Modulation of cytokines by dietary fatty acids in T cells from Type 1 Diabetes-susceptible NOD mice
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Introduction
Type 1 Diabetes is an autoimmune disorder characterized by autoreactive T cells attacking beta cells in the pancreas. This causes insulin levels to decrease throughout an individual’s life until essentially no insulin is being produced. The instances of individuals with low-risk alleles for T1D acquiring the disease has been increasing, which shows that environmental factors have a role in the occurrence of T1D. One such environmental factor is one’s diet, more specifically, the amount of long-chain fatty acids consumed. Experiments have shown that CD4+ and CD8+ T cells produce less IL-10, an anti-inflammatory cytokine, when exposed to linoleic acid, a long chain fatty acid. In this experiment, conditions for CD4+ T cell polarization into Th17 cells were set. Th17 cells have two unique cytokines: IL-17 and IL-21. It has been observed that a high concentration of Th17 cells is associated with disease pathogenesis of T1D.

Objectives
1. To determine if CD4+ T cells would polarize into Th17 cells given various environmental conditions;
2. To evaluate if varying linoleic acid concentration would affect Th17 polarization;
3. To observe how varying linoleic acid concentration would affect IL-17A and IL-21 cytokine production among Th17 cells.

Methods

Three experimental groups were established. CD4+ T cells were isolated. Spleens from two NOD mice were collected. Each experimental group was treated as follows:

P1 group
- 20 ng/mL IL-6
- 10 ng/mL IL-23
- 2 μg/mL TGF-β
- 1 mM CD3
- 5 μg/mL CD28
- 10 μg/mL αIFNγ
- 10 μg/mL αIL-4

P2 group
- 80 ng/mL IL-6
- 20 ng/mL IL-23
- 10 ng/mL TGF-β
- 2 μg/mL CD3
- 5 μg/mL CD28
- 10 μg/mL αIFNγ
- 10 μg/mL αIL-4
- 100 μM Linoleic acid

P3 group
- 50 ng/mL IL-6
- 15 ng/mL IL-23
- 5 μg/mL TGF-β
- 2 μg/mL CD3
- 5 μg/mL CD28
- 10 μg/mL αIFNγ
- 10 μg/mL αIL-4

Also varied concentration of linoleic acid within each experimental group. The flow cytometry panel included the following:

Flow Cytometry Panel
- Surface Stains: CD4, APC/780
- Intracellular Stains: RoRγ, PE
- Live/Dead: Aqua/BV510
- IL-21: APC
- IL-17A: eFluor

In addition to the cytokines measured, Th17 cells were set. Th17 cells have two unique cytokines: IL-17 and IL-21. It has been observed that a high concentration of Th17 cells is associated with disease pathogenesis of T1D.

Results

Gating Strategy:

Control IL-17A FMO:

Control RoRγ FMO:

P2 Conditions:

P3 Conditions:

CD4+/IL-17A P1

CD4+/IL-17A P2

CD4+/IL-17A P3

Data from the P2 group suggested the Affect of linoleic acid on polarization:

- As concentration of linoleic acid increased, expression of RoRγ and IL-17A decreased.

Overall:

- Data from the P2 group suggested the best conditions for polarization.

Future Direction:

- Repeat the experiment with variables similar to the P2 conditions.
- Increase length of incubation period

Conclusion

Slight polarization of CD4+ T cells occurred if RoRγ, a transcription factor crucial for differentiation into Th17 cells, was present, indicating that there was an attempt by the original cells to differentiate.

- Flow cytometry data showed that IL-17A was produced.
- A later ELISA test showed that IL-21 was produced, indicating that something happened between the time of the flow cytometry and the ELISA.
- Production of these two cytokines indicated that Th17 cells were present.

References


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References