ELITE CONTROLLERS WITH DISTINCT PROVIRAL RESERVOIRS EXHIBIT ROBUST CTL REACTIVITY TO NETWORKED HIV EPITOPES

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ABSTRACT

A major objective for HIV cure research is treatment-free suppression of infection, which has been achieved by individuals who spontaneously control HIV to undetectable levels (elite controllers, ECs). Prior analyses of ECs demonstrated that functional cytotoxic T lymphocyte (CTL) responses towards epitopes derived from mutationally constrained ('networked') regions of the HIV proteome were a key distinguishing feature. Recent work has shown that a subset of ECs have distinct viral reservoirs where integrated near full-length HIV genomes are primarily within transcriptionally repressive chromosomal locations, representing an even more promising model of HIV cure, but for which immune correlates remain to be defined. Thus, we characterized HIV-specific CTL responses in ECs with distinct proviral reservoirs (DPRs).

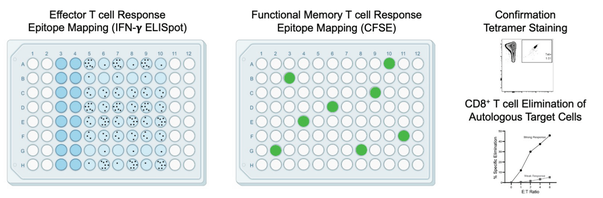
We evaluated the specificity and functionality of HLA-restricted HIV-specific CTL responses in 10 ECs with DPRs (Jiang et al, Nature 585:261) and 30 HIV noncontrollers on long-term ART by IFN-g ELISpot, proliferation assay, T cell phenotyping and CTL elimination assay. Matched integration and proviral sequencing was performed on the entire cohort and was used to evaluate the sequence diversity of targeted epitopes.

Evaluation of CTL immunodominance hierarchies by IFN-g ELISpot and tetramer staning revealed preferential targeting of networked epitopes by ECs with DPRs in comparison to noncontrollers on ART ($p<0.05$). Further assessment of the functionality of CTL responses targeting networked epitopes revealed highly significant differences in proliferative capacity ($p<0.0001$) and primary CD4$^+$ T cell elimination ($p<0.0001$), with some proliferative networked CTL responses being among the highest observed to date. Evaluation of viral sequence diversity in near full-length provirus revealed an absence of immune escape mutations within networked epitopes despite the presence of highly functional CTL responses.

Our findings demonstrate that ECs with distinct viral reservoirs have highly functional CTL responses against networked epitopes in comparison to noncontrollers on long-term ART. Moreover, these networked epitopes resist mutagenesis despite robust CTL pressure. These studies therefore indicate that induction of proliferative and cytotoxic T cell responses targeting mutationally constrained regions of HIV may be a viable strategy to restrict proviruses to transcriptionally repressed genomic regions and thereby durably suppress virus-producing cells.

METHODS

STUDY DESIGN TO EVALUATE CD8$^+$ T CELL RESPONSES



		All (n = 40)	EC (n = 10)	ART (n = 30)	p (EC *vs* ART)
Age (years; median, *IQR*)		55 (*6*)	54 (*10*)	56 (9)	*n.s.*
Sex	Female (n, %)	10 (*25%*)	2 (*20%*)	8 (*27%*)	*n.s.*
	Male (n, %)	30 (*75%*)	8 (*80%*)	22 (*73%*)	
Race	Asian (n, %)	2 (*5%*)	0 (*0%*)	2 (6%)	*n.s.*
	Black (n, %)	9 (*21%*)	2 (*20%*)	7 (*22%*)	
	Multi (n, %)	3 (*7%*)	1 (*10%*)	2 (6%)	
	Unknown (n, %)	1 (*2%*)	0 (*0%*)	1 (3%)	
	White (n, %)	25 (*63%*)	7 (*70%*)	18 (*63%*)	
Plasma HIV Viral Load		*Undetectable*	*Undetectable*	*Undetectable*	*n.s.*
Years Suppressed (median, *IQR*)		15 (*8*)	24 (*12*)	14 (*6*)	*** *0.0002*
CD4 (cells/mL; median, *IQR*)		779 (*347*)	816 (*310*)	779 (*338*)	*n.s.*
Protective *HLA-B* Alleles (n, %)		19 (*45%*)	9 (*90%*)	10 (*31%*)	** *0.002*

Figure 1: Study design. EC, spontaneous elite HIV controllers with distinct proviral reservoirs; ART, HIV noncontrollers on suppressive antiretroviral therapy. Categorical values are summarized as numbers and percentages of participants within each group. Continuous variables are reported as median with interquartile range (IQR). Protective HLA-B alleles include HLA-B*14, B*27, B*52 and B*57. p values were calculated using two-tailed t tests for continuous variables or χ^2 tests for categorical values; n.s., not significant ($p>0.05$).

RESULTS

ECs WITH DPRs HAVE IMMUNODOMINANT CD8$^+$ T CELL RESPONSES TO NETWORKED HIV EPITOPES

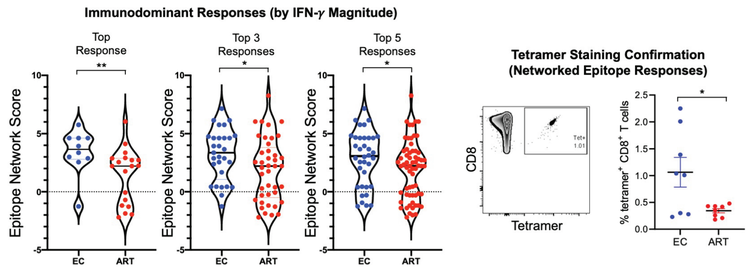


Figure 2: Immunodominant HIV-specific CD8$^+$ T cell responses target highly networked epitopes in ECs with DPRs. Violin plots summarizing epitope network z-scores for top 1 (left, n=10 in EC, n=21 in ART), top 3 (middle, n=25 in EC, n=52 in ART), or top 5 (right, n=35 in EC, n=71 in ART) largest HIV-specific CD8$^+$ T cell responses above background per individual. Representative fluorescent highly networked (epitope network z-score > 3.05) HIV-specific peptide-HLA (pHLA) tetramer response staining among CD8+ T cells. Frequencies of highly networked HIV-specific pHLA tetramer responses among CD8$^+$ T cells (n=8 in EC, n=8 in ART). EC, spontaneous elite HIV controllers with distinct proviral reservoirs; ART, HIV noncontrollers on suppressive antiretroviral therapy. *p < 0.05, **p < 0.01, Mann-Whitney U-tests.

CD8$^+$ T CELL RESPONSES TO NETWORKED EPITOPES ARE HIGHLY PROLIFERATIVE IN ECs WITH DPRs

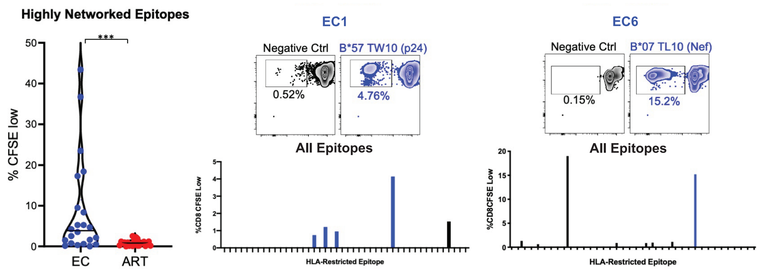


Figure 3: CD8$^+$ T cells from ECs with DPRs exhibit superior antigen-specific proliferation. Violin plot summarizing frequencies of CD8$^+$ T cell proliferation in response to stimulation with individual highly networked HIV peptides (n=22 in EC, n=24 in ART) that were detected above background by IFN-γ ELISPOT (left). Example flow cytometry plots and response frequencies of CFSE-stained CD8$^+$ T cells after 6-day incubation with individual class I HLA-optimal HIV peptides (blue, highly networked) or DMSO solvent controls in two EC participants (right). *** p < 0.001, Mann-Whitney U-test.

HIGHLY NETWORKED CD8$^+$ T CELL RESPONSES HAVE ROBUST CYTOLYTIC CAPACITY IN ECs WITH DPRs

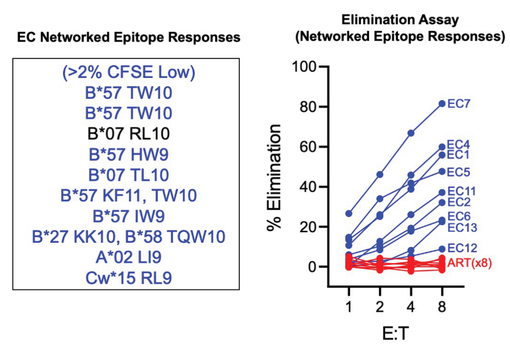


Figure 4: Highly networked HIV-specific CD8$^+$ T cells from ECs with DPRs have robust cytolytic capacity. List of immunodominant HIV-specific CD8$^+$ T cell response epitopes in ECs with DPRs (left; blue, highly networked). Percent elimination of HIV peptide-pulsed targets by autologous peptide-expanded CD8$^+$ T cells at the indicated effector:target (E:T) ratios for EC (n=9, blue) and ART (n=9, red) participants (right).

RESULTS

HIGHLY NETWORKED CD8$^+$ T CELL EPITOPES HAVE LIMITED MUTATIONAL FREQUENCY IN INTACT PROVIRUS

Mutational Sequences of Networked Epitopes in Intact Provirus from ECs

HLA	Protein	Epitope Name	Consensus	Autologous
B57	GAG	TW10	TSTLQEQIGW	TSTLQEQIGW
B57	GAG	TW10	TSTLQEQIGW	TSTLQEQIGW
B07	REV	RL10	RPAEPVPLQL	RPAEPVPLQL
B57	GAG	TW10	TSTLQEQIGW	TSTLQEQIGW
B57	NEF	HW9	HTQGYFPDW	HTQGYFPDW
B07	NEF	TL10	TPGPGIRYPL	TPGPGVRYPL
B57	GAG	TW10	TSTLQEQIGW	TSTLQEQIGW
B57	GAG	KF11	KAFSPEVIPMF	KAFSPEVIPMF
B57	GAG	IW9	ISPRTLNAW	ISPRTLNAW
B58	GAG	TW10	TSTLQEQIGW	TSTLQEQIGW
B27	GAG	KK10	KRWIILGLNK	KRWIILGLNK
A02	POL	LI9	LVGPTPVNI	LVGPTPVNI
A11	GAG	AK11	ACQGVGGPGHK	ACQGVGGPSHK

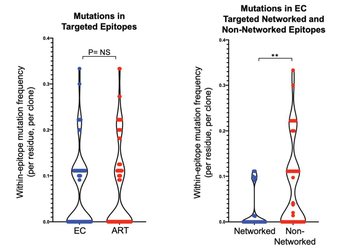


Figure 5: Highly networked HIV epitopes targeted by CD8$^+$ T cells are less frequently mutated in autologous provirus. Summary of autologous HIV epitope sequences translated from proviral DNA for epitopes targeted by immunodominant HIV-specific CD8$^+$ T cell responses in each EC participant (left). Variants relative to clade B consensus epitope sequences are highlighted in red. Violin plot summarizing nonsynonymous epitope mutation frequencies per codon and per proviral clone for each HIV epitope targeted by autologous CD8$^+$ T cells, analyzed by participant group (middle; n=62 in EC, n=90 in ART) or by epitope network z-score in ECs (right; n=24 highly networked, n=36 non-neworked). **p < 0.01, Mann-Whitney U-test.

CD8$^+$ T CELL RESPONSES TO HIGHLY NETWORKED EPITOPES ARE CROSS-REACTIVE TO VARIANTS

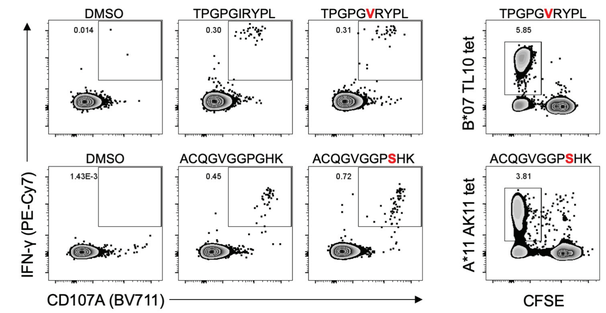


Figure 6: CD8$^+$ T cells from ECs with DPRs cross-recognize autologous proviral HIV epitope variants. CD8$^+$ T cell cross-recognition of consensus and autologous variant peptides as measured by intracellular cytokine staining and flow cytometry for two ECs (rows) relative to solvent (DMSO) control (left). Consensus peptide-HLA tetramer (tet) staining of CFSE-labeled CD8$^+$ T cells after 6-day stimulation with autologous variant peptides.

CONCLUSIONS

ELITE CONTROLLERS WITH DISTINCT PROVIRAL RESERVOIRS HAVE IMMUNODOMINANT CD8$^+$ T CELL RESPONSES TO NETWORKED EPITOPES

NETWORKED CD8$^+$ T CELL RESPONSES ARE HIGHLY PROLIFERATIVE AND CYTOLYTIC IN ELITE CONTROLLERS WITH DPRs

HIGHLY NETWORKED EPITOPES TARGETED BY ROBUST CD8$^+$ T CELL RESPONSES RESIST MUTATION IN ELITE CONTROLLERS WITH DPRs

NETWORKED CD8$^+$ T CELL RESPONSES REMAIN CROSS-REACTIVE TO EPITOPES WITH MINOR POLYMORPHISMS

TARGETING OF HIGHLY NETWORKED CD8$^+$ T CELL EPITOPES PROVIDES A RATIONAL FRAMEWORK TO ACHIEVE A FUNCTIONAL HIV CURE

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