Better defining exhausted TIGIT$^+$KLRG1$^+$ subsets in teplizumab-treated T1D subjects using ATAC-seq: epigenetics and mitochondrial variants

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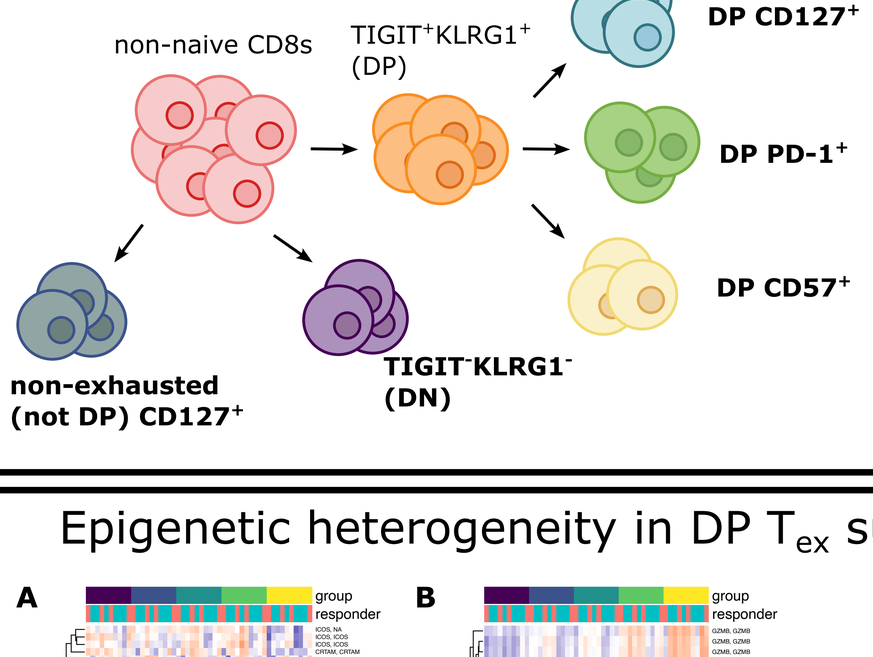
Abstract

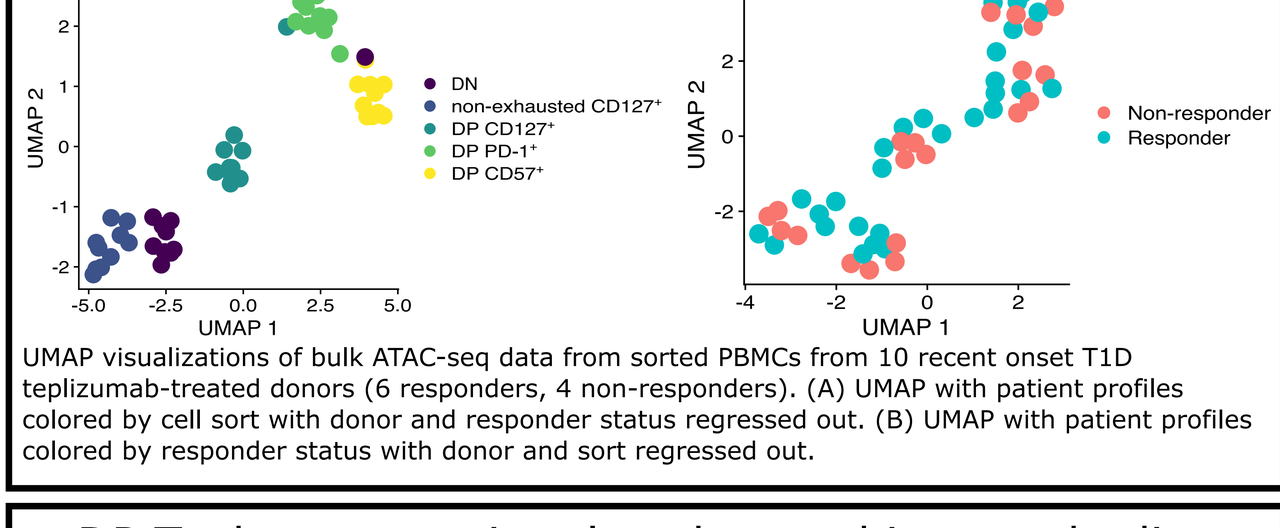
T cell exhaustion, a state of reduced effector function, results from chronic stimulation from antigens that cannot be fully cleared. In recent T1D studies, levels of non-naive, non-exhausted (not TIGIT$^+$KLRG1$^+$, DP) CD127$^+$ CD8s early after treatment correlated with worse response to therapy, consistent with the increased effector function of non-exhausted T cells. To study epigenetic differences between non-exhausted and DP CD127$^+$ CD8s and given the epigenetic changes that characterize T cell exhaustion, here we profiled the epigenetic states of different non-naive CD8 populations from PBMCs of 10 T1D patients treated with teplizumab using bulk ATAC-seq. We found that the epigenetic states of non-exhausted CD127$^+$ and TIGIT$^-$KLRG1$^-$ CD8s were similar and that different DP subsets were more progenitor or effector/terminal exhausted. We also analyzed mitochondrial-mapping ATAC-seq reads and found that the DP PD-1$^+$ and CD57$^+$ CD8s had the most mitochondrial single nucleotide variants. We hypothesize that the non-exhauasted CD127$^+$ CD8s are a parent population to various DP subsets that are more progenitor or effector/terminal exhausted.

Bulk ATACseq of non-naive CD8 populations from PBMCs in recent onset T1D patients



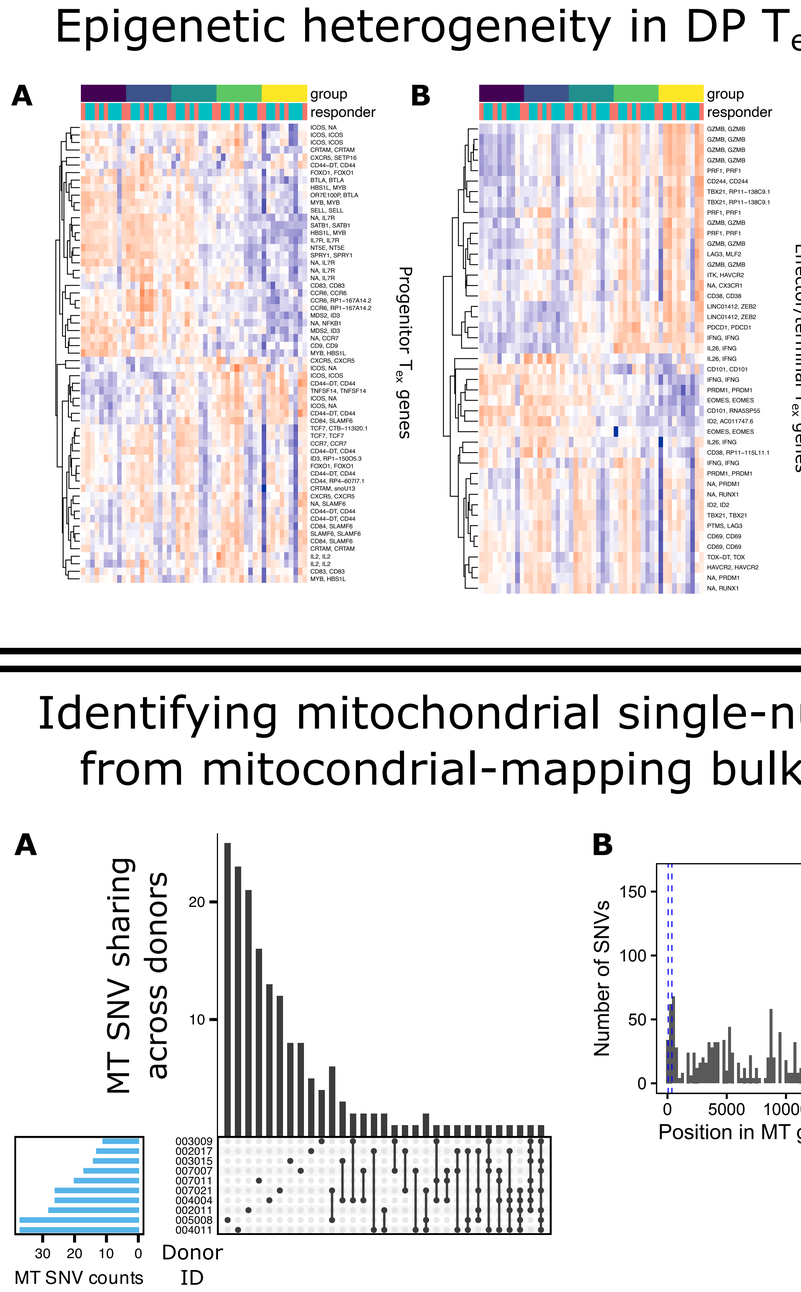
PBMCs from recent onset T1D patients, 6 responders and 4 non-responders to teplizumab, 6 months post-treatment were sorted for bulk ATACseq of the bolded sorts. Rs were chosen for high TIGIT$^+$KLRG1$^+$ fraction of CD8 TEMs, and NRs were chosen for low TIGIT$^+$KLRG1$^+$ fraction of CD8 TEMs.

Epigenetic profiles of different non-naive CD8 populations are distinct



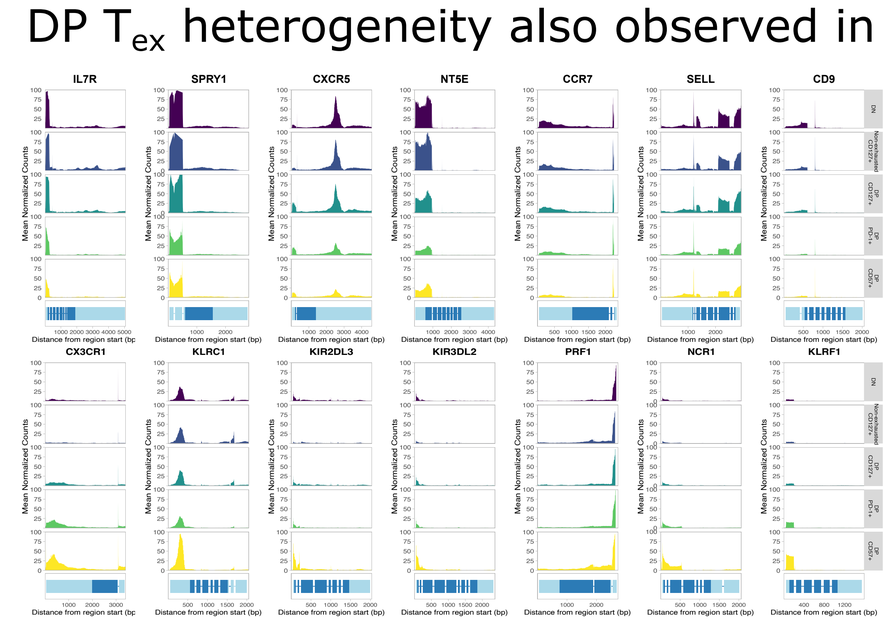
UMAP visualizations of bulk ATAC-seq data from sorted PBMCs from 10 recent onset T1D teplizumab-treated donors (6 responders, 4 non-responders). (A) UMAP with patient profiles colored by cell sort with donor and responder status regressed out. (B) UMAP with patient profiles colored by responder status with donor and sort regressed out.

Epigenetic heterogeneity in DP T$_{ex}$ subpopulations



Heatmaps of log_2-transformed and scaled peak scores for peaks within progenitor (A) and effector/terminal T$_{ex}$ (B) gene sets. Peaks are labeled with the nearest gene and promoter.

DP T$_{ex}$ heterogeneity also observed in gene bodies

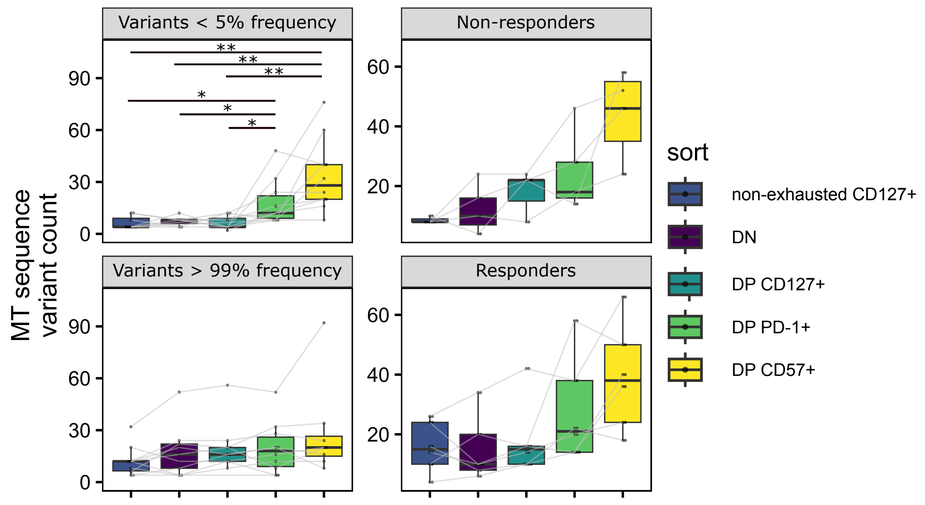


Mean gene coverage plots for selected genes from progenitor T$_{ex}$ genes (top row) and effector/terminal T$_{ex}$ genes (bottom row). Each gene's plot shows the mean gene coverage across all 10 donors for each cell sort. Gene coverage is normalized to read depth.

Identifying mitochondrial single-nucleotide variants from mitocondrial-mapping bulk ATACseq reads

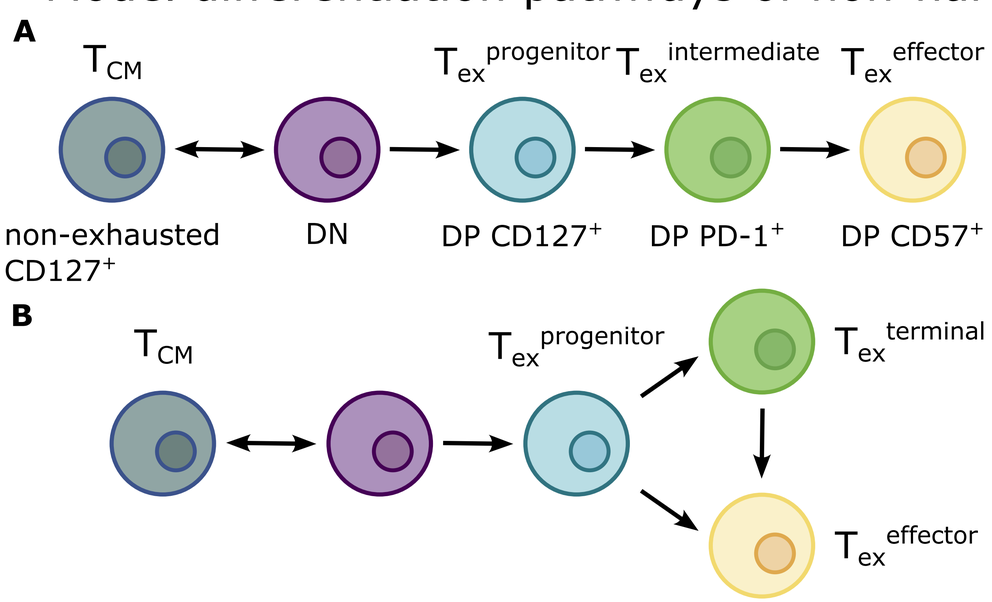
MT SNVs called using VarScan. (A) Upset plot showing sharing of MT SNVs across donors. (B) Distribution of MT SNVs across MT genome with hypervariable regions highlighted (HV1 in red, HV2 in red)

Accumulated over cell divisions, MT SNVs can provide directionality in a differentiation pathway



Mitochondrial single nucleotide variant (MT SNV) counts faceted by variant frequency or responder status. * and ** refer to padj < 0.01 and padj < 0.05, respectively, from Wilcoxon rank sum tests.

Model differentiation pathways of non-naive CD8s



Model differentiation pathways of non-naive CD8s. (A) A linear pathway.(B) A bifurcating pathway

Future directions

1. Identify if DP PD-1$^+$ CD8s here have epigenetic profiles more consistent with T$_{ex}^{intermediate}$ or T$_{ex}^{terminal}$ phenotype (to distinguish between model differentiation pathways)
2. Trace lineages using MT SNVs identified from scATACseq; begin with proof of concept using Allen Institute TEAseq data
3. Confirm that MT SNV counts are proportional to accumulated cell division count in pulse chase mass spectrometry experiment of immune cells in type 1 diabetics
4. Find transcription factors with binding sites specifically enriched in accessible chromatin in specific non-naive CD8 populations here (Homer)
5. Connect results to orthogonal projects of similar cell sorts (RNAseq & TCRseq in P348)

Acknowledgements

Funding: The Immune Tolerance Network

Stephan Pribitzer & Basilin Benson for computational support

John Ray for the idea to investigate MT SNVs

Benaroya Research Institute