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

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Abstract

Type 1 diabetes (T1D) is an autoimmune disease characterized by autoreactive CD8+ T-cell destruction of insulin-producing pancreatic beta-islet cells. T-cell receptor (TCR) signaling is essential for effector T-cell activation and trafficking into target tissues. Interleukin-2-inducible T-cell kinase (ITK), a key tyrosine kinase activated downstream of the TCR, regulates this process, with IRF4 as a critical downstream transcription factor. This study examined the role of ITK and IRF4 in CD8+ T-cell-mediated diabetes pathogenesis using the RIP-mOVA mouse model, where OVA is expressed in beta-islet cells. OT-1 CD8+ T-cells (OVA-specific) from wild-type (WT), ITK-deficient, or IRF4-haploinsufficient mice were adoptively transferred into RIP-mOVA recipients, followed by OVA peptide and LPS immunization. Mice were monitored for diabetes via glycosuria, and tissues were analyzed using flow cytometry and histology.

Results showed nearly all WT OT-1 recipients developed diabetes, while only ~50% of ITK -/- and IRF4 +/fl recipients progressed to disease, with delayed onset. Flow cytometry revealed fewer OT-1 cells in the pancreas, spleen, and lymphoid tissues in ITK -/- and IRF4 +/fl groups compared to WT. CD49d expression, essential for tissue migration, was significantly reduced in these groups as well. Histological staining confirmed OT-1 infiltration into islets in WT mice, though technical limitations precluded analysis in ITK -/- and IRF4 +/fl groups.

These findings support a critical role for ITK-mediated TCR signaling and IRF4 expression in effector CD8+ T-cell trafficking and diabetes development. Targeting ITK or IRF4 may represent a novel therapeutic strategy for autoimmune diseases such as T1D.

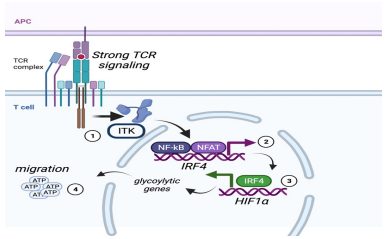

Figure 1: ITK and IRF4's role in the TCR signaling pathway

Materials and Methods

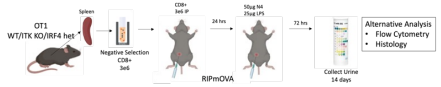

Figure 2: We used the RIP-mOVA/OT-1 transgenic mouse model to study the role of ITK and IRF4 in CD8+ T-cell-mediated Type 1 diabetes. CD8+ T-cells were isolated from OT-1 mice with wild-type (WT), ITK-deficient (ITK -/-), or IRF4 haploinsufficient (IRF4 +/fl) genotypes and adoptively transferred into RIP-mOVA mice. Mice were immunized with OVA peptide and LPS 24 hours post-transfer to activate OT-1 T-cells. Diabetes development was monitored for 14 days via daily urinalysis, with ≥1000 mg/dL glucose indicating disease. Tissues were collected at 72 hours post-transfer for flow cytometry to assess OT-1 cell presence and expression of IRF4, CD49d, and other activation markers. Pancreases from a subset of mice were processed for immunofluorescence to visualize islet infiltration. Data were analyzed using survival curves and group comparisons by ANOVA or t-test. All procedures followed approved animal care protocols (IACUC #00895).

Introduction

Type 1 diabetes (T1D) is an autoimmune disease where autoreactive T-cells destroy insulin-producing pancreatic beta-islet cells, resulting in insulin deficiency and life-threatening hyperglycemia (DiMeglio et al., 2018; Ozougwu et al., 2013). Unlike type 2 diabetes, T1D is not caused by insulin resistance but by immune-mediated beta-cell destruction. Current treatments rely on daily insulin injections, with no available cure (Katsarou et al., 2017). In the U.S., over 2 million people live with T1D, including more than 300,000 children (Bryan et al., 2021). Genetic predisposition, such as HLA genotypes (DR3/4-DQ8), and environmental factors like viral exposures contribute to disease risk (Steck & Rewers, 2011; Laron et al., 2023). Autoreactive CD8+ T-cells play a central role in recognizing and destroying beta cells through activation at the T-cell receptor (TCR) (Xie, Chang, & Zhou, 2014). TCR activation initiates signaling cascades involving interleukin-2-inducible T-cell kinase (ITK), a key mediator of T-cell differentiation and migration (Cho et al., 2020). Downstream of ITK, the transcription factor IRF4 regulates gene expression critical for effector T-cell function (Huber & Lohoff, 2014). In humans, IRF4 haploinsufficiency causes impaired immunity and increased susceptibility to infections like *T. whipplei* (Guérin et al., 2018). While ITK has been shown to regulate CD8+ T-cell migration in viral models, its role in tissue-specific autoimmunity such as T1D remains unclear.

We hypothesize that attenuating ITK and/or IRF4 in a mouse model of Type-1 diabetes will lead to lower incidence of disease due to decreased effector cell migration to the pancreas and may be an important potential therapeutic target of Type 1 diabetes as well as other T-cell mediated autoimmune processes. To test this hypothesis, we took a three-pronged approach looking at rate of diabetes development, flow cytometric analyses, and histological visualization.

Urinalysis: Type 1 Diabetes Incidence is Reduced in ITK -/- and IRF4 +/-

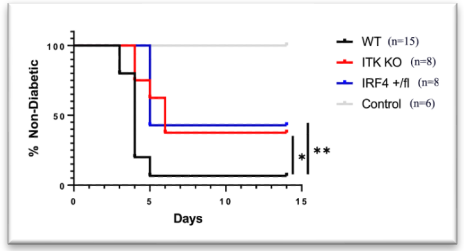

Figure 3: Urinalysis assay survivability curve of time to diabetes between control, WT, ITK -/- , and IRF4 +/fl mice. No control mice developed diabetes as expected, and nearly all WT OT-1 mice developed diabetes. There is a significant difference in rate of diabetes between WT and ITK -/- mice, and WT and IRF4 +/fl mice.

Flow: OT-I IRF4 +/fl Mice Show a Reduction in CD8 T-Cells

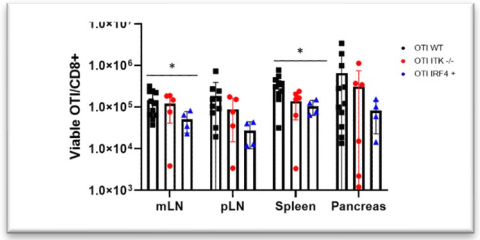

Figure 4: Differences in viable tetramer positive (H2kb +) OT-1 CD8+ T-cells were observed between recipients receiving OT-1 cells of each genotype in the four observed tissues. IRF4 +/fl genotypes have significantly fewer tetramer positive CD8+ T-cells when compared with OT-1 WT CD8+ T-cells in the spleen and mesenteric lymph node (mLN). Other tissue types show a similar trend, though not statistically significant. Additional experimental iterations may yield significance in the future with increased sample sizes.

Flow: OT-I WT T-cells Express High Levels of IRF4 and CD49d

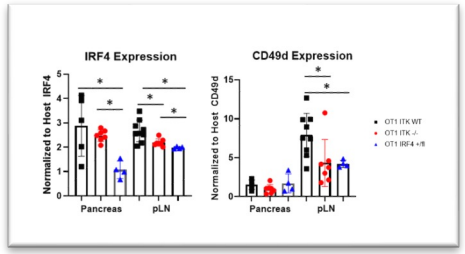

Figure 5: Differences in expression of IRF4 and CD49d in the pancreas and pancreatic lymph nodes (pLN). CD49d (VLA-4) mediates migration of T cells to extravascular spaces. There is significantly lower IRF4 expression in IRF4 +/fl mice compared to WT and ITK -/- in the pancreas and pancreatic lymph nodes. There is significantly lower CD49d expression in ITK -/- and IRF4 +/fl compared to WT in the pancreatic lymph nodes, but no difference observed in the pancreas. Other expression markers including CD25, CD69, CD122/CD44, and CD62L were also evaluated but did not yield significant results.

Histology : Verification of Experimental Model

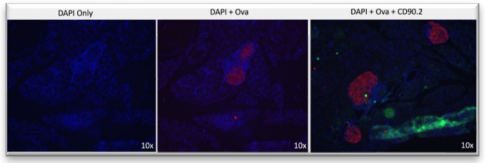

Figure 6: Fluorescent microscope images of histological specimens of mouse pancreases. DAPI stains all cell nuclei (blue), Ova stains ovalbumin (red) allowing for visualization of beta islets, and CD90.2 stains OT-1 WT adoptively transferred CD8+ T-cells (green). There were significant difficulties in obtaining histological samples of other tissue types such as lymph nodes as well as optimizing CD90.2 fluorescence for ITK -/- and IRF4 +/fl mice resulting in limited histological data, though results confirm our experimental model works.

Discussion/Conclusions

• ITK and IRF4 are critical for TCR-mediated signaling in CD8+ T-cells involved in Type 1 diabetes pathogenesis.

Urinalysis revealed:
• WT OT-1 T-cells induced diabetes in nearly all recipient mice, while ITK -/- and IRF4 +/fl T-cells led to significantly fewer cases and delayed onset.
Flow cytometry revealed:
• Reduced numbers of IRF4 +/fl and ITK -/- OT-1 cells in pancreatic, splenic and lymphoid tissues.
• Significantly lower IRF4 and CD49d (a key migration molecule) expression in IRF4 +/fl and ITK -/- groups.
• CD49d reduction in pancreatic lymph nodes suggests impaired extravasation in ITK and IRF4-deficient T-cells.
Histology revealed:
• Pancreatic infiltration by WT OT-1 cells but was technically limited for ITK -/- and IRF4 +/fl visualization.

• These findings support the hypothesis that TCR signaling through ITK and IRF4 promotes effector T-cell migration into target tissues.
Potential therapeutic insight:
• ITK and IRF4 may serve as novel targets in autoimmune diseases like T1D.
• Existing ITK inhibitors and degraders show promise in modulating immune responses in preclinical models (Zhou, Zuo, & Pan, 2023; Kim et al., 2024).
• Study limitations:
• Technical challenges in staining for IRF4 +/fl and ITK -/- histology.
• Small sample sizes for some flow cytometry subsets.
• Results are based on a mice—cannot necessary conclude the same in humans.