Selective JAK Inhibition Ameliorates *Aire* Deficient Driven Autoimmunity

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Abstract

Background: Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is an autosomal recessive monogenic autoimmune disease caused by deficiency in the autoimmune regulator *(AIRE)* gene that manifests with multiple, life-threatening autoimmune manifestations. Deficiency in AIRE results in self-reactive T-cells that escape into the periphery and infiltrate organs causing injury. We recently showed that APECED is an interferon-gammopathy as deletion of *Ifng* or treatment with the FDA-approved JAK 1/2 inhibitor, ruxolitinib, improved autoimmunity in *Aire* KO mice and APECED patients.

Methods: We aimed to evaluate the efficacy of selective JAK1, JAK2, and JAK3 inhibitors relative to that of ruxolitinib to delineate the mechanistic roles of the different Janus kinase pathways in the context of APECED. *Aire* KO mice underwent treatment with three selective JAK inhibitors: JAK1i (itacitinib), JAK2i (CEP-33779), and JAK3i (ritlecitinib). To evaluate the efficacy of these JAK inhibitors in ameliorating autoimmunity, we performed ELISA, qPCR, immunoblot, flow cytometric, and histological analyses.

Results: JAK1-, JAK2-, and JAK3-selective inhibitors significantly decreased the accumulation of pathogenic CD4 and CD8 T cells in *Aire* KO mouse lungs at levels comparable to ruxolitinib. Ruxolitinib and JAK1- and JAK2-selective inhibitors resulted in more pronounced decreases in *Ifng* and *Cxcl9* transcripts and levels of STAT1 and pSTAT1 by immunoblot analyses relative to the JAK3-selective inhibitor in *Aire* KO mouse lung. Concordantly, ruxolitinib and JAK1- and JAK2-selective inhibitors exhibited greater efficacy in ameliorating tissue injury in autoimmunity-affected organs as assessed by histological analysis compared to the JAK3-selective inhibitor in *Aire* KO mice.

Conclusion: We show that JAK1- and JAK2-selective inhibition led to a similarly effective amelioration of IFN-γ-driven inflammation and tissue injury compared to the JAK1/2 inhibitor, ruxolitinib, in *Aire* KO mice. By contrast, although JAK3 inhibition markedly decreased lymphocytic accumulation in *Aire* KO mice, it appeared less effective in reducing IFN-γ-driven inflammation and tissue injury compared to all other tested JAK inhibitors. Our study sheds light on the differential roles of JAK inhibitors in the context of APECED-associated autoimmunity and informs future work that may help develop more selective JAK inhibitor-based treatments in APECED patients with the goal to achieve maximal efficacy and long-term safety.

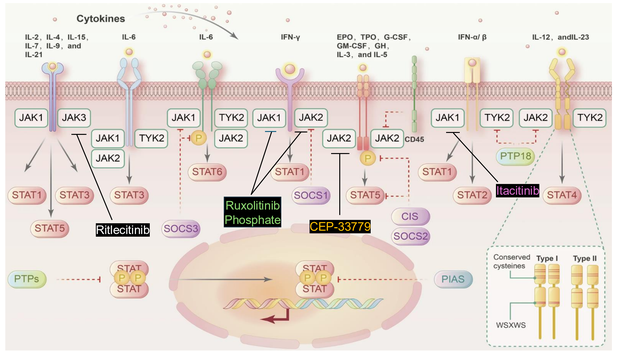

Figure 1. Cytokine signaling (Chen Xue et. al, 2023) adapted. Illustrated above are examples of different cytokine signaling and their respective pathways. Highlighted in black are the selective inhibitors used in this experiment and which JAK proteins they target.

Methods

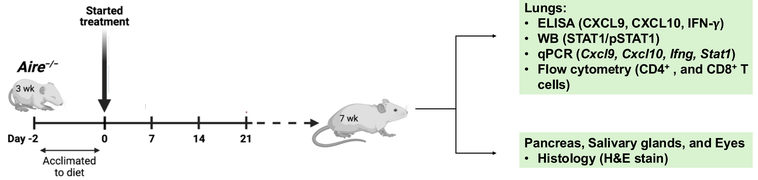

Figure 2. Schematic depiction of JAK selective treatment regimens. Nutragel was mixed with the respective selective JAK inhibitor (0.4g drug/kg food) and used to feed *Aire* KO mice. Treatment was administered to three to four weeks old mice and continued for 28 days. These mice were then used for a terminal study.

Results

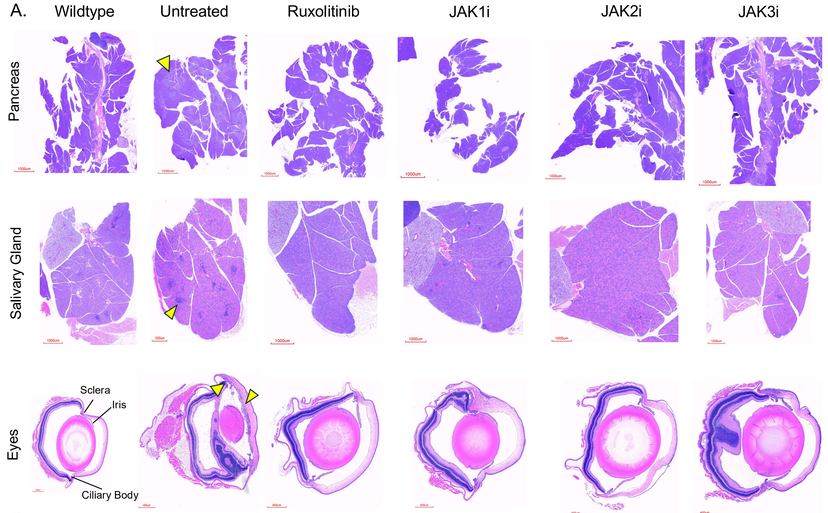

Figure 6. JAK1 and JAK2 selective inhibition ameliorates tissue inflammation of *Aire* deficiency. A) Representative images of hematoxylin and eosin (H&E) staining of the pancreas, salivary glands, and eyes from different treatment groups. Arrowheads point to inflammatory foci. **B**) Pathology scores of the pancreas, salivary glands, and eyes.

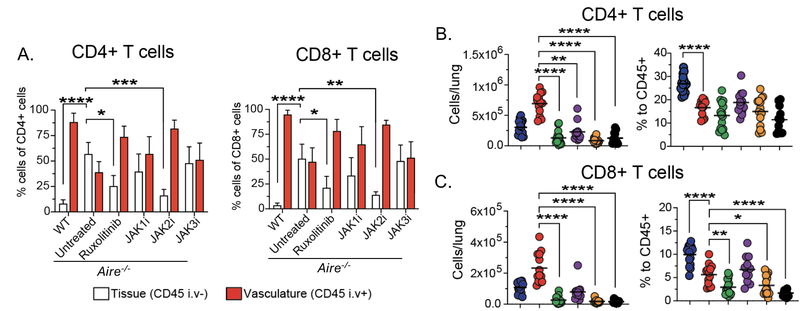

Figure 5. JAK1, JAK2, and JAK3 selective inhibition decreases T cell infiltration in the lungs. A) Proportion of CD4+ and CD8+ cells in the tissue or vasculature of the lungs. **B**) Absolute number of CD4+ cells and percentage of CD4+ to CD45+ cells in the lungs. **C**) Absolute number of CD8+ cells and percentage of CD8+ to CD45+ cells in the lungs.

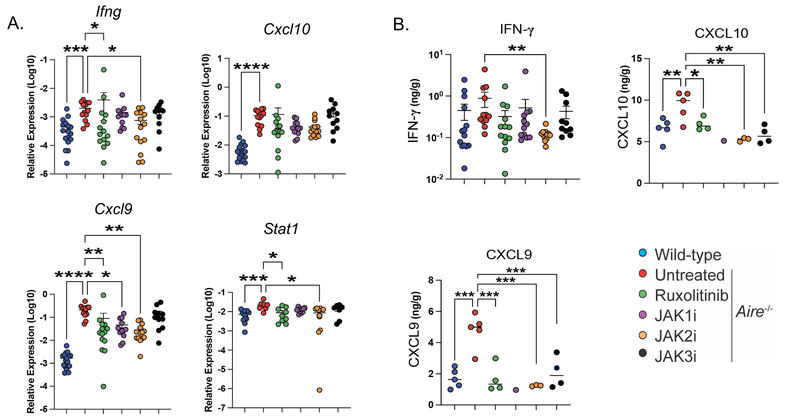

Figure 3. JAK1, JAK2, and JAK3 selective inhibition decrease IFN-γ signaling in the lungs. A) qPCR for *Ifng, Cxcl9, Cxcl10,* and *Stat1* in the lungs. **B**) ELISA for IFN-γ, CXCL9, CXCL10 in the lungs.

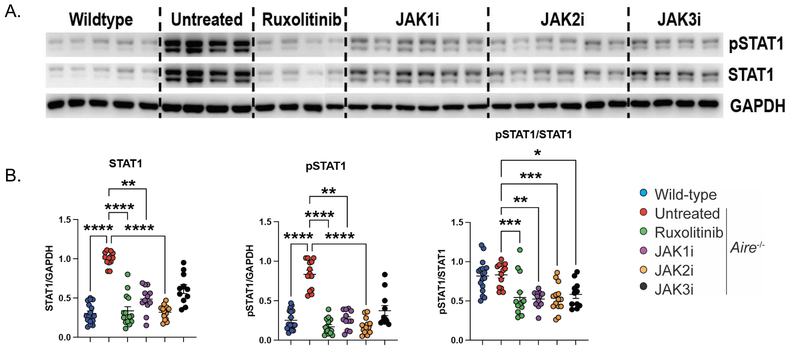

Figure 4. JAK1 and JAK2 selective inhibition diminishes total STAT1 and pSTAT1 in the lungs. A) Representative image of western blot densitometry signals for STAT1, pSTAT1, and GAPDH. **B**) Ratio of pSTAT1 and STAT1 relative to GAPDH or STAT1.

Conclusions

In this comparative study of selective JAK inhibition in *Aire* deficient mice, we have found that:

- Inflammation in different organs and IFN-γ signaling is consistently reduced with selective JAK2 inhibition.
- JAK1 inhibition ameliorated autoimmunity but did not have as powerful effect as JAK2i.
- Even though JAK3 inhibition lowered excess lymphocytes in the lung, it had the least efficacy of the three treatments.

Acknowledgements

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