MSc DEFENCE – Tuesday, May 31st, 2016
Student: Tissa Rahim
Title: **INVESTIGATING THE REGULATION OF ARNT2, A NEUROPROTECTIVE PROTEIN, IN MODELS OF MULTIPLE SCLEROSIS**
Time and location: 1:30pm PDT; Ken Berry Reading Room in the Department of Pathology & Laboratory Medicine Pathology Education Centre, Room 3200, Jim Pattison Pavilion, VGH, 910 West 10th Avenue, Vancouver, BC.
Supervisor: Jacqueline Quandt

ABSTRACT
Background: The process of axonal degeneration and neuronal loss has been described as the major cause of irreversible clinical disability in multiple sclerosis (MS). An ideal neuroprotective strategy would be to focus on inhibition of axonal degeneration and on protection against neuronal cell death in addition to immunomodulation. The aryl-hydrocarbon receptor nuclear translocator 2 (ARNT2) is a protein with neuroprotective properties previously described in ischemic insults and oxidative damage. We hypothesize that alterations in ARNT2 expression are associated with changes in cell viability in in vitro and in vivo models of multiple sclerosis.
Methods: Following exposure to various compounds mimicking MS disease processes, ARNT2protein and mRNA levels were observed in primary cortical neuron-enriched cultures using western blotting, quantitative polymerase chain reaction (qPCR) and immunocytochemistry, alongside cytotoxicity measurements, using a lactate dehydrogenase (LDH) release assay/Live/Dead® Viability/Cytotoxicity assay. ARNT2 protein levels were also evaluated in primary cortical astrocytes using immunocytochemistry. Analyses in an animal model of MS, experimental autoimmune encephalomyelitis (EAE) were conducted, with tissue collected at various stages of the disease course, to examine ARNT2 expression patterns in vivo.
Results: Examination of individual neurons reveals that most cells demonstrate low-medium ARNT2 expression under steady-state conditions. Exposing cells to both low and higher concentrations of hydrogen peroxide (H2O2) to mimic mild to more severe oxidative stress significantly increases ARNT2 protein levels early, as measured via western blotting and immunocytochemistry. At the mRNA level, oxidative stress fails to drive Arnt2. This increased detection of ARNT2 protein is observed in both neurons and reactive astrocytes specifically within the neuronal-enriched mixed populations. Non-reactive astrocytes also express ARNT2 at baseline conditions. Finally, ARNT2 is differentially expressed in healthy versus EAE tissue at peak disease.
Conclusions: This work demonstrates for the first time that ARNT2 can follow
altered expression patterns in vitro in neurons depending on the severity/duration of the stimulus involved in MS disease progression. This lays a foundation for understanding the link between ARNT2 expression and neuronal health in vitro.