Outline of Research:

Project title: Additive effects of prenatal alcohol exposure and early-life adversity on adult immune function.

Project Background:
Children with Fetal Alcohol Spectrum Disorder (FASD) have been shown to display impairments in immune function, including an increased incidence of both major and minor infections, and an increased risk of malignancies. Animal models of prenatal alcohol exposure (PAE) have confirmed and greatly extended these clinical findings, with a wide range of immune disturbances detected following *in utero* alcohol exposure. In addition, children prenatally exposed to alcohol are more likely to encounter stressful and/or abusive environments during early-life, which may also adversely affect immune function. In order to examine the unique and/or interactive effects of PAE and early-life adversity on immune function in adulthood, two rodent models, a model of prenatal alcohol exposure, and an early-life adversity model, were combined. It was hypothesized that the addition of early-life adversity, acting on the already altered immune system of PAE rats, may further exacerbate the immune abnormalities associated with *in utero* alcohol exposure. Overall, it is the aim that these findings will contribute to a better understanding of the immune disturbances associated with FASD.

Methods:
Pregnant Sprague-Dawley dams were assigned to: PAE – ad libitum access to a liquid ethanol diet (36% EtOH-derived calories); Pair-fed (PF) – liquid control diet, maltose-dextrin isocalorically substituted for EtOH; or Control (C) – ad libitum access to control diet. To model early-life adversity, half of the litters in each group were exposed to a limited bedding environment from postnatal day (PD) 8-12. In adulthood (PD 65 – 70), rats received an immune challenge consisting of an intraperitoneal lipopolysaccharide (LPS) injection or a control (saline) injection. Blood was collected 90 min and 4 hr post-injection, and tissue (brain, spleen) collected after 4 hours. Levels of key pro- and anti-inflammatory cytokines will be measured in blood and tissue samples, and immune cell populations characterized in the 4 hr blood sample.

Role:
Ng will play an important role in tissue preparation, cytokine measurements, and data analysis. Tissue preparation will include making homogenization buffers and homogenizing brain (hypothalamus, hippocampus, prefrontal cortex) and spleen using a bead rupter and sonicator (n = 144/tissue). Next, Ng will measure protein levels within the tissue homogenates, using the bicinchoninic acid (BCA) assay. She will then assist with the cytokine assays using Meso Scale Discovery (MSD) multiplex assay kits, with levels of IL-β, IL-4, IL-5, IL-6, IL-10, IL-13, IFN-γ, TNF-α, KC/GRO measured in the above mentioned tissue samples, as well as blood samples (90 min and 4 hr post LPS injection) Due to the large amount of data generated from the multiplex cytokine assays (9 cytokines measured/tissue), after completion of both the protein and cytokine assays, Ng will learn to use the MSD Workbench software to extract cytokine results, adjust cytokine values based on protein levels, make graphs of the results, and perform basic statistics. In addition, Ng will analyze the levels of key immune cells (e.g. monocytes, lymphocytes, eosinophils) to determine whether the immune cell response to the LPS challenge was affected by the prenatal and/or early-life treatments. Finally, Ng will contribute to compiling the data for publication and will be responsible for presenting her results in a lab meeting at the end of the summer.