Robust HIV-Specific CD4+ and CD8+ T Cell Responses in Chronic Progressors on Long-Term ART

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Background

- Recent work has shown that HIV-specific CD8+ T cells from chronic progressors on long-term ART undergo clonal succession and regain controller-like phenotypes.
- CD4+ T cells are critical for HIV control through direct cytotoxic activity, cytokine production, and provision of help to CD8+ T cells.
- HIV-specific CD4+ T cells in untreated progressors are dysfunctional and display signatures of exhaustion.
- Whether CD4+ T cell responses in chronic progressors on long-term ART are similarly rejuvenated has not been investigated.

Objectives

- Compare the magnitude and functionality of Gag-specific CD4+ and CD8+ T cell responses between chronic progressors (CPs) on long-term ART and HIV controllers (HICs).
- Quantify the Gag-specific TCR repertoire in CD4+ and CD8+ T cells using the functional expansion of specific T cells (FEST) assay.
- Characterize the transcriptional profile of Gag-specific CD4+ T cells using single-cell RNA-seq.
- Evaluate the capacity of Gag-specific CD4+ T cells to suppress HIV replication.

HICs and CPs on long-term ART have comparable HIV-specific memory CD4+ and CD8+ T cell responses

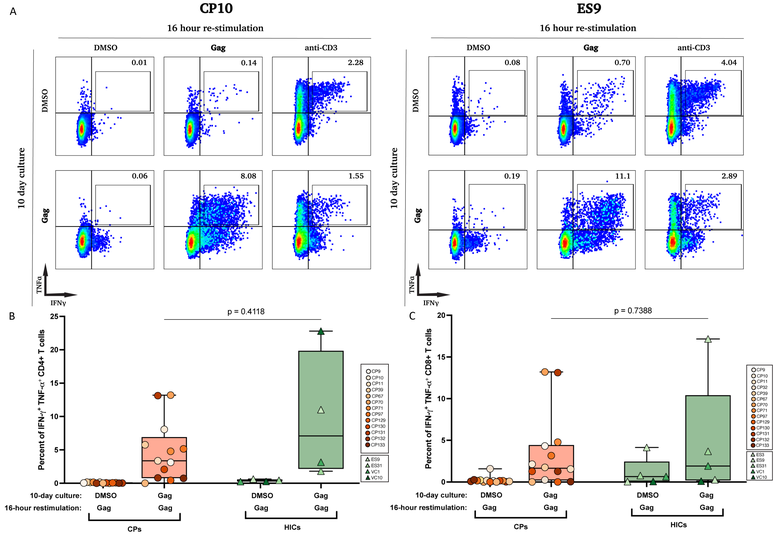


Figure 1: Functional characterization of antigen-specific CD4+ and CD8+ T cell responses following in vitro expansion.
(A) Representative flow cytometry plots showing IFN-γ and TNF-α expression in CD4+ T cells from participants CP10 and ES9 (transmission pair). PBMCs were pre-cultured for 10 days with DMSO (top row) or Gag peptide pool (bottom row), then restimulated for 16 hours with DMSO, Gag peptides, or anti-CD3 antibodies. Numbers indicate the percentage of IFN-γ+TNF-α+ CD4+ T cells. (B) The percentage of IFN-γ+TNF-α+ CD4+ T cells was measured in 14 CPs and 5 HICs after 10-day culture with DMSO or Gag peptides followed by 16-hour restimulation with Gag peptides. Bars indicate median values. Each dot represents one participant. p-values determined by Mann-Whitney U-test. (C) The percentage of IFN-γ+TNF-α+ CD8+ T cells was measured in 14 CPs and 5 HICs after 10-day culture with DMSO or Gag peptides followed by 16-hour restimulation with Gag peptides. Bars indicate median values. Each dot represents one participant. p-values determined by Mann-Whitney U-test.

Summary

- HIV-specific memory CD4+ and CD8+ T cells from chronic progressors on long-term ART display comparable responses to HIV controllers.
- HICs displayed higher frequencies of Gag-specific TCRs in both CD4+ and CD8+ compartments compared to CPs on long-term ART.
- TCR sequencing revealed similar TCR repertoire diversity in both CD4+ and CD8+ compartments between HICs and CPs on long-term ART.
- Gag-specific CD4+ T cells exhibited cytotoxic phenotypes with upregulation of GZMA, GZMB, and GNLY in both CPs and HICs.
- Chronic progressors on long-term ART exhibit comparable CD4+ T cell immune responses to HICs, mirroring recent observations in the CD8+ compartment.

Results

Gag-specific TCR repertoires in HICs and CPs on long-term ART

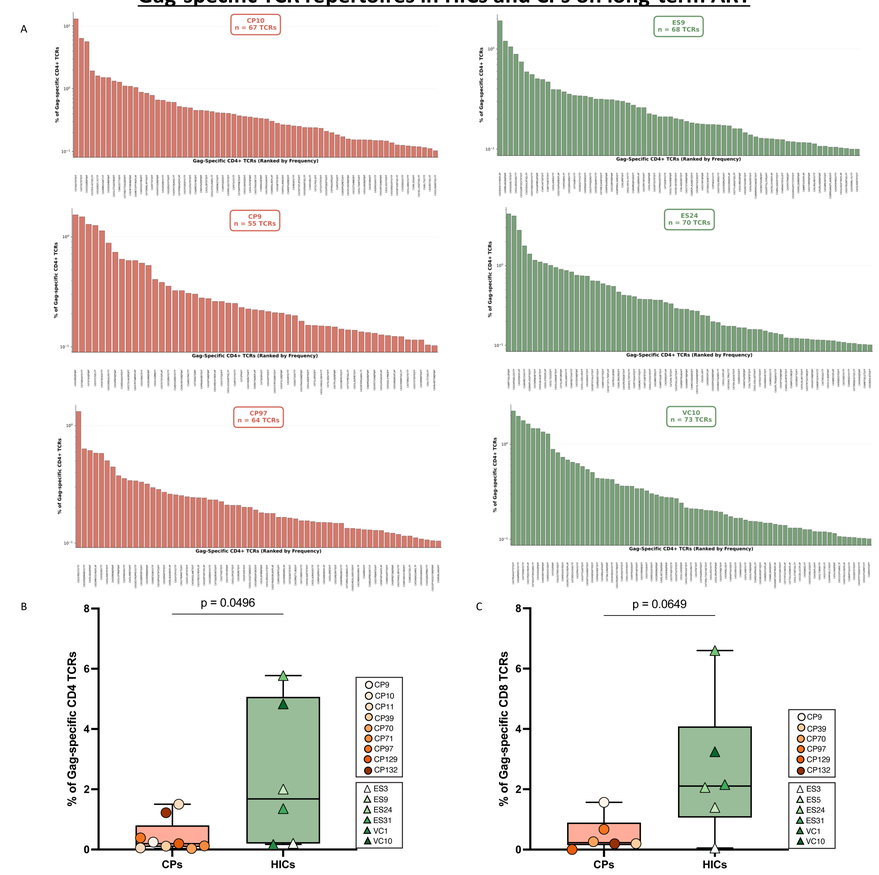


Figure 2: Gag-specific TCR repertoire analysis by FEST assay.
(A) Gag-specific CD4+ and CD8+ TCR clonotypes from representative participants (CP10, CP9, CP97, ES9, ES24, VC10) ranked by frequency. Each bar represents a distinct TCR clonotype identified by FEST assay with paired TCRα/β sequencing. (B) The percentage of Gag-specific CD4+ TCRs was quantified in 14 CPs and 5 HICs. Bars indicate median values. Each dot represents one participant. p-values determined by Mann-Whitney U-test. (C) The percentage of Gag-specific CD8+ TCRs was quantified in 14 CPs and 5 HICs. Bars indicate median values. Each dot represents one participant. p-values determined by Mann-Whitney U-test.

HICs and CPs on long-term ART have comparable Gag-specific CD4+ and CD8+ TCR repertoire diversity

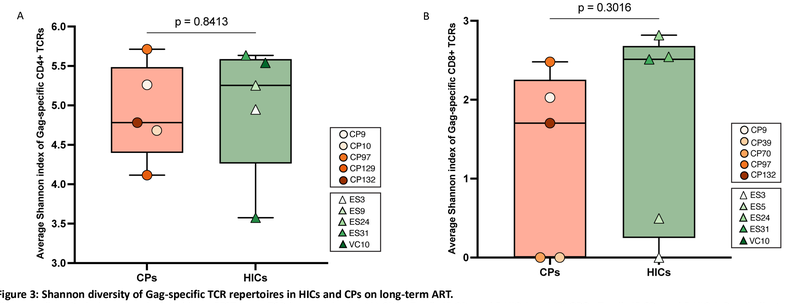


Figure 3: Shannon diversity of Gag-specific TCR repertoires in HICs and CPs on long-term ART.
Shannon diversity index was calculated from FEST assay TCR frequencies for (A) CD4+ T cells (5 CPs and 5 HICs) and (B) CD8+ T cells (5 CPs and 5 HICs). Bars indicate median values. Each dot represents one participant. p-values determined by Mann-Whitney U-test.

Gag-specific CD4+ T cells from HICs and CPs on long-term ART have similar transcriptional profiles

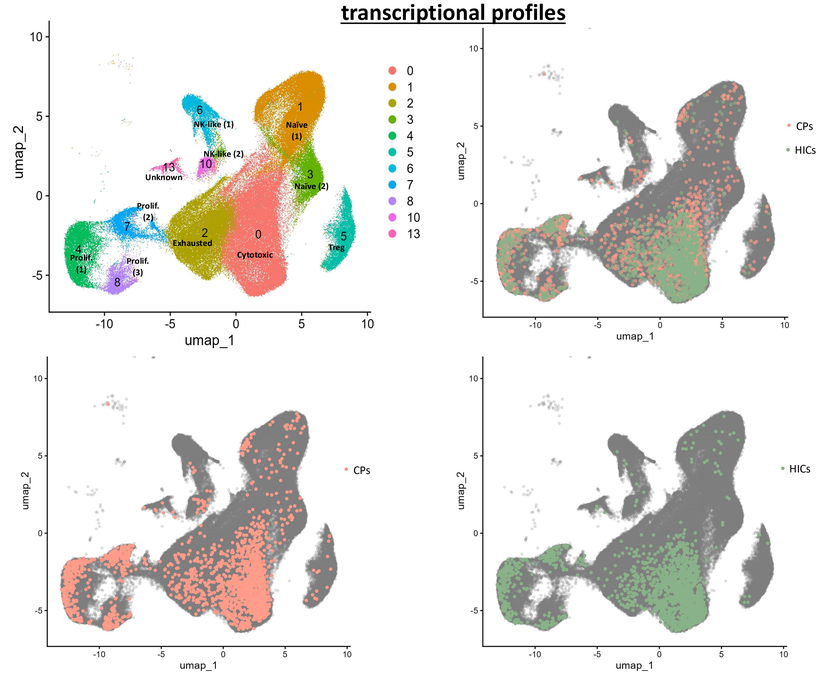


Figure 4: Single-cell transcriptional analysis of Gag-specific CD4+ T cells from CPs and HICs.
CD4+ T cells from 3 CPs (CP10, CP9, CP97) and 3 HICs (ES9, ES24, ES3) were isolated and analyzed by single-cell RNA-seq. TCR sequencing was used to identify Gag-specific clonotypes (figure 2). UMAP projection shows clustering of cells by transcriptional profile. Each dot represents one cell. Right panels show cells colored by participant group (CPs: orange; HICs: green).

Acknowledgments

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