A long-term latent reservoir for HIV-1: discovery and clinical implications

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Despite the remarkable success of highly active antiretroviral therapy (HAART) for the treatment of HIV-1 infection, it now appears that the infection is intrinsically incurable with antiretroviral therapy alone. The major reason is that the virus can persist in a latent form in resting memory CD4 cells. These cells arise when infected CD4+ lymphoblasts carrying an integrated copy of the HIV-1 genome revert back to a resting memory state. In this resting state, CD4 cells are minimally permissive for virus gene expression, and infected memory cells can survive for many years. Following re-exposure to the relevant antigen or other activating stimuli, these cells can begin to produce virus again. The existence of a stable reservoir has altered treatment strategies in several ways. HAART is no longer given with the goal of eradication. In addition, the reservoir serves as a permanent archive for wild-type virus and for drug-resistant variants that arise during treatment. Thus, once resistance to a particular drug arises, the patient will always carry that resistance. Interruption in treatment results in the re-emergence of the original wild-type virus, which often replicates better than drug-resistant virus. Although HAART cannot eradicate the infection, current regimens do come close to stopping virus evolution. Free viruses found in the plasma at low levels in patients on HAART resemble viruses in the latent reservoir and do not contain new drug resistance mutations. Thus although HAART cannot produce eradication, lifetime control of the infection with antiretroviral drugs may be possible.

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Introduction

The past decade has seen remarkable progress in the development of drugs for HIV-1 infection. Twenty antiretroviral drugs belonging to four classes are currently approved for the treatment of the infection. When used in combinations of three or more, these drugs can reduce plasma virus levels to below the limit of detection of extremely sensitive RT-PCR assays. In patients who experience sustained suppression of viraemia on highly active antiretroviral therapy (HAART), progression of the disease is halted and partially reversed. However, despite this extraordinary progress, there is now general agreement that the infection cannot be cured with antiretroviral therapy alone. The principal reason is that the virus can persist in cellular reservoirs. The best understood reservoir is a small pool of latently infected resting memory CD4 cells. This pool shows extraordinary stability and represents a virtually insurmountable barrier to virus eradication. The review will describe the discovery of this latent reservoir and its impact on treatment strategies for HIV-1 infection.

HIV-1 latency and the discovery of a latent reservoir in resting CD4 cells

Latency, the reversibly non-productive infection of host cells by a virus, plays a critical role in the persistence of some viruses. For example, the α-herpesviruses depend on latency as an essential mechanism of persistence between episodes of active disease. At the level of individual cells, HIV-1 can also establish a state of latent infection. However, latency is not essential for HIV-1 persistence. The virus replicates actively throughout the course of the disease, and there is no convincing evidence that latency plays a role in the natural history of HIV-1 infection. Rather, HIV-1 latency appears to be the inadvertent consequence of viral tropism for CD4 cells, cells that undergo profound transitions in activation state that affect permissiveness for viral replication. As is discussed below, these transitions allow a state of latent infection to be established. Although latency is not important in the natural history of HIV-1 infection, it has profound significance in the setting of antiretroviral therapy because
it provides a critical mechanism of viral persistence when active replication is suppressed by drugs.

An early study by Gallo and colleagues\(^6\) suggested that the number of cells carrying HIV-1 DNA \textit{in vivo} might far exceed the number of cells expressing HIV-1 RNA. This study raised the possibility of latent infection. Additional evidence for latency came from \textit{in vitro} experiments using transformed cell lines infected with HIV-1. These studies demonstrated that levels of HIV-1 gene expression could be regulated by stimuli affecting the state of cellular activation.\(^7\) In these cell line models, several potential mechanisms for latency were evaluated. One hypothesis was that latency resulted from the absence in resting CD4 cells of host and viral factors needed for efficient and processive transcription from the HIV-1 LTR.\(^8–14\) This notion is consistent with the observation that HIV-1 gene expression is dependent upon some host transcription factors, including NFAT and NFxB, that are not active in resting CD4 cells.\(^8,10–12\)

Because of the potential relationship between latency and state of cellular activation, initial \textit{in vivo} studies of HIV-1 latency focused on resting CD4 cells.\(^15,16\) Interestingly, direct infection of resting cells did not lead to productive infection. Instead, infection resulted in a labile state known as pre-integration latency. In resting cells, entry and reverse transcription of the viral RNA can occur, but subsequent steps including integration of the viral DNA into the host cell genome and production of progeny virions do not. If the resting cell is activated before the unintegrated viral DNA decays, then virus production can result.\(^17\) Thus, recently infected resting CD4 cells with unintegrated HIV-1 DNA constitute a form of latent infection. However, this state is labile and is unlikely to serve as a mechanism of long-term viral persistence in patients on HAART.

How then does a stable state of latent infection develop in resting CD4 cells? A plausible hypothesis is based on the normal physiology of CD4 cells. At any given time, most CD4\(^+\) T lymphocytes in the body are in a resting G\(_0\) state. Resting lymphocytes are profoundly quiescent cells with a low metabolic rate and a unique morphology characterized by a small cytoplasmic volume. In response to antigen, resting T cells undergo a burst of cellular proliferation and differentiation, giving rise to effector cells. Most effector cells die quickly, but a subset survive and revert to a resting G\(_0\) state. They persist as memory cells, with an altered pattern of gene expression enabling long-term survival and rapid responses to the relevant antigen in the future (for a review of immunological memory, see Ref. 18). Activated CD4 cells are highly susceptible to HIV-1 infection and typically die quickly as a result of the cytopathic effects of the virus or host immune responses. However, some activated CD4 cells may become infected and then survive long enough to revert back to a resting state. This results in a stable form of latency, post-integration latency. Because HIV-1 gene expression is dependent upon inducible host transcription factors that are only transiently activated following exposure to antigen,\(^8,10–12\) HIV-1 gene expression may be extinguished as the cells revert to a resting memory state. The result is a stably integrated but transcriptionally silent form of the virus in a cell whose function it is to survive for long periods of time. If the cell encounters the relevant antigen, then it can begin to produce virus. In the meantime, the virus persists simply as integrated DNA, unaffected by antiretroviral drugs. Therefore, HIV-1 latency inadvertently exploits the most fundamental characteristic of the immune system, the immunological memory that resides in long-lived resting lymphocytes. A prediction of this model is that a stable latent form of HIV-1 should reside in resting memory CD4 cells. Integrated viral DNA in this reservoir is not affected by antiretroviral drugs.

Proving that latently infected cells with integrated HIV-1 DNA were present \textit{in vivo} required the development of methods for isolating extremely pure populations of resting CD4 cells and demonstrating that within these populations were cells carrying the HIV-1 genome stably integrated into host chromosomes. In addition, it was necessary to show that replication-competent virus could be rescued from resting CD4 cells by cellular activation. This was particularly important since the mere presence of viral nucleic acids in resting CD4 cells could also be compatible with the presence of defective viruses. In 1995, the presence of integrated HIV-1 DNA in highly purified populations of resting CD4 cells was demonstrated definitively using inverse PCR.\(^19\) A critical further advance was the development of a culture assay that allowed consistent rescue of replication-competent virus from populations of highly purified resting CD4 cells from infected individuals.\(^20\) In this assay, purified resting CD4 cells are subjected to conditions that activate 100% of the cells, allowing latently infected cells to produce virus. The virus released from these cells is then expanded using CD4\(^+\) lymphoblasts from normal donors. With this assay, resting CD4 cells harbouring replication-competent virus were detected at low frequency in the blood and lymph nodes of all infected individuals studied.\(^20\) Consistent with the hypothesis presented above, latent virus was shown to reside predominantly in the memory subset of resting CD4 cells.\(^20\)

At about the time assays for latently infected cells were being developed, combinations of antiretroviral drugs were first shown to reduce plasma virus levels to below the limit of detection, raising hopes for viral eradication.\(^2–4\) The question then became whether the pool of latently infected cells would persist in patients who had suppression of viraemia to undetectable levels on HAART. Using the culture assay approach described above, three groups simultaneously demonstrated in 1997 the presence of latently infected cells in patients who were responding well to HAART.\(^21–23\) Subsequent longitudinal studies demonstrated that the decay rate of the pool of latently infected cells was extremely slow.\(^24\) A half-life of 44 months was measured. At this rate of decay, over 70 years of treatment would be required for eradication of the latent reservoir. These studies led to the proposal that the latent reservoir in resting memory CD4 cells guarantees lifetime persistence of the virus even in patients on suppressive HAART regimens.\(^25\) Although there was initial hope that more aggressive treatment might accelerate the decay of this reservoir, a recent study has shown that the reservoir is stable even in patients who have had suppression of detectable viraemia for as long as 7 years.\(^25\)

A different form of proof for the stability of the reservoir comes from an analysis of viruses harboured in the reservoir. In patients who have developed drug resistance due to treatment with insufficiently potent one- or two-drug regimens in the pre-HAART era, the reservoir contains the original drug-sensitive, wild-type viruses that the patients were infected with as well as resistant viruses that evolved during non-suppressive pre-HAART therapy.\(^26\) In fact, the reservoir serves as a permanent archive for all wild-type and drug-resistant viruses that have circulated at significant levels during the course of the infection.\(^26\) The fact that the latent reservoir can store forms of the virus...
that are unfit under ambient conditions provides strong genetic evidence for the stability of the latent reservoir.

Clinical implications of the latent reservoir

The existence of a stable reservoir for HIV-1 has at least four important clinical implications. First, because the reservoir makes eradication with antiretroviral therapy an unrealistic goal, treatment strategies have been altered. The ‘hit early’ part of the ‘hit early, hit hard’ treatment paradigm that governed HIV-1 therapy in the years following the advent of HAART has been modified. Recent treatment guidelines\(^1\)\(^2\) include a recommendation to delay the initiation of treatment until the CD4 cell count has fallen to 350 cells/mm\(^3\). This more conservative approach to delay the initiation of therapy reflects both an increasing appreciation of the long-term toxicity of antiretroviral drugs as well as pessimism regarding eradication.

A second important treatment implication is that drug-resistant viruses arising due to inadequately suppressive therapy or poor compliance can be archived in this reservoir, permanently limiting treatment options.\(^{26,27}\) The archiving of viral species allows the virus to ‘remember’ any mistakes that have been made in treatment that have led to the emergence of detectable resistance. The latent reservoir makes HIV-1 evolution unique. In Darwinian terms, it is not simply ‘survival of the fittest’. For HIV-1, it is survival of all the major forms that have been generated within a given individual and active replication of the form that is fittest under the current conditions. If the conditions change, archived variants can re-emerge if they are more fit under the ambient conditions.

A third treatment implication is related to treatment decisions in the setting of failure. Recent work by Deeks and colleagues has raised the possibility that patients failing antiretroviral therapy with multidrug-resistant HIV-1 may derive some benefit from continued treatment.\(^{28}\) This benefit is presumably because drug resistance mutations diminish the fitness of the virus. If therapy is interrupted, then reversion to a more virulent, drug-sensitive form of the virus occurs. This does not represent true genetic reversion or ‘back-mutation’, but rather the re-emergence of an archival wild-type virus, probably from the latent reservoir in resting memory CD4 cells.\(^{29}\) Thus, one benefit of continued therapy in the setting of treatment failure may be the maintenance of selection for a drug-resistant virus with diminished replicative capacity.

A final clinical implication of viral reservoirs is related to the issue of low-level viraemia in patients on HAART. It is becoming increasingly clear that even in patients who have had prolonged suppression of viraemia to below the limit of detection, there is some free virus in the plasma.\(^{29,30}\) This virus could represent a low level of replication that continues despite HAART or virus released from stable reservoirs that is unable to replicate further because of the effects of the drugs. Most likely, it is due to some combination of replication and release. From a clinical perspective, the critical issue is whether the level of replication is sufficient to allow the development of new drug resistance mutations. In recent studies, this low level plasma virus has been characterized genotypically and shown to be similar to viruses in the reservoir and largely devoid of new resistance mutations.\(^{30}\) It appears that wild-type drug-sensitive viruses can be released into the plasma for years without the development of new resistance mutations. Thus, in patients who maintain suppression of viraemia to below the limit of detection, it appears that viral evolution can be largely halted. Therefore, although the latent reservoir guarantees lifetime persistence of the virus, the current HAART regimen can arrest virus evolution and afford patients the chance for lifelong suppression provided that the problems of toxicity can be overcome.

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