Stimulation of broad CTL response is required to clear latent HIV-1 in humanized mice

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Background

Despite antiretroviral therapy (ART), HIV-1 persists in a stable latent reservoir, primarily in resting memory CD4⁺ T cells. This reservoir presents a major barrier to the cure of HIV-1 infection. To purge the reservoir, pharmacological reactivation of latent HIV-1 has been proposed and tested both *in vitro* and *in vivo*. The next step is to eliminate the resting CD4⁺ T cells in which latent HIV-1 has been induced through virus-specific immune mechanisms including cytolytic T lymphocytes (CTL). A key question is whether CTLs in patients on ART can clear infected cells after latency is reversed[1].

Methods

We generated MIS^{KI}TRG[2] mouse in the Rag2−/− Il2rg−/− 129×Balb/c (N2) genetic background harbors knock-in replacement of the endogenous mouse SIRPα, *Csf1, Csf2, Il3 and Tpo genes* with humanized version. All animal experimentations were performed in compliance with Yale Institutional Animal Care and Use Committee protocols. Newborn mice (within first 3 days of life) were sublethally irradiated and engrafted with 250,000 patient bone marrow CD34⁺ cells. 6-8 weeks after engraftment, mice were infected by i.v. with HIV-1 (100ng p24) which was recovered and expanded from the resting CD4⁺ T cells of the same patient (CD34⁺ cell donor). Autologous CD8⁺ T cells were pre-stimulated with Gag peptide(s) in vitro. 20 million autologous CD8⁺ T cells with or without pre-stimulation were injected by i.v. 14 days after infection. Peripheral blood samples were collected by retro-orbital bleeding.

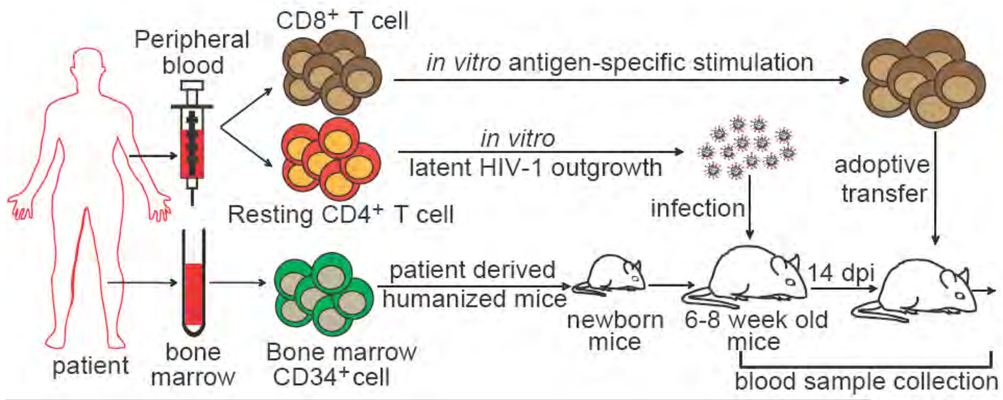


Figure 1. Generation and infection of patient-derived humanized mice.

Results

To investigate whether CTL escape variants are documented in the latent reservoir, we deep sequenced the proviral HIV-1 DNA in resting CD4⁺ T cells from 25 patients. Among them, 10 initiated ART during the acute phase (**AP, within 3 months of** infection) while the other 15 initiated ART during the chronic phase (**CP) of infection.** Our data show that CTL escape variants completely dominate the viral reservoirs of all CP-treated patients. By contrast, few if any CTL escape mutations were archived in AP-treated patients. In CP group, there were only minimal responses to previously documented CTL escape mutants by patient CD8⁺ T cells. In contrast, all tested subjects retained a strong response to peptides representing the wild-type epitopes, suggesting wild-type virus was initially transmitted, with subsequent evolution of CTL escape variants.

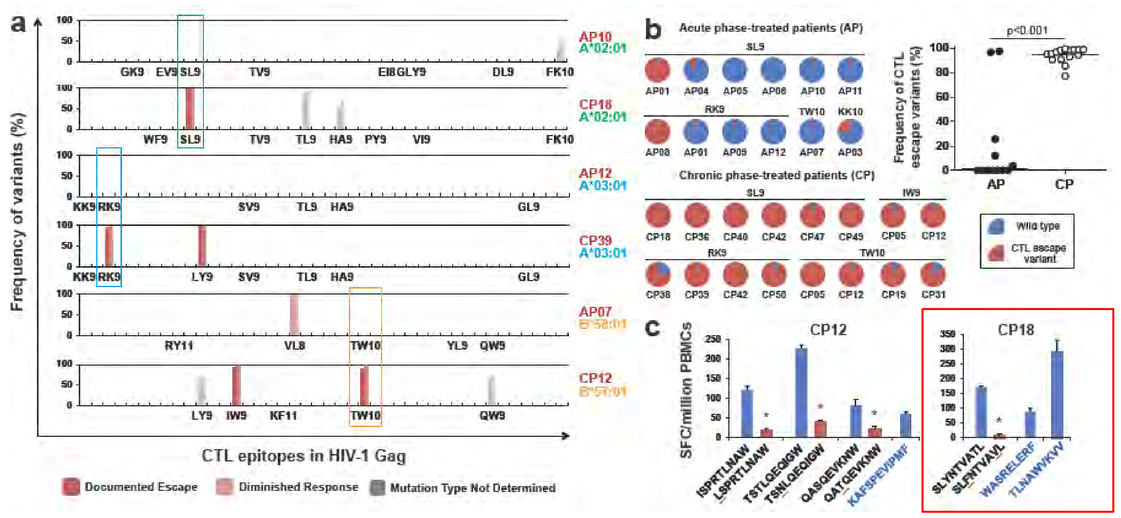


Figure 2. CTL escape variants dominate the latent reservoir in patients chronically infected with HIV-1. Resting CD4⁺ T cells from patients who started cART during chronic (CP) or acute (AP) phase of HIV-1 infection were isolated for viral DNA sequencing. **(a)** Frequency of variants in Gag CTL epitopes in proviruses from resting CD4+ T cells were measured. **(b)** Comparison of CTL escape variant frequency in proviruses between CP- and AP-treated patients. Only well-characterized epitopes are shown. Median and P value from Mann-Whitney test are shown. **(c)** Characterization of CTL responses against wild-type (blue) or mutated (red) HIV-1 Gag epitopes by interferon-γ ELISpot.

To study the function of patient CTLs that recognize unmutated viral epitopes in vivo, we generated patient-derived humanized mice using a recently reported mouse system[2]. MIS^{KI}TRG mice engrafted with patient (CP18) bone marrow CD34⁺ cells successfully developed human T-lymphocyte and monocyte/macrophage subsets (Fig. 3). Plasma HIV-1 RNA levels peaked 20-30 days after infection. Depletion of CD4⁺ T cells was clearly evident 12 days after infection in peripheral blood (Fig. 4).

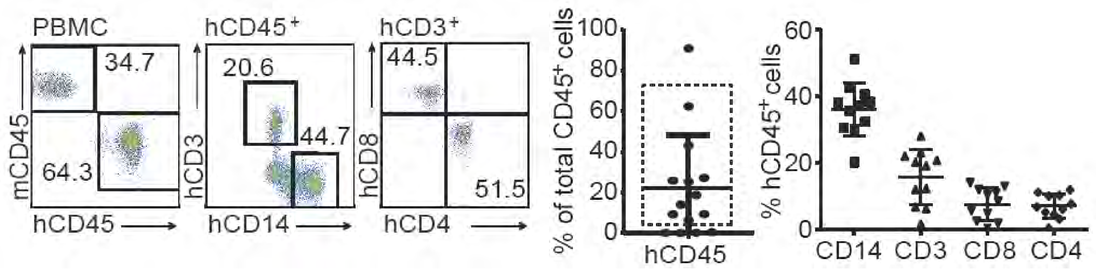


Figure 3. Reconstitution of patient hematopoietic cells in MIS$^{(KI)}$RG mice. Peripheral blood was collected from MIS$^{(KI)}$TRG mice at week 6 post engraftment. Representative flow cytometry analysis and summary of human CD45⁺ cells, human T-lymphocyte and monocyte subsets were shown.

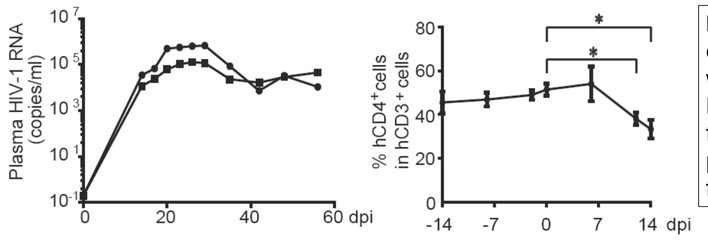


Figure 4. Viral dynamics and depletion of CD4+ T cells in MIS$^{(KI)}$RG mice. Mice were infected with autologous HIV-1. Plasma HIV-1 RNA levels were measured from day 0 to day 56. CD4 to CD8 ratio in peripheral blood was measured by FACS from day 0 to day 14 after infection.

In control mice or mice that received autologous patient CD8⁺ T cells pre-stimulated with a peptide representing the dominant SL9 epitope, levels of plasma HIV-1 RNA and proviral DNA in peripheral blood continued to increase from day 14 to day 29 after infection (Fig. 5). In sharp contrast, mice that received CD8⁺ T cells pre-stimulated with unmutated epitopes (Gag mix or WF9) had a significantly lower level of viral replication (Fig.5). Dramatic decreases in plasma HIV-1 RNA of 100- to 1,000-fold were observed in all three mice that received CD8⁺ T cells pre-stimulated with the mixture of Gag peptides including dominant and subdominant epitopes. Two of three mice had undetectable levels of plasma HIV-1 RNA and proviral DNA in peripheral blood measured at three time points (Fig. 5).

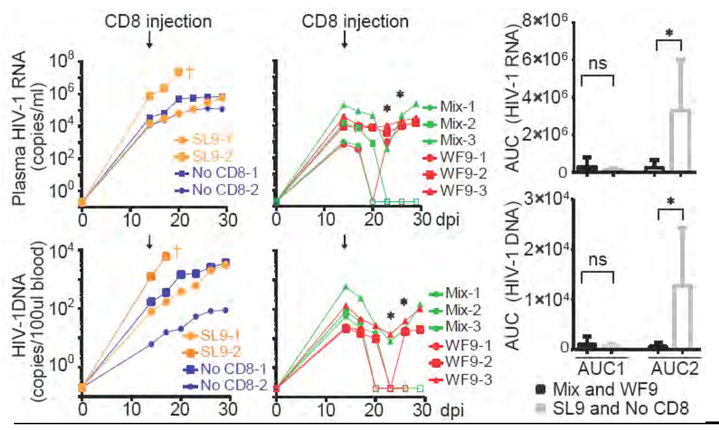


Figure 5. Effect of viral-specific CTLs on the level of viral replication *in vivo.* Peripheral blood was collected for the measurement of plasma HIV-1 RNA and cell-associated viral DNA. The area under the curve of the viremia vs. time plot for each mouse before (AUC1) or after (AUC2) injection of CD8⁺ T cells was calculated to quantitatively represent viral replication over time. Filled: above detection limit;
open: below detection limit.
*: p<0.05, unpaired t test.

Conclusions

1. The vast majority (>98%) of latent viruses carry CTL escape mutations that render infected cells insensitive to patients' dominant CTL clones.
2. Patients retain a broad spectrum viral-specific CTL clones that can recognize and eliminate autologous infected CD4⁺ T cells, despite the presence of CTL escape mutations in dominant epitopes.
3. Future directions in therapeutic vaccine design need to focus on boosting broad CTL responses and/or manipulating immunodominance.

References

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