UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 20, 2022 Mary Gates Hall

Online Proceedings

O-1H

Our War on Pathogens: From Understanding our Enemies to Building Better Defenses

Session Moderator: Celeste Berg, Genome Sciences

MGH 271

1:30 PM to 3:00 PM

* Note: Titles in order of presentation.

Phenotypic Diversity in Chronic *Pseudomonas aeruginosa* Infections Impacting Infection Outcomes after Treatment with Cystic Fibrosis Modulators

Mike Shide (Mike) Zhang, Senior, Microbiology
Mentor: Pradeep Singh, Microbiology
Mentor: Samantha Durfey, Microbiology
Mentor: Sara Klee

Cystic fibrosis (CF) is a genetic disease characterized by defects in lung host defenses against infection. Chronic lung infections by the bacterium *Pseudomonas aeruginosa* (*Pa*) are a major cause of death in CF, and recent work shows that infecting *Pa* strains can evolve extensive genetic diversity *in vivo* during infection. A new class of drugs called modulators markedly improves the CF host defense defect and improves symptoms but does not frequently produce infection eradication. We hypothesize that the extent of phenotypic diversity within *Pa* populations infecting individual subjects will negatively affect *Pa* clearance after modulator treatment. This hypothesis stems from observations in environmental systems showing that diversity generally increases the stress resistance of populations. To test this hypothesis, we sampled the lungs of 9 subjects with bronchoscopy before and 18 months after they started the most effective modulator currently available and collected ˜100 *Pa* isolates from each of the five different lung regions. The collected isolates are being tested for variation in resistance to different classes of antibiotics and their nutritional requirements, motility, capacity to secret virulence factors, and attach to and aggregate on surfaces. These measurements will enable us to compare the phenotypic diversity of *Pa* populations infecting subjects that do and do not eradicate infection. In my preliminary work examining *Pa* populations from two subjects, I found much lower levels of diversity in resistance to three antibiotic classes in the subject that cleared infection as compared to the subject that did not (mean variance in resistance of 0.22 vs 75.3, respectively), which is consistent with my hypothesis. Understanding the consequences of *Pa* diversity in CF infections could shed light on factors causing different infection outcomes and suggest new approaches to treatment.

Characterizing Mechanisms of Antibiotic Resistance in the Pathogen *Staphylococcus aureus*

Tessa Mae Gardiner, Senior, Microbiology
Mentor: Lucas Hoffman, Pediatrics and Microbiology
Mentor: Lauren Gonsalves, Department of Microbiology

Cystic fibrosis (CF) is a genetic disease characterized by polymicrobial lung infections. *Staphylococcus aureus*, a Gram-positive pathogen, is commonly cultured from the secretions of people with CF (PwCF). Treatment for *S. aureus* infections requires antibiotics, such as the bactericidal antibiotic trimethoprim-sulfamethoxazole (SXT). SXT prohibits growth by targeting folate biosynthesis, a pathway important for DNA replication and maintenance and production of cell metabolites. In many PwCF, *S. aureus* generally persists despite antibiotic treatment. Our data shows that *S. aureus* can survive SXT treatment through the accumulation of adaptive mutations. In this project, we examined these adaptive mutations in *S. aureus in vitro* to better understand the mechanisms of resistance. We grew several *S. aureus* isolates with adaptive mutations in Luria Bertani (LB) broth. We sampled the culture tubes at several times in a 24-hour period, measuring viable bacterial counts on chocolate agar. We found that isolates with mutations in the sugar transport gene, *ptsI*, persisted better under SXT selection, relative to wild-type *S. aureus*. Other *S. aureus* isolates with mutations in pathways for aerobic respiration, including menaquinone (*menB, thiN*) and hemin synthesis (*hemB*), also better survived SXT compared to wild-type. Many of these mutations were also identified in *S. aureus* infecting PwCF. These results indicate that adaptive mutations in pathways associated with important metabolic processes may allow survival with folate inhibition. We hypothesize that limiting aerobic respiration may assist in *S. aureus* surviving SXT. As *S. au-*

reus can survive without oxygen, we are currently studying whether limiting oxygen and, consequently, aerobic respiration in wild-type *S. aureus* will improve survival with SXT. This work will help us understand the mechanisms of SXT action and *S. aureus'* response, in an effort to improve treatment for *S. aureus* infections.

Effect of SARS-CoV-2 ORF7a on Human Pluripotent Stem Cell-derived Cardiomyocytes

Flora Abrams, Senior, Bioengineering
Mentor: Charles Murry, Pathology
Mentor: Silvia Marchiano, Laboratory Medicine and Pathology

Since the beginning of the Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2) pandemic, a large number of COVID-19 patients have suffered a variety of cardiovascular complications. In a previous study from the Murry Lab, human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) were infected with live SARS-CoV-2 alpha variant, resulting in increased cell death and functional abnormalities. Once infected with SARS-CoV-2 delta variant, hPSC-CMs showed reduced cell death, compared with the ones infected with alpha. The SARS-CoV-2 genome has at least 14 open reading frames (ORFs), which is the transcribed part of a gene. A difference in expressed ORFs can create new variants. The delta variant has 60 amino acid deletions in the ORF7a sequence, resulting in the absence of the ORF7a protein. Thus, I hypothesized that this protein might play a role in cardiomyocytes' cell death. To demonstrate that this protein has a direct effect on hPSC-CMs cell death, I have created a lentivirus plasmid construct that expresses only ORF7a protein. To assemble the virus, I transfected HEK-293 cells with the ORF7a plasmid with two other plasmids encoding for the packaging and the replication of the lentivirus. Once the virus is ready, I will then infect hPSC-CMs with the lentivirus. If the ORF7a protein is responsible for cell death, we expect to see increased cell death in the hPSC-CMs infected with the ORF7a lentivirus. Showing support of the ORF7a protein inducing harmful effects on cardiomyocytes has the potential to provide valuable insight for scientists to target this particular protein for drug development to combat cardiovascular complications in COVID-19 patients.

Mapping the 5E10 Antibody Epitope on Hemagglutinin from the H3 Strain of Influenza

Kiran Francesca (Kiran) Awatramani, Junior, Biology (General)
UW Honors Program
Mentor: Kelly Lee, Medicinal Chemistry
Mentor: Sally Kephart, Medicinal Chemistry

Hemagglutinin (HA) is a glycoprotein found on the surface of the influenza virus. HA is responsible for binding to sialic acid receptors on host cells and mediating membrane fusion. Because it is involved in these essential functions, HA is targeted by neutralizing antibodies (nAb) against the influenza virus. When the antigen-binding fragment (Fab) domain of a nAb binds to HA, it can neutralize infectivity either by blocking receptor binding of HA, by inhibiting conformational changes required for membrane fusion, or by disrupting HA structure. All of these mechanisms can prohibit the virus from entering the cell. In a hydrogen-deuterium exchange mass spectrometry (HDX-MS) experiment, we attempted to map the epitope– the site on the antigen where the antibody binds– of the 5E10 neutralizing antibody on the HA from the 2011 Victoria H3 influenza strain. A deuterium exchange time course was performed and HDX-MS was used to determine the rate at which deuterium is exchanged for hydrogen on specific peptide segments of the HA protein backbone. We hypothesized that the epitope would be an area that becomes more protected, meaning that it would take up less deuterium, due to local structural ordering from the bound Fab fragment. Through our research, we have identified two potential epitope sites. Identifying the 5E10 epitope on H3 HA will tell us about sites of vulnerability that can be targeted by the immune system. Furthermore, it will help us understand the way the antibody can neutralize the virus as well as predict if the antibody would be able to neutralize other strains of flu based on comparisons of amino acid conservation at the epitope. This information can be used by other scientists to design vaccines that direct an immune response to a specific epitope.

[Unable to Present] Role of Endocytosis in Regulating Signaling of IL-10 Cytokine Family

Alexandra Maria (Alex) Dinu, Senior, Biology (Molecular, Cellular & Developmental), Biochemistry
Mentor: Ram Savan, Immunology

During a microbial infection, cytokine and interferon communication downstream of JAK-STAT signaling molecules are critical for the activation of pro- and anti-inflammatory gene programs. The prevailing model relies on JAKs (Janus Kinases) activation upon cytokine or interferon docking with its receptor at the membrane, which phosphorylates STATs (signal transducers and activators of transcription proteins) and leads to their translocation into the nucleus to either activate or repress the transcription of genes. In this study, we have identified the role of an endocytic pathway responsible for JAK-STAT activation upon interactions between the IL-10 family proteins and the cytokine receptor. Using skin epithelial cell lines, I have performed experiments to show that clathrin-mediated endocytosis is required for the signaling of cytokines that use the IL-10R2 receptor. According to my preliminary results, the inhibition of clathrin-mediated endocytosis using chemical inhibitor dynasore, prior to treatment with IL-26, results in complete shutdown of pSTAT3, pSTAT1, and pErk1/2. These downstream molecules are required for activation of inflammatory gene programming. We

now propose to investigate the roles of other inhibitors within the endocytic pathway including chloroquine, bafilomycin A1, and a Rab7a inhibitor on subsequent JAK-STAT signaling transduction. In order to visualize these results, confocal microscopy studies will be used to investigate trafficking in these vacuoles, followed by colocalization studies of JAK proteins with receptors in early endosomes. Our studies suggest that a change in receptor mobilization controlled through the endosomal trafficking mechanism modulates JAK-STAT signaling and downstream gene expression. This proposal is a paradigm shift which challenges the prevailing dogma that IL-10R2 dependent cytokines can activate JAK-STATs at the cell membrane to induce inflammatory responses. Furthermore, this new signaling model has the potential to impact pharmaceutical interventions as the IL-10 family plays a critical role in inflammatory bowel diseases as well as fungal and bacterial infections.

Investigation of Inflammation and Neuroinflammation During SIV-ZIKV Co-Infection

Skyler Lynn Niemeyer, Senior, Biology (Molecular, Cellular & Developmental), Microbiology
Mentor: Deborah Fuller, Microbiology
Mentor: Megan O'Connor, Microbiology

In this study we developed a nonhuman primate model of simian immunodeficiency virus (SIV)-Zika virus (ZIKV) co-infection to understand how HIV infection impacts ZIKV pathogenesis and test our hypothesis that ZIKV pathogenesis is enhanced in people living with untreated HIV. Previously, we have found delayed viral clearance, as well as delayed and dampened expansion of whole blood monocytes, the ZIKV cellular targets, during SIV infection. Here, we sought to further characterize the innate immune responses of SIV-ZIKV co-infection, by assessing cytokine and chemokine release. Pigtail macaques (n=7) were infected with SIVmac239M and co-infected with ZIKV at 9 weeks post-SIV infection. Co-infected animals were compared to control animals (n=7) infected with ZIKV only. Longitudinal plasma and cerebral spinal fluid (CSF) were collected at timepoints pre- and post-infection and assayed using a multiplex immunoassay to quantify 24 different cytokine and chemokines ex vivo. SIV and ZIKV both induced pro-inflammatory responses, characterized by transient increases in interleukin-17 (IL-17A) and monocyte chemoattractant protein-1 (MCP-1), with no major differences between experimental groups. Plasma MCP-1 concentrations were also found to be consistent with dampened and delayed whole blood monocyte frequencies. Pro-inflammatory interleukin-8 (IL-8), a chemokine needed for recruitment of neutrophils, increased in the plasma during SIV infection but not following ZIKV infection, a result that is in contrast to our previous findings. Transient increases in IL-8 were detected in a few animals in the CSF after ZIKV infection, which may be evidence for neuroinflammation. Overall, no significant differences between SIV+ vs SIV- groups were found for any analytes detected in plasma or CSF during ZIKV infection. Collectively, our results demonstrate that both SIV and ZIKV infections induce a pro-inflammatory response, that is not enhanced by SIV-ZIKV co-infection. This suggests SIV induced immunosuppression does not impair pro-inflammatory cytokine responses during ZIKV infection.

Heterologous Prime/Boost Regimen With DNA and repRNA Vaccine Confer Better Antibody and CTL Responses Against SARS-Cov-2 Spike Protein In BL/6 Mice Compared To Homologous Prime/Boost Regimen

Atharva Bhalerao, Senior, Microbiology
Mentor: Deborah Fuller, Microbiology
Mentor: Justin Ulrich-Lewis

Nucleic-acid based vaccines, including RNA and DNA, provide protective immunity by eliciting antibody (Ab), and cytotoxic T lymphocyte (CTL) responses. FDA approval of mRNA vaccines against SARS-CoV-2 (COVID-19) provide ample evidence that mRNA vaccines are a viable vaccine platform. Once a mRNA vaccine enters a cells cytoplasm, mRNA encoded antigens are produced rapidly inducing an immune response. The production of mRNA encoded antigens will wane over time as mRNA degrades and transfected cells die. Alpha viruses, a RNA virus, have a unique replication process whereby this virus amplifies its RNA genome upon entering a cell. We, as well as others, have developed RNA vaccines that, like alpha viruses, self-amplify once inside of a cell to create more copies of mRNA than entered cell. This self-replicating RNA vaccine is called a replicon RNA vaccine (repRNA). RepRNA induces robust and sustained antigen production and immune responses. Currently, mRNA vaccines are administered as a homologous prime/boost vaccine regimen where the same mRNA vaccine is given as a priming vaccine and boosting vaccine, but antibody titers wane over time allowing for potential infections. This project aims to evaluate If utilizing a heterologous prime/boost regimen with DNA and repRNA vaccines confers more robust and longer lasting antibody and CTL responses than homologous regimens with DNA or repRNA. Mice will be vaccinated with DNA as a priming vaccine and repRNA as a boosting vaccine that encode SARS-CoV-2 spike protein. Anti-SARS-CoV-2 spike IgG antibody titers will be measured at 3-4 different timepoints, and CTL responses will be evaluated using an interferon gamma (IFN-γ) enzyme-linked immune absorbent spot (ELISpot) assay. The insights gained from this project will help to inform future DNA vaccine formulations and regimens as more DNA vaccines and repRNA vaccines enter clinical trials.

Mucosal Vaccination Provides Protection from Herpes Simplex Virus Type 2(HSV-2) Infection and Disease

Kiersten Piper (Kikki) Tucker, Senior, Neuroscience
Levinson Emerging Scholar, Mary Gates Scholar
Mentor: Jennifer Lund, Vaccine and Infectious Disease Division and Global Health, Fred Hutchinson Cancer Research Center and University of Washington

Herpes simplex virus type 2 (HSV-2) is a sexually transmitted pathogen that is estimated to infect around 23 million people per year. Despite high global prevalence, there are not any approved vaccines that are therapeutic or preventative. Most vaccines that we use today rely on injecting antigens intramuscularly in order to elicit an adaptive immune response. However, given that most pathogens gain entry to the host across barrier surfaces, a focus on eliciting mucosal immunity may enhance protection; vaccine-induced local immunity at the site of first pathogen exposure may have the best chance at preventing the spread of infection beyond the pathogen portal of entry. We hypothesized that a mucosal immunization would prime memory T cells to reside in vaginal tissues and provide better protection against vaginal HSV-2 exposure than other routes of immunization. Our initial data suggests that intranasal immunization is effective in protecting mice from vaginal HSV-2 infection. Ongoing work focuses on characterizing the role of vaccine-elicited mucosal CD8+ T cells in preventing infection to provide insight into the mechanisms of protection induced by mucosal immunization for HSV-2. These findings contribute to the efforts to generate an effective vaccine to prevent HSV-2 infection and disease.