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Online Proceedings

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CENTRAL NERVOUS SYSTEM DISEASE MODELS

Session Moderator: Gwenn Garden, Neurology

JHN 026

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Glial Cell Characterization in an mGluR5 Knockout Rat Model of Autism Spectrum Disorders

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Amgen Scholar, Mary Gates Scholar, UW Honors Program, Undergraduate Research Conference Travel Awardee
Mentor: Elizabeth Nance, Chemical Engineering

Autism Spectrum Disorder (ASD) affects 1 in 68 children and has been steadily growing in incidence in recent years. As a developmental disability, ASD causes social, communication, and behavioral challenges. ASD is as mysterious as it is diverse—no two cases are the same. This not only complicates treatment methods but also makes physical characterization of the autistic brain a tedious challenge. As the number of ASD cases begins to increase, so does the urgency for answers at the developmental and biological level that may unravel this condition and lead to more effective and targeted treatment. Microglia and astrocytes are key non-neuronal cells that exhibit altered activity levels in the autistic brain. The changes in behavior and the role these cells play in ASD, and many other neurological diseases, make them potential therapeutic targets. Leveraging this fact, the focus of my research is to study the morphology and distribution of glial cells in wild type and knock-out (autistic-like) mGluR5 rats using confocal microscopy. Regions of interest for changes in cell behavior and morphology include the hippocampus, cortex, and thalamus. Rats with ASD have microglial cells with enlarged soma, retracted and thickened processes, and processes encircling neurons. Fluorescent antibody stains are used to visualize these cells and structures. By defining a physical distinction between healthy and autistic brains, a new wave of therapeutics that target and mitigate the overactivation and inflammation of microglia and astrocytes can be explored.

Quantum Dot Localization in Glia and Neuronal Cells in the Developing Brain

Binh Dang, Senior, Chemical Engineering
Mary Gates Scholar
Mentor: Elizabeth Nance, Chemical Engineering
Mentor: Mengying Zhang, Molecular Engineering and Science

Quantum dot (QD) semiconductor nanocrystals offer significant advantages over conventional fluorescent markers due to broad excitation spectrum, narrow emission spectrum, and very high photo stability, which enables long term visualization. Because of this, there is great interest in using QDs as biomarkers, where cellular uptake of QDs play an important role. In this study, we aim to characterize QD uptake in neuronal and glial populations in the developing brain as a function of QD surface functionality. Previous research has shown that the behavior of QDs in biological systems can be dictated by surface functionality; however, this has not been systematically studied, particularly in the developing brain. Here, we used cadmium-selenide (CdSe) QDs with either cadmium-sulfide (CdS) or zinc-sulfide (ZnS) shell. These QDs are coated with a layer of surface ligands to protect the core-shell and minimize their hydrophobicity. We set out to examine five different surface coatings, including: mercaptoundecanoic acid (MUA), mercaptopropionic acid (MPA), polyethylene glycol-amine (PEG-NH2), polyethylene glycol-methoxy (mPEG) and carboxylic acid (COOH). We incubated organotypic neonatal rat brain slices in 0.1 and 0.01 μM of QDs over a 24 h period. Using immunohistochemistry and co-localization analysis, we observed QDs with PEG-NH2 functionality localize in neurons, whereas QDs with MUA or MPA surface functionality remain in the brain extracellular space. High resolution confocal imaging is used to visually assess the stained brain slices, and the degree of colocalization with cell stains can be quantified using ImageJ. Furthermore, we investigated the region-dependent of QD-PEG-NH2 localization in neurons. This provides insight into the mechanism of uptake for future studies, which include using QDs

as biomarkers of inflammatory processes, mediated by glia, in the developing brain.

Ischemia/Reperfusion-Induced Type 1 Interferon Signaling in Microglia

Chungeun (Chloe) Lee, Senior, Neurobiology
Levinson Emerging Scholar, Mary Gates Scholar, UW Honors Program, Undergraduate Research Conference Travel Awardee
Mentor: Jonathan Weinstein, Neurology

Ischemic preconditioning (IPC) refers to a neuroprotective phenomenon in which a brief ischemic episode confers robust neuroprotection against subsequent prolonged ischemia. Microglia, the resident immune cells in the brain, play a central role in ischemia/reperfusion-induced neuroinflammation and IPC-induced neuroprotection. Previous work in our laboratory has demonstrated that type 1 interferon signaling, specifically in microglia, is critical for IPC-mediated neuroprotection in white matter. We have also demonstrated that both hypoxic/hypoglycemic (ischemia-like) followed by normoxic/normoglycemic (H/H-N/N) conditions in vitro and transient ischemia in vivo leads to robust expression of interferon stimulated genes (ISGs) in microglia that is completely dependent on expression of type 1 interferon receptor (IFNAR1). In vitro, the H/H-N/N-induced ISG expression in microglia is also dependent on Toll-like receptor-4 (TLR4). However, the mechanism(s) of H/H-N/N-induced ISG response is unknown. We hypothesize that TLR activation by damage-associated molecular patterns (DAMPs) induces a signal transduction cascade that leads to phosphorylation of STAT1, which is also dependent on IFNAR1. Phosphorylation of STAT1 activates specific transcription factors that induce transcription of ISGs, which in turn skews the phenotype of microglia toward a neuroprotective state. We sought to test this hypothesis first by culturing primary microglia from wild-type (WT), TLR4-/- and IFNAR1-/- mice, exposing the microglia to type 1 IFNs or TLR4 agonists and quantifying STAT1 phosphorylation using flow cytometry. Next we exposed WT, TLR4-/- or IFNAR1-/- microglia to H/H-N/N and assessed levels of STAT1 phosphorylation at multiple time points. Our preliminary results suggest dynamic and complex temporal changes in the level of STAT1 phosphorylation in microglia with differential effects in the setting of genetic depletion of TLR4 and IFNAR1. These findings will help elucidate the mechanism of the ischemia/reperfusion-induced ISG response in microglia and may identify molecular targets for modulation of the neuroimmune response in stroke.

Deciphering the Requirements for Binding of a Key Virulence Factor, PfEMP1, to Brain Microvasculature in Pediatric Cerebral Malaria

Cristian Ovadiuc, Senior, Microbiology, Biochemistry
Mary Gates Scholar, McNair Scholar
Mentor: Joe Smith, Center for Infectious Disease Research
Mentor: Selasi Dankwa, Center for Infectious Disease Research

Cerebral malaria (CM) is a multifactorial disease with lethal neurological complications plus increased risks of cognitive and neurological impairments making CM a leading cause of childhood neuro-disability. The malaria parasite, Plasmodium falciparum, accounts for most mortality from malaria, and primarily kills children under five in sub-Saharan Africa. The deadly CM complication develops when red blood cells infected with parasites (iRBCs) bind to the endothelial cell wall in brain microvasculature. This process of parasite sequestration via cytoadhesion is mediated by the parasite ligand, P. falciparum erythrocyte membrane protein 1 (PfEMP1) encoded by a family of 60 var genes. An individual parasite, however, strategically undergoes allelic exclusion to express only a single distinct PfEMP1 variant. PfEMP1 proteins possess the ability to bind relatively few host receptors despite seemingly limitless sequence diversity. Requirements for maintaining binding surfaces conserved in structure and binding potential despite significant sequence diversity remain a mystery. Understanding these requirements will be critical for developing therapeutics to mitigate disease severity. A subset of PfEMP1 proteins associated with severe/cerebral malaria bind highly conserved regions of the host receptor, endothelial protein C receptor (EPCR), and inhibit binding of its natural ligand, activated protein C (APC). There are six PfEMP1 subdomains that bind EPCR (CIDRα1.1 and CIDRα1.4–1.8 domains), which share limited sequence identity among PfEMP1 variants. The data so far suggests the presence or otherwise absence of a coiled coil domain within CIDRα1 determines binding to EPCR. Our study uses computational and molecular biology tools to investigate the molecular and structural determinants for malaria parasite adhesion to EPCR.

Engineering a 3D Brain Microvessel Model for Study of *Plasmodium falciparum* Cerebral Malaria Pathogenesis

Celina Ebba Gunnarsson, Senior, Bioengineering
NASA Space Grant Scholar, UW Honors Program
Mentor: Ying Zheng, Bioengineering

In *Plasmodium falciparum* malaria, infected erythrocytes (IEs) adhere to vascular endothelium, causing occlusion, coagulopathy, organ dysfunction and inflammation. While IE cytoadhesion occurs in many organs, cerebral malaria in particular is associated with high IE cytoadhesion and life-threatening complications. However, IE cytoadhesion in cerebral malaria remains incompletely understood. Research

into human cerebral malaria pathogenesis has been limited by the inaccessibility of the human brain, and existing experimental platforms fail to recapitulate human vascular conditions and disease pathophysiology. *In vitro* microfluidic models lack appropriate geometry and materials to mimic *in vivo* flow conditions and matrix, while animal models do not adequately replicate human disease pathology due to use of non-human malaria parasites and non-human tissues. To address these limitations, we propose a 3D *in vitro* brain microvascular model to study *P. falciparum* cerebral malaria pathogenesis. Microvessels are created by molding collagen I with micropatterned stamps and incorporating brain endothelium and supporting cells into the collagen channel. Micropatterned stamps allow specification of complex physiological vessel network structure, and when these networks are designed using computational flow simulation, they allow specification of physiological flow properties in the fabricated microvessel. Collagen comprises the vascular extracellular matrix *in vivo* and regulates cell morphology, lumen formation and vessel stability, so its use as a biomaterial scaffold increases physiological relevance of the *in vitro* model. By constructing a physiologically relevant brain microvascular bed, we will characterize the molecular and biophysical mechanisms of cytoadhesion and the resulting changes in local endothelial function. The completion of this project will improve understanding of IE cytoadhesion, thereby improving understanding of *P. falciparum* pathophysiology to guide the development of treatments targeting disease mechanisms.

Examining the Activation of c-Jun N-terminal Kinase during the Development of Chronic Epilepsy

Amisha Nitin (Amisha) Parikh, Senior, Biochemistry, Neurobiology

Mary Gates Scholar, UW Honors Program, Washington Research Foundation Fellow

Mentor: Nicholas Poolos, Neurology

Chronic epilepsy is thought to develop due to dysfunctions in various ion channels. Previous researchers in the Poolos lab manipulated a signaling cascade regulating these channels and discovered an increase in activation of c-Jun N-terminal kinase (JNK) in animals experiencing seizures. My project aims to understand this connection and the timeline of JNK activity during the development of epilepsy, hypothesizing that JNK activity will precede epilepsy onset and may be a cause of chronic epilepsy. To study epilepsy, we administer research-bred rats a pilocarpine injection. Shortly after, the rat is in status epilepticus (SE), a state of continuous seizures. After one hour, a phenobarbital injection is administered to halt SE. Seizures begin around one week post-SE and achieve steady-state frequency around four weeks. I analyze brain tissue samples taken after one hour, one day, and one week post-SE using Western-blotting to obtain a relationship between phosphorylated-JNK (pJNK) levels from pilocarpine-treated rats to control rats. There are three JNK isoforms, separated into two bands: 54kDa and 46kDa. JNK 1 is primarily in the 46kDa band, JNK 2 is present in both bands, and JNK 3 is predominately in the 54kDa band. For the 54kDa band, I have discovered a significant increase of pJNK one hour post-SE ($124\pm8.1\%$, $p<0.05$, n=7), one day post-SE ($132\pm9.2\%$, $p<0.05$, n=14), an insignificant change at one week post-SE ($100\pm9.6\%$, $p>0.05$, n=10) and a significant increase in chronic epilepsy ($126\pm7.6\%$, $p<0.05$, n=12). For the 46kDa band, I have discovered an insignificant change of pJNK at one hour post-SE ($101\pm7.1\%$, $p>0.05$, n=8), a significant increase one day post-SE ($123\pm12.8\%$, $p<0.05$, n=12), one week post-SE ($122\pm7.6\%$, $p<0.05$, n=10) and in chronic epilepsy ($116\pm5.9\%$, $p<0.05$, n=12). We hope that, by understanding JNK activation, we can explore avenues for medicine and treatments for epilepsy, through antiepileptic drugs and other therapeutic options.