Introduction

Although autoantibodies against β-cell antigens are used to define type 1 diabetes (T1D) their pathogenic role is unclear. In non-obese diabetic (NOD) mice lacking B cells there’s a decreased incidence of T1D suggesting B cells play a role in disease development. Despite this, little is known regarding the phenotype, specificity and function of B cells in the onset of T1D. Previous studies have found that naive B cells (Bnd1) are decreased in pediatric recent-onsets compared to healthy controls. Their decrease suggests they may play a pathogenic role in the more aggressive form of disease.

Therefore, we have developed a 36-marker B-cell mass cytometry (CyTOF) panel to further elucidate what subsets of B cells play a role in range of recent-onsets compared to age/sex matched healthy controls. Combined with the ability to enrich for multiple antigen specific B cells (Insulin and Tetanus), we use manual gating and algorithm-based analyses to find new targets for therapeutics to treat T1D.

Purpose

Our findings using dimensional reduction, clustering, and trajectory inference algorithms indicate Bnd cells can be divided into two subpopulations based on varying expression of CD11c, we term Bnd1 (CD11c+ B cells) and Bnd2 (CD11c-) Bnd2 cells are phenotypically similar to the previously identified DN2 B cell subset, which are a source of extra-follicular plasmablasts, but Bnd2 cells still express IgD. We are therefore interested in exploring the following:

- How does the frequency and activation status of Insulin specific B cells change over time?

- What is the pathogenic role of these B cells across the range of recent-onsets compared to age/sex matched healthy controls?

- Do these B cells give rise to DN2 cells?

- What is the pathogenic role of these B cells in the induction of disease development?

Figure 1: Enrichment and detection of antigen binding B cells

Methods

Figure 3: Dimensional reduction, clustering, and MST reveal relations between B cell subsets.

A. Dimensional Reduction: UMAP Clustering: Phenograph

- Insulin Binding B cells
- DN2 B cells
- Bnd2 B cells

B. Minimum Spanning Tree: FlowSom

Figure 4: Manual gating of Bnd2 and DN2 + Heatmap of phenotypic differences

- Identify clusters using multiple algorithms
- Identify clusters using heatmaps and manual gating
- Normalization by down-sampling to lowest events
- Concatenate and form UMAP projection
- Trajectory analysis and functional annotations
- Assign to individual samples

Figure 5: Germinal center vs. Extra-follicular pathway for antibody secreting cells

A. B. C.

Figure 6: Enrichment of Insulin and Tetanus Binding B cells for mass cytometry

A. CD45 enriched
B. CD45 depleted
C. Hashing by CD45

Figure 7: Potentially pathogenic Naïve B cells are specific to insulin pediatric recent onset type 1 diabetics compared to healthy controls

Conclusions:

- Bnd2 cells are a unique cell population.
- Bnd2 cells are highly activated in the periphery of recent-onsets compared to healthy controls.
- Unlike DN2 cells, Bnd2 cells are decreased in frequency in Insulin reactive B cells from pediatric recent-onsets. This suggests they’ve matured into a new cell type or migration to target tissue.
- Trajectory and phenotypic similarity suggests Bnd2 cells give rise to DN2 cells which become a source of extra-follicular antibody secreting cells.

Future Directions:

- ELISPOT assays to assess cytokine production of Bnd2 and DN2 B cells.
- surface antigen analysis by mass cytometry.
- Analysis spleen and pancreatic lymph node from nPOD donors for presence of Bnd2 and DN2 B cells.

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Unsupervised clustering identifies an activated, Insulin specific, novel B cell subset in peripheral blood & tissue of young-onset T1D

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