Elevated Expression of Anti-HIV-1 Restriction Factors in Effector Memory CD4+ T cells *in vivo*

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BACKGROUND

- Recent observations suggest that there may be less viral outgrowth from effector memory than central memory CD4+ T cells from ART-suppressed individuals, despite similar levels of HIV-1 DNA in these cellular subsets.
- This may be driven by cell-intrinsic factors suppressing viral production, and/or the disproportionate presence of replication-incompetent HIV-1 variants in effector memory cells.
- In this study, we comprehensively compare anti-HIV-1 restriction factor gene expression patterns between effector memory and central memory CD4+ T cells from individuals of different HIV-1 disease states, to address the hypothesis that host restriction mechanisms suppress viral outgrowth in effector memory CD4+ T cells.

OBJECTIVE

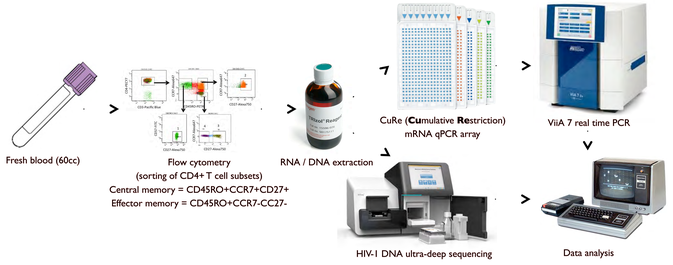
To determine the relative gene expression of anti-HIV-1 host restriction factors in peripheral central memory and effector memory CD4+ T cells.

METHODS

- Fresh blood was collected from 20 individuals enrolled in the SCOPE cohort:

5 HIV negative	5 Elite controllers	5 ART Suppressed	5 Non-controllers
	Antiretroviral therapy-naive subjects who have at least one year duration of documented viral loads that are below the level of detection of conventional assays	Subjects taking antiretroviral therapy with at least three months of documented viral loads below the level of detection of conventional assays	Subjects who have had at least one documented viral load above 10,000 copies/ml

- The following schematic illustrates our experimental workflow:



- To represent overall HIV-1 inhibitory potential, we defined the CuRe score as the cumulative fold-difference in restriction factor expression with respect to a control individual, calculated using the following formula (expression value for the ith gene is notated as e_i, i=1,2,…,n, assuming there are n genes; the reference expression value for the ith gene is notated as r_i, i=1,2,…,n):

$$CuRe = \sum_{i=1}^{n} e_i / r_i$$

- The CuRe quantitative PCR array measures the mRNA expression of the following list of anti-HIV-1 host restriction factors:

NAME	NCBI ENTREZ GENE DESCRIPTION	KEY ANTI-HIV-1 ROLE(S)
APOBEC3 (A-H)	Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like3	Hypermutation; lethal mutations in viral DNA Inhibition of reverse transcription Inhibition of integration
TRIM family (11 members)	Tripartite motif family	Targeting of viral capsid Inhibition of viral transcription
BST-2/Tetherin	Bone marrow stromal cell antigen 2	Blocks release of enveloped viruses
BRD4	Bromodomain-containing protein 4	Transcriptional repression of HIV-1
CDKN1A (P21)	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	Blocks reverse transcription Blocks RNA transcription by reducing activity of CDK9
PAF1	Paf1, RNA polymerase II associated factor	Inhibits early events of viral life cycle from reverse transcription to integration
CTR9	Ctr9, Paf1/RNA polymerase II complex component	Inhibits early events of viral life cycle from reverse transcription to integration
RTF1	Rtf1, Paf1/RNA polymerase II complex component	Inhibits early events of viral life cycle from reverse transcription to integration
EIF2AK2 (PKR)	Eukaryotic translation initiation factor 2-alpha kinase 2	Inhibits viral protein translation by protein phosphorylation; promotes innate immune signaling
HERC5	HECT domain and RLD 5	Blocks early stage of retroviral particle assembly
IFITM Family (3 members)	Interferon induced transmembrane protein	Inhibition of cytosolic entry
ISG15	ISG15 ubiquitin-like modifier	Blocks interaction between HIV-1 Gag and Tsg101 (ESCRT-I) required for efficient budding of HIV-1
MOV10	Mov10, Moloney leukemia virus 10, homolog	Inhibits proteolytic processing of Gag and reverse transcription
RNASEL	Ribonuclease L (2',5'-oligoisoadenylate synthetase-dependent)	Cleave single-stranded RNA in U-rich sequences; activate antiviral innate immunity
RSAD2 (Viperin)	Radical S-adenosyl methionine domain containing 2	Inhibits viral production
SAMHD1	SAM domain and HD domain 1	Inhibits HIV replication in myeloid cells, probably by regulating cellular dNTP supply
SLFN11	Schlafen family member 11	Inhibits viral protein synthesis

RESULTS

- Most anti-HIV-1 restriction factors are significantly elevated in effector memory CD4+ T cells in comparison to central memory CD4+ T cells in HIV negative individuals, elite controllers, and viremic non-controllers (Table 1, Figure 1)
- Within the ART-suppressed group, Four restriction factors were significantly elevated in effector memory cells: APOBEC3F, APOBEC3H, TRIM11, and PML / TRIM19 (Table 1, Figure 2).

Table 1. p-values for statistical comparisons of restriction factor expression in cellular subsets. Blue highlighting indicates significantly elevated expression in effector memory CD4+ T cells, orange indicates elevated expression in central memory CD4+ T cells.

	APOBEC3C	APOBEC3D	APOBEC3F	APOBEC3G	APOBEC3H	BRD4	BST2	CDKN1A (P21)	CTR9	EIF2AK2 (PKR)	HERC5	IFITM1	IFITM2	IFITM3	ISG15	MOV10	PAF1	PML / TRIM19	RNASEL	RSAD2 (VIPERIN)	RTF1	SAMHD1	SLFN11	TRIM11	TRIM14	TRIM21	TRIM22	TRIM26	TRIM28	TRIM3	TRIM5
HIV negative	0.007	0.043	0.194	0.025	0.005	0.108	0.119	0.101	0.614	0.079	0.032	0.621	0.137	0.016	0.049	0.799	0.243	0.004	0.125	0.187	0.056	0.184	0	0.003	0.018	0.006	0.406	0.032	0.044	0.314	0.064
Elite controllers	0.009	0.01	0.101	0.02	0.034	0.082	0.023	0.272	0.046	0.019	0.081	0.08	0.011	0.408	0.074	0.674	0.252	0.003	0.106	0.23	0.064	0.266	0.021	0.005	0.067	0.068	0.077	0.239	0.993	0.017	0.109
ART suppressed	0.085	0.066	0.039	0.068	0.001	0.228	0.181	0.132	0.175	0.167	0.163	0.667	0.201	0.393	0.111	0.338	0.191	0.003	0.721	0.263	0.059	0.283	0.081	0.04	0.071	0.069	0.111	0.077	0.234	0.036	0.066
Non-controllers	0.007	0.003	NA	0.011	0.008	0.462	0.029	0.058	0.02	0.243	0.281	0.949	0.101	0.09	0.011	0.005	0.989	0.003	0.698	0.159	0.492	0.081	0.012	0.002	0.03	0.009	0.831	0.052	0.741	0.002	0.915

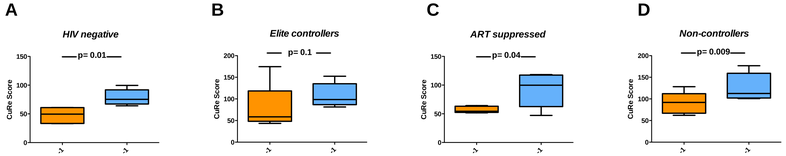


Figure 1. CuRe scores in central memory and effector memory CD4+ T cells. CuRe scores, representing the cumulative expression of anti-HIV-1 restriction factors, were significantly higher in effector memory CD4+ T cells in comparison to central memory CD4+ T cells in (A) HIV-1-negative,, (B) HIV-1-infected untreated, and (C) HIV-1 infected ART-suppressed individuals (based on paired T tests).

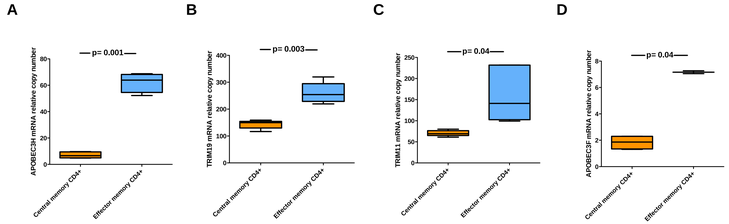


Figure 2. Restriction factors elevated in effector memory CD4+ T cells from ART-suppressed individuals. Within the ART-suppressed group, four restriction factors were significantly elevated in effector memory CD4+ T cells with respect to central memory CD4+ T cells: (A) APOBEC3F, (B) APOBEC3H, (C) TRIM11, and (D) PML / TRIM19. (based on paired T tests).

CONCLUSIONS

- The majority of anti-HIV-1 restriction factors are expressed at higher levels in the effector memory CD4+ T cells of ART-untreated subjects, as compared to central memory CD4+ T cells
- Restriction factors that are elevated in effector memory CD4+ T cells of ART-suppressed individuals include particular TRIM family members that attack HIV-1 at the post-integration phase of the viral life cycle, and APOBEC3 cytidine deaminases that hypermutate HIV-1 genomes.
- Expression data from our exploratory analyses of sorted T cell subsets indicate that enhanced host restriction factor expression may contribute to the reduced viral outgrowth observed in effector memory CD4+ T cells.
- Variation in selection pressure from cell-intrinsic immune mechanisms may drive viral genetic compartmentalization between tissues and cell types.

FUTURE DIRECTIONS

- Gene expression patterns will be characterized in additional subjects to increase sample size and validate observations
- Protein-level analyses will be implemented to complement mRNA expression data
- Ultra-deep sequencing of HIV-1 DNA from CD4+ T cell subsets will be performed to examine viral genetic features associated with each cell type, focusing on evidence of APOBEC3-mediated hypermutation
- Expression profiling of cellular subsets from lymphoid tissues will be performed

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