SCHOOL OF MEDICINE & DENTISTRY
UNIVERSITY of ROCHESTER MEDICAL CENTER

2018 PREP Symposium

Auditorium K-307 (3-6408)

Tuesday, June 12, 2018

Please join us for refreshments immediately following the presentations in CEL Room 2-7534

Post-baccalaureate Research and Education Program

Program Directors:

Edith M. Lord, Ph.D., Jacques Robert, Ph.D., & Elaine M. Smolock, Ph.D.

2:00pm-2:15pm	**Nicole Fernandez**	**Checkpoint Molecule Expression in the Lungs is Affected by *Pneumocystis* Pneumonia Immunopathogenesis** ***Mentor: Terry Wright, Ph.D.***
2:15pm-2:30pm	**Amber George**	**The DNA phosphorothioation System of *Mycobacterium abscessus*** ***Mentor: Martin Pavelko, Ph.D.***
2:30pm-2:45pm	**Xavier Gonzalez**	**An Innate-like T cell Subset Critical for Viral and Tumor Immunity in the Amphibian *Xenopus*** ***Mentor: Jacques Robert, Ph.D.***
2:45pm-3:00pm	**Melvin King**	**Editing the Human Cytomegalovirus Genome with the CRISPR/Cas9 System** **Mentor: Joshua Munger, Ph.D.**
3:00pm-3:15pm	**Cody McKee**	**Modifying Lysosomal Function in an *in Vitro* Alzheimer's Model** ***Mentor: Chris Pröschel, Ph.D.***
3:15pm-3:30pm	**Danielle Reid**	**Investigating the Ability of Human Immunodeficiency Virus-1 (HIV-1) to Infect Pericytes and Astrocytes** ***Mentor: Sanjay Maggirwar Ph.D.***
3:30pm-3:45pm	**Lauren Roberts**	**Russian and American LAIV Mutations Influence Temperature Sensitivity in pdm 09 H1N1 RNA Polymerase Differently** ***Mentor: Steven Dewhurst, Ph.D.***
3:45pm-4:00pm	**Carlos Rodriguez**	**Development of an Orthotopic Colorectal Cancer Mouse Model for Radiation Therapy Studies** ***Mentor: Edith Lord, Ph.D.***

Thank you to everyone involved in PREP!

Mentors, Bench Mentors & Committee Members

PREP Council Leader:

Americo Lopez Yglesias, Ph.D.

Skills Workshop Leaders:

Nicholas Battaglia, Melvin King, Americo Lopez Yglesias, Ph.D., & Janelle Veazey

GRE Study Session Leader:

Melvin King

Administrative Assistance:

Daisy Bird, Stephanie Corbitt & Benjamin Lovell

Life Sciences Learning Center:

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

SCHOOL OF MEDICINE & DENTISTRY
UNIVERSITY of ROCHESTER MEDICAL CENTER

Checkpoint Molecule Expression in the Lungs is Affected by *Pneumocystis* Pneumonia Immunopathogenesis

Nicole Fernandez, Jane Malone, Justin Cobb, Francis Gigliotti & Terry Wright

Pneumocystis pneumonia (PCP) is an opportunistic fungal infection that causes life-threatening complications in immunocompromised, such as those with HIV/AIDS, and immunosuppressed individuals. The morbidity and mortality rates caused by PCP remains significantly high due to the growing issue of antibiotic resistance against *Pneumocystis*, creating difficulty for patients to seek effective treatment. Novel treatments utilizing host-mediated immune defenses are necessary to reduce the increasing PCP-related mortality rates in patients. Impaired or absent CD4 T cell has been found to prompt *Pneumocystis* infection and subsequent pneumonia in hosts. Prior research in *Pneumocystis* infected mice lacking CD4 T cells has shown a large accumulation of impaired CD8 T cells present in the lungs. These compromised cells are unable to perform antifungal effector functions thus cannot clear the pathogen from the host. We hypothesize that the CD8 T cells present in infected hosts lacking CD4 T cells become functionally exhausted and contribute to harmful PCP-associated inflammation. Our research focuses on determining the inhibitory receptors and their ligands that contribute most to this suppressive phenotype. We utilized a CD4 T cell depleted mouse model of PCP to investigate this hypothesis. Mice were sacrificed over the course of a 5-week infection to characterize differences in checkpoint receptor and ligand expression in regards to disease severity. The differential gene expression levels were quantified utilizing mice lung tissue mRNA with primers of selected receptors and ligands. Our study suggests that the checkpoint inhibitors PD-1, PD-L1, B7-H4, CD200R, and CD86 have expression levels affected, either through upregulation or downregulation, through the pathogenesis of PCP. Using this data, the future direction for our project would be to treat *Pneumocystis* infected CD4 T cell deficient mice with antibodies to block expression of these inhibitors to reverse CD8 T cell exhaustion.

The DNA Phosphorothioation System of *Mycobacterium abscessus*

Amber George & Martin Pavelka

Mycobacterium abscessus is a non-tuberculosis mycobacterium (ntm) that causes soft tissue and bronchopulmonary infections and is often acquired in the environment from contaminated soil or water. Research conducted on *M. abscessus* typically focuses on addressing its resistance to a wide range of antimicrobials as well as a variety of disinfectant agents. Treatment of *M. abscessus* is very difficult as it involves combination antibiotic therapy, which is ineffective at clearing the bacteria. This course of antibiotic treatment is administered for a long period of time and is often expensive, especially in the case of a severe infection.

The goal of this study is to construct and analyze a mutant strain of *M. abscessus* lacking the DNA phosphorothioation (dnd) system. A cluster of dnd genes control how sulfur is incorporated into the DNA backbone by replacing a non-bridging oxygen with an atom of sulfur. We hypothesize that the loss of this DNA backbone modification will have an influence on gene expression and DNA stability in the bacteria, and may make it more susceptible to oxidative, chemical and antibiotic stresses. The loss of the dnd system may result in decreased fitness of the mutant in an *in vivo* mouse model. A greater understanding of this system and its effects on DNA stability and gene expression could lead to new treatment targets for this pathogenic mycobacterium.

An Innate-like T cell Subset Critical for Viral and Tumor Immunity in the Amphibian *Xenopus*

Xavier Gonzalez, Eva-Stina Edholm, Maureen Banach & Jacques Robert

Innate-like (i)T cells express an invariant TCRα and are restricted by MHC class-like molecules. Although iT cells are gaining attention owing to their potential to regulate immune responses to a broad range of pathogens, their functions *in vivo* are still not fully understood. As such, there is a need for an alternative animal model. Recent findings have identified an MHC-class-I-like molecule, XNC10, in the amphibian *Xenopus* that is required for the development and function of an innate-like T cell subset (iVα6 T cells) that expresses the invariant TCR α-chain iVα6-Jα1.43. To explore XNC10/iVα6 axis; we are using a multipronged approach, including viral infection and tumor challenge. Both XNC10 and iVα6 T cells play important roles in an effective immune response against Frog Virus 3 (FV3), a pathogen that harms natural amphibian populations worldwide. We hypothesize that during early stages of FV3 infection, the expression of XNC10 molecules increases at the surface of immune cell effectors such as neutrophils and macrophages, and that iVα6 T cells are activated by these effectors. However, it is not known whether iVα6 T-cells require activation by XNC10 to elicit an antiviral response. Although treatment with an anti-XNC 10 pAb before or just after FV3 infection did not affect survival or viral replication, it may be that this Ab is not sufficiently efficient in impairing XNC10 positive cells *in vivo*. Notably, previous studies revealed that in one of *Xenopus* thymic lymphoid tumors - 15/0, XNC10 depletion leads to tumor rejection in syngeneic tadpoles. To further explore the role of XNC10 during tumor development, we focused on a similar but genetically distinct lymphoid tumor, ff-2. Remarkably, CRISPR-Cas9-mediated disruption of XNC10 in ff-2 tumors also results in their acute rejection in syngeneic tadpoles. Our work using *Xenopus* and an evolutionary approach contributes to better understanding the relevance of MHC class I-like molecules and iT cells in host defense.

Editing the Human Cytomegalovirus Genome with the CRISPR/Cas9 System

Melvin King, Xenia Schafer & Joshua Munger

Human Cytomegalovirus is an opportunistic, widespread pathogen that is a major cause of birth defects and can be deadly to immunocompromised individuals. Recombinant human cytomegalovirus (HCMV) virions, generated by Bacterial Artificial Chromosomes (BAC), are common tools for determining which viral gene products can be targets of therapeutic intervention. However, BAC recombineering is bottlenecked by nonspecific recombination events and the lengthy, low efficiency process of regenerating virus from BAC vectors. We present an alternative method of producing recombinant HCMV using the CRISPR/Cas9 system in primary fibroblasts. Using CRISPR-mediated Homologous Recombination (HR), we demonstrate the introduction of genomic modifications up to 1.5kb in size. We also utilize HR and Non-homologous end-joining (NHEJ) to make smaller modifications and efficiently silence viral gene expression. These results provide a framework for using CRISPR/Cas9 to investigate the role of individual open reading frames (ORFs) in HCMV molecular pathology.

Modifying Lysosomal Function in an *in Vitro* Alzheimer's Model

Cody McKee, Gail Johnson & Chris Pröschel

Alzheimer's Disease (AD) is a neurodegenerative disease that currently affects over 5 million Americans with prevalence expecting to increase exponentially in the near future. The two factors that epitomize this disease are the hyper-phosphorylation of microtubule tau proteins and the aggregation of amyloid beta (Aβ) plaques. Lysosomes, a key player in the cell's autophagy system, bind with autophagic vacuoles providing the acidic environment required to break down and recycle lipids and proteins to maintain homeostasis. It has been shown that some mutations associated with familial AD (FAD) result in impaired lysosomal function that leads to exacerbated disease pathology. Here I have established a FAD model using primary fetal neurons. This model incorporates various mutations that have been associated with FAD pathology. The goal of this project is to study these mutations in the context of lysosome functionality. We hypothesize that additional FAD associated mutations result in alkalization and dysfunction of the lysosome. I can assess lysosomal functionality using LysoTracker, a marker used to track acidified compartments within the cell. Here I have preliminary evidence that suggests that a specific amyloid precursor protein mutation known to result in accumulated amyloid beta within the lysosome, has a decreased number of acidified vacuoles when compared to the control. If this trend proves to be significant I can then treat with FDA approved drugs that have been previously identified by the lab to re-acidify the lysosome and mitigate lipid accumulation in the context of a lysosomal storage disorder model. If treatment with these lyso-restorative drugs leads to a decrease in AD pathology, we can further investigate them as a potential therapeutic to treat this devastating disease.

Investigating the Ability of Human Immunodeficiency Virus-1 (HIV-1) to Infect Pericytes and Astrocytes

Danielle Reid & Sanjay Maggirwar

The human immunodeficiency virus-1 (HIV-1) is a retrovirus that attacks the immune system and brain. Early during infection, HIV-1 can cross the blood brain barrier (BBB), infect brain cells, and induce inflammation. The BBB prevents and regulates the passage of materials between the peripheral and cerebrospinal compartments. Through passive and active transport, materials are carried in and out of the cerebrospinal fluid via efflux mechanisms, for which HIV-1 is a substrate. The efflux mechanisms may explain why some drugs are unable to reach therapeutic concentrations in the central nervous system. Induced inflammation caused by infection can lead to the development of HIV-associated neurocognitive disorder (HAND), characterized by neuronal damage and loss of pericytes and astrocytes. HIV can interact with factors that recognize and repair DNA damage, and as a result the DNA damage response (DDR), and likely telomere maintenance, are less efficient. There is insufficient information concerning how silenced HIV-1 infection in latent cells affects DDR in memory T cells and brain cells, where infected cells are exposed to neuroinflammatory conditions and chronic oxidative stress. Previously, we showed that HIV latency in memory T cells increases cellular susceptibility to DNA damage. In this study, we investigated whether latent and non-latent infections of pericytes and astrocytes affect cell susceptibility to neuroinflammation by increasing pro-inflammatory factors, extracellular glutamate, nitric oxide, and sCD40L. Previously we showed that HIV-1 infected pericytes and astrocytes significantly reduced cell count. When non-latently infected pericytes were exposed to TNFα, and infected pericytes to glutamate, γH2AX levels increased, signifying increases in the steady-state levels of DNA damage. Our data indicate that pericytes and astrocytes silence HIV expression at a similar rate and time frame as primary memory T cells. However, latently infected pericytes and astrocytes are more susceptible to glutamate and inhibitors targeting repair of single and double strand DNA breaks. TNFα and IL-1β can reactivate HIV-1 in latently infected pericytes, but not in astrocytes, suggesting that disease progression and neuroinflammation may facilitate virus reactivation. In conclusion, our results indicate that infected pericytes and astrocytes that survive infection will be affected by neuroinflammation due to virus interference with the DDR, subsequently resulting in reduced cellular viability.

Russian and American LAIV Mutations Influence Temperature Sensitivity in pdm 09 H1N1 RNA Polymerase Differently

Andrew Smith, Lauren Roberts, Laura Rodriguez-Garcia, Aitor Nogales, Luis Martinez-Sobrido & Stephen Dewhurst

The influenza A virus (IAV) infects 10-20% of the world population annually, but current vaccine approaches continue to show poor (50% or less) levels of efficacy. Our long-term goal is therefore to improve the safety and efficacy of Live-Attenuated Influenza Vaccine (LAIV). As a first step towards this objective, we wish to understand the underlying molecular basis for the attenuation of LAIV – initially by characterizing how the mutations in the viral RNA-dependent RNA polymerase (RdRp) contribute to the temperature sensitive (*ts*) phenotype of LAIV. We performed viral minigenome assays in human 293 T cells at 33, 37 and 39°C, to assess the functional activity of the viral RdRp, and we also performed virus growth assays at the same temperatures, along with *in vivo* assays of viral virulence. For these studies, we compared both the U.S (A/Ann Arbor/6/60 [H2N2]) and Russian (A/Leningrad/134/17/57) LAIVs – by introducing the corresponding polymerase mutations into the genetic background of the pandemic A/California/04/09 [H1N1] virus (CAL). The US LAIV mutations placed in a CAL background the polymerase exhibits only a slight a *ts* phenotype during *in vitro* replication at 39C. The Russian LAIV mutations further inactivates the RdRp more than the US LAIV at 39C in the CAL background. Overall, these data indicate that the Russian LAIV polymerase mutations induce a more pronounced *ts* and attenuated phenotype on influenza A viruses than do the US LAIV polymerase mutations. These findings have important implications for the development of new, improved LAIVs

Development of an Orthotopic Colorectal Cancer Mouse Model for Radiation Therapy Studies

Carlos J. Rodriguez Hernandez, Nicholas G. Battaglia, Scott A. Gerber & Edith M. Lord

Radiation is commonly used before surgery to reduce tumor burden in rectal cancer. It has been suggested that radiation therapy induces an immune response against the tumor. Previous work using mice ectopic models has shown a correlation between tumor regression and the production of IFN-γ and the induction of cytotoxic T cells after radiotherapy. Ectopic models, such as the use of colon cell lines in leg muscle, provide us with some information about the response to radiation, but this response may differ if the tumor is grown orthotopically in the tissue from which it originates. To assess this potential difference in response, we have developed an orthotopic model to study the growth of colorectal cancer in mice and the response to radiation treatment. In our model, MC38 adenocarcinoma murine cells expressing luciferase are implanted in the rectal wall of C57BL/6 mice through the serous membrane. Tumor growth, assessed using an in vivo imaging system (IVIS), revealed tumor masses that steadily increase in size reaching approximately 1 gram 21 days post-injection of 50,000 cells. Importantly, tumor growth in most mice was limited to the site of injection, as mesenteric and omental sites were free of tumor burden. The cellular composition and the morphology of these tumors is being examined using flow cytometry and immunohistochemistry, respectively. Furthermore, studies are underway to determine the response of these tumors to fractionated radiation and to determine if there are differences from the ectopic models of colon cancer. This more clinically relevant model will improve our understanding of response to radiotherapy in colorectal cancer and help develop more effective treatments for this disease.