Effective Control of Virus Replication in HIV-1 Elite Controllers by Active SAMHD1

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ABSTRACT

Background: Elite controllers are a small number of HIV-1 positive individuals who control infection and remain healthy without the need for antiretroviral therapy. The basis of this elite control of infection remains unclear. SAMHD1 has been identified as an HIV-1 restriction factor that controls the intracellular deoxynucleotide pool, limiting HIV-1 reverse transcription. SAMHD1-mediated restriction is counteracted by HIV-2 Vpx and is deactivated by cyclin-dependent kinase (CDK)-mediated phosphorylation.

Methodology: Purified CD4+ T cells from healthy donors (n=11), viremic HIV+ patients (n=10) and elite controllers (n=12) were stimulated and infected with HIV-1 or HIV-2 strains in the presence or absence of coexpressed Vpx. Replication levels were measured by flow cytometry and proviral DNA measured by quantitative PCR. Gene and protein expression were measured by mRNA qPCR and Western blot analysis respectively. Cell activation was measured by analysis of CD25 and CD69 expression and proliferation by Ki67 expression and absolute cell count. Unpaired t test was used for statistical analysis.

Results: Despite similar levels of CD4+ T cell activation and proliferation than control cells, anti-CD3 or PHA/IL-2 stimulation of CD4+T from elite controllers showed an intrinsic resistance to infection ($p<0.0001$) at the level of unintegrated proviral DNA formation ($p<0.0023$) that was overcome by coexpression of HIV-2 Vpx, indicating that restriction was mediated by SAMHD1. HIV-1 infection of CD4+ T cells from healthy donors correlated with the degree of phosphorylation of SAMHD1 ($r2=0.7437$) measured by Western blot. Moreover, cells from elite controllers showed significantly ($p<0.0001$) lower levels of SAMHD1 phosphorylation compared to healthy donors or cells from viremic HIV+ individuals. RNA interference or pharmacological intervention showed that CDK2 and CDK6 control SAMHD1 phosphorylation in primary hematopoietic cells. Moreover, CD4+ T cells from elite controllers showed lower mRNA expression of CDK2 ($p<0.0001$) and CDK6 ($p=0.0085$) concomitant to reduced activation of CDK2 as measured by CDK2 Thr160 phosphorylation.

Conclusions: These results provide a mechanism of viral control common to HIV-1 elite controllers linking virus replication and cell cycle regulation, and suggest that SAMHD1-mediated restriction may be relevant *in vivo* as a determinant of HIV-1 disease progression and control.

1. Similar levels of activation and proliferation of activated lymphocytes from healthy donors and elite controllers

A

Phenotype	number (n)	Viral load at set point (copies/ml)	CD4+ count at set point (cells/µl)	Protective HLA-B alleles
HD	14	ND	ND	ND
EC	12	<40	706 (498-880)	5701 (n:2) 5703 (n:1) 2702 (n:1) 3501 (n:1) 3503 (n:1)
VP	10	491,531 (384,261-730,000)	398 (370-544)	ND

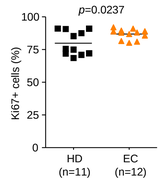
HD, seronegative healthy donors; EC, elite controllers; VP, viremic patients; SD, IQR, interquartile range. ND; not determined.

B

	Mean (SD)		
	HD (n=12)	**EC (n=9)**	**VP (n=8)**
CD4$^+$ (%)	88.0 (6.0)	78.1 (11.6)	81.0 (9.0)
CD4$^+$/CD69$^+$ (%)	35.7 (31.9)	28.2 (15.4)	58.6 (14.1)
CD4$^+$/CD25$^+$ (%)	98.3 (1.0)	92.3 (5.4)	99.2 (1.3)
Ki67$^+$ (%)	84.9 (9.9)	84.4 (6.4)	88.2 (3.7)
Naïve (CD45RA$^+$ CCR7$^+$)	67.1 (12.9)	60.4 (16.7)	ND
$T_{(CM)}$ (CD45RA$^-$ CCR7$^+$)	15.7 (8.3)	10.1 (2.9)	ND
$T_{(EM)}$ (CD45RA$^-$ CCR7$^-$)	7.1 (5.9)	7.0 (6.2)	ND
CXCR4$^+$ (% T cells)	47.3 (15.7)	37.2 (8.3)	ND
CCR5$^+$ (% T cells)	5.8 (4.1)	3.7 (3.7)	ND

HD, seronegative healthy donors; EC, elite controllers; VP, viremic patients; CM, control memory; EM, effector memory; SD, standard deviation; IQR, interquartile range. ND; not determined.

C



D

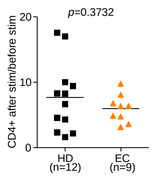
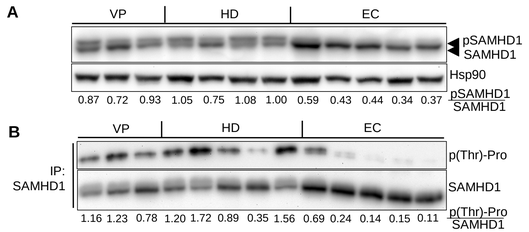


Figure 1. (A) Phenotype data of patients included in the study. **(B)** Phenotype markers of PBMC after stimulation with a CD3-CD8 bispecific antibody. **(C)** Percentage of Ki67+ CD4+ T cells in healthy donors (HD) and elite controllers (EC). Horizontal bars indicate the mean value. **(D)** Proliferation ratio was obtained dividing the total number of CD4+ T cells recovered 5-days after stimulation between the initial number of CD4+ T cells. Horizontal bars indicate the mean value. Statistical significance was calculated by unpaired two-tailed Student's t test.

3. Activated CD4+ T cells from elite controllers showed significantly lower levels of SAMHD1 phosphorylation compared to cells from healthy donors



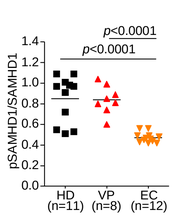
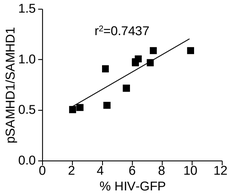


Figure 3. (A) Lysates of stimulated cells were run in 6% polyacrylamide gel, transferred and blotted with SAMHD1 and Hsp90 antibodies. One representative blot with samples from the three studied groups is shown. The ratio between the density of the corresponding phosphorylated band of SAMHD1 and the unphosphorylated form (pSAMHD1/SAMHD1) is indicated for each sample. **(B)** Lysates were subjected to overnight immunoprecipitation of SAMHD1 and blotted with an anti-phospho-threonine-proline antibody (p(Thr)-Pro) and SAMHD1 antibody. The ratio between the density of the corresponding phosphorylated band of SAMHD1 and the unphosphorylated form (p(Thr)-Pro/SAMHD1) is indicated for each sample. One representative blot with samples from the three studied groups is shown. **(C)** pSAMHD1/SAMHD1 ratio for HD, VP and EC. Horizontal bars indicate mean values. Each dot represents the mean density ratio of at least two independent blots for each sample obtained as shown in (A). Statistical significance was calculated by unpaired two-tailed Student's t test.

4. Infection of CD4+ T cells from healthy donors correlated with SAMHD1 phosphorylation

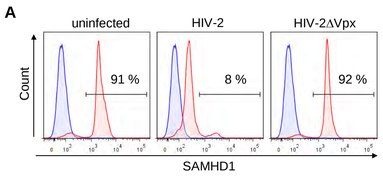
Infection values from Figure 2 corresponding to healthy donors correlated with the pSAMHD1/SAMHD1 ratio calculated for the different lysates of this population as shown in Figure 3.

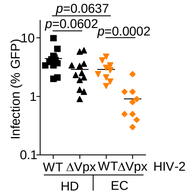


CONCLUSIONS

- As previously reported (Saez-Cirion A *et al*. 2011, *Blood*; Chen H *et al*. 2011, *JCI*), CD4+ T cells from elite controllers shoappear to have an intrinsic resistance to HIV-1 infection.
- Blockade of proviral DNA formation in cells from elite controllers correlated with a reduced degree of SAMHD1 phosphorylation and CDK2 expression/activation and could be overcome by Vpx-expressing HIV-2.

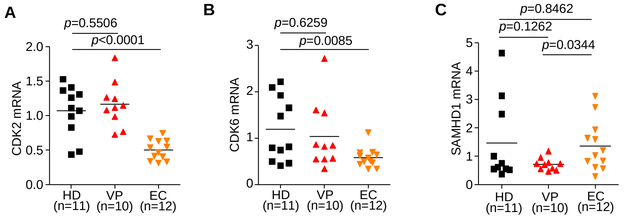
5. Vpx counteracts HIV-1 restriction in CD4+ T cells





(A) Purified CD4+ T lymphocytes were left uninfected or infected with complete HIV-2 or HIV-2 lacking Vpx protein (HIV-2ΔVpx). Three days after infection, cells were stained using a SAMHD1 antibody (red histogram). The secondary antibody alone was used as a control (blue histogram). Representative histograms of one experiment are shown. Experiment was performed in three independent donors. **(B)** CD4+ T cells from HD (n=12) and EC (n=9) were infected with HIV-2 wild-type (WT) and HIV-2ΔVpx and infection measured as percentage of GFP+ cells by flow cytometry. Horizontal bars indicate mean values. Statistical significance was calculated by unpaired two-tailed Student's t test.

6. Reduced CDK2 expression in cells from elite controllers



(A) mRNA levels of CDK2, **(B)** CDK6 and **(C)** SAMHD1 in stimulated CD4+ T cells from seronegative healthy donors (HD), viremic patients (VP) or elite controllers (EC). mRNA was normalized to the mean of the HD population. Horizontal bars indicate mean values. Statistical significance was calculated by unpaired two-tailed Student's t test.