SCHOOL OF MEDICINE & DENTISTRY
UNIVERSITY of ROCHESTER MEDICAL CENTER

2020 PREP Symposium

June 3, 2020

Via Zoom from 2:00-4:00p

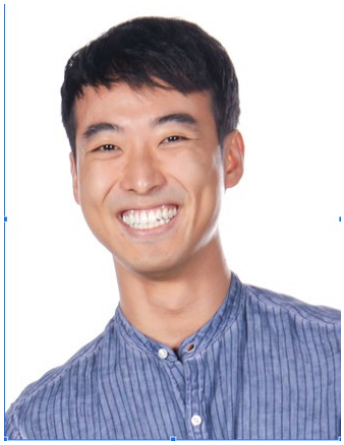
Please stay on zoom and join us for a virtual reception following the presentations.

Program Directors:

Jacques Robert, PhD & Elaine M. Smolock, PhD

Presentations

Time	Name	Title; *Advisor*
2:00pm-2:15pm		**Introduction**
2:15pm-2:30pm	**Jong Keon Shin**	**Significance of the MHC class I-like XNC10 and Innate-Like T Cell iVα6 in immunity to *Xenopus* Thymic Lymphoid Tumor Using CRISPR/Cas9-mediated Reverse Genetics.** ***Advisor: Jacques Robert, PhD***
2:30pm-2:45pm	**Madison Armstrong**	**Characterization of IDO1 Expression in Human Placenta throughout Pregnancy** ***Advisor: Martha Susiarjo, PhD***
2:45pm-3:00pm	**Darline Castro-Meléndez**	**Influence of AHR Activation on Dendritic Cell Responses During Influenza A Virus Infection** ***Advisor: B. Paige Lawrence, PhD***
3:00pm-3:15pm	**Victoria Popov**	**High-density Electrical Mapping of Inhibitory Processes During Dual-Task Walking in Schizophrenia** ***Advisors: John Foxe, PhD & Ed Freedman, PhD***
3:15pm-3:30pm	**Adilene Realivazquez Pena**	**Evaluating the Regulation and Function of MocR in *Staphylococcus aureus* CC30** ***Advisor: Steven Gill, PhD***
3:30pm-3:45pm	**Kimberly Scofield**	**Iron-Sulfur Contaminants as a Cause of Air Pollution-Induced Developmental Neuropathology** ***Advisor: Deborah Cory-Slechta, PhD***
3:45pm-4:00pm	**Francesca Agobe**	**Nanoparticle Mediated Immunosuppression in Contact Hypersensitivity** ***Advisor: Lisa DeLouise, PhD***



Significance of the MHC class I-like XNC10 and Innate-Like T Cell iVa6 in immunity to *Xenopus* Thymic Lymphoid Tumor Using CRISPR/Cas9-mediated reverse genetics

Jong Keon Shin, Adil Khan, Matthieu Paiola, Francisco De Jesús Andino, and Jacques Robert

Major histocompatibility complex (MHC) molecules are encoded by a set of genes that play a critical role in adaptive immunity of vertebrates. From our previous study in the amphibian *Xenopus laevis,* the MHC class I-like XNC10 was found to be a required genetic component for the development of a subset of innate-like T(iT) cells expressing the T cell receptor (TCR) rearrangement iVα6-Jα1.43, which exhibits similarities with mammalian invariant natural killer T(iNKT) cells. The antitumor immune function of iVα6-Jα1.43 was evaluated by transplanting the *Xenopus* thymic lymphoid tumor, ff-2, into histocompatible F inbred tadpoles. Notably, transitory depletion of iVα6 T cells by XNC10 tetramer treatment in wild type (WT) F strain tadpoles resulted in increased growth of transplanted WT ff-2 tumors. The effect of XNC10 deficiency was further investigated either at the organism or the tumor cell levels. Interestingly, tumor rejection was induced both when ff-2 cells with disrupted XNC10 gene were transplanted in WT F tadpoles or when WT ff-2 cells were transplanted into XNC10-deficient transgenic F tadpoles. These unexpected results suggest that the absence of XNC10 either at the organism or at tumor cell level leads to tumor rejection. To further explore this issue, we transplanted homozygous $XNC10^{-/-}$ and heterozygous $XNC10^{+/-}$ KO ff-2 tumors into XNC10-deficent transgenic tadpoles. Although our result was inconclusive due to small sample size, we have observed a trend of increased tumor growth in XNC10 deficient tadpoles transplanted with $XNC10^{+/-}$ ff-2 tumor, whereas transplanted $XNC10^{-/-}$ ff-2 tumor tadpoles did not show major growth difference compared to ff-2 WT control.

Characterization of IDO1 Expression in human placenta throughout pregnancy

Madison Armstrong, Shawn P. Murphy, and Martha Susiarjo

The human placenta plays an important role in pregnancy, facilitating the exchange of oxygen and nutrients while also protecting the developing fetus from the maternal immune system and infections. Failure in establishing maternal-fetal immune tolerance is linked to pregnancy loss and other complications including preeclampsia. Previous studies showed that indolamine-2,3 dioxygenase (IDO)1 is involved in regulating maternal immunity. This enzyme catabolizes the essential amino acid tryptophan along the kynurenine pathway. In mice, kynurenine metabolites suppress maternal T-cell activity at the maternal-fetal interface, providing protection for the developing fetus. Perturbed IDO1 activity results in elevated levels of maternal tryptophan that have been more frequently observed in women with recurrent pregnancy loss and preeclampsia. Neither the window of activation for IDO1 expression within the human placenta has been well defined, nor how IDO1 expression changes throughout gestation. Furthermore, interferon gamma (IFNγ) was previously shown to upregulate IDO1 expression in the placenta, but the effects of IFN alpha (IFNα) and IFN lambda (IFNλ) have not been reported. Thus, the goals of this study are to define the temporal window of IDO1 expression within the human placenta, and evaluate the effects of interferons on IDO1 expression across gestation.

Human placentas were collected from elective terminations of 1st trimester (9-12 weeks) and 2nd trimester (13-22 weeks) pregnancies and Caesarean sections of term (39-41 weeks) pregnancies. Placental villous explants were cultured *in vitro* +/- IFNs to examine the effects on IDO1 expression. Protein lysates were extracted from placental villi and levels of IDO1 protein assayed using Western Blot. IDO1 levels were normalized to 14-3-3/YWHAZ.

Our study shows that IDO1 expression is not detectable by Western Blot in first trimester placental villi, but it increases progressively as pregnancy advances to term. IDO1 expression begins to increase during the thirteenth week of gestation and plateaus towards the end of gestation. IFNγ significantly increased IDO1 expression at all stages of gestation, while neither IFNα nor IFNλ had any effect.

Our results show the IDO1 expression is induced early during the 2nd trimester and increases further as pregnancy continues to term. IFNγ substantially increased IDO1 expression in human placenta, while IFNα and IFNλ had no effect. Identifying the window of activation for IDO1 expression within the placenta provides a foundation for future studies to define the molecular mechanisms accounting for regulation of expression and the precise functional roles of IDO1 during gestation.

Influence of AHR activation on Dendritic Cell responses during Influenza A virus infection

Darline Castro-Meléndez, Anthony M. Franchini, and B. Paige Lawrence

Influenza A viruses (IAV) cause severe disease in humans and are a significant public health problem. Epidemiology studies have shown that human exposure to dioxins and PCBs exacerbate influenza and other respiratory diseases. These compounds bind the aryl hydrocarbon receptor (AHR), and by activating the AHR they hinder effective host response to infection by negatively regulating the ability of dendritic cells (DCs) to prime naive CD8+ T cells. Thus, AHR activation reduces the generation of cytotoxic T cells (CTLs), which are critical for controlling viral infections. Yet, how environmental AHR ligands regulate antiviral defenses in DCs is not fully understood. Recently, transcriptomic analysis of DCs isolated from the lungs of IAV infected mice revealed that AHR activation changed expression of several gene families associated with viral uptake and processing. Specifically, AHR activation significantly decreased expression of *Cd209a* and *Clec9a*, receptors that detect and bind carbohydrate antigens, influence DC phagocytic activity, and antigen processing pathways. The present study tests the hypothesis that AHR-driven changes in gene expression enhance direct infection of dendritic cells, reducing their ability to sense and uptake virus particles for processing and presentation to naïve T cells. To test this, we used both *in vitro* and *in vivo* systems to determine if AHR ligands alter DC expression of C-type lectin receptor family members and if AHR alters direct IAV infection of DCs using flow cytometry. *In vitro* treatment of bone marrow derived dendritic cells (BMDC) with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or kynurenic acid (KYNA) did not show any significant differences. However, *in vivo* treatment with TCDD in C57Bl/6 mice caused a statistically significant increase in the amount of IAV in DCs from lung-draining mediastinal lymph nodes (MLN) compared to DCs from MLNs of infected vehicle control treated mice. These data show that following AHR activation immune responses to virus infections are altered through changes in function of DCs. This study provides further evidence that the AHR is a multifaceted regulator of the immune system and understanding the effects of its activation will give us further insight into how environmental exposures modify anti-viral immune responses.

High-density Electrical Mapping of Inhibitory Processes During Dual-Task Walking in Schizophrenia

Victoria A. Popov, Edward G. Freedman, and John J. Foxe

Background Schizophrenia spectrum disorders (SSDs) are chronic, debilitating psychiatric disorders. These multi-dimensional disorders are characterized by impulsivity and deficiencies in planned behavior. Disruptions in sensory and cognitive processing pathways in people with diagnoses of SSDs are well-established (Barch and Sheffield, 2014; Javitt, 2009; Sehatpour et al., 2010). However, to our knowledge, no studies to date have examined changes in neural activity in individuals with SSDs when walking and cognitive tasks co-occur. Physical activity and the resultant increase in functional connectivity between the default mode network and frontal executive control networks may ameliorate symptoms and improve cognitive performance in individuals with SSDs (Lebiecka et al., 2019; Maurus et al., 2019). This study is critical for identifying and characterizing neural endophenotypes in individuals with SSDs, that are the result of altered neural pathways, during walking. Knowledge of such changes in cognitive function during walking could aid in the establishment of optimal treatments for improving cognitive functioning.

Methods We aim to include 30 neurotypical, age-matched healthy controls and 30 individuals with SSDs. High density electroencephalography (EEG), motion capture, and Go/NoGo response inhibition cognitive task performance will be recorded across 7 dual-task walking blocks and 7 single-task seated blocks. Neural endophenotypes associated with cognitive function can be identified and characterized through the utilization of high density EEG. Clinical rating scales will include the Positive and Negative Syndrome Scale (PANSS), Brief Psychiatric Rating Scale (BPRS), and the Structured Clinical Interview for DSM-V Disorders (SCID-V), to diagnose and quantify current symptom severity and ensure correct placement of subjects into their associated groups. Daily physical activity will be recorded through the International Physical Activity Questionnaire (IPAQ) and cognition will be assessed through the Wechsler Abbreviated Scale of Intelligence, Second Edition (WASI-II).

Expected Results/Conclusions We hypothesize that decreased response inhibition in individuals with SSDs will be related to the single-task (De Sanctis et al., 2014) compared to dual-task and neurotypical, age-matched control subjects. This can be portrayed in individuals with SSDs as greater commission errors and reduced reaction times when making these errors. We also hypothesize that individuals with SSDs will reveal a unique event-related potential (ERP) pattern, with enhanced N2 and reduced P3 (Krakowski et al., 2016), which are neural response inhibitory components, when completing a single-task compared to dual-task and neurotypical, age-matched control subjects. It is critical to acknowledge that the opposite may be true, where findings may be due to dual-task rather than single-task. Dual-task allocates cognitive-motor processes and an increase in cognitive load, due to walking, may decrease task performance and portray cognitive deficits in individuals with SSDs (Malcolm, Foxe, Butler, and De Sanctis 2015). Identification of alterations in inhibitory processes, specifically N2 and P3 ERP components, could lead to intriguing endophenotypes for schizophrenia. Such findings could allow for establishment of therapies to improve cognitive functioning, impulsivity, and deficiently planned behavior through targeting identified endophenotypes.

Evaluating the regulation and function of MocR in *Staphylococcus aureus* CC30

Adilene Realivazquez, Christie Gilbert, and Steven Gill

Staphylococcus aureus is a Gram-positive bacterium that colonizes approximately 30% of the population. The microorganism may cause cutaneous and soft tissue infections, and even more severe infections such as bacteremia, endocarditis, and osteomyelitis. The pathology of *S. aureus* diseases is characterized by the bacterium's ability to colonize nutrient deficient environments, and strategic evasion of the human immune system. Different *S. aureus* strains can be grouped into clonal complexes (CC) that are based on genomic content. Among these, CC30 is frequent in nosocomial and community acquired infections. In this project we focus on the CC30 lineage, which is commonly found in healthcare facilities and often associated with persistent bacteremia and infective endocarditis. The goal of this project is to further characterize the regulation and function of MocR, a transcriptional regulator within the unique genomic islet, LFR. The LFR islet contains a putative LysE translocator (**L**ysE), fatty acid desaturase (**F**ad), and a Moc**R** regulator. Preliminary data suggests that the LFR islet plays a key role in metabolic regulation during *S. aureus* infections. We hypothesize that MocR acts as an autoregulator and plays a significant role in adapting to nutrient deficient environments. To assess MocR activity, promoter-*lacZ* fusion constructs with putative *mocR* promoter sites were transformed into UAMS-1 wild-type and UAMS-1 Δ*mocR* strains. *mocR* promoter activity was evaluated at early exponential, late exponential, and early stationary phase by measuring the hydrolysis of ortho-nitrophenyl-β-galactoside, and quantified as Miller units. In both wild type and Δ*mocR* strains, MocR activity increased with time, showing the most activity during early stationary phase. Additionally, MocR expression in the wild-type strain was significantly higher than in Δ*mocR*, suggesting that MocR acts as an auto regulator by activating its own transcription. To evaluate the role of MocR in *S. aureus* metabolic plasticity in biologically relevant environments, UAMS-1 wild-type and UAMS-1 Δ*mocR* strains were grown in human blood and serum. Our results demonstrated that the presence of MocR did not significantly enhance the survival of *S. aureus* in human blood or serum. Altogether, preliminary data and the results from this project suggest that MocR is an autoregulator and may contribute to enhancing *S. aureus* survival by regulating genes involved in nutrient transport and metabolism. Understanding the intricate process of metabolic regulation in CC30 strains may elucidate the mechanisms of adaptation that enable *S. aureus* to successfully colonize nutrient deficient environments and cause persistent infections.

Iron-Sulfur Contaminants as a Cause of Air Pollution-Induced Developmental Neuropathology

Kimberly Scofield, Katherine Conrad, Elena Marvin, Alyssa Merrill, Timothy Anderson, Marissa Sobolewski, Gunter Oberdorster, and Deborah A. Cory-Slechta

Increasing evidence suggests that the central nervous system (CNS) is susceptible to life-long damage following exposure to air pollution during neurodevelopment. Our previous inhalation studies in mice have identified ambient ultrafine particles (UFPs) as a toxic component of particulate air pollution following either gestational or postnatal exposures. Exposures to ambient UFPs in the early postnatal period, equivalent to human 3rd trimester brain development, resulted in one particularly dramatic neuropathological effect, i.e., male-biased ventriculomegaly (i.e., lateral ventricle dilation, a clinical predictor of adverse developmental outcome), accompanied by corpus callosum demyelination. While accumulating evidence suggests exposure to air pollution may cause neurotoxicity via a multitude of neurodevelopmental mechanisms, less is known about what actual components underlie this damage. However, analyses of particulate filter measurements revealed high levels of multiple trace elements, particularly iron (Fe), and sulfur (S). Fe is critical to myelination, and both Fe and S can act via a ferroptic mechanism of oxidative insult and are neurotoxic in excess. To investigate the potential involvement of Fe and S in the developmental neurotoxicity of UFPs, C57/Bl6J mice were exposed to Fe_xO_y and SO_2 (Fe: 1.0 μg/m^3, SO_2: 0.66 mg/m^3, representative of human exposures) from postnatal days (PND) 4-7 and 10-13, for 4 hours/day. Like UFPs, Fe_xO4-SO_2 combined exposure produced a significant ventriculomegaly, but simultaneously in both the male and female cohorts, as measured at PND14. Additionally, a trend towards increased astrocyte population density in the Fe_xO4-SO_2 combined exposure, but a stable microglial population density was observed in both sexes. Increased myelination however was solely found in the nucleus accumbens of the female Fe_xO4-SO_2 combined exposure group. Collectively, these findings suggest that specific trace element contamination contributes significantly to the neuropathological consequences of ambient UFP exposure during development, a notable finding given the nature of neurodevelopmental disorders such as autism and schizophrenia that have already been linked to air pollution in epidemiological studies. Such findings suggest a potential need for regulation of Fe emissions in air pollution for public health protection and underscore the need to further evaluate elemental contaminants of air pollution.

Nanoparticle Mediated Immunosuppression in Contact Hypersensitivity

Francesca Agobe and Lisa DeLouise

Amorphous silicon nanoparticles (SiNPs) can be found in a wide variety of everyday products. SiNPs are used as food preservatives and can also be found in cosmetics. Due to their large surface area and easily altered surface chemistry, SiNPs can also be used for targetable drugs. The prevalence of SiNPs makes them relevant to study. An immunosuppressive effect has been observed when SiNPs are applied to mouse skin that has been treated with a hapten to induce contact hypersensitivity, which is the mouse model for allergic contact dermatitis. This effect is believed to be mediated by cell trafficking which is assisted by collagen fiber alignment in the skin. Additional research will examine the degree of penetration of SiNPs in mouse skin. Future studies will also examine the role of extracellular vesicles in cell-to-cell communication.

Thank you to everyone involved in PREP!

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