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

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Regulatory T cell (Treg) dysfunction is implicated in type 1 diabetes (T1D), contributing to beta cell destruction by islet autoreactive conventional T cells (IAR Tconv). However, little is known about IAR Tregs. Previously, we observed limited TCR sharing between IAR Tconv and Treg. To investigate if this is unique to IAR T cells, we compared foreign antigen (ag) vs IAR CD4 Tconv and Treg in 3 HC and 3 T1D subjects using multimodal 10X sequencing. PBMC were stimulated with islet peptides, CEFX, or CD3/CD28 antibodies (ab) for 20 h. After, each donor and stimulation were stained with a unique hashtag ab, combined by stimulus, and stained for CD154 and CD137. Activated cells were enriched, stained with CITE-seq and flow abs and sorted for CD154+ and/or CD137+ cells. Polyclonal activated cells were added to the ag activated cells to enable clustering of ag reactive cells against the total CD4 landscape and run using 10X GEM-X XLEAP chemistry. The IAR and polyclonal activated Tconv and Treg cells showed similar transcriptomic signatures and cell recovery, while CEFX cells displayed a distinct transcriptomic profile and were recovered in greater numbers. Naïve and memory Treg and Tconv cells were distinguished by RNA-seq and CITE-seq. Ag specific cells were stringently gated using protein tags for ongoing investigation of transcript profiles and TCR repertoires of Treg vs Tconv cells between stimulations. This represents a technical advance to analyze both IAR Tconv and Tregs in relation to other specificities in T1D.