenic bacteria. With this information we can begin to study ways to halt plasmid transfer to slow the global spread of antimicrobial resistance.

POSTER SESSION 2

MGH 241, Easel 151

1:00 PM to 2:30 PM

KSHV Modulates the Expression of Genes Involved in Peroxisome Biogenesis

Mira Naidoo, Senior, Microbiology
Mary Gates Scholar, UW Honors Program
Mentor: Michael Lagunoff, Microbiology

Kaposi's sarcoma-associated herpesvirus (KSHV) is the causative agent of Kaposi's Sarcoma (KS), a cancer of endothelial cell origin that commonly presents in immunocompromised individuals. While relatively rare in the United States, KS remains endemic to certain parts of Africa, where it is the most common cancer. Previous research has established that the number of peroxisomes is altered during latent KSHV infection. Peroxisomes are multifunctional cellular organelles involved in a variety of metabolic pathways important to KSHV pathogenesis. We propose to evaluate the cellular mechanism by which KSHV induces peroxisome biogenesis. I hypothesized that KSHV increases the transcription of specific regulatory genes responsible for the increase of peroxisome biogenesis. After mock and KSHV infecting cells, I evaluated gene expression of a known transcription factor, peroxisome proliferator-activated receptor alpha (PPARA), that has been shown to be responsible for peroxisome biogenesis. I used real time PCR to evaluate gene expression of the transcription factor PPARA. Our preliminary data shows that PPARA is increased, suggesting that PPARA regulates the expression of peroxisome associated genes and peroxisome biogenesis. Currently, I am evaluating if other peroxisome genes are increased, including those involved in peroxisome formation and enzyme activity. In addition, I am using small interfering RNA (siRNA) to transiently knock down PPARA. Silencing the expression of PPARA will determine its specific role in peroxisome biogenesis. I will mock and KSHV infect the cells in which PPARA is knocked down and use real time PCR to evaluate peroxisome biogenesis. In the absence of PPARA, I expect that PEX gene expression will be downregulated, which would suggest that PPARA regulates PEX genes at the gene transcription level. These results will help elucidate one of the key pathways involved in KSHV latency, and potentially contribute to the development of novel therapeutic avenues for KS treatment. ???

POSTER SESSION 2

MGH 241, Easel 152

1:00 PM to 2:30 PM

The Role of Translesion Synthesis Polymerases at Replication-Transcription Conflicts

Chris Hsu, Sophomore, Pre-Major (Arts & Sciences)
Mentor: Houra Merrikh, Microbiology
Mentor: Patrick Nugent, Microbiology

Since DNA replication and transcription are concurrent in most bacteria, collisions (or conflicts) occur between these two processes. Conflicts have deleterious effects on cells, including double strand breaks and mutagenesis, especially when a gene is transcribed head-on to replication. Because conflicts increase mutagenesis specifically in genes that are oriented head-on to replication, this process may be a conserved strategy for targeted evolution. Consistent with this, a bioinformatics analysis determined that many genes in the head-on orientation are involved in stress response. We previously showed in *Bacillus subtilis* that the error prone translesion synthesis (TLS) polymerase YqjH is required for the asymmetric mutagenesis of genes in the two orientations and that transcription-coupled nucleotide excision repair (TC-NER) is also involved in this repair pathway. Under DNA damaging conditions, however, a different TLS polymerase, YqjW appeared to be responsible for mutational asymmetry, though TC-NER appears to still be involved. To show this, I used RecA-GFP microscopy to measure rates of replisome stalling in Wildtype, YqjW deletion mutant, and YqjW/UvrA double-deletion mutant (WT, ΔyqjW, and ΔyqjW/ΔuvrA) in B. subtilis, along with measuring these same strains' survival rates in response to two types of DNA damage. The data I generated was supported by mutation rate data generated through reversion assays done by a technician in the lab, and these data led us to propose that during conflicts in the presence of DNA damage, YqjW outcompetes YqjH to fill in gaps in DNA during TCR.

POSTER SESSION 2

MGH 241, Easel 153

1:00 PM to 2:30 PM

Inhibition of *Staphylococcus aureus* Protein A Expression by Nitric Oxide

Helen Ivory (Helen) Warheit Niemi, Senior, Microbiology
UW Honors Program
Mentor: Ferric Fang, Laboratory Medicine & Microbiology
Mentor: Rodolfo Urbano, Microbiology

Staphylococcus aureus is a global human pathogen and a major cause of wound infections, food poisoning, and invasive disease. Its success as a pathogen requires virulence factors used to evade the human immune system and subvert host defenses. Protein A is a multifunctional immune evasion factor that inhibits bacterial uptake by immune cells, modulates inflammatory responses, and disrupts development of adaptive immunity. We found that protein A expression decreased when cultures of *S. aureus* were exposed to the antimicrobial molecule nitric oxide (NO). NO is a reactive radical produced at high levels by macrophages and other host cells during infection. Reverse transcriptase quantitative PCR (RT-qPCR) showed that transcription of the *spa* gene, encoding protein A, was significantly decreased in the presence of NO. Dose response curves were generated comparing the sensitivity of *spa* and other genes to NO as well as to hydrogen peroxide (H_2O_2), another host-produced antimicrobial compound. Western blots confirmed that NO inhibition of *spa* transcription resulted in reduced protein A levels. Through allelic exchange mutagenesis, we mutated multiple *spa* transcription factors of *S. aureus* and identified the regulator protein XdrA as a likely NO target. Given that NO modifies cysteine thiols of proteins, we hypothesized that XdrA is S-nitrosylated by NO, leading to inhibition of *spa* transcription and concurring decrease in protein A. Single cysteine mutants of XdrA (C54S, C73S) have shown partial resistance to NO inhibition. A double cysteine XdrA mutant will be tested to assess the cumulative effect that multiple NO modifications have on *spa* transcription. This will allow us to understand the molecular mechanism of NO dependent protein A inhibition. Additional experiments demonstrating that NO produced by macrophages inhibits production of an important *S. aureus* virulence factor would characterize one mechanism by which innate immunity controls bacterial infections.

SESSION 2M

CELLULAR MECHANISMS MEDIATING DISEASE

Session Moderator: Ian Sweet, Medicine

MGH 389

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Elucidating the Viral Mechanisms of Vascular Endothelial Growth Factor Receptor Upregulation by KSHV

Emily Caroline (Emily) Anderson, Senior, Microbiology
Mary Gates Scholar, UW Honors Program
Mentor: Michael Lagunoff, Microbiology

Kaposi's sarcoma-associated herpesvirus (KSHV) is the etiologic agent for Kaposi's sarcoma (KS)—the most common malignancy of AIDS patients worldwide. Because KS is an endothelial cell based cancer, it relies on mechanisms unique to endothelial cells to proliferate. One such way is through expression of vascular endothelial growth factor receptors 1 and 3 (R1 and R3). The goal of my project is to determine the viral mechanisms by which KSHV upregulates these receptors. Because the majority of KSHV-infected cells are in latent phase, I hypothesized that latent genes are respon-

sible for R1 and R3 upregulation. By infecting cells with an adenovirus modified to express only genes within KSHV latency-associated region (KLAR), I was able to confirm that KLAR genes are sufficient to upregulate R1, but not R3. To determine which KLAR genes are required for R1 upregulation, I created lentiviruses to express one viral gene at a time. By infecting cells with these lentiviruses and monitoring R1 mRNA transcript production by PCR, I was able to rule out three of the four genes in KLAR. Using a cell line modified to constituently express the remaining KLAR gene, latency-associated nuclear antigen (LANA), I found mRNA transcript levels for R1 were significantly higher in LANA-expressing cells than in control cells. This suggests LANA is responsible for R1 upregulation. I am currently conducting a luciferase assay to determine if LANA interacts with the R1 promoter to modify R1 expression. To narrow the search for genes involved in R3 upregulation, I will continue to make lentiviruses to test the involvement of viral genes not located in KLAR. I will present current work examining if conditioned media induces R3 to determine if virus induced secreted factors could be responsible for R3 upregulation. My research will contribute to the ongoing search for treatment strategies for KSHV and KS.

SESSION 2M

CELLULAR MECHANISMS MEDIATING DISEASE

Session Moderator: Ian Sweet, Medicine

MGH 389

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Elucidating the Role of HIF2alpha in KSHV-Induced Angiogenesis

Mona Ahmed, Recent Graduate, Biology, University of Washington

UW Post-Baccalaureate Research Education Program

Mentor: Michael Lagunoff, Microbiology

Kaposi's sarcoma (KS) is the most common tumor found in sub-Saharan Africa and in AIDS patients worldwide. It is a highly angiogenic tumor caused by Kaposi's sarcoma-associated herpesvirus (KSHV), which establishes a latent infection in endothelial cells. KSHV infected endothelial cells display many angiogenic phenotypes, including vascular tube formation and stabilization. Recent data shows a link between KSHV angiogenesis and the hypoxia inducible factors (HIF) pathway. HIFs are a family of heterodimeric transcription factors that respond to low levels of oxygen in the cell. The HIF pathway has been shown to activate pro-angiogenic genes, including vascular endothelial growth factor (VEGF). Our lab has previously shown that both HIF-1alpha and HIF-2alpha are upregulated during KSHV infection. Although HIF-1alpha is expressed ubiquitously, HIF-2alpha is specific to endothelial cells. I hypothesize that HIF-2alpha regulates angiogenesis during KSHV infection. To test this hypothesis, I knocked down HIF-2alpha expression in endothelial cells using an shRNA lentivirus, which successfully knocked down expression by 80%. I then conducted a capillary tube assay on mock and KSHV infected cells. This assay involves homogenous seeding of cells onto Matrigel, a basement membrane matrix for studying angiogenesis *in vitro*. The cells attach and migrate on the Matrigel to organize into capillary-like structures, which mimics the later stages of angiogenesis. Latent KSHV infection has been shown to form tubes at 6 hours post-plating in Matrigel and to stabilize tube formation at 24 hours, a time point when capillary-like formation normally regresses in uninfected cells. My preliminary data shows that when HIF-2alpha is knocked down, KSHV-induced tube formation is inhibited. Future work will explore the viral mechanism and the cellular significance of HIF-2alpha upregulation during latent KSHV infection. If HIF-2alpha is required for KSHV-induced angiogenesis, this transcription factor may be a promising therapeutic target for KS tumors.

POSTER SESSION 3

Balcony, Easel 114

2:30 PM to 4:00 PM

Identifying and Characterizing Host c-di-AMP Sensing Proteins

Lisie Thayer, Senior, Biochemistry, Microbiology

Mary Gates Scholar

Mentor: Adelle McFarland, Molecular and Cellular Biology/Microbiology

Mentor: Joshua Woodward, Microbiology

Cyclic diadenosine monophosphate (c-di-AMP) is an essential nucleotide for bacterial virulence. C-di-AMP has been extensively studied in *Listeria monocytogenes* (*Lm*), but is also produced by other pathogens including *E. coli*, C. trachomatis, and *M. tuberculosis*. Although c-di-AMP is a signaling molecule in bacteria, host cells detect it as a pathogen-associated molecular pattern. During infection, the host receptor STING senses c-di-AMP and stimulates expression of interferon-beta, an anti-viral molecule. To date, STING is the only known sensor of c-di-AMP, and its promotion of an anti-viral versus anti-bacterial response has been somewhat perplexing. However, our lab has recently discovered another host c-di-AMP sensor, the murine aldo-keto reductase1C13 (AKR1C13) or "RECON", that induces an alternative, anti-bacterial cellular response to infection. Based on the importance of bacterial clearance and the ubiquity of AKRs across species, we began to investigate whether other murine and human AKRs respond similarly to c-di-AMP. Sequence alignment of AKR genes and data on tissue-specific expression of

these genes returned six candidates: the murine AKR1C12 as a potential paralogue, and human AKRs 1C1, 1C2, 1C3, and 1C4 as potential homologues. Cloning techniques allowed me to insert these genes into an *E. coli* expression vector that I used to purify recombinant protein via affinity chromatography. The binding affinity with c-di-AMP of each protein was tested with radio-ligand binding assays to determine dissociation constants (K_d) and we additionally used enzyme activity assays to quantify inhibition of protein activity. AKRs that bind c-di-AMP will be further characterized *in vivo* in mammalian cell lines in which expression has been knocked down via lentiviral transduction. Further work in cell lines may elucidate novel pathways that are activated downstream of c-di-AMP detection. Understanding the pathways that naturally clear infections is important to potential medical advances in bacterial infection management and prevention.

POSTER SESSION 4

MGH 241, Easel 132

4:00 PM to 6:00 PM

Prevalence and Stability of *Escherichia coli* Clonal Types in Routinely Collected Urine Samples

Diana Wen (Diana) Chan, Senior, Microbiology
UW Honors Program
Mentor: Evgeni Sokurenko, Microbiology
Mentor: Veronika Tchesnokova, Microbiology

Escherichia coli is a major causative agent of extra-intestinal infections and highly clonal. Our goal was to use the newly developed septatyping method to determine the clonal structure of *E. coli* population in routinely collected urine samples from a clinical microbiology lab, evaluate the patterns of antibiotic resistance of individual clones, their temporal stability and prevalence in samples with clinically significant load. Isolated *E. coli* were assigned a clonotype using septatyping, which is a quantitative real-time PCR test consisting of seven reactions that identify the absence or presence of clone-specific SNP's in two loci – *fumC* and *fimH*. The eighth reaction is a reference control, detecting *E. coli* specific loci, *uidA*. We identified 40 (out of 128 possible) clonotypes from 647 *E. coli* isolates. The most prevalent was the 561 clonotype, aka H30 clone (114 isolates, 17.6%), followed by clones 571, 760, 620 and 271 (7-8% of isolates per clone). Clonal structure of *E. coli* remained relatively stable over the 3-year period (less than 2% difference). Furthermore, clones varied significantly in their antibiotic susceptibility profiles. For example, 561 (H30) is a highly multi-drug resistant clone, whereas 760 and 530 are highly susceptible to all tested antibiotics. Such differential susceptibility can be extremely useful for choosing the proper course of treatment for the patient. When we compared the clonal structure of *E. coli* isolated from samples with low bacterial load ($<10^4$cfu/ml, usually discarded as contamination) and high load ($\geq 10^4$, clinically significant), most of the clonotypes were evenly split between the two groups. The fact that the same clones can grow both to low and to high numbers in patients' urine indicates that the cutoff of 10^4 cfu/ml may need revision as a clinically significant level.

POSTER SESSION 4

MGH 241, Easel 133

4:00 PM to 6:00 PM

Detection of Pandemic Multidrug-Resistant Clone of *Escherichia coli*, H30, in Fecal Samples from Healthy Women

Huxley Ann (Huxley) Smart, Senior, Microbiology
Mentor: Evgeni Sokurenko, Microbiology
Mentor: Veronika Tchesnokova, Microbiology

Escherichia coli (E. coli) is the major causative agent of urinary tract infections (UTI) and is highly clonal. One of the major clones is a multidrug-resistant H30 clone, which has spread worldwide and is almost exclusively resistant to fluoroquinolones, accounting for 50% of all ciprofloxacin-resistant uropathogenic *E. coli*. The goal of this study was to determine if H30 clone is prevalent among fecal *E. coli*, which might explain its high occurrence in extraintestinal infections. We obtained 555 fecal samples from female volunteers and screened them for the H30 clone using a multiplex quantitative PCR test, ST200. This test contains primers for *uidA* (*E. coli* specific loci), and primer-probe combinations for ST131, ST69, and *fimH30*. The combination of positive ST131 and *fimH30* probes identifies the H30 clone. The results of fecal screenings were compared to the data on H30 found among urine isolates from women with UTIs. Out of 555 fecal samples, 531 (96%) had detectable presence of *E. coli* ranging from 10^4 to 10^7 cfu/ml. H30 clone was found only in 25/555 samples (4.5%), while it was significantly more prevalent in urine (7.7%, P=0.008). The prevalence of H30 clone in younger (<50 years old) versus older (>50 years old) women didn't differ significantly for fecal samples (6.1% vs 8.1%, P=0.24). However H30 was significantly more prevalent in older women's urine samples (5.9% vs 8.6 %, P=0.014). Overall, 44/555 (7.9 %) fecal samples contained ciprofloxacin-resistant isolates. Similarly to urine, H30 clone accounted for almost half of isolates (21/44, 47.7 %), although resistance of fecal H30 isolates to fluorquinolones was slightly decreased in comparison to urine H30 isolates (84% vs 95%). In summary, the H30 clone preferentially causes UTIs in elderly women and is the major reservoir of ciprofloxacin-resistant *E. coli* in gut microflora, potentially leading to higher infection rates due to mismatched antibiotic therapy.

POSTER SESSION 4

MGH 241, Easel 131

4:00 PM to 6:00 PM

The Influence of Conserved Elements Responses during the Acute Infection of HIV on the Response to ART and DNA Therapeutic Vaccination

Nika Hajari, Senior, Microbiology
Mary Gates Scholar, UW Honors Program
Mentor: Deborah Fuller, Microbiology
Mentor: Paul Munson, Microbiology

While HIV treatment is improving, there are still problems associated with it. Antiretroviral therapy (ART) has unfavorable side effects, is expensive, and requires a lifelong daily pill intake. However, it has been proposed that a therapeutic vaccine could provide durable control of viremia in the absence of ART. This is a difficult approach due to the high mutation rate of the virus. Research has shown that there are conserved elements (CE) within the virus that do not change because they are important for the viral fitness. Accordingly a therapeutic DNA vaccine is being investigated that targets the CE epitopes. To address this, rhesus macaques were infected with SIV (Simian Immunodeficiency Virus), put on ART, vaccinated with four doses of DNA vaccine expressing CE, removed from ART, and their viral loads monitored. A successful vaccine is expected to reduce the viral loads to negligible amounts post ART cessation. My research project investigates the role of CE responses developed during acute infection. *I hypothesize that macaques that naturally develop higher CD4+ and CD8+ T cells (types of white blood cells) with CE specificity in response to the acute SIV infection will respond better to ART and CE vaccinations*. To investigate this I collected plasma and lymphocytes from the blood during acute infection and analyzed the CD4+ and CD8+ T cells using enzyme linked immunospot (ELISpot) and intracellular cytokine stain (ICS) assays. The viral loads were monitored by measuring the concentration of viral RNA in the plasma. T cell responses measured at each time point was then correlated to viral loads post ART-pre vaccinations or to CE responses measured after the final DNA vaccination by Spearmann Rank correlative test. My findings will help us better understand the significance of conserved elements in controlling the HIV and thus design better human therapeutic vaccines.