Abstract

Type 1 Diabetes (T1D) is an autoimmune disease in which the immune system develops autoreactive T-cells that attack the beta cells of the pancreas. As a possible treatment for T1D, we inhibited Interleukin 7 (IL-7) receptors in autoreactive T-cells. Previous research has shown that IL-7 receptor inhibition can delay the onset of T1D in mice, but the mechanism behind this effect is still unknown. We hypothesized that blocking the IL-7 receptors would decrease the mitochondrial activity in these T-cells. To test this, two groups of mice were used; one was given the IL-7 receptor inhibition therapy while the other was given RAtIgG as a control. Three days later, the spleens were removed from the mice, and T-cells were isolated. After two days of stimulation with anti-CD3 and anti-CD28, the supernatant produced by the cells was collected to test for cytokine production while the cells were used to measure oxygen consumption. The cells were plated and placed in a respirometer to measure metabolic activity and oxygen consumption. The respiration measurements displayed a significant decrease in basal respiration, oxygen used to produce ATP, and proton leak in cells with inhibited IL-7 signaling. The ELISA assay was run to determine the concentrations of cytokines such as IL-2 and IFNγ. The results showed that blocking the IL-7 receptor reduced the mitochondrial activity of T-cells, and altered cytokine production, inhibiting their overall function. The results from these experiments could be one of the first steps in describing a mechanism for a possible treatment for T1D and other T-cell based autoimmune diseases.

Methods

Preparing the Cells: Two groups of mice were treated. The anti-IL-7Rα Group was given .5 mg of anti-IL-7Ra treatment, and the RlgG group was given .5 mg of the RlgG control. After three days, the CD4+ T-cells were harvested from the spleens and stimulated with 10 mg of anti-CD3 and 4 mg of anti-CD28.

Seahorse Respirometry: CD4+ T-cells were isolated and diluted to a concentration of 450,000 ceils per mL and plated. Respiration was measured after exposure to Oligomycin, FCCP, and Antimycin.

ELISA Assays: The supernatants from the CD4+ T-cells were collected after two days. These supernatants were analyzed using enzymes and antibodies to isolate specific cytokines. The approximate concentration of IL-2, IFNγ, and IL-10 were determined from these tests.

Conclusion

We found a significant difference between the respiration rate of the RlgG mice and the anti-IL-7Ra mice. Overall, the anti-IL-7Ra mice had less mitochondrial activity than the RlgG mice across the board. There was a clear difference in the Basal respiration, oxygen used to produce ATP, and Proton Leak. We can conclude from these results that the anti-IL-7Ra cells were less metabolically active than the RlgG cells. This could mean that the lowered T-Cell activity delays the onset of diabetes.

The ELISA assays showed that the anti-IL-7Ra mice had increased cytokine production for the IL-2, IFNγ and IL-10. This leads us to conclude that the anti-IL-7 receptor therapy altered the cytokine production of the T-cells, changing the overall function of these cells.

The reason for the increased protein concentration in the anti-IL-7Ra cells is still unknown and more research is necessary. A possible model would be to allow the T-cells to incubate for 7 to 12 days as opposed to 3 days. These steps could yield a possible explanation for effect blocking the IL-7 receptor has on the onset of Type 1 Diabetes.

References