Enrichment and detection of antigen-binding B cells for mass cytometry in T1D

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Introduction
Type 1 diabetes (T1D) is an autoimmune disorder characterized by the destruction of the pancreatic beta cells by immune cells. Recent findings show that in T1D B cells are:
• Increased in the blood
• Increased in frequency in the pancreas
• Play a pathogenic role in individuals who develop T1D at an earlier age. Importantly, this age-specific signature is associated with rapid progression of disease. Despite the recent evidence for B cell participation, little is known regarding the phenotype and function of B cells in subjects at different ages of onset. To help fill in these gaps, we have developed a 38+ B cell panel for high dimensional single cell mass cytometry to simultaneously identify Insulin reactive and Tetanus reactive B cells, the various B cell subpopulations, and their activation and functional status, allowing for more granular characterization of B cells.

Purpose
Using this comprehensive B cell panel, we aim to determine whether a specific B cell subset/phenotype exists in the peripheral blood of young onset T1D subjects and a portion of at-risk individuals. The potential impact of these studies includes:
• Identification of the pathogenic B cells responsible for the aggressive disease seen in young onset individuals.
• Increase our understanding of T1D.
• Increase the precision of future age appropriate therapeutics.

Methods
Here we demonstrate for the first time to our knowledge that identification and enrichment of antigen-binding B cells can be accomplished using Mass cytometry.

Figure 1: Enrichment and detection of antigen binding B cells

Figure 2: Gating strategy for identifying Insulin, Tetanus, and established subpopulations of B cells.

Figure 3: Heatmap showing expression patterns of major cell types

Figure 4: Phenograph of global populations in periphery

Figure 5: Unsupervised clustering of antigen-reactive B cell populations and their expression patterns

Figure 6: AVID-Seq proof of concept using spiked in MD4 splenocytes

Future Directions
We currently enroll a range of participants with recent onset T1D to explore what populations of B cells contribute to the aggressive form seen in young individuals. Current limitations include low throughput compared to flow cytometry, the limited availability of cleavable beads (Miltenyi Biotech), inability to study the repertoire and function of B cells in the process preventing downstream study.

To overcome the limitations we are working to make our method compatible with 10x sequencing which will further enhance our ability to identify targets for future age appropriate therapeutics.

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Figure 7: Cell type and distribution of AVID signal.