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COMMENTARY

# Towards a cure for HIV: the identification and characterization of HIV reservoirs in optimally treated people

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**Currently available anti-HIV-1 drugs suppress viral replication and maintain viral levels below the detection threshold of most assays but do not eliminate cellular reservoirs. As a result, very low levels of circulating virus can be detected in most people despite long–term treatment with potent anti–HIV drug combinations. Not surprisingly, viral levels rebound with discontinuation of treatment. New evidence indicates that there is a viral reservoir in bone marrow progenitor cells.**

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Over ten years ago, it was demonstrated that HIV establishes cellular reservoirs of latent virus in resting CD4$^+$ T cells [1]. However, subsequent studies in which residual viral genomes were sequenced and compared to cellular proviral sequences suggested that reactivation of latently infected CD4$^+$ T cells could not account for the majority of the residual virus [2, 3]. Recent studies of viral load decay kinetics after the initiation of highly active antiviral therapy have also suggested the existence of additional reservoirs, including at least one reservoir that did not appreciably decay after seven years [4]. The identification and characterization of HIV-1 cellular reservoir(s) in effectively treated patients (HIV-1 RNA below 50–75 copies) is now a high priority as a first step towards the development of a strategy for virus eradication.

Recently, several studies have demonstrated that multipotent hematopoietic stem/precursor cells (HSPCs) can become infected with HIV-1 [5, 6]. Moreover, results from one study suggest that these cell types may be an important cellular reservoir for HIV-1 [5].

Most multipotent hematopoietic precursor cells express CD34, a cell-surface marker found on HSPCs ranging from hematopoietic stem cells (HSCs) to progenitor cells committed to differentiation. CD34$^+$ populations that lack CD38 and express CD133 are enriched for multipotent progenitor cells. However, functional assays demonstrating the capacity of cells to differentiate are necessary to definitively identify multipotent cells. Multipotent cells (HSCs, multipotent progenitor cells (MPPs), and common myeloid progenitor cells (CMPs)) are capable of forming colonies containing cells of all myeloid lineages in methylcellulose. Because lymphoid cells do not grow under these conditions, these assays do not distinguish HSCs and MPPs from CMPs.

A proportion of CD34$^+$ cells express the HIV receptors CD4, CXCR4, and CCR5, making these cells potentially susceptible to HIV-1 infection (reviewed in [7]). Beginning more than 20 years ago, some studies suggested that rare infection of CD34$^+$ cells could occur both *in vitro* and *in vivo* [8-11]; however, the authors could not rule out the possibility of contamination by other cell types. Furthermore, studies assessing HIV-1 infection of multipotent colony–forming or CD133$^+$ HSPCs failed to detect either HIV-1 infection or expression of CD4, CXCR4 and CCR5 in these cells [12-14].

Reexamination of this topic has provided important new evidence that a percentage of multipotent HSPCs are susceptible to HIV infection. A 2007 study examined the ability of HIV-1 subtype C to infect multipotent CD34$^+$ HSPCs *in vitro* and *in vivo* [6]. The authors found that

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several isolates of HIV-1C could infect multipotent cells *in vitro* and that HIV was detected in most (12 of 19) CD34$^+$ cell samples isolated from HIV-infected donors with AIDS. Moreover, levels of provirus in the CD34$^+$ cells were higher than levels observed in total peripheral blood mononuclear cells in 11 of the 12 with detectable provirus in their CD34$^+$ HSPCs. Notably, these authors could not detect HIV DNA in multilineage colonies generated from HSPCs exposed to HIV-1B isolates — however the authors could not rule out the possibility that HIV-1B infection is cytotoxic leading to cell death rather than infected colony formation.

To further explore HIV-1B infection of HSPCs, we used flow cytometric approaches to detect the expression of the HIV-1 Gag protein in CD34$^+$ cells after very short incubation periods (three days) [5] and demonstrated that five full-length viruses had the capacity to infect CD34$^+$ cells. Notably, the use of flow cytometry avoided potential contamination problems that plagued prior studies because it allowed the simultaneous examination of individual cells for expression of both CD34 and viral proteins. Moreover, the use of five different full length live viruses (some of which were initially isolated directly from patient samples) demonstrated the physiological relevance of this result.

Intracellular Gag staining allows simultaneous analysis of multiple markers on individual cells, but there is potential for false positive results. However, HIV-1 Gag expression was dramatically reduced by pre-treatment of the cells with anti-retroviral drugs such as AZT [5]. Gag$^+$ cells could be detected in freshly isolated cells from some donors with high viral loads but not in cells from donors optimally treated with antiretroviral drugs [5]. Additionally, Gag-positive cells expressed functional HIV Nef based on their ability to downmodulate MHC-I [15, 16]. Similar results were obtained using reporter viruses that expressed marker genes such as GFP or placental alkaline phosphatase. Infection was further confirmed by the detection of viral particles released from CD34$^+$ cells, by the demonstration that viral particles released from CD34$^+$ cells had the capacity to infect T cells and by the detection of integrated viral genomes in CD34$^+$ cells [5]. Based on these controls and confirmatory experiments, Gag staining represented bona fide infection.

All five of the full length HIV-1s we tested *in vitro* were cytopathic and the number of infected CD34$^+$ cells declined dramatically over time with continued cell culture. Therefore to measure the capacity of infected cells to form mutilineage colonies, CD34$^+$ cells were infected with a non-cytotoxic, GFP-expressing HIV-1B that lacked expression of potentially toxic viral gene products [5]. Using these conditions, we could detect multilineage colonies that were uniformly GFP$^+$, demonstrating that HIV-1B envelopes can target multipotent HSPCs and that HIV integration can occur in multipotent HSPCs *in vitro.* However, this assay is not able to determine whether these cells were HSCs, MPPs and/or CMPs.

Importantly, HIV could establish latency in HSPCs infected *in vitro*. After the initial infection had declined and active infection could no longer be observed, agents that stimulated myeloid differentiation resulted in a resurgence of viral gene expression [5]. This observation was easiest to demonstrate using viruses that were able to amplify the signal by spreading infection to the differentiating macrophages, but we were able to detect reactivation of virus even in the presence of antiretroviral drugs that blocked viral spread [5]. We also developed an HIV that allowed us to directly detect a population of latently infected cells (GFP$^+$, HIV Gag$^-$) that was stable in culture [5]. Notably, some latently infected cells were lin$^-$, CD38$^-$ and CD34$^+$, consistent with multipotent cells. Together, these data provide evidence that latent HIV-1 infection of primitive HSPCs is possible *in vitro*.

To determine whether HIV infects CD34$^+$ bone marrow HSPCs *in vivo*, we obtained bone marrow from HIV–infected individuals. Three out of six HIV$^+$ individuals with high viral loads (> 50 000 copies/ml) had detectable Gag$^+$CD34$^+$ (and CD34$^-$) cells. The use of flow cytometry was crucial for these studies because Gag could be detected within both CD34$^+$ and CD34$^-$ cells in this cohort. In contrast, CD34$^+$ cells from donors with clinically undetectable viral loads for more than six months did not express detectable amounts of HIV Gag. In the three high viral load donors that were initially negative for Gag$^+$CD34$^+$ cells and in one donor with undetectable viral load, HIV Gag expression was induced upon myeloid differentiation of CD34$^+$. This finding provides evidence that CD34$^+$ cells could also harbor latent HIV in some donors, even those with clinically undetectable viral loads for more than six months on optimal therapy.

To examine whether CD34$^+$ cells harboring HIV genomes persisted after more than six months of optimal therapy, CD34$^+$ bone marrow cells were obtained from optimally treated individuals with clinically undetectable viral loads (< 48 copies/ml). As expected, optimally treated donors had no evidence of Gag expression in freshly isolated CD34$^+$ cells. However, HIV-1 DNA could be amplified from CD34$^+$ cells from more than 40% of these donors. In contrast, we were unable to detect comparable amounts of HIV DNA from bone marrow cells depleted for CD34$^+$ cells, indicating that infected CD34$^-$ bone marrow cells do not persist at similar levels in people on HAART [5]. The ability of CD34$^+$ cells to

harbor latent HIV *in vivo*, even in patients on optimal therapy, indicates that $CD34^{+}$ cells can act as a long-lived reservoir of HIV.

Additional studies are now needed to clarify which subsets of $CD34^{+}$ cells harbor HIV genomes *in vivo* and whether latently infected $CD34^{+}$ cells contribute to residual viremia in patients on HAART. More research will also be needed to determine whether different types of HIVs target different subsets of $CD34^{+}$ cells.

For many years it has been unclear whether multipotent HSPCs could be infected with HIV-1. Recent studies have now provided evidence that both active and latent infection can happen *in vitro* and *in vivo*. Infection of these HSPCs may contribute to hematopoietic defects noted in late stage HIV disease. Moreover, latent infection of HSPCs may create a stable long-lived reservoir that contributes to residual plasma viremia in optimally treated patients. Successful eradication of HIV will require clearance of virus from all cellular reservoirs.

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