



Expanded cellular clones carrying replication-competent HIV-1 persist, wax, and wane

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The latent reservoir for HIV-1 in resting CD4⁺ T cells is a major barrier to cure. Several lines of evidence suggest that the latent reservoir is maintained through cellular proliferation. Analysis of this proliferative process is complicated by the fact that most infected cells carry defective proviruses. Additional complications are that stimuli that drive T cell proliferation can also induce virus production from latently infected cells and productively infected cells have a short in vivo half-life. In this ex vivo study, we show that latently infected cells containing replication-competent HIV-1 can proliferate in response to T cell receptor agonists or cytokines that are known to induce homeostatic proliferation and that this can occur without virus production. Some cells that have proliferated in response to these stimuli can survive for 7 d while retaining the ability to produce virus. This finding supports the hypothesis that both antigen-driven and cytokine-induced proliferation may contribute to the stability of the latent reservoir. Sequencing of replication-competent proviruses isolated from patients at different time points confirmed the presence of expanded clones and demonstrated that while some clones harboring replication-competent virus persist longitudinally on a scale of years, others wax and wane. A similar pattern is observed in longitudinal sampling of residual viremia in patients. The observed patterns are not consistent with a continuous, cell-autonomous, proliferative process related to the HIV-1 integration site. The fact that the latent reservoir can be maintained, in part, by cellular proliferation without viral reactivation poses challenges to cure.

HIV persistence | latent reservoir | clonal expansion

The latent reservoir for HIV-1 in resting memory CD4⁺ T cells persists even in patients on optimal antiretroviral therapy (ART) and is the major barrier to cure (1–3). The reservoir is established early in infection (4, 5) and is extremely stable, with an estimated half-life of 44 mo (6–8), making cure with ART alone unlikely. One strategy to eliminate the latent reservoir is termed shock-and-kill (9, 10). It depends on inducing proviral expression in latently infected cells to allow their elimination by immune mechanisms or viral cytopathic effects.

Previous studies suggested that the proliferation of infected cells might contribute to HIV-1 persistence (11–19). At least three mechanisms may explain the proliferation of infected cells: antigen-driven T cell proliferation (16), homeostatic proliferation (13), and proliferation driven by effects related to the site of HIV-1 integration (14, 15). Proliferation of infected cells driven by antigen or cytokines is unexpected because these stimuli also induce viral gene expression in latently infected cells (20, 21), which exposes the cells to viral cytopathic effects and immune clearance. In vivo, most productively infected cells have a short half-life (22, 23). Interestingly, it has been shown in a model system that latently infected CD4⁺ T cells can proliferate in response to cytokines such as IL-2 and IL-7 without viral reactivation (24).

Early evidence for the in vivo proliferation of HIV-1-infected cells came from detailed phylogenetic studies showing the presence in plasma of viruses with identical sequences, termed pre-

dominant plasma clones (PPCs), in some infected individuals on prolonged ART (11, 12). In treated patients who have suppression of viremia to below the limit of detection of clinical assays, this residual viremia can be detected with very sensitive assays (25–28). The residual viremia consists of archival viral sequences that are sensitive to the current drug regimen and do not show evolution over time (12, 29–32). Numerous studies have shown that levels of residual viremia cannot be reduced by treatment intensification (33–35). All of these results suggest that residual viremia results from the daily activation of a small number of latently infected cells rather than ongoing cycles of replication. The clonal nature of residual viremia suggested that it is produced by a limited subset of infected cells carrying identical HIV-1 sequences, likely arising as a result of cellular proliferation (11, 12).

Further support for the role of T cell proliferation in HIV-1 reservoir dynamics came from a landmark study that defined the distribution of latent HIV-1 among the central memory, transitional memory, and effector memory CD4⁺ T cell subsets and the role of cytokine-driven homeostatic proliferation in reservoir stability (13).

More recent studies used HIV-1 integration site analysis to provide direct and definitive evidence for clonal expansion in infected cells in individuals on long-term ART (14, 15, 36). A large fraction of the infected cells appear to have undergone clonal expansion based on the presence of identical HIV-1 integration

Significance

The HIV-1 latent reservoir cannot be eradicated by antiretroviral therapy (ART). The reservoir is a major barrier to cure. To characterize the mechanisms that contribute to persistence of the latent reservoir, we examined clonally expanded cell populations carrying replication-competent HIV-1 and followed them longitudinally. Expanded clones harboring replication-competent HIV-1 were identified in all study participants, but these clones emerge and wane on a time scale of years. A similar pattern was identified in viruses sampled from residual viremia. The findings suggest that the latent reservoir is likely to be maintained through expansion driven by antigens and cytokines, and that the expansion is balanced with a constant cell loss.

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sites in different infected peripheral blood lymphocytes (14, 15, 36). One study described identical viral sequences integrated at exactly the same position in the human genome in multiple cells, consistent with infected cell proliferation (15). Two studies suggested that HIV-1 integration into genes associated with cell growth and survival might drive proliferation of infected cells (14, 15).

While clonal expansion of infected CD4⁺ T cells is prevalent in infected individuals, most HIV-1 proviruses are defective (37, 38). Since integration site analysis captures only a small part of the HIV-1 genome, it is likely that many of the expanded cellular clones detected by this method harbor defective proviruses (36). However, a highly expanded clonal population of CD4⁺ T cells harboring a replication-competent provirus was recently characterized in an infected individual with metastatic squamous cell carcinoma, suggesting that cells carrying replication-competent proviruses can expand and persist (16). In addition, three recent studies demonstrated that clonal CD4⁺ T cell populations carrying replication-competent viruses are common in infected individuals, accounting for over 50% of the latently infected cells examined (17–19). Therefore, it is crucial to evaluate how cells containing replication-competent provirus proliferate. This information may help us to understand the forces that shape and preserve the latent reservoir.

To address these issues, we asked whether infected CD4⁺ T cells harboring replication-competent provirus could proliferate in response to T cell receptor (TCR) stimulation or cytokines that are known to drive homeostatic proliferation and, if so, whether this proliferation can occur without viral reactivation. In addition, we examined the *in vivo* dynamics of clonal populations of infected cells carrying replication-competent HIV-1 to determine whether and how expanded clones harboring replication-competent provirus persist over time.

Results

CD4⁺ T Cells Containing Replication-Competent Virus Can Proliferate in Response to TCR Activation and Cytokine Treatment. The immune system maintains normal levels of T cells, in part, through a process of homeostatic proliferation. For CD4⁺ T cells, the gamma chain cytokine IL-7 is an important stimulus for homeostatic proliferation *in vivo* (39, 40). A previous study evaluated the role of IL-7–driven proliferation in HIV-1 reservoir stability and demonstrated a relationship between plasma IL-7 concentration and reservoir size (13). Bosque et al. (24) used a primary cell model of HIV-1 latency to demonstrate that latently infected central memory T cells could proliferate in the presence of IL-7 without viral reactivation. To explore the stimuli and conditions associated with proliferation of infected cells *in vivo*, we purified resting CD4⁺ T cells from 10 HIV-1–infected donors on ART and stained the cells with carboxy-fluorescein succinimidyl ester (CFSE) dye. The cells were then treated with anti-CD3/CD28 antibodies or with an optimal concentration of IL-7 (24) for 7 d in the presence of antiretroviral drugs (Fig. 1A). IL-2 was included in all of the cultures to maintain cell viability and match the conditions described by Bosque et al. (24). Most cells proliferated in response to anti-CD3/CD28 antibodies, while proliferation induced by IL-7 was more limited (Fig. 1B). We also examined the activation status of cells cultured for 7 d with anti-CD3/CD28 or IL-7 by flow cytometry. Cells were stained for CD69, CD25, and HLA-antigen D related (DR). With anti-CD3/CD28 stimulation, most cells were positive for CD25, while only 20% of the cells treated with IL-7 were positive for CD25 (Fig. 1B).

The cells receiving each stimulus were then sorted based on whether they had proliferated in response to anti-CD3/CD28 or IL-7. This allowed us to examine the properties and fate of infected cells that had recently gone through the cell cycle. We sequenced the TCRs in the sorted CFSE low cell populations that proliferated in response to each treatment and compared the sequences with those in the resting T cell population. With anti-CD3/CD28 stimulation, the proportions of cells with particular TCRs remained the same as in unstimulated resting CD4⁺ T cells ($P > 0.001$), while some clones achieved a higher frequency in the population that proliferated in response to IL-7 compared with that in unstimulated resting CD4⁺ T cells ($P < 0.001$) (Fig. 1C).

To explore the nonuniform expansion induced by IL-7, we then analyzed IL-7 receptor (CD127) expression on resting CD4⁺ T cells from infected individuals and found that 90% of the cells expressed CD127 (Fig. 1D). This observation indicates that not all resting CD4⁺ T cells are able to proliferate in response to cytokines, which may provide a partial explanation for the preferential expansion of certain clones in response to IL-7 (Fig. 1C). In any event, the sorting of cells that had proliferated in response to anti-CD3/CD28 or IL-7 allowed us to directly examine the clonal expansion of HIV-1–infected cells.

We next asked whether either stimulation induced rapid virus release from freshly isolated resting CD4⁺ T cells. We collected culture supernatants from resting CD4⁺ T cells from 10 infected individuals after 7 d of stimulation with media alone, anti-CD3/CD28, or IL-7. Anti-CD3/CD28 induced virus production, as measured by HIV-1 mRNA in the supernatants. IL-7 treatment induced detectable HIV-1 mRNA in the supernatant of cells from five of 10 infected individuals, but the HIV-1 mRNA levels for those patients were at least two logs lower than those observed with anti-CD3/CD28 (Fig. 1E). The finding that latently infected CD4⁺ T cells treated with IL-7 can proliferate with little or no virus production suggests that homeostatic proliferation induced by cytokines could potentially expand the latent reservoir without exposing the infected cells to immune clearance. Anti-CD3/CD28 induced much higher levels of T cell proliferation and latency reversal.

Most infected cells harbor defective proviruses that may not interfere with clonal expansion (37, 38). To determine whether cells harboring replication-competent HIV-1 genomes can proliferate in response to these stimuli, we plated the sorted cells that had proliferated in response to anti-CD3/CD28 or IL-7 in a limiting dilution quantitative viral outgrowth assay (QVOA) (Fig. 1A). In this assay, the cells are stimulated with phytohemagglutinin (PHA) and γ -irradiated allogeneic peripheral blood mononuclear cells (PBMCs) to reverse latency as previously described (41, 42). Infectious virus particles released following reversal of latency are detected by adding MOLT4/CCR5⁺ cells to the culture. These cells lack class II MHC expression and do not cause allogeneic stimulation of patient cells (42). However, they are highly susceptible to infection and allow exponential growth of infectious virus released from patient cells. With this assay, we detected viral outgrowth by p24 ELISA of supernatants from cultures of unstimulated resting CD4⁺ T cells or cell populations that had proliferated in response to stimulation with either anti-CD3/CD28 or IL-7 (Fig. 1F). The frequencies of cells that were able to produce replication-competent virus were similar in all three cases (Fig. 1F). Thus, cells harboring replication-competent virus can proliferate in response to either TCR stimulation or cytokines, survive for at least 7 d, and retain the ability to produce infectious virus upon subsequent stimulation.

In the same experiment, cell populations that had proliferated in response to either anti-CD3/CD28 stimulation or cytokine treatment were also plated in the QVOA without additional PHA stimulation. Viral outgrowth was observed in cultures set up with MOLT-4 cells and cells that had proliferated in response to anti-CD3/CD28 without another round of PHA activation, suggesting that CD4⁺ T cells carrying replication-competent provirus can proliferate with anti-CD3/CD28 stimulation and continue to produce virus for at least 7 d. However, no viral outgrowth was observed in cultures set up with cells that had proliferated in response to IL-7 unless PHA and irradiated allogeneic PBMCs were added (Fig. 1F). This result suggests that the concentrations of cytokines used in this culture assay were sufficient to induce proliferation of some resting CD4⁺ T cells without latency reversal. The progeny cells could produce virus with additional stimulation. We have previously shown that CD4⁺ T cells harboring replication-competent provirus can proliferate in response to global T cell activation without producing virus while retaining the ability to do so upon subsequent stimulation (18, 37). Thus, both antigen and cytokines provide a potential explanation for the presence of expanded cellular clones in the latent reservoir.

Some Expanded Clones Harboring Replication-Competent Virus Persist Over Time, While Others Wax and Wane. Three recent studies showed that expanded cellular clones containing replication-competent HIV-1 are common in infected individuals (17–19). However, little is known about the dynamics of these clonal populations. To examine whether these clones persist, we recovered the infectious virus from eight treated individuals at two or three time points spanning 2–3 y (Fig. 2A). Limiting dilutions of resting CD4⁺ T cells were subjected to stimulation in the QVOA with the T cell mitogen PHA and γ -irradiated allogeneic PBMCs. MOLT-4/CCR5⁺ cells were added on day 2 of the culture to expand virus released from cells in which latency was reversed, and the culture supernatants were tested for virus production by p24 ELISA on day 14 (42). We amplified the highly variable V3–V4 region of the *env* gene by reverse transcriptase (RT)-PCR from viral RNA in the supernatants of all p24⁺ wells from the QVOA. As expected from the limiting dilution format of the QVOA, sequences from individual p24⁺ wells should represent independent isolates of replication-competent virus. Sequences from each individual were compared by phylogenetic analysis. All eight individuals had one or more sets of independent isolates with identical *env* sequences at two or three time points (Fig. 2A). To determine whether these isolates with identical *env* sequences were identical throughout the genome, a previously described clonal prediction score was used (43). The *env* amplicon had a clonal prediction score of 96, indicating that 96% of the sequences identical in this region are identical throughout the entire HIV-1 genome. In addition, we previously established using full-genome sequencing that a subset of these sequences obtained at the first time point were identical throughout the entire HIV-1 genome (18) (Fig. 2A). Phylogenetic analysis established that identical proviruses in these patients reflect expanded cellular clones rather than infection of a large number of cells by a dominant viral species (18). Therefore, each set of identical *env* sequences is very likely to represent a clonal population of infected cells derived from a single initially infected cell by extensive *in vivo* proliferation.

Longitudinal sampling over a time span of 1–3 y allowed us to address the question of whether clones harboring replication-competent virus persist over time. In seven of eight individuals, we observed sequences that were present and prevalent at one time point at other time points (Fig. 2A and B), suggesting some cellular clones persisted on a time scale of years and comprised a substantial fraction of the total population of resting CD4⁺ T cells with replication-competent proviruses over these time periods. However, in seven of eight individuals, we also found clonal populations carrying different replication-competent viruses at time point 2 and time point 3 that were not present at time point 1 (Fig. 2A and B). In addition to the appearance of new clones, we observed the disappearance of other clones. In subject S01, one large expanded clone was only observed at time point 1 and then disappeared, while another expanded clone appeared and was only seen at time point 2 (Fig. 2A and B). The same dramatic appearance and disappearance pattern was observed in subject S04 (Fig. 2A and B). Of all of the sequences collected, 65.6% were seen only at one time point. Of 17 clones observed at two or more time points, 10 clones showed an increasing frequency over time, while seven clones decreased in frequency over time. Overall, these results are consistent with a homeostatic process in which individual clones increase and decrease in frequency, while the size of the total pool of latently infected cells decreases only very slowly (1, 7, 8).

Due to the low frequency of cells harboring inducible replication-competent proviruses, the number of infected cells sampled at any given time point is limited. We therefore analyzed whether a difference in observed clone frequencies between time points within the same patient could be attributed to sampling error alone (Fig. 2B). In most comparisons between pairs of time points, the observed difference in clone frequencies was highly significant, supporting the notion that the difference in clone frequencies cannot be accounted for by sampling alone (Fig. 2B). These findings demonstrate that clonally expanded cells harboring replication-

competent virus wax and wane on a time scale of years. Our results do not support a process of continuous expansion.

Clonal Populations of Free Plasma Virus Change Over Time. To provide further insight into the dynamics of expanded cellular clones carrying replication-competent HIV-1, we analyzed residual viremia in patients on suppressive ART over time. Most of the defects found in HIV-1 proviruses are major defects that would preclude the production of virus particles (37, 38, 44). Therefore, the dynamics of the cells that produce the residual viremia are likely to reflect the dynamics of cells that carry replication-competent proviruses. However, analysis of residual viremia provides additional information because virus production requires latency reversal.

Fig. 3 shows phylogenetic analysis of *env* gene sequences from plasma virus and from proviruses in resting CD4⁺ T cells from a representative patient on ART. This phylogenetic tree illustrates previously described features of residual viremia, including an intermingling of plasma and cellular sequences (12), the lack of temporal structure (degree of divergence is not correlated with time of sampling) (45), the presence of PPCs (12), and a lack of correlation between the frequency of clonal sequences in plasma and resting CD4⁺ T cells (12). All of these features are consistent with the hypothesis that a stable reservoir of HIV-1 in resting CD4⁺ T cells contributes to the residual viremia as cells in the reservoir become activated. One large clonal population was detected at time point 1 and persisted through time point 3, which occurred 5 mo later, but very few matching proviral sequences were found. The lack of correlation between the frequency of clonal sequences in plasma and resting CD4⁺ T cells reflects the fact that most of the proviruses in resting CD4⁺ T cells are defective and incapable of producing plasma virus (37, 38). Therefore, extensive sampling is required to find the matching proviral sequences. The presence of a large clonal population in plasma reflects not only the proliferation of a clone of infected cells but also the activation of at least some of those cells, presumably by some antigen, to a degree that reverses latency.

In four of eight patients sampled, we found dominant plasma virus populations, as evidenced by the identical viral sequences obtained in multiple, independent, single-genome amplifications. Interestingly, we observed the same pattern of appearance and disappearance of these plasma virus clones as we observed with proviruses in resting CD4⁺ T cells (Fig. 4). Subject 14 was studied at four time points over a 2-y period. We identified one large clonal population that was first detected at time point 2, became dominant at time point 3, and persisted through time point 4, which occurred 1.2 y later (Figs. 4 and 5). Another distinct clonal population was dominant at time point 2 but was not seen in the plasma at later time points. Another distinct clonal population of plasma virus was identified only at the last time point in this patient. This study participant also had populations of clonal proviruses from resting CD4⁺ T cells. However, we only found matching proviral sequences for one of the clonal plasma viruses (Fig. 4), consistent with the fact that the vast majority of proviral sequences are defective. These results show that clonal populations of infected cells producing residual viremia wax and wane on a time scale of years. This likely reflects changes in the frequency of individual clones, as indicated by viral outgrowth studies (Fig. 2). This, in turn, may reflect changes in the exposure to cognate antigens that could drive both proliferation and virus production.

In subject 13, we sampled plasma viruses at four points over a 4-y period (Figs. 4 and 5). We observed multiple distinct clonal populations of plasma viruses, the abundance of which changed over time. In subject 15, we sampled plasma viruses at six time points over a 4-y period (Fig. 4). Three distinct clonal populations of plasma viruses were identified. Two populations were observed at earlier time points but not after the third time point. Another population was only observed at time point 5 and time point 6 (Figs. 4 and 5). The pattern of emergence and disappearance of clonal populations of plasma virus is summarized in Fig. 5. This pattern is consistent with our finding that clonal populations of replication-competent provirus wax and wane

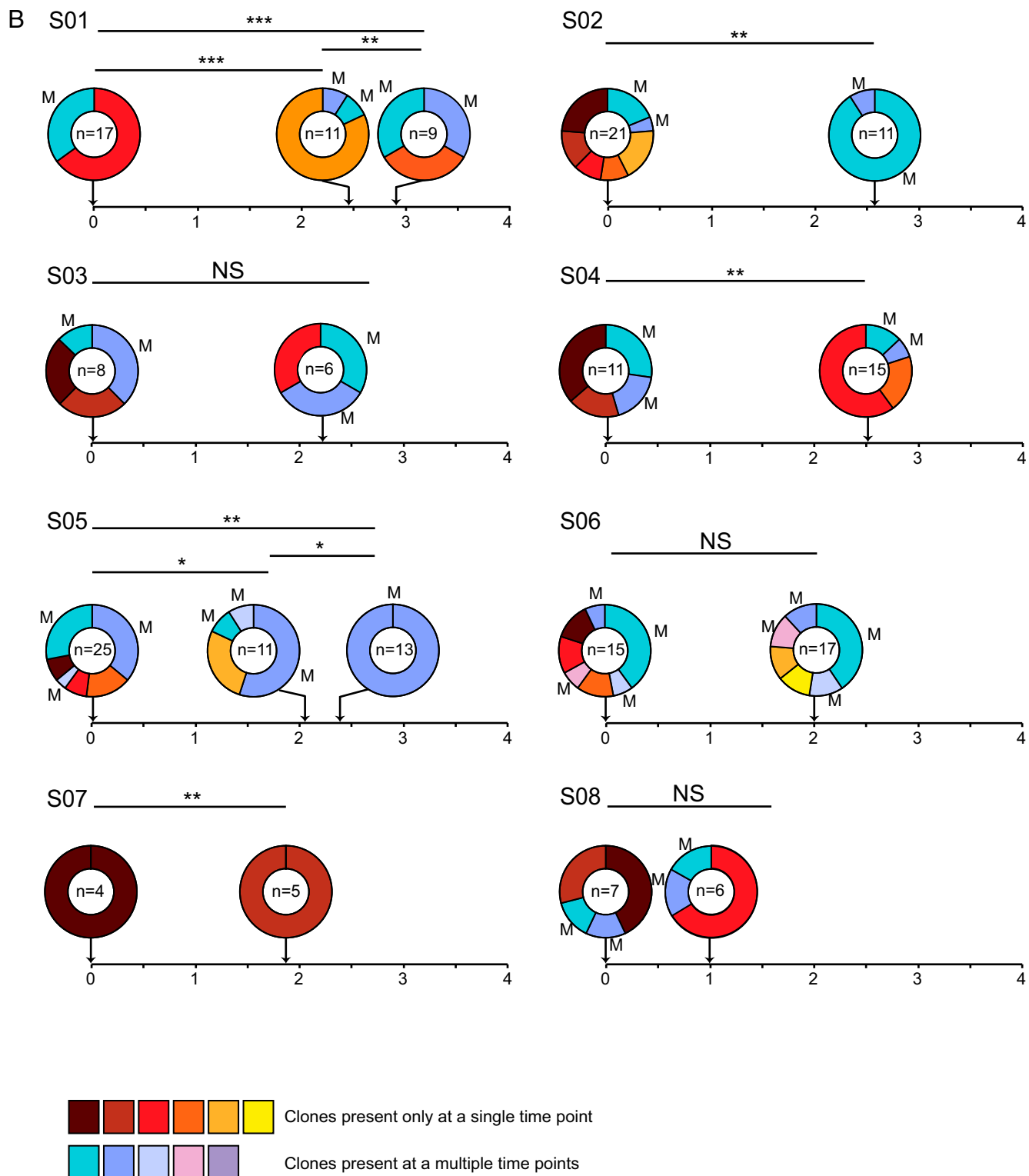


Fig. 2. Expanded clones carrying replication-competent HIV-1 emerge and wane over time. (A) Phylogenetic trees of *env* sequences of independent isolates of replication-competent virus from eight subjects on ART (S01–S08) are shown. Sequencing was performed on genomic viral RNA in supernatants of p24⁺ wells. Different colors correspond to viruses recovered from different time points as indicated under the time line. Groups of identical sequences are indicated by symbols present on the same vertical “rake.” Sequences for the first time point were included in a previous study (18). Sequences that were previously shown to be identical by full-genome sequencing are grouped in boxes (18). The time scale indicates time in years from study entry. All patients were on suppressive ART for >6 mo before study entry. Black squares indicate the reference sequence HXB2. (B) Dynamics of expanded clones containing replication-competent HIV-1. Each pie figure shows how all of the replication-competent viruses (*n*) collected at a specific time point (shown on the x axis) are divided into clonal populations, with distinct colors representing different clones. Clones marked by M were identified at multiple time points. Starred lines indicate samples that are significantly different according to a test for difference in clone proportions when the null model is a random partition of the aggregated samples (*Materials and Methods*) (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. NS, $P > 0.05$).

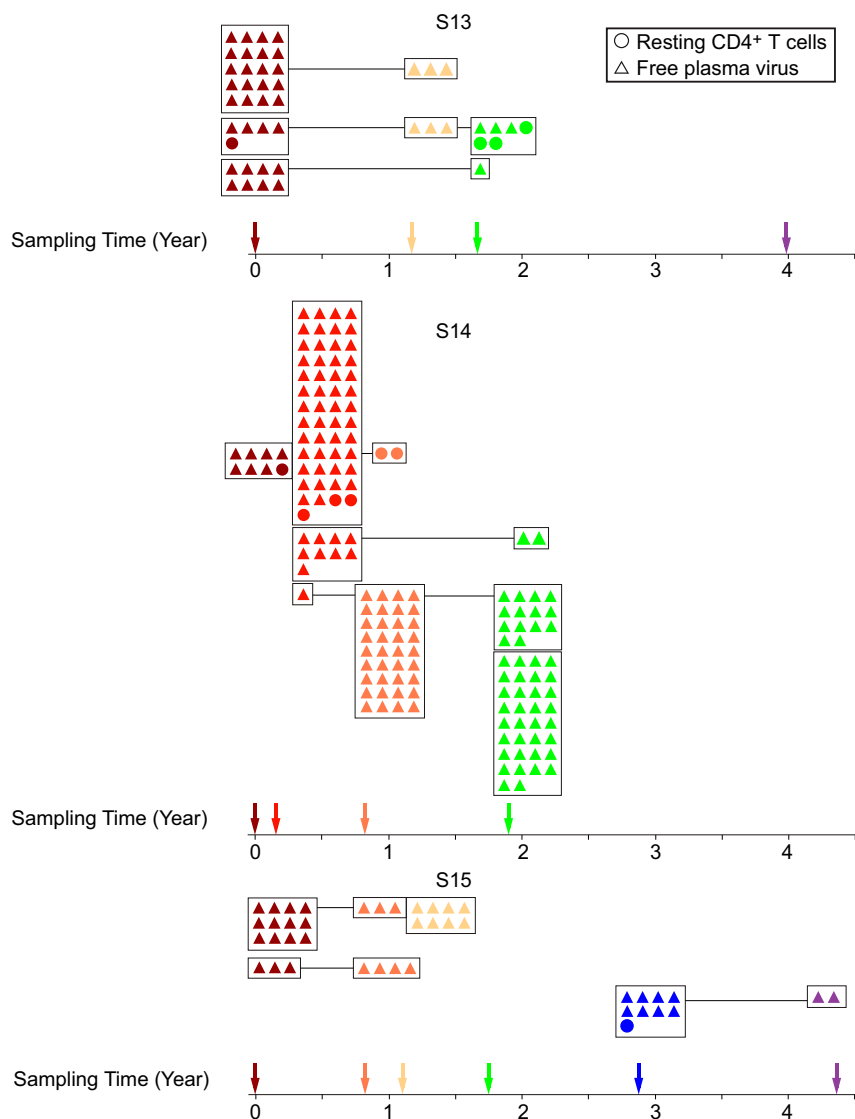


Fig. 5. Composite graph illustrating the appearance and longevity of clonal populations of free plasma virus and provirus derived from resting CD4⁺ T cells. Colors correspond to time of sampling and are derived directly from the corresponding phylogenies.

PBS (Invitrogen) and lysed, and the RNA was extracted via a silica bead-based RNA isolation protocol implemented on an EZ1 Biorobot (Qiagen). The RNA was eluted in 60 μ L of elution buffer and subsequently treated with amplification grade DNase I (Invitrogen) according to the manufacturer's instructions. To amplify the C2-V4 region of the *env* gene from RNA isolated from free plasma virus, the RNA was subjected to a one-step RT-PCR assay using a SuperScript III RT/Platinum Taq High-Fidelity DNA polymerase one-step RT-PCR kit (Invitrogen) followed by a nested PCR assay, using Platinum Taq High-Fidelity DNA polymerase, and 2.5 μ L of the outer reaction as a template. Control reactions were carried out for all experimental amplifications, including a no-RT control to rule out DNA contamination and a no-template control. Primers for the outer and nested reactions were as follows: (outer forward) 5'-CTGTTAAATGGCAGTCTAGC-3', (outer reverse) 5'-CACTTC TCCA-ATTGTCCTCA-3', (nested forward) 5'-ACAATGCTAAAACATAATAGT-3', and (nested reverse) 5'-CATACATTGCTTTTCTACT-3'. PCR conditions for the one-step RT-PCR assay were as follows: reverse transcription at 50 $^{\circ}$ C for 30 min and denaturation at 94 $^{\circ}$ C for 3 min, followed by 40 cycles of 94 $^{\circ}$ C for 30 s, 55 $^{\circ}$ C for 30 s, and 68 $^{\circ}$ C for 1 min. The PCR conditions for the nested reaction were as follows: denaturation at 94 $^{\circ}$ C for 3 min, followed by 40 cycles of 94 $^{\circ}$ C for 30 s, 55 $^{\circ}$ C for 30 s, and 68 $^{\circ}$ C for 1 min. Products of the nested reaction were separated on 1% agarose gels. Bands of appropriate size were excised, and the corresponding amplicons were eluted using QIAquick Gel Extraction Kits. Isolated amplicons were subsequently cloned using a PCR2.1 TOPO cloning vector (Invitrogen), and at least six clones were sequenced from each PCR

assay using an ABI Prism 3700 DNA analyzer (Applied Biosystems). Only sequences that could be shown to be derived from independent templates were analyzed. Phylogenetic analysis by neighbor joining was performed on previously published (12, 50) and novel sequences as discussed below. Novel sequences have been deposited in Genbank. Accession nos.: GQ256402-GQ256627, GQ261350-GQ261724, DQ391282-DQ391351, KF878519-KF878571, KF878848-KF878929, MG680752-MG680913, and MG751469-MG751764.

Phylogenetic Analysis. A consensus sequence for each sample was generated with forward and reverse sequences using default assembly parameters on CodonCode Aligner software (CodonCode Corporation). Each consensus sequence was aligned with an HXB2 reference HIV-1 sequence from the Los Alamos National Laboratory HIV sequence database using BioEdit software. Aligned sequences were trimmed to the same length for phylogenetic tree generation. Genetic distances were calculated, and neighbor-joining trees (51) were generated using a maximum composite likelihood algorithm and default parameters using MEGA7 software (Molecular Evolutionary Genetics Analysis Program) (52). The conclusions were not sensitive to the method of tree generation, and neighbor-joining trees are shown in the figures.

Statistical Analysis of Clones. A statistical test was designed to see if changes in the frequency distribution of clones carrying sequence-identical, replication-competent virus were significantly different between time points or if they

could be attributed to sampling error alone. Under this null model, samples from two different time points are drawn from the same underlying distribution. Physiologically, this corresponds to a scenario in which the clonal composition of the latent reservoir does not change over time. To construct a null distribution of data for this model, data from two time points were aggregated and repartitioned randomly, while all margins were fixed [implemented using the R package *vegan* (53)]. To test whether the observed data supported a more extreme difference in proportions than the repartitioned data, the likelihood ratio test statistic was calculated for the observed data and the distribution of repartitioned data under a multinomial model. The likelihood ratio test statistic T was derived by finding the maximum likelihood parameter estimate vector \hat{p} for a multinomial model with the combined clone size data (n_{1+2}) and a multinomial model with separated data (n_1, n_2), and then calculating their respective likelihoods:

$$T = -2 \log \left(\frac{L(n_{1+2} | \hat{p}_{1+2})}{L(n_1 | \hat{p}_1) L(n_2 | \hat{p}_2)} \right).$$

The P values corresponding to the proportion of random partitions with statistic T were at least as large as that of the true observation. This test is equivalent to a Fisher's exact test when the permuted matrices are ordered by the above statistic.

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