ROBUST CD8⁺ T CELL PROLIFERATION AND STEM-LIKE MEMORY PHENOTYPE LINKED TO HIV POST-TREATMENT CONTROL

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ABSTRACT

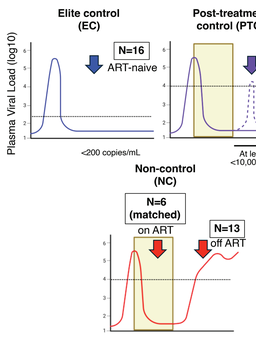
Post-treatment controllers (PTCs) of HIV control viremia to low or undetectable levels following discontinuation of ART. Previous studies have suggested a lack of association between *ex vivo* HIV-specific CD8+ T cell responses and post-treatment control. Here we extended beyond *ex vivo* evaluations to assess the proliferative capacity of HIV-specific CD8⁺ T cell responses in PTCs and the immune phenotypes driving these responses.

We evaluated HIV-specific CD8⁺ T cell responses of 14 PTCs, in comparison to 16 ART-naive elite controllers (ECs) and 13 post-treatment non-controllers (NCs), 6 of whom also had matched on-ART samples. PTC were defined as having plasma viral loads <10,000 copies/mL for at least 52 weeks post-treatment interuption (TI), while EC were defined as having ART-free viral suppression <200 copies/mL for at least 52 weeks. PBMCs from each participant were stimulated with overlapping peptide pools to HIV proteins in two stimulation conditions – a short 6-hour stimulation to identify reactive T cells *ex vivo* (day 0) and a 6-day stimulation to evaluate T cell proliferation (day 6). Samples were subsequently analyzed by flow cytometry.

There was no significant difference in the frequency of *ex vivo* pre-expansion HIV-specific (defined as interferon (IFN)-γ⁺ CD107a⁺) CD8⁺ T cells between the three groups post-TI. However, both EC and PTC demonstrated a significant and marked fold-expansion of HIV-specific cells following 6-day peptide stimulation compared to NC. Pre-expansion HIV-specific CD8⁺ T cells in EC and PTC had a significantly higher proportion expressing the transcription factor TCF1, a regulator of stem-like memory potential, than NC. Conversely, HIV-specific CD8⁺ T cells from NC had higher expression of the exhaustion-associated transcription factor TOX, cytolytic effector molecules perforin and granzyme B, and the inhibitory receptors PD-1 and CD39. Notably, there were no significant differences in the T cell phenotypes of NC on or off ART, suggesting this signature of T cell dysfunction preceeds the failure to control viral rebound. These findings establish key correlates of post-treatment control which can be leveraged as biomarkers to evaluate therapeutic vaccines and immunotherapies for HIV cure.

METHODS

STUDY COHORT AND METHODS



	PTC	NC	EC
N	**14**	**13**	**16**
Treatment Timing			
Early	11	6	-
Chronic	3	7	-
Pre-ART VL (log10)			
Median	**4.41**	**4.92**	-
Q1	*3.64*	*4.42*	-
Q3	*5.50*	*5.20*	-
Weeks on ART			
Median	**141**	**166**	-
Q1	*63*	*89*	-
Q3	*246*	*319*	-
Setpoint VL post-TI (gMean)			
Median	**2.12**	**4.34**	**1.64**
Q1	*1.73*	*3.95*	1.45
Q3	*2.67*	*4.85*	1.74
Protective HLA	**5**	**0**	**9**
B14	2	-	-
B27	1	-	4
B57	3	-	8

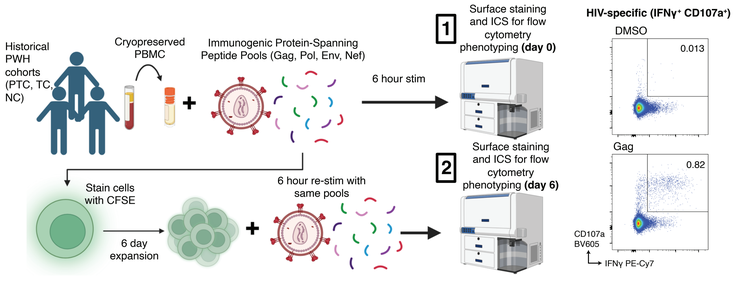


Figure 1: Cohort overview and methods. Top, description of study cohort and definitions of EC, PTC, and NC used. Cohort includes participants from the Ragon Institute/Massachussetts General Hospital HIV+ cohort, the ACTG CHAMP studies (Brigham and Women's Hospital), and the University of California, San Francisco OPTIONS study. Bottom, schematic for methods used to enumerate and characterize HIV-specific CD8⁺ T cells. PTC, post-treatment controller. EC, elite controller. NC, post-treatment non-controller. TI, treatment interruption. VL, viral load. HLA, human leukocyte antigen. CFSE, carboxyfluorescein succinimidyl ester. IFN, interferon. DMSO, dimethyl sulfoxide. PBMC, peripheral blood mononuclear cells. PWH, people with HIV. ART, antiretroviral therapy. ICS, intracellular cytokine staining.

RESULTS

PTC AND EC HAVE FEWER HIV-SPECIFIC CD8⁺ T CELLS WHICH EXPAND ROBUSTLY UPON ANTIGEN ENCOUNTER

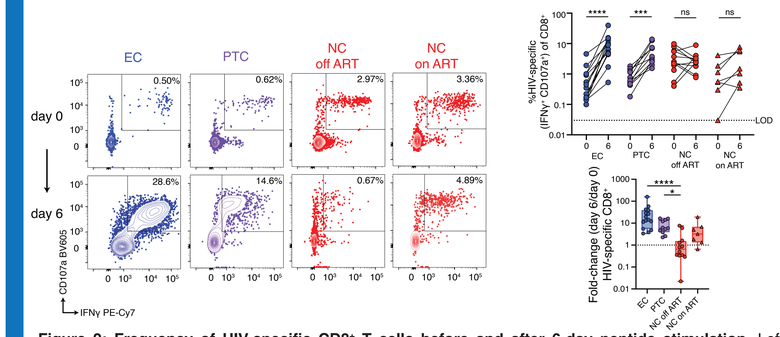


Figure 2: Frequency of HIV-specific CD8⁺ T cells before and after 6-day peptide stimulation. Left, representative flow cytometry data showing frequency of antigen-specific cells before (day 0) and after (day 6) 6-day peptide stimulation. Right top, Total frequency of HIV-specific CD8⁺ T cells summed across four HIV peptide pools (Gag, Pol, Env, Nef) before and after 6-day peptide stimulation. Right bottom, fold increase of HIV-specific cells over the 6-day stimulation. *P<0.05, ***P<0.001, ****P<0.0001, Wilcoxon matched-pairs signed rank test (top) or Kruskal-Wallis test followed by Dunn's multiple comparisons test (bottom).

NC CD8⁺ T CELLS DEMONSTRATE REDUCED PROLIFERATION WHEN STIMULATED OFF ART

Figure 3: Proliferation and expansion of HIV-specific CD8⁺ T cells following 6-day stimulation. Left, representative flow cytometry plots showing frequency of HIV-specific CD8⁺ T cells post-expansion (day 6) as well as proliferation of CD8⁺ T cells by day 6 as measured by dilution of the cell tracing dye CFSE. Right, frequency of total HIV-specific CD8⁺ T cells at day 6 that were CFSE^low, indicating cell proliferation.*P<0.05, ****P<0.0001. Kruskal-Wallis test followed by Dunn's multiple comparisons test.

PTC AND EC CD8⁺ T CELLS DISPLAY PHENOTYPIC MARKERS OF STEM-LIKE MEMORY PRE-EXPANSION

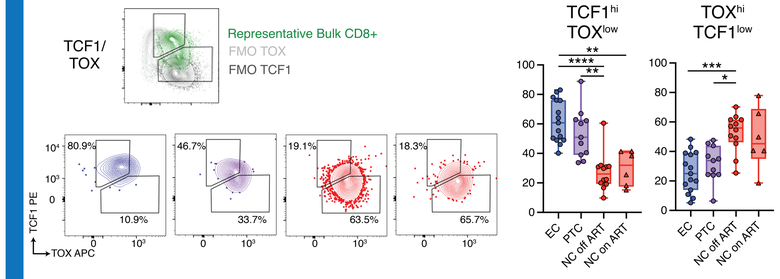


Figure 4: Expression of TCF1 and TOX in day 0 (pre-expansion) HIV-specific CD8⁺ T cells. Left, representative flow cytometry plots showing staining for the stem-like memory transcription factor TCF1 and exhaustion/terminal differentiation-associated transcription factor TOX, gated on HIV-specific CD8⁺ T cells. Right middle, frequency of pre-expansion HIV-specific CD8⁺ T cells expressing high for TCF1. Far right, frequency of pre-expansion HIV-specific CD8⁺ T cells high for TOX. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. Kruskal-Wallis test followed by Dunn's multiple comparisons test.

RESULTS

NC HIV-SPECIFIC CD8⁺ T CELLS EXHIBIT A TERMINALLY DIFFERENTIATED EFFECTOR PHENOTYPE

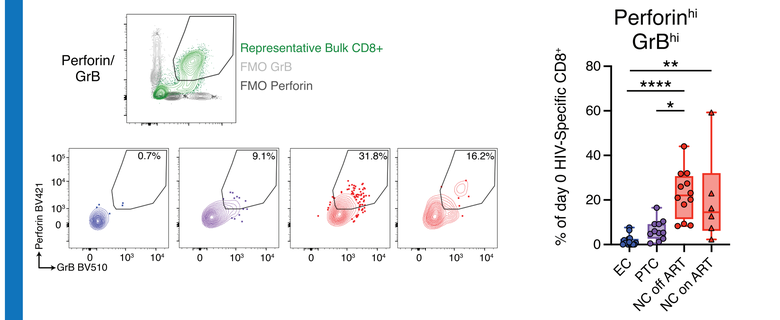


Figure 5: Expression of the cytolytic effector molecules perforin and granzyme B. Left, representative flow cytometry plots showing staining of perforin and granzyme B (GrB). Expression of cytolytic effectors in unstimulated CD8⁺ T cells suggests a more terminal effector differentiation. Right, frequency of pre-expansion HIV-specific CD8⁺ T cells with high expression of perforin and GrB. *P<0.05 **P<0.01, ****P<0.0001. Kruskal-Wallis test followed by Dunn's multiple comparisons test.

NC HIV-SPECIFIC CD8⁺ T CELLS DEMONSTRATE HIGHER INHIBITORY RECEPTOR EXPRESSION

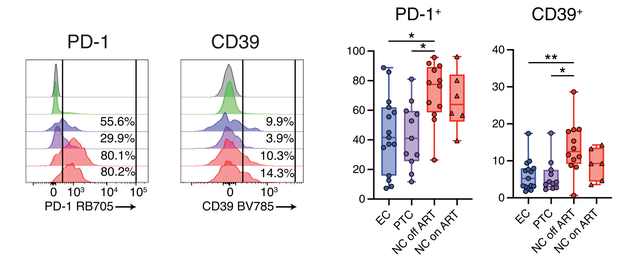


Figure 6: Expression of the inhibitory receptors PD-1 and CD39, associated with T cell dysfunction. Left, representative flow cytometry plots showing staining of PD-1 and CD39, two inhibitory receptors associated with T cell exhaustion and dysfunction. Right, frequency of pre-expansion HIV-specific CD8⁺ T cells expressing either PD-1 or CD39. *P<0.05, **P<0.01. Kruskal-Wallis test followed by Dunn's multiple comparisons test.

CONCLUSIONS

	PTC	NC
We observed:	Robust proliferative response, similar to EC	Abortive or insufficient proliferative response
Which led us to measure:	• TCF1↑ • TOX ↓ • GzB/PRF↓ • PD1/CD39↓	Stem-like memory ⟷ Terminal differentiation/ exhaustion markers: • TCF1↓ • TOX ↑ • GzB/PRF ↑ • PD1/CD39 ↑
We hypothesize this leads to:	Quick viral clearance and suppression, control is established	Unable to mount immune response before viral replication overwhelms immune system

Induction and maintenance of functional, stem-like memory HIV-specific CD8⁺ T cells is likely to be an integral part of HIV functional cure efforts.

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