

HIV: Persistence through division

A long-lived latent reservoir for HIV-1 persists in CD4⁺ T cells despite antiretroviral therapy and is the major barrier to cure. In this issue of JEM, Hosmane et al. show that T cell proliferation could explain the long-term persistence of this reservoir.

HIV infection continues to be a global epidemic, with almost 37 million individuals infected worldwide. Despite considerable effort, there is no vaccine and, once contracted, there is no cure for HIV infection (Escolano et al., 2017).

HIV-1 infection can be suppressed for decades by combination antiretroviral therapy, which targets multiple steps in the viral life cycle. However, HIV-1 persists in a latent state as a stably integrated provirus, and it rapidly reemerges from this latent reservoir within 2–3 wk after therapy is interrupted. The reservoir is established very early in infection and has an estimated half-life of 44 mo, making it the major barrier to curing HIV-1 infection (Siliciano and Greene, 2011). How the reservoir is maintained for such long periods of time and the precise cellular and molecular nature of the reservoir are not well defined. In this issue of JEM, Hosmane et al. describe a new technique for investigating the reservoir and use it to uncover a fundamental element that contributes to its persistence.

Understanding the reservoir has been a difficult challenge in part because replication-competent latent cells are only a small fraction of all infected cells. It is estimated that $0.1\text{--}10 \times 10^6$ CD4⁺ T cells in individuals on suppressive combination therapy harbor intact latent viruses. The most accurate measurements are made by viral outgrowth assays (VOA), in which peripheral CD4⁺ T cells from virally suppressed patients are activated to produce virus *in vitro*, and the number of latent cells quantified by limiting dilution. However, this assay can vary by up to 1 log (Laird et al., 2013), and in addition, it significantly underestimates the size of the latent reservoir because not all latent cells are reactivated to produce virus *in vitro* (Ho et al., 2013; Bruner et al., 2016). In addition, the cells

circulating in blood may or may not be entirely representative of what could be a diverse latent compartment.

Although faster and simpler assays that detect HIV-1 nucleic acids have been developed to measure the reservoir, they are problematic because only a very small percentage of the integrated viral DNA in antiretrovirally suppressed individuals is intact and able to produce infectious particles. Thus, DNA measurements that only account for a small segment of the virus cannot easily distinguish between intact and defective proviruses. Moreover, the defective proviruses can be transcribed, and therefore RNA-based assays are similarly flawed (Imamichi et al., 2016).

Viral outgrowth assays indicate that most latent cells reside in resting CD4⁺ memory T cells. This finding is consistent with the idea that HIV reservoir persistence is associated with long-lived CD4⁺ T cells and with the observation that actively infected T cells die by apoptosis or pyroptosis (Doitsh et al., 2014). However, several recent studies, including Hosmane et al. (2017) in this issue, suggest that clonal proliferation of infected cells may also play a role in maintaining the reservoir (Maldarelli et al., 2014; Wagner et al., 2014; Cohn et al., 2015; Kearney et al., 2015; Simonetti et al., 2016).

Examination of the HIV-1 integration sites in circulating CD4⁺ T cells showed that a large number of HIV-1 proviruses are found in expanded clones of cells (Maldarelli et al., 2014; Wagner et al., 2014; Cohn et al., 2015). As might be expected based on the relative rarity of intact proviruses integrated into CD4⁺ T cell DNA, the majority of the CD4⁺ T cell clones examined contained defective proviruses (Imamichi et al., 2014; Cohn et al., 2015). However, a unique



Insight from Lillian B. Cohn and Michel C. Nussenzweig

patient with a history of metastatic squamous cell carcinoma had what appeared to be an expanded clone of CD4⁺ T cells carrying a replication-competent virus (Simonetti et al., 2016). Unfortunately, definitive proof of clonal expansion of CD4⁺ T cells carrying latent virus could not be obtained in that case because the virus was integrated in a region of the genome that could not be mapped with certainty. Therefore, although the possibility of multiple independent integration events was very unlikely, it could not be formally ruled out.

Given that isolation of latent cells is not yet feasible, Hosmane et al. (2017) and Lorenzi et al. (2016) independently developed methods that combine limiting dilution viral outgrowth with viral sequencing to simultaneously quantitatively and qualitatively characterize replication-competent viruses in the latent reservoir (Q²VOA; Lorenzi et al., 2016; Hosmane et al., 2017). By sequencing a large number of unique, replication-competent viruses, both groups found that >50% of the viruses emerging from the reservoir are identical to other viral isolates from the same individual. Moreover, the same groups of viruses could be found at two time points separated by 6 mo, indicating they are stable over time (Lorenzi et al., 2016). Because HIV-1 mutates rapidly, finding identical viruses in multiple single cells is strongly

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suggestive that they emerge from clones of expanded T cells. Consistent with the observation that HIV-1 virus production in activated T cells leads to cell death and that large expanded clones generally contain inactive proviruses, clone size was inversely correlated with the probability of finding a replication-competent virus (Lorenzi et al., 2016).

Hosmane et al. (2017) also show that latent cells can divide without producing virus, dying, or infecting other cells. They used a modified limiting dilution viral outgrowth assay that uses a transwell system with patient cells in one chamber and target cells for viral amplification in the other for separate manipulation of the two populations of cells. This allowed the authors to track patient cell proliferation through sequential rounds of stimulation while providing fresh target cells for each round of stimulation. Using this updated viral outgrowth system, the authors were able to formally demonstrate that latent cells are able to divide without producing infectious virus *in vitro*. They found that CD4⁺ T cells containing an intact integrated HIV-1 provirus could divide multiple times before producing virions. Thus, retroviral emergence from the reservoir appears to be at least in part stochastic.

The finding that identical latent proviruses occur in multiple individual CD4⁺ T cells and that latent cells can be stimulated to divide without producing virus are strongly supportive of the hypothesis that the HIV-1 latent reservoir is maintained at least in part by T cell proliferation. These observations have important implications for ongoing efforts to develop strategies to cure HIV-1.

One of the proposed methods to cure HIV-1 involves a "shock and kill" approach whereby latent cells are acti-

vated to produce the virus by a shock with an activator of viral transcription, followed by targeting the infected cells by using specific antibodies or killer T cells, for example. If reactivation is in part stochastic, then this would require multiple cycles of shock and kill.

However, the idea that the HIV-1 reservoir is maintained, at least in part, by cell division opens up new ways of thinking about how to eliminate the reservoir because it strongly implies that the reservoir is dynamic. Multiple groups have measured the reservoir over time and found it to be stable (Finzi et al., 1999; Crooks et al., 2015). If the reservoir is composed of cells that are dividing, then it must also contain an equal number of latent cells that are dying. The Q²VOA assay developed by Hosmane et al. (2017) and Lorenzi et al. (2016) facilitates the investigation of whether and how specific groups of infected cells grow or contract during infection in the context of quantitatively stable reservoir.

The dividing cells found in clones are likely expanding in response to antigen. Why and how the latent cells in the reservoir die is unknown, but solving this problem is likely to lead to new insights into HIV-1 treatment and cure.

Note added in proof: A recent study (Descours et al., 2017) suggested that CD32a may be a marker for latent cells harboring replication-competent virus in virally suppressed patients and can be used to isolate latent cells.

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