Poster Session 2

Commons West, Easel 21

1:00 PM to 2:30 PM

Gait Assessment in Wild Type and mdx^4cv Mice

Indu Tejasa Vanteru, Junior, Pre Engineering
UW Honors Program
Mentor: Jeffrey S Chamberlain, Neurology
Mentor: Katrin Hollinger, Neurology

Duchenne Muscular Dystrophy (DMD) is an X linked, recessive genetic disease caused by mutations in the dystrophin gene. The disease is characterized by progressive muscle degeneration. Dystrophic mice (mdx^4cv) are a DMD animal model. Here, we compared mdx^4cv and WT mice to quantitatively assess motor performance. We hypothesized that the mdx^4cvmice would show differences in gait compared to WT as a result of muscle weakening. To test our hypothesis, mice were assessed at 1.5, 3 and 6 months of age using the Noldus CatWalk XT system to analyze gait. The mice voluntarily moved across an enclosed walkway on a backlit glass plate. The prints were captured by a camera and later analyzed using the CatWalk software. Our results show that there are significant differences in gait between the WT and mdx^4cvmice. The mdx^4cv mice were slower to cross the runway than WT at 1.5 (mdx^4cv: 3.60.8s WT: 2.80.5s) and 3 months (mdx^4cv: 3.80.9s, WT 2.51.2s). The distance between the front paws of the mdx^4cv mice were wider than WT at 1.5 months (mdx^4cv: 3.60.8cm WT: 2.80.5cm), 3 months (mdx^4cv: 3.80.9cm WT: 2.51.2cm) and 6 months (mdx^4cv: 1.50.2cm WT: 1.20.2cm). Print position is the distance between the position of the hind paw and the position of the previously placed front paw on the same side. In mdx^4cv mice the print position was larger than WT at 1.5 (mdx^4cv: 1.30.8cm WT: 0.40.2cm) and 3 months (mdx^4cv: 1.40.8cm WT: 0.50.4cm), indicating that the hind leg moved a shorter distance in a single stride. The gait analysis assay can be extended to assess gene therapy based treatments that our lab is currently developing. This will allow us to determine if the treatments are successfully able to mitigate the effects of DMD.

Session 2Q

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.

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.

SESSION 2Q

CENTRAL NERVOUS SYSTEM DISEASE MODELS

Session Moderator: Gwenn Garden, Neurology
JHN 026
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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 ($124 \pm 8.1\%$, $p<0.05$, n=7), one day post-SE ($132 \pm 9.2\%$, $p<0.05$, n=14), an insignificant change at one week post-SE ($100 \pm 9.6\%$, $p>0.05$, n=10) and a significant increase in chronic epilepsy ($126 \pm 7.6\%$, $p<0.05$, n=12). For the 46kDa band, I have discovered an insignificant change of pJNK at one hour post-SE ($101 \pm 7.1\%$, $p>0.05$, n=8), a significant increase one day post-SE ($123 \pm 12.8\%$, $p<0.05$, n=12), one week post-SE ($122 \pm 7.6\%$, $p<0.05$, n=10) and in chronic epilepsy ($116 \pm 5.9\%$, $p<0.05$, 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.

POSTER SESSION 3

MGH 241, Easel 161
2:30 PM to 4:00 PM

Mechanisms of Alzheimer's Disease Mediated by Mutations in Presenilin 2

Michelle Hong, Senior, Neurobiology, Psychology
Mary Gates Scholar, UW Honors Program
Mentor: Suman Jayadev, Neurology
Mentor: Carole Smith

There is an urgent need to clarify the causes of Alzheimer's Disease (AD) so targeted treatments can be developed. Mutations in Presenilin 2 (PSEN2) cause an inherited form of AD, but the mechanism for this relationship is not fully understood. PSEN2 protein (PS2) has been found to regulate pro-inflammatory microglial response and the expression of microRNA miR146, an innate immune regulator. These findings led us to pursue the effects of PSEN2 mutations on microglia function, peripheral immunity, and AD. Our aim is to study mechanisms of microglia-induced neuron injury in AD. We hypothesize that PS2 mutation-expressing microglia contribute to neuroinflammation in AD by cytotoxicity and synaptic pruning mechanisms. To study the impact of stimulated microglia on neurons, we employ co-cultures, where microglial (BV2) and neuronal (SY5Y) cell lines are cultured together. We used a lactose dehydrogenase (LDH) cytotoxicity assay to measure levels of microglia-induced neuronal death. After initial assay optimizations, we measured LDH of a BV2/SY5Y coculture to quantify baseline LDH and optimize the ratio of neuron to microglia plating. Fluorescent microscopy was used to observe microglia and neuron interactions morphologically and flow cytometry quantified the levels of neuron phagocytosis of microglia. Using these methods, we were able to demonstrate interactions between the cells and these same methods can be used to later study synaptic pruning. From here, we will determine whether there is a difference in synaptic pruning levels and cytotoxicity between microglia from a control mouse and a mouse expressing mutated PSEN2 transgene when co-cultured with neurons. If our hypothesis holds true, we expect to see increases in both cytotoxicity and synaptic pruning induced by mutated microglia. Determining and understanding PSEN2's role in causing AD could lead to the development of targeted

treatment for patients with these mutations.

POSTER SESSION 3

MGH 241, Easel 162

2:30 PM to 4:00 PM

Examining Mutations in Presenilin 2 Associated with Alzheimer's Disease

Leah Ariel Osnis, Senior, Biochemistry
UW Honors Program
Mentor: Suman Jayadev, Neurology
Mentor: Susan Fung, Neurology

Mutations in the gene Presenilin 2 (PSEN2) cause familial Alzheimer's disease (fAD). FAD shares clinical and pathological features of sporadic, late onset AD thus fAD cell models can be useful to study mechanisms relevant to all forms of AD which is critical to developing effective AD therapeutics. Presenilin 2 protein (PS2) forms the catalytic subunit of the γ-secretase complex, which cleaves amyloid precursor protein and releases Aβ1-42, considered a pathogenic contributor to Alzheimer's disease (AD). Our laboratory is interested in a fAD associated PSEN2 mutation, a frameshift two base-pair deletion (PSEN2 K115Fx) . The PSEN2 K115Fx is predicted to either lead to a truncated protein suggesting that the mutation may create a shortened peptide that interferes with normal cellular function (dominant negative or toxic gain of function) or result in degradation of the RNA transcript and subsequent loss of normal amount of PS2 protein (loss of function). To better understand how PSEN2 mutations cause disease, we have two objectives. The first aim of this project is to determine if the PSEN2 K115Fx does indeed result in a truncated protein or influence levels of wildtype PS2. I will be collecting human cultured fibroblasts isolated from AD patients with the PSEN2 mutations or controls and prepare cell lysate for analysis by Western blot. My colleague will also be analyzing mRNA levels from those same samples to determine the stability of the PSEN2 mutant and wildtype transcripts in all cases. The second aim is to determine the impact of the mutation on PS2 enzymatic activity. I will culture the cells described above, then infect with a luciferase based enzyme reporter assay to compare the impact of PSEN2 mutations on γ-secretase mediated cleavage of APP. My work will help identify the candidate mechanisms by which the PSEN2 K115Fx mutation causes AD.

POSTER SESSION 3

MGH 206, Easel 171

2:30 PM to 4:00 PM

The Effect of cMaf Overexpression and p53 Excision in Murine Microglia

Lewis Wenbo Yin Luo, Senior, Business Administration (Finance), Neurobiology
Mary Gates Scholar
Mentor: Gwenn Garden, Neurology

Microglia are the resident immune cells of the central nervous system, and are responsible for acute inflammatory responses when injury or infection is detected. However, chronic neuroinflammation has been implicated as a key precursor to neurotoxicity, and thus microglia play crucial roles in neurodegenerative disorders. cMaf is an anti-inflammatory transcription factor that mediates neuroprotective functions of microglia such as tissue repair and phagocytosis of cell debris. In 2014, the Garden Lab published results detailing a hypothesized pathway in which cMaf is downregulated by the cell-cycle regulator p53. However, these results were observed in p53 knockout murine microglia that likely have developed compensatory molecular responses to the lack of this critical transcription factor. To determine if acute p53 deletion by Cre-recombinase in floxed-p53 murine microglia influences cMaf expression we utilized AAV-Cre to infect floxed-p53 microglia, which sheds new light upon the current hypothesis regarding the relationship between p53 and cMaf. In this project, we also explored the effect of overexpression of cMaf by infecting wild-type microglia with exogenous cMaf to overexpress cMaf and examine the resulting impact on microglial activation and phagocytosis of apoptotic bodies using flow cytometry. We use quantitative real-time PCR and Western Blot to analyze the immunological impacts of cMaf overexpression in microglia. The implications of this research extend to a wide range of neurodegenerative disorders, like ALS and Alzheimer's disease, where dysfunctional inflammatory responses may contribute to disease pathology.