Type-1 diabetes (T1D) is the most common autoimmune disease (AID) in which insulin producing β-cells in the pancreatic islets are destroyed resulting in hyperglycemia with no cure in sight. While a defective thymic selection contributes to T1D pathogenesis by failing to delete pancreatic β-cell antigen specific T cells, a defective peripheral tolerance contributes by failing to control those pathogenic T cells in the periphery. Hitherto, it is believed that little to nothing can be done to correct the thymic selection defect. Therefore, many different approaches have been used to suppress pathogenic immune responses either directly by inducing anergy/apoptosis of self-reactive effector T-cells (Teff) and/or indirectly by expanding peripheral regulatory cells (pTreg) to suppress proliferation and effector functions of $Foxp3^{-}CD4^{+}/CD8^{+}$ Teff and B cells. However, many of the current approaches are unsuitable for routine clinical use. Therefore, an approach that can cause TCR-independent and selective expansion of lineage stable Tregs with sustained suppressor function *in vivo* is highly desired. While absence of non-canonical NF-kB signaling in Tregs can result in loss of suppressive functions, a deficient canonical NF-kB signaling can cause substantial reduction in thymic (t) Tregs and accelerate T1D. These findings show the critical importance of NF-kB and IL-2 signaling in Treg function.

OX40L and Jagged-1 (JAG1) can selectively expand Tregs without expanding Teffs by a TCR-independent mechanism by interacting with their cognate receptors OX40 and Notch3, which are constitutively expressed on Tregs and not on Teff cells. These ligands expanded lineage-stable Tregs suppressed T1D in NOD mice, Hashimoto's thyroiditis in CBAj mice and lupus in NZB/NZW F1 mice. While OX40L-induced non-canonical NF-kB signaling drove TCR-independent proliferation of pTregs, canonical NF-KB, cooperatively regulated by OX40L/JAG1 co-signaling, was critical for sustained Foxp3 expression. Most intriguingly, OX40L/JAG1, synergized with IL-2, to increase thymic Treg (tTreg) precursor differentiation, tTreg maturation and proliferation. Significance also comes from our finding of expansion of human tTregs and pTregs by human OX40L/JAG1. Based on these novel findings, we will plan to determine the therapeutic efficacy of OX40L/JAG1 in NOD mice, its mechanism of action in the suppression of autoimmune response without causing global immune suppression; We will determine the effect of OX40L/JAG1 on thymic selection and tTreg differentiation, and on NF-kB activation required for Treg expansion and lineage stability. Additionally, we will determine whether OX40L/JAG1 can similarly affect tTreg generation and expand lineage stable human Tregs.

Our project is novel and highly significant, and will provide mechanistic insights into factors regulating tTreg and pTreg homeostasis, lineage stability and mode of action in suppressing T1D. Establishing that OX40L/JAG1 can suppress T1D in NOD mice without causing general immune suppression, and expand human Tregs could pave the way for developing a novel, safe, effective and easy to use treatment for T1D and other AIDs.