Cytotoxic T lymphocytes may shape the HIV-1 proviral landscape

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Abstract

Background. The majority of the HIV-1 proviruses are defective, making it irrelevant to use HIV-1 DNA quantitation to measure the size of the latent reservoir. However, a decrease in HIV-1 proviral DNA levels has been reported *in vivo* using the shock-and-kill strategy. We have previously shown that defective HIV-1 proviruses can be transcribed and translated, making them potential targets for cytotoxic T lymphocyte (CTL) elimination. We hypothesize that HIV-1 proviral landscape is dynamic, and may be shaped by CTL selection pressure.

Methods. We examined the HIV-1 proviral landscape in virally suppressed HIV-1-infected individuals. We then examined whether defective HIV-1 proviruses can be recognized by CTLs *in vitro* and *ex vivo*.

Results. In the HIV-1 proviral landscape of virally suppressed HIV-1-infected individuals, we found that the frequency of hypermutated proviruses correlates negatively with the duration of infection, while the frequency of proviruses containing large internal deletions correlates positively with the duration of infection, implying a dynamic process.

To examine whether defective HIV-1 proviruses can be recognized by CTLs *in vitro*, we co-cultured CD4+ T cells transfected with defective HIV-1 proviruses with autologous CTL clones known to recognize specific CTL epitopes. CTL recognition of cells containing defective HIV-1 proviruses were measured by CD107a loading. Surprisingly, we found that cells containing hypermutated proviruses which has a premature stop codon or mutated start codon 5' to an intact CTL epitope can be recognized by CTLs, while cells containing large internal deletions cannot be recognized by CTLs.

To understand whether hypermutated HIV-1 proviruses may be recognized by CTLs *ex vivo*, we used targeted deep-sequencing to examine the frequency of APOBEC-mediated nonsense mutations in *gag*. We compared resting and activated CD4+ T cells treated with and without autologous prestimulated CTLs. We found that the frequency of HIV-1 RNA containing premature stop codons 5' to the CTL recognition site decreased upon CTL co-culture, indicating that cells containing hypermutated proviruses can be eliminated by CTLs *ex vivo*.

Conclusions. We found that hypermutated HIV-1 proviruses may be targeted by CTLs. HIV-1 DNA measurement may in part reflect the effect of HIV-1 elimination strategies. HIV-1 proviral landscape may be shaped, at least in part, by CTL selection pressure.

Background

The majority of HIV-1 proviruses in infected cells are defective, including those containing packaging signal (ψ)/major splice donor (MSD) defects, APOBEC-mediated G-to-A hypermutations, large internal deletions and point mutations. In HIV-1-infected individuals treated with antiretroviral therapy (ART) for >4 years, the frequency of HIV-1 proviral DNA **remains constant over time** (Besson *et al.*, CID 2014). The frequency of cells containing induced HIV-1 proviruses, i.e. the size of the latent reservoir, **decays minimally over time** (Siliciano *et al.*, Nature Med 2003; Crooks *et al.*, JID 2015). However, recent studies showed that HIV-1-infected cells may undergo HIV-1-driven aberrant proliferation through integration into proliferation-related genes (Maldarelli *et al.*, Science 2014; Wagner *et al.*, Science 2014; Cohn *et al.*, Cell 2014), and the frequency of HIV-1-infected cells which undergo clonal expansion **increases over time**. Given that the frequency of clonally expanded Hiv-1-infected cells increases over time but the total HIV-1 proviral DNA level remains constant, we hypothesize that the HIV-1 proviral landscape is dynamic.

While clonal expansion may contribute to the dynamic HIV-1 proviral landscape, it Is recently suggested that cytotoxic T lymphocytes (CTLs) may contribute to viral suppression in the presence of ART (Cartwright *et al.*, Immunity 2016). We hypothesize that CTLs may shape the landscape of HIV-1 proviruses through different selection pressure on different subsets of HIV-1 proviruses.

Methods/results

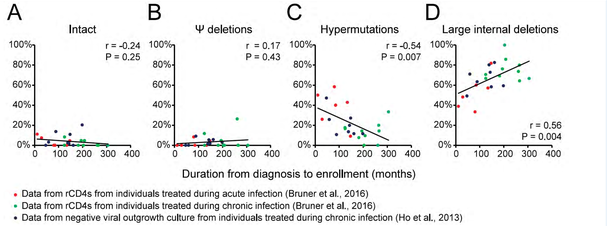


Figure 1. The landscape of HIV-1 proviruses may be dynamic. Correlation between the duration from diagnosis to enrollment and the proportion of different subsets of HIV-1 proviruses containing (**A**) intact genome, (**B**) Ψ/MSD deletions/mutations, (**C**) hypermutations, (**D**) large internal deletions. r and P values are calculated by Pearson correlation.

Methods/results

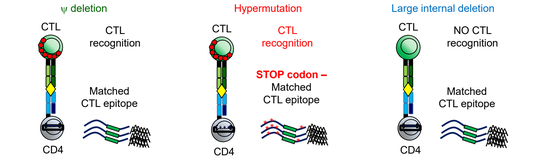


Figure 2. Experimental scheme of CTL recognition of cells containing defective HIV-1 proviruses. CTL clones known to recognized specific CTL epitopes (yellow) were cultured with autologous CD4+ T cells transfected with defective proviruses. CTL recognition of cells containing defective HIV-1 proviruses was measured by CD107a expression (red dots) by flow cytometry.

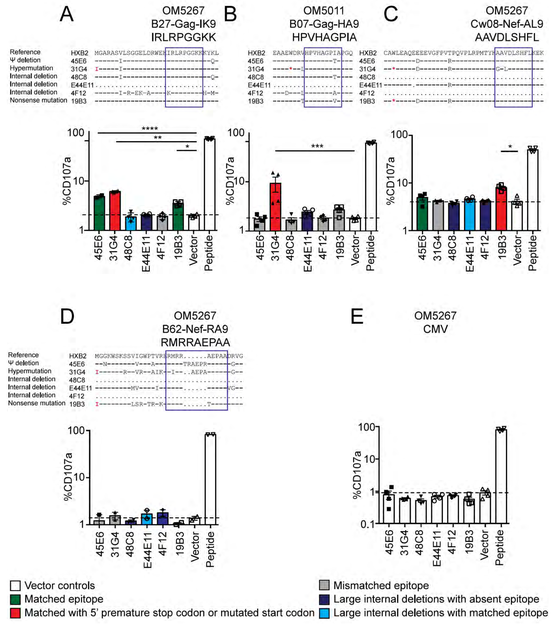


Figure 3. Cells containing defective HIV-1 proviruses can be recognized by HIV-1-specific CTLs. Flow cytometry showing %degranulated CTLs (CD8+CD107a+) from 2 Gag-specific clones (**A–B**), 2 Nef-specific clones (**C–D**) and a CMV-specific clone (**E**) upon co-culture with autologous CD4+ T cells transfected with defective proviral plasmids. Each target was tested in independent quadruplets shown in mean ± SEM. P values were calculated by one-way ANOVA with Dunnett's multiple comparison test.
*P<0.05, **P<0.01, ***P<0.001, **** P<0.0001. See also Figure S5.

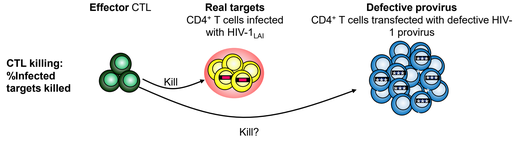


Figure 4. Experimental scheme of a competition assay measuring CTL recognition of cells containing defective HIV-1 proviruses. CTL clones known to recognized specific CTL epitopes were cultured with autologous CD4+ T cells infected with a infectious HIV-1 reference strain. CD4+ T cells were labeled with a fluorescent dye Cell Trace Far Red. Autologous CD4+ T cells transfected with defective proviruses were added to the culture to examine competition of CTL killing with cells containing infectious HIV-1 proviruses. %Infected targets was measured by %Gag expression among cells stained with Cell Trace Far Red.

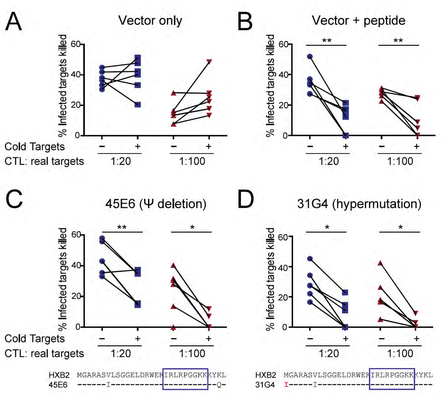


Figure 5. Cells containing defective HIV-1 proviruses competes with cells containing intact proviruses for CTL recognition. (**A**) Negative control using CD4+ T cells transfected with vector plasmid. (**B**) Positive control using CD4+ T cells transfected with vector plasmid and loaded with cognate peptide. (**C–D**) Cold-target inhibition assay showing HIV-1-specific CTL recognition of cells containing defective HIV-1 proviruses. Six paired replicates per condition were shown. P values were calculated by paired Student *t*-test.

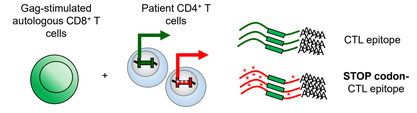
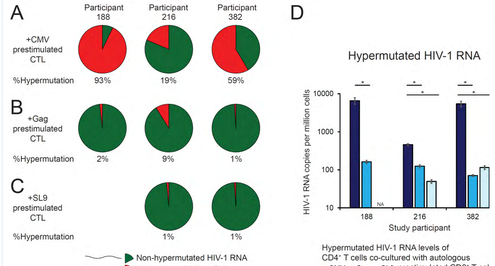


Figure 6. Experimental scheme of CTL recognition of cells containing hypermutated HIV-1 proviruses *ex vivo*. Study participants were HLA-typed to map out corresponding CTL epitopes. Patient resting CD4+ T cells were activated with αCD3/CD28 for 3 days in the presence of ART. Patient CD8+ T cells were stimulated with Group B Gag peptide mixture for 6 days in the presence of peripheral blood mononuclear cells (PBMC) and ART. Pre-stimulated CD8+ T cells and activated CD4+ T cells were co-cultured at 1:1 ratio in the presence of ART for 2 days. CD8+ T cells were then removed by magnetic depletion. Frequency of cell-associated hypermutated HIV-1 RNA was measured by targeted *gag* deep sequencing and qRT-PCR.



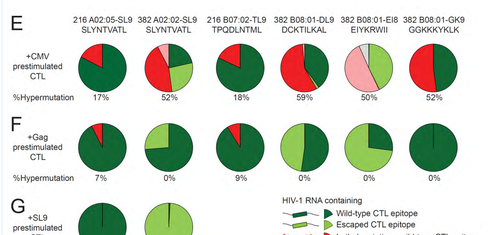


Figure 7. Cells containing hypermutated HIV-1 proviruses can be recognized by CD8+ T cells *ex vivo*. Resting CD4+ T cells were activated with anti-CD3/CD28 in the presence of enfuvirtide and co-cultured with autologous CD8+ T cells prestimulated with a Gag peptide mixture, a CMV peptide mixture and a HLA-A2-restricted SL9 peptide. Targeted *gag* RNA deep sequencing and qRT-PCR was used to measure the proportion and frequency of HIV-1 RNA containing lethal hypermutations. (**A–D**) Proportion (**A–C**) and frequency (**D**) of HIV-1 RNA containing lethal hypermutations from CD4+ T cells upon co-culture with autologous (**A**) CMV-, (**B**) Gag-, (**C**) SL9-prestimulated CD8+ T cells. (**E–G**) Proportion of HIV-1 RNA with or without lethal hypermutations 5' to wild-type or escaped CTL epitopes upon co-culture with autologous (**E**) CMV-, (**F**) Gag-, and (**G**) SL9-prestimulated CD8+ T cells. Data represent mean ± SEM. *, P value <0.05 by two-tailed Student *t*-test.

Conclusions

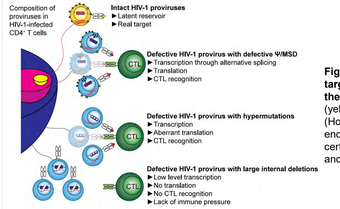


Figure 8. Proposed model of the scope of potential targets of HIV-1-specific CTLs compared with the size of the latent reservoir. The provirus population includes intact (yellow and pink) and defective (blue) proviruses as described (Ho et al., 2013). Following reversal of latency by endogenous stimuli or latency reversing agents, cells with certain types of defective proviruses may express HIV-1 RNA and protein and may become targets for HIV-1-specific CTLs.

Acknowledgements

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