Name of Student: **Dominika Nackiewicz**

Research Supervisor: **Dr. Jan A. Ehses**

Title of Presentation: **Resident islet macrophages are M2 skewed in models of islet inflammation and type 2 diabetes in an effort to limit inflammation and β cell damage.**

Introduction: Annually around 4.6 million deaths worldwide are attributable to type 2 diabetes. Chronic inflammation is a characteristic of type 2 diabetes, and it is becoming increasingly clear that disturbances in the resolution of inflammation are an underlying feature of chronic inflammatory conditions. One of the features of type 2 diabetes is an increased number of macrophages infiltrating pancreatic islets, contributing to IL-1-mediated β cell dysfunction in this disease. While previous work has focused on the detrimental role of pro-inflammatory M1 macrophages, little attention has been given to the role of M2 macrophages during islet inflammation. Understanding the role of macrophages during islet inflammation will be critical for targeting these cells to treat diabetes.

Objective: To characterize the role of islet M2 MΦs during islet inflammation, and determine how they affect β cell function and survival.

Methods: Islet M2 MΦs were studied in the Goto-Kakizaki (GK) rat model of type 2 diabetes and multiple low-dose streptozotocin (MLD-STZ) mouse model of β cell death. Clodronate-loaded liposomes (clo-lip) were used to deplete islet M2 MΦs *ex vivo* and pro- and anti-inflammatory cytokine gene expression was assessed. Flow cytometry was used to sort islet M2 MΦs, followed by gene expression analysis via qPCR. To study the role of M2 MΦs *in vitro* we generated bone marrow derived MΦs (BMDMs) and stimulated them with IL-4, IL-10 and TGFβ1 in the absence and presence of lipopolysaccharide (LPS)+/-ATP.

Results: Pancreatic islets isolated from 8-12 wk old GK rats displayed marked up-regulation of genes involved in pro-IL-1β processing (*Casp1*), genes of pro-inflammatory cytokines (*Il1b, Il6, Tnf*), and anti-inflammatory cytokines (*Il1rn, Il*10) compared to age-matched Wistar rats. Depletion of resident islet MΦs from GK islets *ex vivo* resulted in reduced *Il1rn* (IL-1Ra) expression relative to Wistar controls, and a 4-fold increase in tissue *Il1b* expression, with no changes in other cytokine genes. Following STZ-induced β cell death, resident mouse islet $F4/80^{+}CD11b^{+}CD11c^{+}$ MΦs were increased as % total islet cells, and had up-regulated *Il1rn*, *Il10*, and *Igf1* mRNA on day 1, and increased *Vegfa* mRNA on day 10 post-STZ. No changes in pro-inflammatory cytokine (*Il1b, Tnf*) mRNA expression were detected. Finally, generating M2 MΦs *in vitro* with IL-4, IL-10, and TGFβ1 resulted in increased production of IL-1Ra in response to LPS+ATP, and increased PDGFa and TGFβi secretion in response to LPS only. Skewing MΦs to M2 also partly reversed the effect of M1 macrophages to reduce *Pdx1* expression in mouse islets.

Conclusion: Resident islet MΦs in the GK rat and resident islet MΦs following STZ-induced β cell death are M2 skewed and are likely major contributors of functional islet IL-1Ra secretion, in an attempt to antagonize IL-1 effects on β cells. Generating M2 MΦs *in vitro* with IL-4, IL-10, and TGFβ1 also resulted in increased IL-1Ra production, and ongoing studies will determine the role of these cells on β cell function and survival. Future studies will elucidate the role of M2 MΦs following STZ-induced β cell death *in vivo*. Strategies aimed at skewing MΦs to M2 may be used to improve glycemia and treat type 2 diabetes.