UNIVERSITY of WASHINGTON

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Online Proceedings

2D

## MICROBIOME AND VACCINES

*Session Moderator: James Mullins, Microbiology*

**MGH 234**

*3:30 PM to 5:15 PM*

* Note: Titles in order of presentation.

**Vaginal Dysbiosis Induces Altered Pre-Exposure Prophylaxis Pharmacokinetics**

*Avie Ha, Senior, Biology (Physiology)*
*Casey Yaejin Kim, Senior, Biology (Molecular, Cellular & Developmental)*
*Mentor: Nichole Klatt, Pharmaceutics*
*Mentor: Ryan Cheu*

Human immunodeficiency virus (HIV) infects over one million women annually. Pre-exposure prophylaxis (PrEP) drugs are developed to prevent HIV acquisition and have yielded varying results in women. Despite adherence, recent evidence suggests biological factors, such as the vaginal microbiome, contributes to its variability. Such drugs are tenofovir (TFV), tenofovir alafenamide (TAF), tenofovir disoproxil fumarate (TDF), and dapivirine (DPV) and their efficacy in HIV prevention are vital to public health. There is recent evidence demonstrating vaginal dysbiosis is associated with an increased risk of HIV transmission by bacterial metabolism, altering PrEP pharmacokinetics (PK). Vaginal dysbiosis occurs when the dominant *Lactobacillus* is replaced with a more diverse anaerobic community, including *Gardnerella*. To investigate the impact of the vaginal microbiome on PrEP PK, I incubated target Jurkat cells, with various PrEP drugs in the presence of *Lactobacillus* and *Gardnerella vaginalis*. *Lactobacillus* spp. represents the healthy commensal species, while *G. vaginalis* represents dysbiosis. Abiotic and drug free samples were used as negative controls. Using Liquid Chromatography-tandem Mass Spectrometry, I measured residual PrEP drugs, bacteria mediated metabolites, and pharmacodynamically active metabolites representing target cell uptake at different time points. In the presence of *G. vaginalis*, we saw a significant decrease in extracellular TFV ($p<0.0001$) and DPV ($p<0.001$) when compared to *Lactobacillus* and abiotic controls. We saw a milder effect with TDF ($p=0.004$) and TAF ($p=0.03$), most likely due to the pro-drug formulation. When comparing bacterial metabolites, we observed an increase in the presence of *G. vaginalis* for TFV ($p=0.0001$), but not in abiotic and *Lactobacillus* samples. Similarly, we saw a decrease in pharmacodynamically active metabolites in the presence of *G. vaginalis* for TFV ($p=0.0004$) and DPV ($p<0.0001$) and an increase in abiotic and *Lactobacillus* samples. By improving the health of the vaginal microbial communities, we can improve the adherence of PrEP drugs and improve HIV prevention.

**Gut Microbiome Dysbiosis After Zika Virus Infection is Associated with Increased Peripheral and Nervous System Inflammation Macaques**

*Claudya Allicia Evandy, Senior, Biology (Molecular, Cellular & Developmental)*
*Cindy Anggelica Evandy, Senior, Biology (Molecular, Cellular & Developmental)*
*Mentor: Nichole Klatt, Pharmaceutics*
*Mentor: Charlene Miller*

Originating from an outbreak in Brazil, with an estimated 440,000-1,300,000 Zika cases in 2015, Zika virus infection (ZIKV) has made its way into the United States. The objective of this study was to examine the changes in the gastrointestinal microbiota after ZIKV infection and investigate how this contributes to microbial translocation, immune activation and inflammation in the periphery and CNS. Eight pigtail macaques (PTM) and eight rhesus macaques (RM) were infected with a Brazilian isolate of ZIKV. DNA extractions were carried out from PTM rectal swabs and were used to analyze microbial shifts through 16S rRNA analysis. Production of short chain fatty acids (SCFAs) in the gut were measured by GC-MS. Expression of microbial translocation markers (LBP) were analyzed by ELISA in PTMs. Inflammation and immune activation were determined from neopterin production and kynurenine:tryptophan ratio (KTR) in the plasma and CSF of both species by tandem LC-MS/MS. We found that ZIKV infection results in microbial dysbiosis in the rectum of PTMs, including loss of beneficial Firmicutes phyla and an increase in opportunistic pathogen associated bacteria such as Proteobacteria and Spirochaetes. A significant decrease was seen in total SCFA production in PTMs

7 days post-inoculation (dpi) (p=0.0125). Plasma LBP was significantly increased 3 dpi in PTMs (p=0.0305). A statistical increase in neopterin and the KTR was detected in plasma and CSF of both species 7 dpi (all p values $<$0.0001). Moreover, the loss of Firmicutes *Solobacterium* in the male PTM after ZIKV infection was negatively associated with microbial translocation (p$<$0.0001, LBP) and inflammation (p=0.0374, KTR). These data indicate that microbial dysbiosis after ZIKV infection is associated with microbial translocation and inflammation, which may underlie the localized inflammation and immune activation occurring in the CNS during ZIKV infection and provide possible evidence for a mechanism on how CNS inflammation occurs in ZIKV infection.

**Alterations in Microbial Communities and Function from Cannabis Use amongst HIV+ Individuals**

*James Bryceson Hummer, Senior, Microbiology*
*Mentor: Nichole Klatt, Pharmaceutics*
*Mentor: Ryan Cheu*
*Mentor: Andrew Gustin, Global Health - Pathobiology*

Human immunodeficiency virus (HIV) infections are often paired with microbial translocation within the gastrointestinal tract (GI). In-vivo studies show that HIV-associated microbial translocation results from multiple immunopathological events occurring at the GI mucosa: early, detrimental, severe CD4(+) depletion in mucosal regions, mucosal immune hyperactivation and inflammation, and intestinal epithelium damage. Previous human studies have shown anti-inflammatory effects of cannabis, along with slowed GI tract disease progression. This project aims to further understand HIV infection and its response to patients' cannabis use. We hypothesize the anti-inflammatory effects of cannabis should decrease the microbial translocation within HIV+ individuals. Using stool from HIV+ and HIV- patients, DNA and short chain fatty acid (SCFA) extractions were performed. The DNA was extracted using Power Fecal DNA extraction kits. In order to understand microbial community and how it relates to SCFA production, the extracted genomic DNA was PCR-amplified and will be sequenced with an Illumina MISEQ. SCFA extraction was done with ethyl acetate. We analyzed the samples using gas chromatography-mass spectrometry, and it showed HIV+ patients had a decrease in acetic (p=0.0057), propionic (p=0.0325), butyric (p=0.0007), iso-butyric (p=0.0039), and valeric acid (p=0.0133). When compared with HIV- patients, there's an overall decrease in total SCFA (p=0.0012). These results demonstrate the detrimental effects HIV infection has on SCFA production, which can lead to a decrease in available energy for colon cells and disrupt gut microbial community health. However, amongst HIV+ with cannabis use, we saw an increase in acetic (p=0.0173), propionic (p=0.0087), butyric (p=0.0173), iso-butyric (p=0.0043), and total SCFA (p=0.0057). The HIV+ patients that used cannabis saw improvement in microbial community health, which is a promising sign that cannabis assists with the recovery of antiretroviral treatment.

**Vaccine for East Coast Fever**

*Brian Hyung Chan Kim, Senior, Biochemistry*
*Mary Gates Scholar, UW Honors Program*
*Mentor: Neil King, Biochemistry*

East Coast Fever (ECF) is a tick-transmitted disease caused by *Theileria parva* in cattle that is detrimental to the economic well-being of Eastern and Southern Africa. Current disease control involves pesticides, antibiotics, and a commercial live parasite vaccine, but they are insufficient and thus developing an effective, affordable, and protective vaccine for ECF is a pressing need. Studies by the Nene group at International Livestock Research Institute have demonstrated that the *T. parva* sporozoite stage-specific surface coat protein p67 provides partial protective immunity to cattle when used as a subunit vaccine. Within the King group at the University of Washington, I generated novel p67C nanoparticle immunogens intended to induce more potent and durable immune responses in immunized cattle. I genetically fused the p67C epitope to a variety of self-assembling protein nanoparticle subunits and screened for stability and expression levels. Based on these data, I selected three best-performing p67C nanoparticles for larger-scale expression and purification: I32-19, I32-28, and I53-50. Each of them, with 60 copies of the p67C, was expressed, purified, and extensively quality-controlled to confirm monodispersity, purity, and low endotoxin levels for immunization studies. Immunogenicity data from the ILRI show that the nanoparticles with p67C induce a similar level of p67C-specific antibodies as a combination of HepB core antigen and mesoporous silica nanoparticles containing more than twice as much p67C antigen, and far higher antibody levels than p67C alone. It is an outstanding vaccine candidate to help those suffering from ECF. These preliminary results will be confirmed by parasite challenge studies in 2018.