Evaluating Environmental Influence On T-Cell Development In Type One Diabetes

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BACKGROUND

- Type One Diabetes (T1D), sometimes known as juvenile diabetes, is a chronic autoimmune disease in which the pancreas produces little to no insulin.
- This happens with the damaging of the beta-cells, insulin-producing cells, in the islets of Langerhans by the immune system.
- This immune attack occurs due to the activation of T cells, specifically CD4 and CD8 T cells, that recognize the islet autoantigens, which leads to apoptosis of beta cells
- Through central and peripheral tolerance our immune system can eliminate developing T cells that are made to attack self-peptides
- In the case of T1D, there is a loss of tolerance to tissue autoantigens which is attributed by defects in both central and peripheral tolerance.
- Factors in the environment, such as parasitic infections, induce chemical mediators called cytokines
- The five main antigens that auto reactive T cells bind to that are known to cause T1D are BDC 2.5 V1, BDC 2.5 V2, GAD 65 V1, GAD 65 V2 and Insulin
- Through positive selection, and lack of negative selection in the thymus, T cells that respond to these antigens escape the thymus and cause T1D

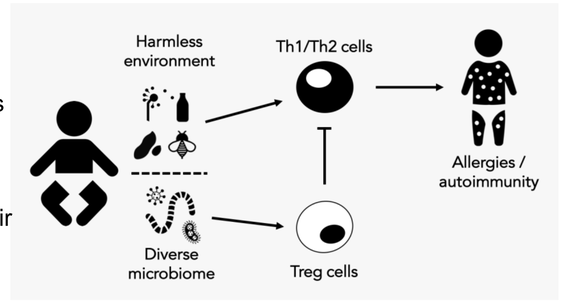


Figure 1: This is the hygiene hypothesis which suggests that there is a benefit to infectious agents and their composites on immunological diseases

HYPOTHESES

If we inject IL-4 cytokines to act as an environmental factor in the thymus, then that will alter the central tolerance and stop the targeting of beta cells by T cells.

METHODS

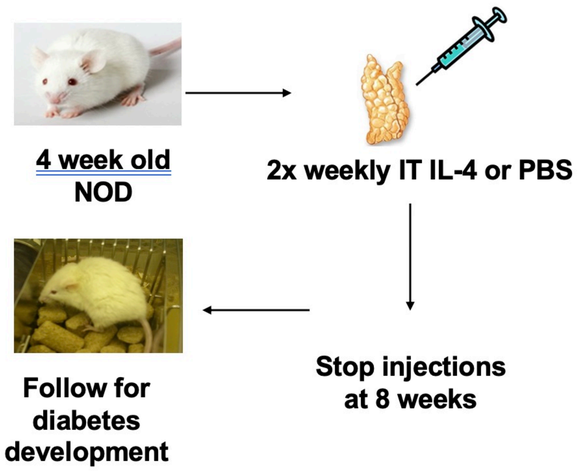


Figure 2: This image depicts the carrying out of our procedure with the NOD mouse and their intrathymic IL-4 and saline treatments for our control group

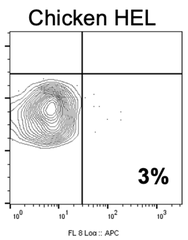
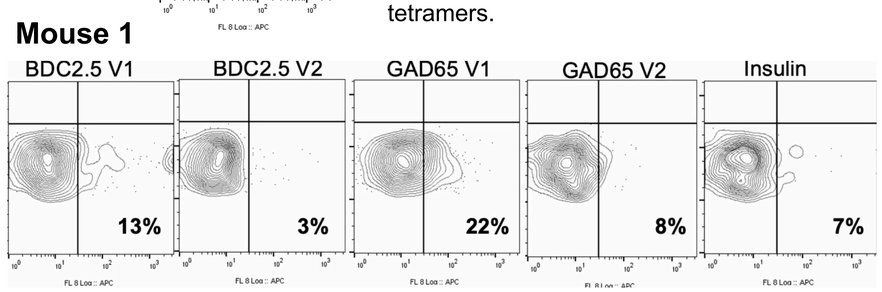


Figure 3: The ability of pancreatic T cells to bind to the indicated MHC tetramers was measured by flow cytometry. Shown here are the results of our control NOD mouse. The percentage values show the amount of CD4 T Cells that are targeting each tetramer. The Chicken HEL group shows us that there is a 3% margin of error for all the other tetramers.



METHODS

- We used female mouse from the age range of 12-14, and in that age range we have NOD mice treated with either saline or IL-4
- Break down pancreatic tissue using collagenase and centrifuge the remaining cells
- Use CD4 T cell microbeads to only isolate the CD4 T cells from the rest of the pancreatic cells
- Isolate CD4 T cells them through the MACS column magnet
- Distribute the sample of CD4 T cells into different tubes
- Stain each CD4 T cell sample tube in the dark with one of 6 tetramers being BDC 2.5 V1, BDC 2.5 V2, Insulin, Chicken HEL, GAD 65 V1, GAD 65 V2
- As a negative control group, we used Chicken HEL to give us a margin of error
- Run samples through a flow cytometer instrument
- Analyze sample results through FlowJo software

RESULTS

We found in our preliminary data that these NOD mice that weren't treated with IL-4 had a high amount of T cells produced that have targeted BDC 2.5 V1, and GAD 65 V1

SIGNIFICANCE

- From the data we have gathered we, hypothesize that we will have significantly lower percentage values from the mice that are also of 12-14 weeks of age, but are treated with IL-4
- We will also compare these percentage values to the ones of mice at 6-8, 12-14 and 20 plus weeks of age that have been treated with saline and IL-4
- Future directions consist of lowering the percentage of T Cells that target BDC 2.5, GAD 65 and Insulin with IL-4 treatments

ACKNOWLEDGEMENTS

The project described was supported by the MARC Fellows Program via grant number T34 GM 136493 from the National Institute of General Medical Science (NIGMS), a component of the National Institutes of Health (NIH)