Type 2 diabetes mellitus is rapidly becoming the most common chronic disease in the US, with more than 1,500,000 new cases diagnosed per year. Type 2 diabetes is also accompanied by several long-term complications that ultimately cause more adult cases of blindness, renal failure, cardiovascular and peripheral vascular disease, and stroke.

Type 2 diabetes is characterized by insulin resistance and impaired insulin secretion due to B-cell dysfunction. There are also several reports indicating that islets from patients with type 2 diabetes are infiltrated with macrophages, and human islets exposed to metabolic stress release increased levels of cytokines. Thus, chronic innate inflammation due to local cytokine generation is a potentially important pathway mediating pancreatic B-cell damage in type 2 diabetes. However, the cell types and activation state of the immune cells in human islets in type 2 diabetes remain poorly defined. This is the major scientific challenge that will be addressed in this proposal. This feat will be accomplished primarily through flow cytometric analyses, designed to examine the immune response in human islets through the following specific aims:

Aim 1: Investigate to what extent the development of type 2 diabetes affects the immune cell composition of human islets. Aim 2: Characterize the immune response to possible mediators of type 2 diabetes in human islets.

Several studies have utilized flow cytometry to describe leukocyte infiltration in animal models of type 1 diabetes. However, the application of flow cytometry to the field of islet biology in type 2 diabetes has been limited. The difficulty of accessing human islets in vivo has been the major hurdle in understanding the pathogenesis of islet failure in type 2 diabetes. Neither diagnostic nor therapeutic probing is feasible for islets due to their location and distribution. The research opportunity has increased as Beta-Pro Inc., and the Integrated Islet Distribution Program have recently started to provide human islets from both healthy and diabetic donors. We have a unique opportunity to perform a flow-cytometry-based method to analyze the immune composition of human healthy and type 2 diabetic islets and to characterize the effects of possible mediators of the islet dysfunction on the activation of islet-located immune cells.

Our preliminary results using fluorescence activated cell sorting (BD FACS™) analysis demonstrate an existence of distinct populations of lymphocytes and CD11c⁺CD11b⁺ cells within the islets and suggest an importance of the local immune response at the initial stages of type 2 diabetes development. This preliminary data also highlights the utility of the FACS approach for analyzing the immune response in islets. Based on our preliminary data we hypothesize that immune cells already reside within healthy human islets, and that type 2 diabetes induces an additional recruitment of various leukocytes into islets. This, in turn, further exacerbates the local immune response and islet dysfunction.
To test this hypothesis, we propose to investigate tissue leukocytes that are present in healthy and type 2 diabetic islets by 8-color FACS. We will measure the population of T cells (CD3, CD4, and CD8), B cells (B-220, CD19, and IgM), NK and NKT cells (NK1.1, CD3, CD4, CD8), dendritic cells (CD11c, CD205), and macrophages (Mac-1, CD68, F4/80) in the human islets. To gain a more comprehensive picture of T-cell subsets in human islets, we will also analyze tissues for the presence of Treg (CD4+CD25+Foxp3+) and Th1 (CD4+IFN-g+) in human healthy and type 2 diabetic islets. Immunohistological evidence has indicated the presence of CD68+ Mf in type 2 diabetic islets, but the subtype and function of these Mf are unclear. Therefore, we will compare the subpopulation of Mf (M1- IL-1, IL-6, IL-12-secreting Mf and M2- IL-10+CD206+ Mf) in human islets.

Several mediators are proposed for the activation of innate immune response in human islets of type 2 diabetes. Our preliminary data reports increased production of IL-1b, TNF-a, and IFN-g in human type 2 diabetic islets. Therefore, to further understand the impact of pro-inflammatory mediators on the immune cell activation, we will perform FACS to test whether 1) a combination of the islet-associated pro-inflammatory cytokines (IL-1b, TNF-a, and IFN-g), 2) PA induce the activation of the resident immune cells in islets. To determine to what extent the treatment with the pro-inflammatory stimuli will activate resident islet immune cells, we will perform flow cytometry analysis of activation markers for T cells (CD69, CD62L), DCs (CD11c, MHC-II, CD80, and CD86), and for MHC-II, CD86 on human non-diabetic and type 2 diabetic islets. This approach will highlight potential candidates from the immune system that might participate in islet inflammation in type 2 diabetes.

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