HIV PROVIRUSES MIMIC THE CLONALITY OF HIGHLY EXPANDED CD4⁺ T CELLS IN PEOPLE ON ART

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Background

- Clonal proliferation of HIV-infected CD4⁺ T cells is a major contributor to viral persistence in people on ART.
- We previously demonstrated that the clonal distribution of HIV proviruses becomes **highly skewed** by ~10 years on ART, such that the rank-abundance (clonality) distribution of HIV-infected cells is characterized by **a few very large clone families and many smaller clone families or singlets**.

Central Question

- Is the highly skewed clonality distribution of HIV-infected cells at 10+ years on ART a natural product of the proliferative dynamics of memory CD4⁺ T cells in PWH on ART or is it suggestive of HIV-specific selection over time?

Study Design & Methods

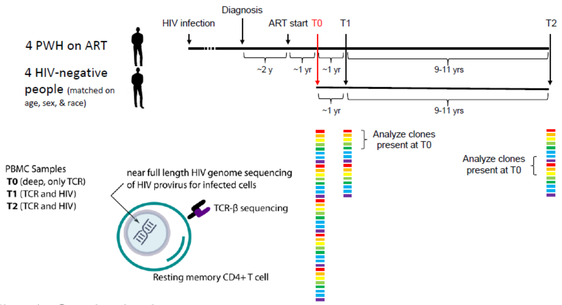


Fig. 1. Study design.

- Four PWH on ART (3 men, 1 woman) with consistent viral load suppression for 10+ years on ART were chosen for analyses.
- At t1 and t2, an average of 57 HIV proviruses were sequenced from resting CD4⁺ T cells.
- Most provirus sequences were defective. Provirus sequences were used to identify clonal families.
- At t0, t1, and t2, an average of 115,000 T cell receptor (TCR)-β CDR3 regions were sequenced from resting memory CD4⁺ T cells (Adaptive Biotech).
- Four HIV seronegative individuals (age, sex, and race-matched to PWH) were chosen and an average of 184,000 TCRs were sequenced from resting memory CD4⁺ T cells.
- TCR-β CDR3 region sequences were used to estimate clonality distributions for primarily HIV-uninfected cells. ddPCR analysis revealed that >99.9% of resting CD4⁺ T cells are HIV-uninfected in these samples.
- Sample size-normalized ecological metrics including the slope of clone rank-abundance distributions were used to compare clonality distributions in HIV-infected and primarily HIV-uninfected resting CD4⁺ T cells from the same blood samples.
- To provide a fair comparison of clonal dynamics between HIV-infected and HIV-uninfected CD4s, we controlled for the lack of new diversity entering the HIV-infected pool of cells in PWH on ART by analyzing at t1 and t2 only the specific TCRβ sequences that were also observed at t0 in PWH and HIV seronegative individuals.

Results

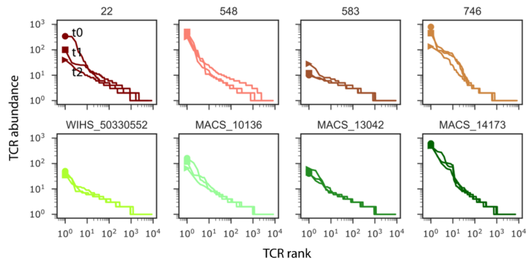


Fig. 2. rmCD4 TCRβ rank-abundance curves at t0, t1, and t2. Variation between people is greater than intra-person variation over a decade timespan.

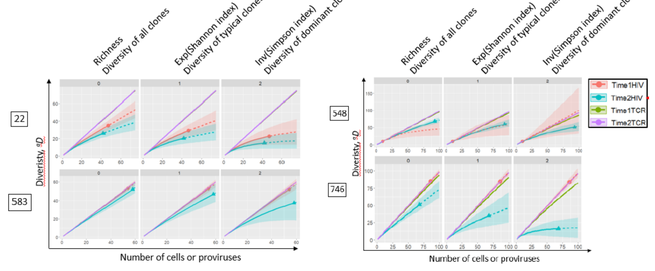


Fig. 3. Decreased diversity (interpolated Hill numbers D^0, D^1, and D^2) in HIV-infected rCD4s as compared to primarily uninfected rmCD4s from the same samples, controlling for new diversity after t0.

- During long-term viral suppression on ART:
  - HIV proviruses become concentrated into a small number of extremely large rCD4 T cell clones over time.
  - The emergence of infected clone dominance is not typical of the underlying population of uninfected rmCD4s but instead mirrors that of the most highly expanded rmCD4s in circulation.
- Primarily uninfected rmCD4 clones persist in the peripheral circulation for 10+ years in people with and without HIV.
- Defective proviruses decay at a rate indistinguishable from the underlying rCD4 population, which is distinct from the intact provirus and QVOA decay rates.

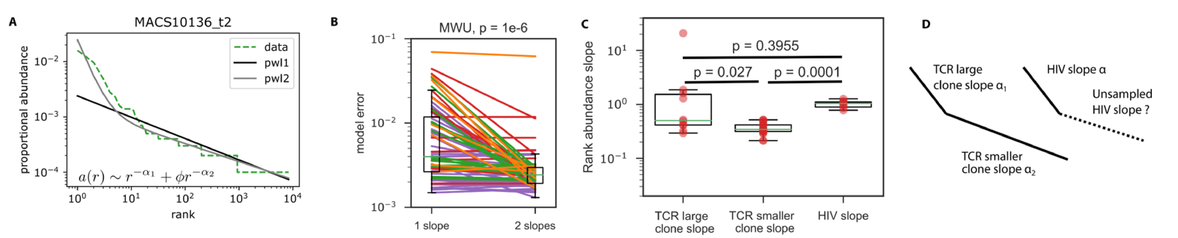


Fig. 4. HIV rank-abundance distributions mimic those of highly expanded rmCD4 TCRβ clones. A, Example two-slope model fit of a TCR rank-abundance distribution. B, Two-slope model outperforms one-slope model for PWH (yellow, red) and HIV-seronegative people's (green, purple) TCRs. C, Slope of model fit of HIV provirus rank-abundance distribution is indistinguishable from that of the top ~10-15 highly expanded rmC4s. P values, nonparametric Mann-Whitney U test. D, Cartoon depiction of distribution shapes.

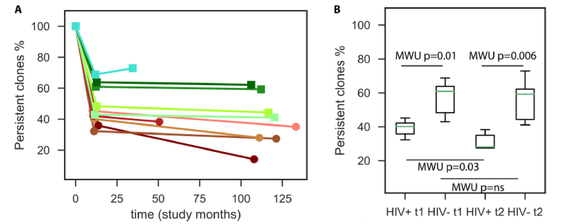


Fig. 5. Resting memory CD4s persist for 10+ years in PWH (oranges, browns) and HIV-seronegative people (greens, blues). PWH on ART have fewer persisting clones from t0 at t1 and t2, and they experience a drop from t1 to t2. P values, Mann-Whitney U test.

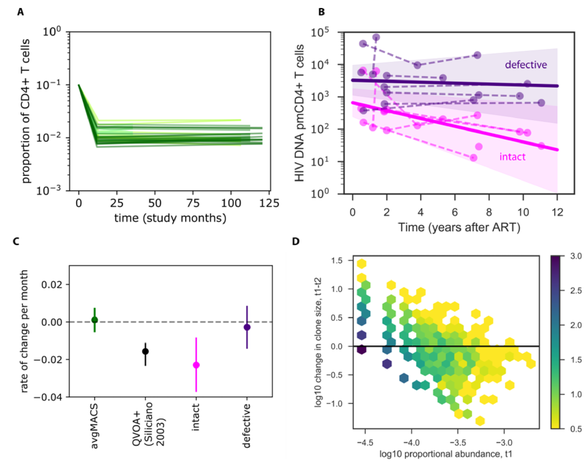


Fig. 6. rmCD4⁺ T cell clones decay with similar rates to defective HIV proviruses that are distinct from intact provirus clones. A, 10 replicate simulations assuming 10% of TCR are HIV-infected, tracking the proportional abundance of these clones over time. B, Decay of intact and defective HIV proviruses among rCD4s by IPDA. C, rCD4 clone decay (TCRs) is similar to defective but not intact provirus or QVOA decay rates. D, An example illustrating individual TCR clones waxing and waning. A high proportional abundance at the first time point is highly predictive (Spearman ρ=0.8) of a decrease in clone size at the next time point. The colorbar denotes $\log_{10}$ number of events observed. Most clones have small abundance and stay small over time (dark blue), whereas observed highly expanded clones rarely expand more.

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