# Interleukin-2 Kinase-Mediated T-Cell Receptor Signaling Is Critical In The Development Of Type 1 Diabetes By OT-1 T-Cells


## ABSTRACT

*Purpose of Study*

When CD8+ T-cells are activated at the T-cell receptor (TCR), changes in cell trafficking are induced, helping to promote effector T-cell extravasation and migration into target tissues. Interleuken-2 Kinase (ITK), a tyrosine kinase activated by TCR signaling, has been shown in previous studies to be necessary for CD8+ T-cell migration into virally infected gastrointestinal tract tissue. The purpose of our study was to determine if attenuation of ITK signaling and downstream IRF4 expression also affects CD8+ T-cell trafficking into uninfected tissue in the context of tissue-specific autoimmune disease.

*Methods Used*

We utilized the rat insulin promoter membrane ovalbumin (RIP-mOVA) mouse model, in which OVA protein is expressed in β-islet cells of the pancreas to examine the ability of OT-1 T-cells, which express a TCR specific for OVA peptide, to migrate to the pancreas and cause Type 1 diabetes through β-islet destruction. WT, ITK-deficient, or interferon regulatory factor 4 (IRF4) heterozygous OT-1 T-cells were adoptively transferred into RIP-mOVA recipient mice. IRF4 is an important transcription factor downstream of ITK and IRF4 haploinsufficiency in humans has been shown to result in defective control of the bacteria *T. whipplei* leading to severe gastrointestinal inflammation. 24 hours post-adoptive transfer, mice were immunized with OVA peptide and LPS, and then monitored for glycosuria as an indicator of Type 1 diabetes induction. We monitored mice for diabetes up to 14 days post-transfer and immunization as well as harvested the spleen, pancreas, mesenteric lymph nodes, and pancreatic lymph nodes of a subset of mice 72 hours post-transfer to examine cells through flow cytometry and histology.

*Summary of Results*

We found that attenuation of ITK-mediated TCR signaling in both the absence of ITK and reduced IRF4 expression results in impaired or delayed ability of the transferred T-cells to induce Type 1 diabetes. Almost 100% of WT OT-1 transferred mice developed diabetes whereas only about 50% of ITK -/- and IRF4 +/fl transferred mice progressed to disease at an overall slower rate compared to WT mice. Additionally, the numbers of ITK -/- and IRF4 +/fl OT-1 T-cells in the pancreas were reduced compared to WT OT-1 T-cells and there were differences in molecular marker expression.

*Conclusions*

Our results indicate that ITK and IRF4 expression have important roles in TCR signaling to generate effector T-cells capable of migrating into β-islet cells of the mouse pancreas. While more research must be conducted to elucidate the underlying mechanism of impaired migration, our data provide support for the potential of ITK inhibitors to be used as a new therapeutic target for patients with tissue-specific autoimmune diseases.