LN CXCR5+ HIV-Specific CD8 T Cells of Controllers Express GZMK to Mediate Rapid Effector Functions

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Introduction

- HIV-specific CD8 T cells from Elite and Viremic controllers (EC and VC) are central to viral control, and reproducing their properties may be crucial to achieve ART-free HIV suppression
- Lymphoid tissues and lymph nodes (LN) are major sites of HIV replication, but the mechanisms enabling viral control in this compartment remain unclear
- Previous evidence indicates that granzyme B (GZMB) or perforin-mediated cytotoxicity may be reduced in LN
- We hypothesized that in-depth profiling of LN HIV-specific CD8 T cells from EC and VC could uncover additional effector functions linked to viral control in LN

Methods

➢ Study participants donating paired blood and LN samples:
❑ 5 individuals with chronic HIV infection (**CHI**)
❑ 4 Elite controllers (**EC**)
❑ 5 Viremic controllers (**VC**)
❑ 4 ART-treated individuals (**ART**)

➢ HIV-specific CD8 T cells were sorted for transcriptomic profiling
❑ Barcoded dextramers identified each CD8 T cell restriction
❑ Anti-CXCR5 CITE-Seq enabled CXCR5 protein detection

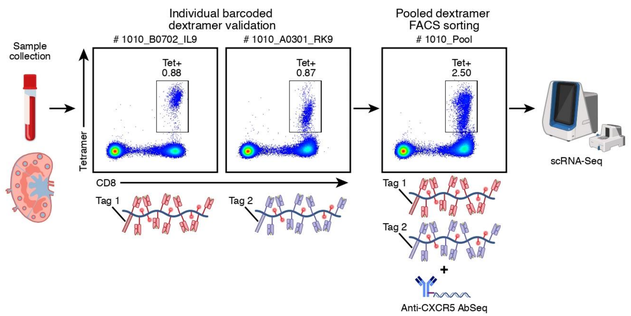


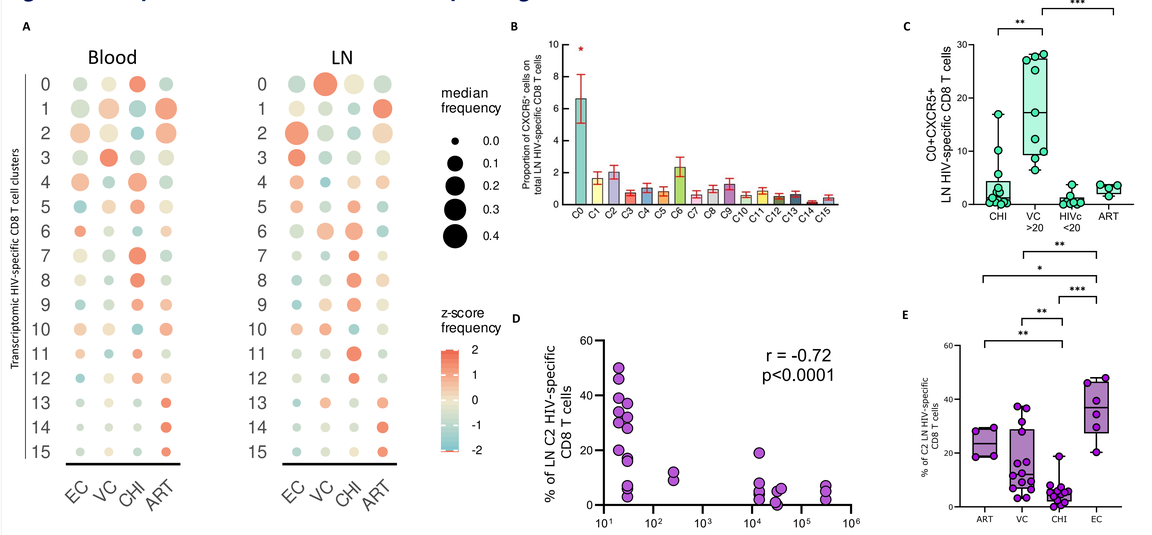
Figure 1. Experimental strategy for scRNA-Seq and CXCR5 CITE-Seq.

➢ High-dimensional mass cytometry was used to validate transcriptomic findings at a protein level

➢ Reconstructive *in vitro* experiments were performed to test the functional features of HIV-specific CD8 T cells

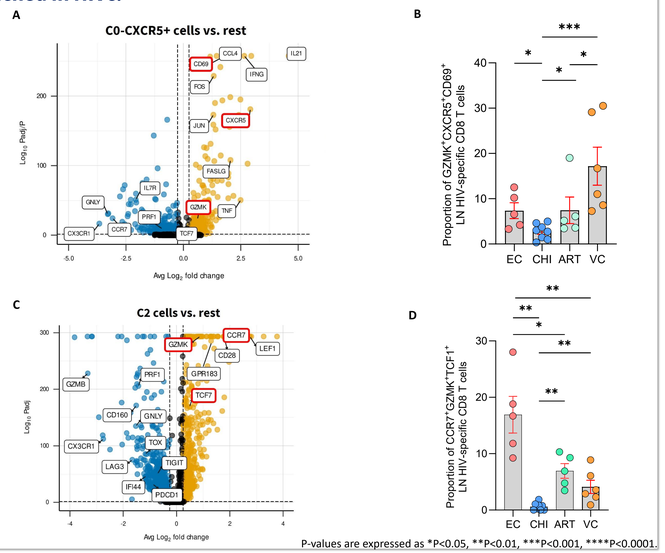
Results

Figure 2. HIV-specific CD8 T cells in C2 and C0 expressing CXCR5 are enriched in HIVc.



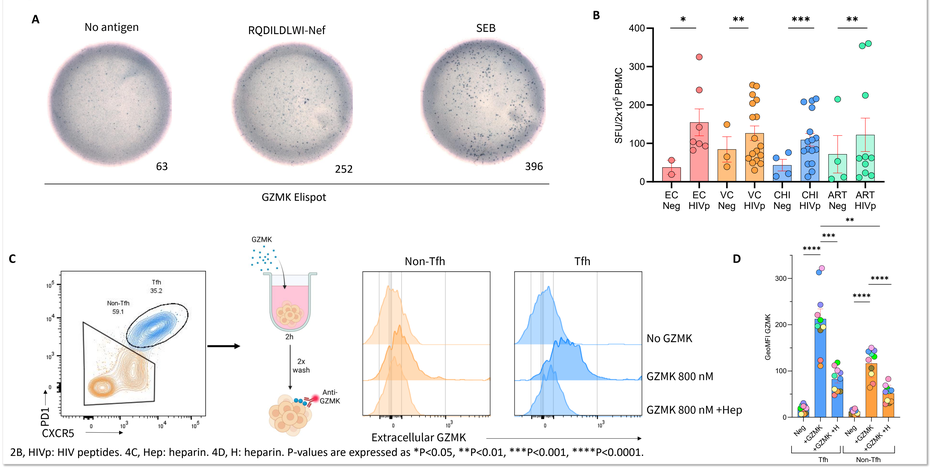
2C, HIVc: HIV controllers. P-values are expressed as *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Figure 3. Two LN HIV-specific CD8 T cell subsets expressing GZMK are enriched in HIVc.



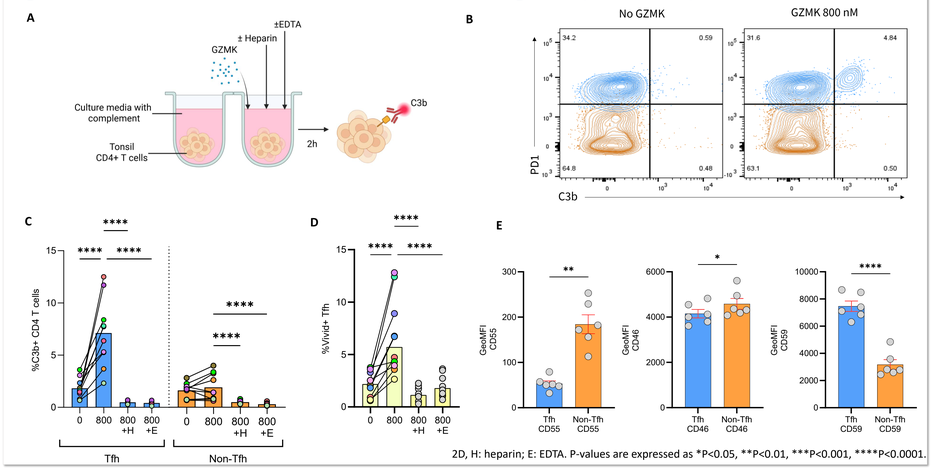
P-values are expressed as *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Figure 4. GZMK can be released by HIV-specific CD8 T cells and binds preferentially to Tfh



2B, HIVp: HIV peptides. 4C, Hep: heparin. 4D, H: heparin. P-values are expressed as *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Figure 5. Membrane-bound GZMK eliminates Tfh through complement deposition



2D, H: heparin; E: EDTA. P-values are expressed as *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Conclusions

- HIVc display peculiar HIV-specific CD8 T cell populations in LN, possibly associated to HIV control
- EC are enriched in a "stem-like" population expressing GZMK, suggesting both immediate effector potential and proliferative capacity. VC are enriched in CXCR5$^+$ polyfunctional cells expressing GZMK
- GZMK is released by HIV-specific CD8 T cells, binds CD4 T cells and triggers complement-mediated killing, possibly representing a novel mechanism of HIV control in LN in addition to classical cytotoxicity
- Tfh bind more GZMK, express less complement regulators, and are particularly sensitive to GZMK/complement-mediated cell death

Plain Language Summary

- Rare individuals with HIV, called HIV controllers, can control the virus without treatment. Understanding the mechanisms behind this capacity could help develop new cure strategies.
- CD8 T cells of HIV controllers may control HIV by positioning at key sites of viral replication in lymph nodes, and by rapidly releasing the non-canonical cytotoxic molecule Granzyme K.
- Granzyme K binds to target cells and marks them for elimination through the complement system. This mechanism may be especially effective against Tfh, a key CD4 T-cell subtype infected by HIV in lymph nodes, possibly increasing the chances of controlling HIV in tissues.

Acknowledgements

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