Foreign and autoreactive CD4 conventional and regulatory T cells in T1D and healthy subjects

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Dysfunction in regulatory T cells has been implicated in Type 1 Diabetes (T1D). It is thought that regulatory T cells (Treg) play a crucial role in preventing autoimmune damage by autoreactive conventional T cells (Tconv) to the pancreatic Islet beta cells, which produce insulin. The precise mechanism by which Treg moderate Islet antigen-reactive (IAR) Tconv remains an open question. A prior investigation revealed limited TCR sharing between IAR Tconv and Treg. Here, we investigate whether this is also the case for foreign antigen-reactive T cells. This goal of this experiment is to compare the TCR repertoire and transcript phenotypes of foreign antigen- vs autoantigen-reactive CD4 Tconv and Treg to determine if features of autoreactive cells differ from foreign reactive T cells, particularly for Treg cells. To investigate these potential differences, we compared CD4 Tconv and Treg cells reactive to a microbial peptide pool (CEFX) to Tconv and Treg cells reactive to an islet peptide pool in 3 HC and 3 T1D subjects.

Two 10X GEM-X XLEAP captures consisting of 3 subjects each for single cell sequencing for RNA-seq, TCR-seq, and a limited set of CITEseq antibodies were run. Fresh blood was processed to PBMCs that was stimulated with 1) islet peptides, 2) CEFX, and 3) anti-CD3/anti-CD28 (polyclonal stimulation) for 20 hours. After stimulation, each donor and stimulation was stained with a unique hashtag antibody and combined by stimulus. Activated cells were enriched for CD154+ and CD137+ cells, surface stained with CITE-seq and flow antibodies, and sorted for CD154+CD137+ cells. All islet and CEFX stimulated cells were combined into a single tube and polyclonally stimulated cells will be in a separate tube. The polyclonal cells were added to the antigen reactive cells to enable clustering of antigen reactive cells against the total CD4 landscape.

The IAR Tconv and Treg cells showed similar transcriptomic signatures and cell recovery, while CEFX cells displayed a distinct transcriptomic profile and were recovered in much greater numbers. Across all three stimulations, Treg and Tconv cells are distinguishable by scRNA-seq and CITE-seq. Approximately 85% of recovered cells contained TCR pairs.

Investigation of TCR clonality and expansion between Treg and Tconv cells and between stimulations may help to determine if IAR Tconvs bear a distinct relationship to Treg cells compared with foreign antigen-reactive Tconv cells.

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