have compared neutron and x-ray diffraction data from both species. The authors first replaced exchangeable protons with deuterons in large (1 mm) crystals of CcP, and then treated a CcP crystal with hydrogen peroxide to produce CcP-I. The results show that the catalytic imidazole of His^{52} is not protonated in resting CcP, as expected (see the figure). In CcP-I, both nitrogen atoms in His^{52} are protonated (deuterated), which was unexpected according to the generally accepted mechanism. This means that the protons required for O-O bond cleavage in CcP-0 (see the figure) must have come from another source, such as an adjacent water molecule. In this snapshot of the catalytic cycle, the ferryl oxygen, Fe(IV)=O, of CcP-I is not protonated, and the short iron-oxygen distance expected for the ferryl is confirmed.

Visualizing the mechanistically pertinent protons has important implications for the mechanism of O-O bond scission mediated by CcP and other heme proteins. A "wet" version of the peroxidase mechanism has been proposed, in which a water molecule adjacent to His^{52} mediates O-O bond cleavage (*1*, *8*, *9*). Retention of the His^{52} proton adjacent to the ferryl heme after O-O bond heterolysis, as revealed by Casadei *et al.*, suggests that another proton, likely traveling through an aqueduct of water molecules leading to the active-site cavity, is also necessary (see the figure).

CcP-catalyzed peroxide bond heterolysis thus seems to occur via a proton relay mechanism similar to that of cytochrome P450 (*10*), with electrons arriving through Trp^{191} (see the figure). In this scenario, deprotonation of His^{52} would occur during subsequent reduction of CcP-I by another enzyme, ferrocytochrome c. This realization points to a water-mediated, acid-catalyzed process for O-O bond heterolysis, which is mechanistically satisfying because of its analogies to other proton relay mechanisms, such as that of cytochrome P450. Further, the need for a water channel and an external proton in peroxidase catalysis are highly informative for the design and construction of new heme-iron biocatalysts. ■

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ILLUSTRATION: V. ALTOUNIAN/*SCIENCE*

HIV/AIDS

Persistence by proliferation?

Latently HIV-infected cells driven to proliferate may raise a further challenge for eradication strategies

By **David Margolis**[1] *and* **Frederic Bushman**[2]

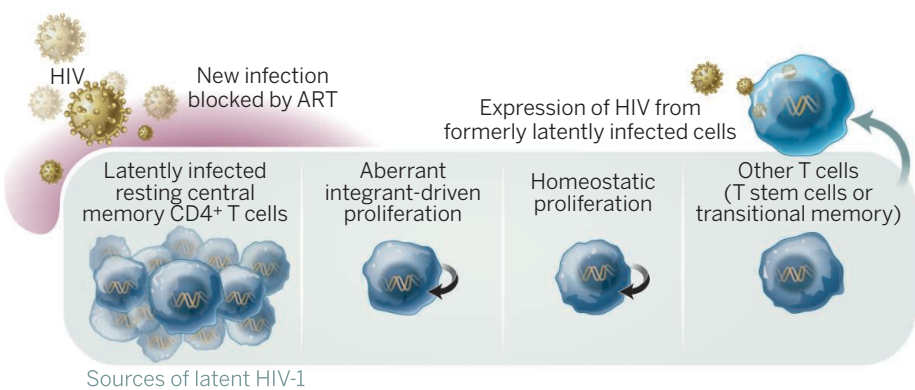
The persistence of HIV-1 infected cells in individuals on antiretroviral therapy (ART) presents an obstacle for cure of infection. ART is the best available remedy for millions of infected people, but treatment must be life-long because HIV establishes latent infection that is unaffected by antiretrovirals and is invisible to immune surveillance. Because decades of treatment may be unsustainable, there is intense interest in reversing latency. If quiescent HIV in $CD4^+$ T cells can be identified and activated without enhancing new infection, HIV-targeted immune response might be able to control or even clear infection. On page 179 in this issue and in this week's *Science* Express, Maldarelli *et al.* (*1*) and Wagner *et al.* (*2*), respectively, raise a new challenge for these efforts suggesting that proliferation of latently infected cells may be a key factor in sustaining this durable viral reservoir.

Latent HIV proviruses (viral genome integrated into the host cell DNA) are found most often in resting $CD4^+$ T cells within the central memory arm of the immune system (*3*–*5*), although other T cell subpopulations have been implicated (*6*, *7*). The latent pool shows minimal or absent decay (*8*), which is not fully understood. One possible explanation is that ongoing low-level HIV replication during ART replenishes the pool. However, viral genetic diversity does not increase over time in individuals during ART (*9*), at odds with this view.

Other evidence suggests that virus emerges from the pool of latently infected cells periodically. Even patients whose viral load is well suppressed show intermittent bursts of viremia ("blips"), and in many patients viremia is detectable in specialized assays (*10*, *11*). Given that the pool of latently infected cells must be primarily established before ART, it is difficult to understand why such periodic induction of the pool does not lead to it running dry.

Homeostatic proliferation of infected transitional memory T cells (*6*) has been proposed as a source that could maintain the pool, but this does not explain persistence in the dominant central memory reservoir. Latently infected stem cell–like memory T cells could proliferate (*7*), and it will be of great interest to compare integration patterns seen in these cells and in more differentiated cell populations.

Maldarelli *et al.* and Wagner *et al.* harvested DNA from the blood cells of HIV-infected individuals after a decade of successful ART, and analyzed the distribution of sites of proviral integration in the human genome. Typically, HIV favors integration in regions of the genome that are transcriptionally active (*12*), but a unique pattern was seen in rare proviruses from well-suppressed patients. Both groups found expanded proviral clones that were enriched for proviruses in or near a limited set of cellular genes, some of which



The persistent pool of HIV-1. Antiretroviral therapy can prevent the creation of new latently infected cells, but it does not affect cells in which latency was initially established. Intermittent bursts of viremia originate in part from this latent reservoir. Forcing these cells to exit the latent state without enhancing new infection could make the virus vulnerable to clearance by an HIV-targeted immune response. Blocking the proliferation of these latently infected T cells could deplete the pool if its stability is driven by such multiplication.

encode products involved in controlling the cell division cycle or cancer progression. This supports the hypothesis that disruption of these genes by proviral insertion promotes growth or persistence of the host cell (*13*). Maldarelli *et al.* and Wagner *et al.* identify the host gene encoding the basic leucine zipper transcription factor 2 (BACH2) as a frequent site of HIV integration. BACH2 is a transcriptional regulator that controls $CD4^+$ T cell senescence and cytokine homeostasis (*14*). Thus, the new findings suggest a link between the persistence of latently infected cells and proviral integration in genes related to cell proliferation and cancer.

"...findings suggest a link between the persistence of latently infected cells and proviral integration in genes related to cell proliferation ..."

Further experiments should strengthen these ideas. There is as yet no molecular evidence that such integrations of HIV-1 lead directly to the proliferation of latently infected cells, but it should be possible to engineer viral integration into specific sites of the host cell genome and demonstrate cell proliferation. In addition, there is as yet no proof that the proviruses encode for replication-competent HIV genomes. Maldarelli *et al.* did carry out the Herculean task of single-genome amplification and sequencing tiny amounts of HIV RNA recovered from the plasma of some patients studied. This verified a close similarity of circulating viral envelope sequences to those found in integrated proviral genomes in expanded clones. However, like prior studies (*11*), such sequencing is limited to a small portion of the HIV genome, and cannot eliminate the possibility of inactivating mutations in other parts of the proviral genome, making the virus incompetent to replicate. Given that the cells harboring quiescent HIV-1 are only a tiny minority of the total $CD4^+$ T cell population examined by Maldarelli *et al.* or Wagner *et al.,* and that years of ART have allowed for years of selection, alternative interpretations of the data are possible. For example, it is not yet ruled out that the expanded T cell clones detected could be expanding for other reasons (e.g., in response to stimulation by a specific antigen). There may be other reasons for preferential viral integration into the genes described as well. There may also be "survivor bias" in the detection of replication-incompetent genomes. Indeed, given the model, it is puzzling that no increase in the total number of HIV DNA-positive cells was observed.

The findings of Maldarelli *et al.* and Wagner *et al.* raise additional issues. Lentiviral vectors are used extensively in therapeutic gene transfer, so monitoring for related events of proliferation-promoting integration with these vectors during gene therapy is important. Indeed, clonal expansion was observed in the case of a lentiviral-based gene correction of the blood disorder beta-thalassemia in which integration at the site of a proto-oncogene increased cell proliferation (*15*). In this case, the host gene encoding high-mobility group AT-hook 2 (HMGA2) produced a truncated mRNA due to vector insertion within the gene. HMGA2 is a transcription regulatory protein. The truncated mRNA removed a binding site for a microRNA that negatively controls HMGA2 expression. The result was increased accumulation of HMG2A mRNA and protein. HMGA2 was not an integration target in the cells studied by Maldarelli *et al.* or Wagner *et al.*, raising questions about the differences between latent HIV infections and beta-thalassemia gene therapy.

Both studies also mention the concern that HIV integration could contribute to the development of cancers by insertional mutagenesis. However most HIV-related malignancies are not T cell cancers, and even most HIV-related lymphomas are of B cell origin. HIV cancers are not thought to harbor integrated HIV DNA, although this could be reinvestigated.

If blocking proliferation of latently infected cells proves to be necessary, it will complicate efforts to clear the latent reservoir. But clearance of this reservoir is crucial to achieve a cure of HIV infection. ■

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OCEANS

Microplastics in the seas

Concern is rising about widespread contamination of the marine environment by microplastics

By **Kara Lavender Law**[1] *and* **Richard C. Thompson**[2]

Plastic debris in the marine environment is more than just an unsightly problem. Images of beach litter and large floating debris may first come to mind, but much recent concern about plastic pollution has focused on microplastic particles too small to be easily detected by eye (see the figure). Microplastics are likely the most numerically abundant items of plastic debris in the ocean today, and quantities will inevitably increase, in part because large, single plastic items ultimately degrade into millions of microplastic pieces. Microplastics are of environmental concern because their size (millimeters or smaller) renders them accessible to a wide range of organisms at least as small as zooplankton, with potential for physical and toxicological harm.

Since its introduction in the published literature in 2004 (*1*), the term microplastic has been widely used to describe plastic fragments in the marine environment. Typically considered to be smaller than 5 mm in diameter, microplastics are ill defined by size, with ranges that vary between studies. In most open-water studies, microplastics are measured with plankton nets, and particles smaller than the net mesh (typically ~0.33 mm) can evade capture. In marine sediment, bulk sampling can retain particles of all sizes; however, efficient identification is a serious challenge in quantifying microplastic loads, especially with decreasing size. Spectroscopic analysis has identified individual fragments of common plastics as small as 20 μm in diameter.

The sources of microplastic include fragmentation of larger items entering by rivers, runoff, tides, winds, and catastrophic events, together with at-sea sources, including lost cargo and fishing and aquaculture gear. There are also direct inputs of microplastics as micrometer-sized particles, such as cosmetic beads and clothing fibers

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