Single-Cell, Multi-Omics Analysis of SIV-Specific CD8⁺ T Cells Across Multiple Anatomical Sites

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BACKGROUND

Antigen-specific CD8⁺ T cells play a key role in the host's antiviral response. T cells recognize viral epitopes via the T cell receptor (TCR). During chronic simian immunodeficiency virus (SIV) infection of rhesus macaque nonhuman primates, tissue specific TCR clonotypes were identified among SIV-specific CD8⁺ T cells. We sought to answer the following questions:

- Do SIV-specific CD8⁺ T cells exhibit altered transcriptional profiles and TCR repertoires in different anatomical sites?
- Do SIV-specific CD8⁺ T cells with tissue-specific TCR sequences show distinct transcriptional profiles compared to cells with TCR sequences shared across multiple sites?

METHODS

- SIV gag CM9 (CTPYDINQM)-specific CD8⁺ T cells were sorted from the peripheral blood mononuclear cells (PBMCs), multiple non-gut draining lymph nodes (LNs), mesenteric lymph nodes (MeLN), spleen and liver of chronically SIV infected Mamu-A*01⁺ rhesus macaques.
- Single-cell RNA and TCR sequencing libraries were generated from the CM9-specific CD8⁺ T cells via the 10x Genomics pipelines.
- Sequencing libraries were sequenced via the NovaSeq or NextSeq platforms and data was analyzed using software programs CellRanger, Seurat and scRepertoire.

RESULTS: Overlap of RNAseq profiles of multiple anatomical sites.

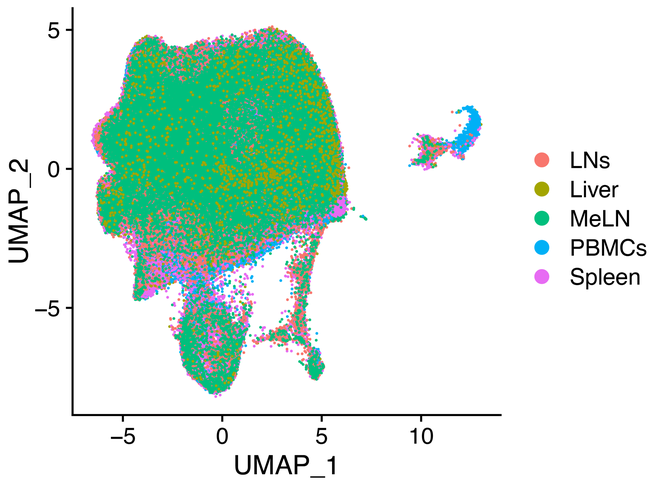


Figure 1: RNAseq profiles of CM9 specific CD8⁺ T cells isolated from PBMCs, LNs, MeLN, spleen and liver. n= 6.

SIV-specific CD8⁺ T cells with tissue-specific TCR sequences show distinct transcriptional profiles compared to cells that share TCR sequences across multiple anatomical sites.

RESULTS: TCRseq data shows similar TCR repertoire diversity and TCR repertoire composition across anatomical sites.

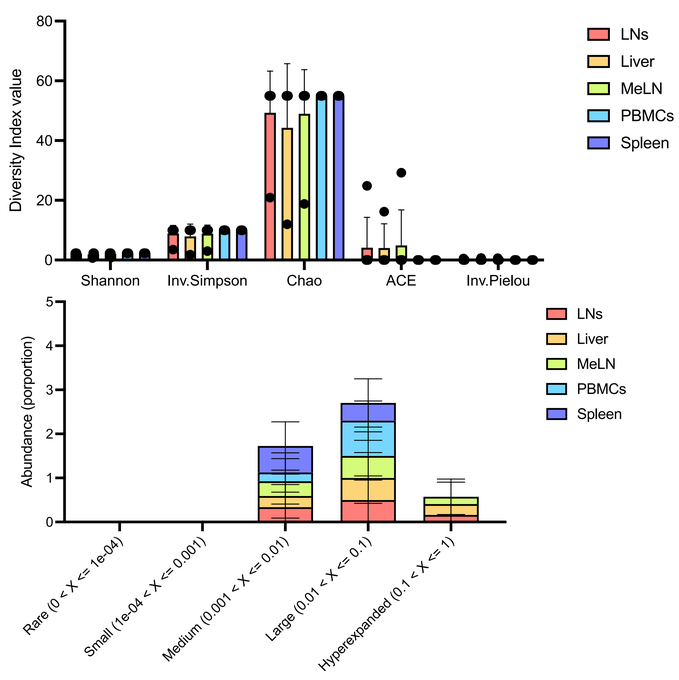


Figure 2: TCR repertoire diversity and composition of CM9 specific CD8+ T cells isolated from PBMCs, LNs, MeLN, spleen and liver. (A) TCR repertoire diversity index values. (B) TCR sequence abundance. n= 6.

RESULTS: GSEA identified distinct transcriptional profiles between cells with shared/tissue specific TCRs.

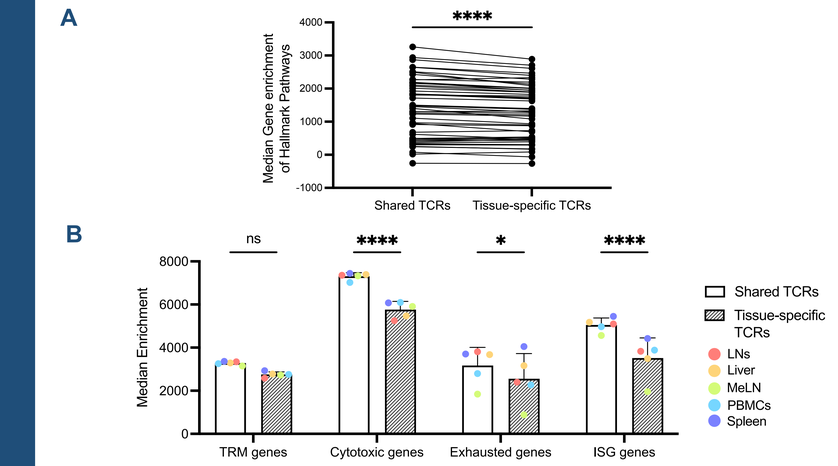


Figure 3: Gene set enrichment analysis (GSEA) of CM9 specific CD8+ T cells isolated from PBMCs, LNs, MeLN, spleen and liver. (A) Median enrichment scores for GSEA using Hallmark gene sets. (B) Median enrichment scores for GSEA using targeted (TRM, Cytotoxic, Exhausted and ISG) gene sets. n= 6.

RESULTS: Average gene expression analysis showed distinct transcriptional profiles between cells with shared/tissue specific TCRs in the liver.

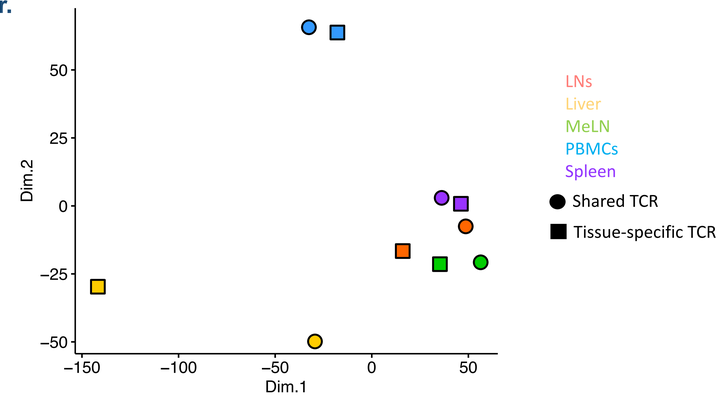


Figure 4: MDS plot of average gene expression of CM9 specific CD8+ T cells isolated from PBMCs, LNs, MeLN, spleen and liver. n= 6.

CONCLUSIONS

- CM9 specific CD8+ T cells expressing tissue specific TCR sequences exhibit distinct transcriptional profiles, including reduced expression of cytotoxic and exhausted genes compared to cells with shared TCRs.
- These data illustrate the presence of distinct tissue-specific antiviral responses, which may lead to novel therapeutic strategies to enhance T cell mediated defenses against viruses.

ADDITIONAL KEY INFORMATION

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