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INSIGHTS

Proliferation of HIV-1 reservoir cells: The delusion of infinite growth

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Proliferation of HIV-1–infected cells contributes to viral persistence despite antiretroviral therapy. A new study by Kufera et al. (https://doi.org/10.1084/jem.20231511) demonstrates that proliferative growth of cells infected with genome-intact HIV-1 is not limitless; rather, these cells seem to be at least partially refractory to TCR stimulation, restricting their ability to proliferate in response to antigenic challenge.

Although HIV-1 was first discovered more than 40 years ago, it largely remains the "epidemic of our time," with ∼39 million people living with HIV-1 worldwide for whom no curative treatment approaches are available; lifelong antiretroviral suppression treatment represents the only available therapeutic option for all people living with HIV-1. Persistence of HIV-1 despite antiretroviral treatment is due to small numbers of virally infected CD4 T cells harboring chromosomally integrated, genome-intact viral DNA that can drive viral rebound once treatment is interrupted (Finzi et al., 1997). Considerable progress has been made recently in understanding the mechanisms that permit long-term survival of these so-called "viral reservoir cells": By infecting CD4 T cells, which are designed to persist long-term to provide sustained cellular immune memory, HIV-1 hijacks a cell population that is intrinsically highly durable and able to proliferate in response to antigenic stimulation or cytokine signals. This proliferative ability of infected cells seems to represent one of the principal mechanisms supporting HIV-1 persistence. Indeed, molecular profiling of infected cells with single-genome near full-length sequencing frequently revealed large clusters of cells that contain identical proviral sequences and corresponding chromosomal integration sites, reflecting clonal proliferation of infected CD4 T cells during which viral genomes are passed on to daughter cells (Einkauf, 2019; Lian, 2023). First and foremost, this proliferation seems driven by engagement of the T cell receptor (TCR), the canonical mechanism for T cell activation. However, some studies suggest that homeostatic proliferation in response to cytokine signals may also play a role (Chomont et al., 2009). Interestingly, in some cases, proliferation of infected cells seems to be induced by insertional mutagenesis of proviruses integrating into proliferation-associated genes (Liu et al., 2020). Of note, activation and proliferation of infected T cells can frequently lead to higher levels of viral gene expression and increase the immunological vulnerability of HIV reservoir cells. Therefore, it appears that selected subsets of cells containing intact proviruses integrated into heterochromatin regions with repressive epigenetic modifications may be better equipped to proliferate in the setting of ongoing antiviral immune surveillance in the host (Jiang, 2020). In addition, the proliferation of infected cells by itself can be regarded as a viral strategy to resist host immunity—elevated proliferative activity of HIV-1–infected cells may outcompete negative host selection mechanisms and promote viral persistence despite antiviral immune responses. Supporting this view, recent studies suggest that proliferation of infected cells may be up to 10 times faster than the decay rate of virally infected cells (Reeves et al., 2023). Of note, proliferation can occur in CD4 T cells infected with genome-intact proviruses. However, there is a much larger fraction of proliferating CD4 T cells that harbor replication-defective HIV-1 DNA sequences, due to the error-prone viral reverse transcriptase that frequently introduces lethal mutations when genomic viral RNA is reverse transcribed into DNA. Considering that HIV-1–infected T cells can proliferate in response to antigen stimulation, therapeutic inhibition of T cell proliferation may appear as one possible modality to destabilize the viral reservoir cell pool. However,

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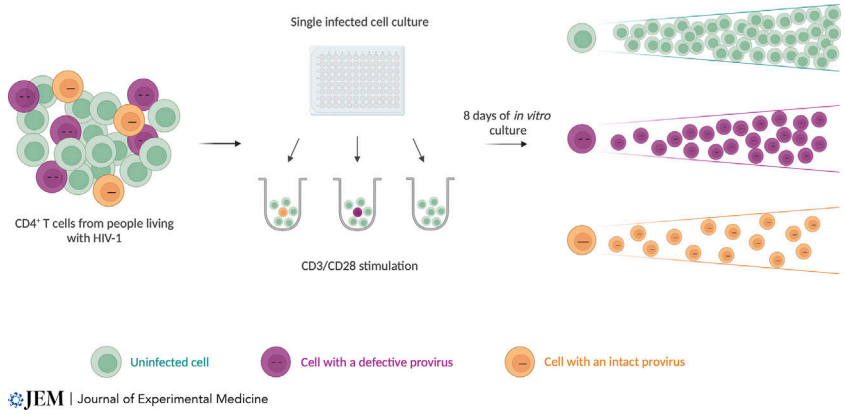
is it likely that such a strategy would indeed translate into clinical benefits? And is proliferation of virally infected T cells really a process without limits and restraints?

In this *JEM* issue, Kufera et al. (2024) described an experimental in vitro tissue culture model to evaluate the proliferative behavior of HIV-1–infected cells in great detail. For this purpose, they isolated resting CD4⁺ T cells from persons living with HIV-1 who had received suppressive antiretroviral therapy for more than 10 years. These cells were then diluted to a single virally infected cell per culture well and stimulated with CD3/CD28 beads that activate TCR signaling. After 8 days of in vitro culture with IL-2 and antiretroviral agents (to block new cycles of infection), the authors used PCR assays to evaluate the expansion of cells harboring intact, defective, or no HIV-1 proviruses. Interestingly, experiments in many of these microcultures demonstrated that the cells harboring intact proviruses proliferated significantly less than uninfected cells. The proliferation of cells harboring a defective provirus was also reduced in comparison to uninfected cells, but not to the same extent as in cells harboring intact HIV-1. To explore underlying mechanisms for this observation, the authors analyzed HIV-1 virion production by quantifying viral poly-A–tailed RNA, hypothesizing that production of viral RNA and proteins may impair cellular proliferation through cytopathic effects. However, there was no clear statistical correlation between cellular proliferation and the quantity of virions produced. Moreover, proliferation of HIV-1–infected cells in this experimental system was not associated with specific chromosomal integration site locations. Remarkably, the authors found that the clone size of HIV-1–infected cells, determined through proviral integration site-specific PCRs directly ex vivo, was inversely correlated to the ability of infected cells to proliferate in vitro in the experimental microculture system, prompting the authors to hypothesize that proliferative defects of HIV-1–infected cells in vitro may reflect T cell exhaustion resulting from prior high-level proliferation in vivo.

In sum, these data suggest that proliferation of HIV-1–infected cells over many years of treatment with suppressive antiviral therapy leaves a notable functional footprint in these cells, characterized by a more limited proliferative responsiveness to



Reduced proliferation of cells harboring intact HIV-1 proviruses. Using an in vitro model permitting analysis of the proliferation of individual HIV-1–infected and uninfected CD4⁺ T cells in microcultures, Kufera et al. (2024) show that cells harboring intact HIV-1 proviruses proliferate less than uninfected cells after TCR stimulation. The proliferation of cells harboring defective HIV-1 proviruses was also decreased but to a more limited degree. These results suggest an intrinsic defect of cells harboring intact proviruses to proliferate in response to antigenic challenges. Future studies will be necessary to identify mechanisms underlying this functional deficit.

de novo TCR stimulation in vitro. Exploring the reasons for this functional impairment in subsequent studies may be very helpful to better understand the biological behavior of HIV-1 reservoir cells. One possibility is that HIV-1–infected cells upregulate immune checkpoint markers and negative immunoregulatory molecules that inhibit cell proliferation; upregulation of these markers may reduce proviral transcription and limit the immunological visibility of infected cells. However, this improved adaptation to host immune surveillance may come at the cost of a reduced ability to proliferate in response to TCR stimulation. Recent work describing new methods to profile the surface phenotype of viral reservoir cells indeed demonstrated elevated expression of such surface markers on unperturbed ex vivo isolated cells, specifically on those harboring genome-intact HIV-1 DNA (Sun et al., 2023). In addition, it is possible that viral reservoir cells, after years of continuous proliferation in vivo, may have reached a state of "replicative senescence," characterized by limited responsiveness to TCR stimulation, paired with upregulation of anti-apoptosis markers, telomere attrition, and long-term persistence in a functionally impaired state (Campisi, 1996). Such a cellular condition has frequently been described in senescent T cells specific for CMV, and CMV-specific CD4 T cells may indeed encompass considerable proportions of viral reservoir cells (Mendoza et al., 2020; Simonetti et al., 2021). However, replicative senescence is unlikely to represent the only underlying mechanism of the observed findings, since cellular senescence would likely be present in all virally infected cells, and not just in the subset of cells infected with genome-intact HIV-1. Finally, it is possible that viral RNA and possibly viral protein production may limit proliferation of cells, despite the fact that Kufera et al. (2024) did not find evidence for an association between poly-A–tailed viral RNA production and proliferative activities. In particular, analysis of cell death in single infected cells was technically not possible in the study by Kufera et al. (2024); therefore, the authors were unable to evaluate how cell demise during in vitro culture conditions may have impacted their findings. This would be of particular importance in future studies, as a series of prior investigations suggested that some virally infected cells rely on sophisticated molecular mechanisms to maintain long-term cellular survival, while others presumably do not (Collora et al., 2022; Ren et al., 2020). Of note, the proliferative defect of HIV-infected cells that Kufera et al. (2024) propose is supported by in vitro tissue culture experiments. However, the authors propose that the partial unresponsiveness to TCR stimulation in vitro likely reflects a general proliferative defect in infected cells that influences their growth behavior in vivo. The development of technologies that assess the functional profile

of these cells in direct ex vivo assays may help to address this further.

In conclusion, Kufera et al. (2024) suggest that clonal proliferation of HIV-1–infected cells is not limitless; rather, continuous in vivo proliferation may render these cells partially refractory to TCR stimulation. As outlined by the authors, one important implication of this work is that pharmaceuticals designed to unselectively inhibit T cell proliferation may be more likely to block total T cell proliferation, rather than reducing the small pool of infected cells that have impaired proliferative activities. The fact that HIV-1–infected cells cannot proliferate endlessly can, from our perspective, be viewed as a positive signal, indicating that physiological resources to sustain HIV-1 persistence are not unlimited. A greater understanding of the physiological processes that influence and drive HIV-1 persistence may ultimately lead to improved clinical strategies to target HIV-1 reservoir cells more effectively.

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