Short and Long Term Dynamics of SIV-infected Cell Populations on ART

388

Emily J. Fray[1], Fengting Wu[1], Francesco R. Simonetti1, Carolin Zitzmann[2], Narmada Sambaturu[2], John D. Ventura[3], Thomas Leitner[2], Ruy M. Ribeiro[2], Alan S. Perelson[2], Dan H. Barouch[3], Janet D. Siliciano[1], Robert F. Siliciano[1,4]

[1]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA, [2]Los Alamos National Laboratory, Los Alamos, NM, USA, [3]Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Boston, MA, USA, [4]Howard Hughes Medical Institute and Department of Medicine, Baltimore, MD, USA

BACKGROUND

Antiretroviral therapy (ART) potently suppresses HIV-1 replication and prevents infection of new cells. Understanding changes in HIV-infected cell populations following ART initiation can inform design and evaluation of cure strategies by providing insights into the cells that enter the long-term reservoir. Recently, White et al. explored the decay kinetics of infected CD4+ T cells in people living with HIV-1 (PLWH) on ART and found that intact genomes decayed with biphasic kinetics characterized by a rapid initial phase, and a slower 2nd phase that was faster than the decay in PLWH on long-term ART. The decay of SIV-infected cells in macaques on ART has not been described. Analogous studies using the SIV/non-human primate model offer an opportunity for insights difficult to obtain in PLWH.

METHODS

We characterized quantitative and qualitative changes in SIV-infected CD4⁺ T cells for 4 years following ART initiation. We used digital droplet PCR to compare the decay of intact and defective proviruses, as well as single-genome sequencing (SGS) of *env* from plasma virus and proviral DNA to look for qualitative changes during ART.

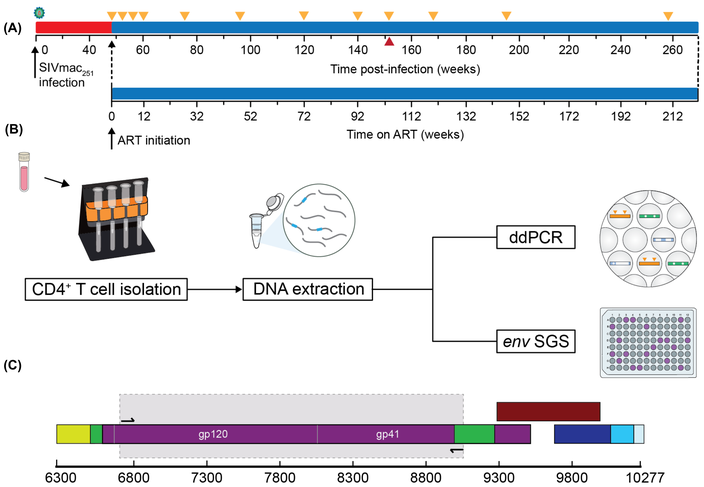
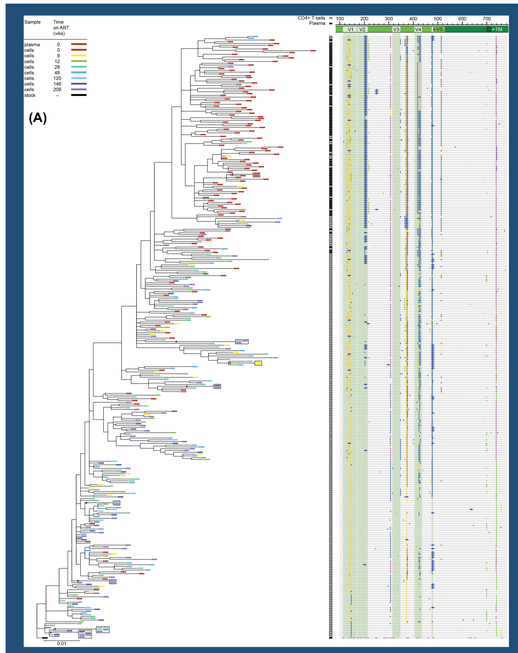


Figure 1. Study overview and methods. (A) Timeline of sample collection. Animals were infected by repeated intrarectal challenge with an $SIVmac_{251}$ swarm until detection of SIV RNA in the plasma. The cohort (n=10) was infected for 48 weeks after initial challenge (red) before initiation of combination antiretroviral therapy (ART) with tenofovir, emtricitabine and dolutegravir (blue). Orange triangles indicate sampling times. The red triangle indicates the final time point available for 2 animals (T545 and T628), which were necropsied due to unrelated complications. (B) Overview of methodology. CD4+ T cells were isolated from viably frozen PBMCs using negative depletion, after which genomic DNA was extracted. DNA was used as the input for ddPCR and *env* SGS via limiting-dilution PCR using the amplicon indicated in (C).



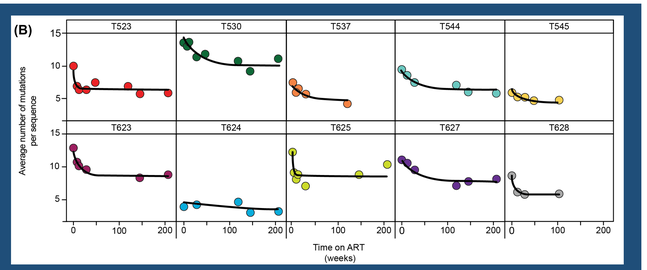


Figure 2. Qualitative changes to the SIV latent reservoir during ART. (A) Sequencing analysis of *env* diversity in plasma virus and proviral DNA from CD4⁺ T cells for animal T530. Phylogenetic tree and highlighter plot of *env* sequences collected from either plasma or CD4⁺ T cell DNA from animal T530. Highlighter plot was generated using Los Alamos National Lab's Highlighter program. The tree is rooted on a consensus sequence of the stock. Sequence analysis includes 2054 nt. (B) Decay of non-synonymous mutations in *env* sequences during ART. Data were fitted to a biphasic model (black line), with an initial phase of decay with an average half-life of 11.4 weeks (95% CI: 4.6-28.1 weeks), and a second phase that is so slow as to be statistically indistinguishable from "no decay."

RESULTS

By following treated macaques for 4 years, we observed both early and late phase dynamics of the latent reservoir. We found that the decay kinetics of cells harboring intact SIV proviruses are similar to those reported for HIV-1, characterized by multiphasic decay and a final phase representing stable persistence of a latent reservoir. *env* SGS revealed that ART potently stops viral evolution and that the composition of infected cell populations changes during ART. We found that the average pairwise distance from the stock decreased over time on ART, reflecting decay of recently infected cells. Ancestral variants with fewer mutations that had been archived in the latent reservoir were not present in the plasma at ART initiation and became prominent over time on treatment.

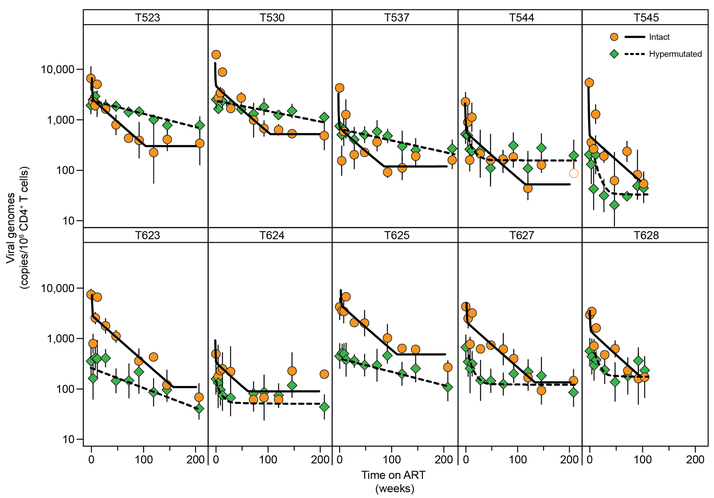


Figure 3. Decay of intact vs. hypermutated proviruses during ART. Data were fitted to a triphasic model (black line) for intact proviruses, or biphasic or monophasic models (dotted lines) for hypermutated proviruses. Vertical lines indicate the standard deviations. Intact proviruses decayed with triphasic kinetics, with a 1st phase with an average $t_{1/2}$ of 3.3 days (95% CI: 2.5-4.5 days), a 2nd phase with an average $t_{1/2}$ of 8.1 months (95% CI: 6.5-10.0 months) and a stable 3rd phase with no decay, reached after an average of 2.3 years (95% CI: 1.6-2.9 years). Hypermutated proviruses decayed with half-lives that were very different from those described for intact proviruses, such that the best-fit model was not uniform. For 5 animals, there was biphasic decay with a 1st phase $t_{1/2}$ of 42 days, 95% CI: 31-55 days; followed by a 2nd phase with no decay), and monophasic decay for the other 5 – $t_{1/2}$ of 27.8 months, 95% CI: 19.5-39.9 months).

CONCLUSIONS

These data provide a baseline decay rate for SIV-infected cells in macaques on ART, which is critical to accurately evaluate cure strategies aimed at the latent reservoir. Our results show that variants enter the reservoir throughout untreated infection and not just at ART initiation. The population of SIV-infected CD4⁺ T cells is dynamic and changes both quantitatively and qualitatively for several years following ART initiation. Perhaps most importantly, our decay analyses demonstrate that in the absence of additional intervention, there is a clear and consistent decrease in the number of SIV-infected cells during the first 3 years on ART. The strikingly different decay kinetics described for cells harboring intact vs. defective proviruses suggests that these populations are under different selective pressures during ART. Additionally, our findings suggest that the populations that decay most rapidly after treatment are largely made up of recently infected cells, although it remains unclear whether their decay is due to viral cytopathic effects, killing by host effector cells, or other mechanisms of clearance.

These findings should be taken into consideration when designing future animal trials utilizing the SIV model. Any intervention aimed at reducing the size of the SIV latent reservoir will need to improve on the baseline decay that we have described here. Our results provide a framework for evaluating and interpreting intervention trials utilizing the SIV/NHP model.

ACKNOWLEDGEMENTS

This work was supported by the NIH Martin Delaney Collaboratory grant UM1AI164556 to D.H.B and by Howard Hughes Medical Institute to R.F.S. Animal studies were supported by AI124377, AI128751, AI149670, AI164556, AI169615 for D.H.B. F.R.S is supported by the Office of the NIH Director and National Institute of Dental & Craniofacial Research (DP5OD031834). Portions of this work were performed under the auspices of the U.S. Department of Energy through Los Alamos National Laboratory, which is operated by Triad National Security, LLC, for the National Nuclear Security Administration of the US Department of Energy (Contract 89233218CNA000001). Support was also provided by NIH Grants R01-AI028433, R01-OD011095, P01-AI131365 (to A.S.P.), R01-AI15270301; and UM1-AI164561 (to R.M.R.).