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 in T1D. 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 single cell sequencing. PBMCs were stimulated with islet peptides, CEFX, or CD3/CD28 antibodies (ab) for 20 hours. 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.

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compared using an AIM assay coupled with single cell RNA-seq in T1D and healthy control (HC) subjects,

may help to determine if IAR Tconvs bear a distinct relationship to Treg cells compared with foreign antigen-reactive Tconv cells.