COMPARTMENTALIZATION OF THE HIV-SPECIFIC CD8+ T+CELLS RESPONSE REVEALED BY SINGLE CELL T-CELL RECEPTOR AND WHOLE TRANSCRIPTOME SEQUENCING

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Background
Although antiretroviral therapy (ART) blocks human immunodeficiency virus (HIV) replication and reduces plasma viremia to undetectable levels in peripheral blood of infected individuals, viral replication and HIV evolution persists in lymphoid tissue. $CD8^+$ T-cells play an important role in identifying and eradicating infected cells within lymphoid tissue. The specificity of each T-cell is determined by the T-cell receptor (TCR) that recognizes processed peptides in complex with major histocompatibility complex (MHC) presented on the surface of the infected target cell. Despite the cellular heterogeneity that exists within T-cells, most studies have only focused on circulating T-cells at the population level. To better understand the TCR dynamics that define tissue resident HIV specific $CD8^+$ T-cells, we set out to identify the TCR clonotype usage and the underlying transcriptional signatures at the single cell level from a secondary lymphoid structure (tonsil) of HIV-infected individuals.

Methods
Tonsils and matched blood were obtained from HIV-infected individuals. Following HLA class I typing, tetramers were used to isolate single HIV or cytomegalovirus (CMV) specific $CD8^+$ T-cells from 6 subjects by Fluorescence Activated Cell Sorting (FACS). SMART-Seq-II was used to amplify the whole transcriptome followed by targeted PCR amplification of the TCR. cDNA libraries and TCR products were sequenced on the illumina Hiseq platform.

Results
We identified HIV and CMV specific $CD8^+$ T-cells in both blood and tonsils. Some were present in both compartments, whereas others only were present in either blood or tonsil tissue. Higher frequencies of antigen specific $CD8^+$ T-cells were found in the blood compartment, and tonsil cells expressed more PD-1, CD69 and CD103 indicative of tissue resident cells. Moreover, HIV and CMV specific $CD8^+$ T-cells within the same tonsil showed major differences in PD-1, CD103 and CD127 expression indicating distinct functional and intra-tissue location of resident virus specific $CD8^+$ T-cells. Single cell paired alpha and beta (α and β) chain TCR was used to construct complete clonotypes. We found at least one preferential pairing with the highest diversity originating from the β- chain. Moreover, the β- chain CDR3 region: CASSLSITTEAFF was more frequently shared in both blood and tonsil tissue, while the α-chain was unique within either compartment. Complete α-β paired clonotypes showed no clonal overlap between tonsil and circulating HIV specific $CD8^+$ T-cells suggesting compartmentalization of the HIV specific $CD8^+$ T-cell response. Full-length cDNA libraries for single cell whole transcriptome analysis was optimized and ready for deep sequencing.

Conclusion
These data demonstrate compartmentalization between circulating and tissue resident HIV and CMV specific CD8+ T-cell responses both at the phenotype and clonotype level. This work in progress is of direct relevance for HIV cure strategies that have the overall goal of removing viral reservoirs from lymphoid tissue.