OPINION PIECE

Eradication of HIV from the brain: reasons for pause

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Introduction

Eradication of HIV was unthinkable a few years ago, but now with a better understanding of the T-cell reservoirs and the development of new strategies that activate the virus but not cells, the idea is gaining traction. Confidence has also been gained as this was seemingly achieved in a single patient who received a bone marrow transplant from a donor with a mutation in chemokine receptor CCR5, although the reasons for the 'cure' are still unclear [1]. Major granting agencies such as the National Institutes of Health, the Gates foundation, and the American Foundation for AIDS Research have all set aside funds to develop strategies for eradication of HIV. Clearly, eradication of HIV is a laudable goal. However, this cannot be achieved unless HIV is also eliminated from all the tissue reservoirs. The purpose of this article is to examine the current strategies and how they may impact the HIV reservoirs in the brain.

Brain as a reservoir for HIV

Although in the periphery the central memory T cell is the major reservoir of the virus [2], T cells do not normally reside in the brain for long periods of time. Perivascular macrophages, microglial cells, and astrocytes are major cell types infected with HIV (reviewed in [3]). These cell types can be actively, persistently, or latently infected. Nearly 5–20% of perivascular astrocytes may be infected and they amount of infection correlates with the severity of encephalitis and dementia [4]. Glial cells in the brain have a very low turnover rate [5,6] and, thus, the virus could potentially reside in these cells for extended periods of time spanning several years. In support of this possibility, evidence for immune activation can be found in the cerebrospinal fluid (CSF) of HIV-infected patients despite undetectable HIV RNA (<50 copies/ml) in plasma for greater than 4 years [7]. In fact, patients in whom the CSF viral load is greater than that in plasma may develop an antigenic gradient, whereby cytotoxic T cells may infiltrate the brain causing an encephalitis, which in some may be very severe [8]. In-vitro studies show that a subset of macrophages and astrocytes can serve as a long-term reservoir for HIV infection and can produce fully replicative virus following stimulation with cytokines even after several months [9,10]. Importantly, animal studies suggest that the virus enters the brain and infects resident macrophages soon after systemic infection [11–14]. Further, the level of viral DNA in the brain did not diminish on combined antiretroviral therapy (cART) [15]. The virus may also evolve in the brain and adapt to this environment resulting in specific mutations in both the regulatory [16] and structural regions of the virus [17–19]. Signature mutations specific to the CSF in the V3 loop of env region of HIV have also been identified [20]. Hence, eradication strategies focused on T-cell reservoirs alone may not be sufficient, and consideration needs to be given to the reservoirs within the brain that involve other cell types.

Current strategies for HIV eradication

The major strategy is the suppression of viral transmission with cART, reactivation of viral reservoirs, and

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elimination by cytotoxic T-cell responses. Several different approaches for each of these steps have been proposed. For viral suppression in the brain, the choice of antiretroviral drugs should include those that have the best penetration into the brain and are not neurotoxic [21]. Further it remains unknown the extent to which these drugs can prevent HIV replication in macrophages/microglia and astrocytes. In general, it appears that ARTs are not as effective in macrophages [22] and the effect of ARTs on HIV replication in astrocytes has not been explored.

Several approaches have been used to reactivate HIV infection (reviewed in [23]). These include cytokines such as IL-2 and IL-7. However, clinical trials with IL-2 and cART or anti-CD3 have failed. IL-7 appears promising, as it can activate both memory and naive T cells. Chemical compounds are being developed that activate protein kinase C, or transcription factors, nuclear factor-κB (NF-κB), and SP-1. One such compound, prostratin is being considered for clinical trials. Several compounds are also being considered that block histone deacytelase (HDAC). However, recent trials with valproic acid, a weak HDAC inhibitor, have not been successful. Another compound, vorinostat or suberoylanilide hydroxamic acid (SAHA), is clinically approved for cancer therapy, is also a HDAC inhibitor, and is being considered for such studies. Methyltransferase inhibitors and other novel compounds are also being developed to reactivate HIV. It is critical that before these approaches are applied to humans, their effects on central nervous system (CNS) reservoirs be studied. Reactivation of the virus in the brain could potentially have devastating consequences by causing neuronal injury. Even in the presence of cART, early viral proteins are expected to be formed by the proviral DNA, and within the brain these proteins can cause neuronal injury not only at the site of infection but also at distant regions [24,25].

It is clear that the patient's cytotoxic immune responses are insufficient to control the infection; hence, several approaches are being pursued, including the generation of T cells that would encode T-cell receptors derived from potent HIV-specific clones of cytotoxic T cells. For this strategy to be successful, it would be important that the repertoire of T-cell receptors include those that recognize antigens expressed in brain, as viral evolution may occur in this compartment independent of lymphoid organs [16,17,26]. Another strategy includes the insertion of a mutation in the CCR5 gene in patient's T cells, thus making them resistant to HIV infection. Although repeated injections of these cells may eventually replace the T cells in the patient, it is unlikely to impact the cellular reservoirs in the brain.

Another strategy is to increase the number of integrated copies of HIV proviral DNA in a cell by dissociating the Rev-integrase complex. The incorporation of multiple copies of HIV into the chromosomal DNA leads to death of the cell. This strategy is based on the observation that only one or two copies of HIV proviral DNA get integrated in an infected cell despite the fact that a large number of unintegrated copies of the DNA are present in the cell. This is because the integrase gets complexed with Rev and thus prevents its activity. Dissociating this complex allows multiple copies of HIV proviral DNA to get integrated [27]. This strategy could potentially be effective in a variety of cell types; however, death of a substantial number of cells within the brain within a short period of time could lead to disruption of the blood–brain barrier with edema and impairment of cerebral function.

Intensification of HAART was one of the earliest strategies proposed; however, to date this has failed to impact the reservoirs [28]. The availability of newer drugs that target CCR5 and integrase has raised renewed hope for this approach. However, for this approach to be successful, it would need to block HIV replication in CNS reservoirs. Infection of glial cells can occur independent of CCR5 [29] and these cells have large amounts of unintegrated virus [30] that may be capable of forming viral proteins. Further, the duration of treatment would need to take into account the slow turnover rate of glial cells within the brain. Initiation of HAART soon after infection may decrease the establishment of viral reservoirs [31]; however, its impact on CNS reservoirs needs to be determined.

Suggestions for future directions

It is imperative that a better understanding of the viral reservoir in the brain be achieved in the antiretroviral era. An estimate of the number of cells infected in the brain will be important, as an immune attack against a large number of cells would be very detrimental. Unlike other organs, the brain is encased in a bony cavity with little or no room for expansion; hence, any inflammation in the brain could lead to substantial brain injury. For example, activated T cells can cause neuronal injury by the extracellular release of granzyme B [32,33]. Microglia and astrocytes are long lived with very little turnover; hence, elimination of a significant number of these cells could have profound effects on cerebral function. Nonetheless, if an immune-mediated elimination of reservoirs in the brain is to go forward, then strategies need to be developed for gradual elimination of the reservoirs. Anti-inflammatory approaches to block the secondary effects of cytotoxic T-cell-mediated neuronal injury need to be developed. In-vitro studies are needed to determine whether cytotoxic T cells can eliminate viral infection in macrophages/microglia and in astrocytes. The diversity and evolution of the virus in the brain needs to be understood, if we are to engineer T cells that will

recognize the diversity of viral epitopes in the brain. For pharmacological approaches or other biological but nonimmune-mediated approaches that target the virus, the viral load with the amounts of integrated and unintegrated proviral DNA in the brain needs to be determined in patients on prolonged ART. Further, the timing of these approaches in relationship to the duration of infections needs consideration as well. Early in the course of infection, the viral reservoirs may be small, and the virus may not have had a chance to evolve to a significant degree in various compartments, although this remains speculative. Further, it is likely that the chances of developing an immune reconstitution syndrome would also be minimized.

In summary, phenomenal progress has been made in recent years in understanding the biology of HIV infection; however, if eradication of this virus is to be achieved, some very fundamental questions of the reservoir in the brain need to be addressed. Or else the CNS reservoirs may not be cleared and more importantly, several of the approaches being considered could potentially have devastating consequences on the brain.

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