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Where does HIV hide? A focus on the central nervous system

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Abstract

Purpose of review—To review the literature on infection and evolution of HIV within the brain in the context for understanding the nature of the brain reservoir and its consequences.

Recent findings—HIV-1 in the brain can evolve in separate compartments within macrophage/microglia and astrocytes. The virus adapts to the brain environment to infect these cells and brain-specific mutations can be found in nearly all genes of the virus. The virus evolves to become more neurovirulent.

Summary—The brain is an ideal reservoir for the HIV. The brain is a relatively immune privileged site and the blood–brain barrier prevents easy access to antiretroviral drugs. Further, the virus infects resident macrophages and astrocytes which are long-lived cells and causes minimal cytopathology in these cells. Hence as we move towards developing strategies for eradication of the virus from the peripheral reservoirs, it is critical that we pay close attention to the virus in the brain and develop strategies for maintaining it in a latent state failure of which could result in dire consequences.

Keywords

astrocytes; brain; dementia; HIV; microglia; mutations; reservoirs; viral evolution

INTRODUCTION

HIV compartmentalization is well known to occur in the brain, cerebrospinal fluid (CSF), and genital tract, although viral compartmentalization also occurs within the gut, lung, liver, kidney, and breast milk [1]. Although it was recognized soon after the discovery of the virus that HIV establishes a reservoir in the brain and causes neurocognitive dysfunction, much emphasis was put on determining the mechanisms of neuronal injury and many approaches to protecting neurons were developed. However, clinical studies with neuroprotective agents have been of little or no clinical benefit. Although combined antiretroviral therapy (cART)

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Conflicts of interest

There are no conflicts of interest.

has had a significant impact in decreasing the severity of neuronal injury, milder yet clinically significant neurocognitive impairment persists despite optimal treatment with cART [2■■]. In these individuals the cells in the CSF show evidence of immune activation [3■]. A likely possibility is that the viral reservoir in the brain is not fully controlled; however, intensification of cART does not seem to have impact on the low-level viral production [4]. For these reasons, a better understanding of the viral reservoirs in the brain is critical so that new approaches may be developed to control the infection.

Recently, much enthusiasm has developed for the possibility of developing a cure for HIV infection. The successful eradication of the virus in at least one individual who in the course of treatment for leukemia received chemotherapy, radiation, and stem cell transplant from a donor with CCR5 mutation has suggested the possibility that an approach could potentially be developed to rid the body of the virus [5]. However, such an approach requires a cautionary note, as several of the proposed approaches could potentially have a devastating effect on the brain [6]. For such approaches to be successful, a thorough understanding of the timing of HIV entry into the brain, the cell types infected, and the rate of turnover of the infected cells is important so that tailored approach to viral control or eradication may be considered.

TIMING OF VIRAL ENTRY INTO THE BRAIN

In the absence of technology for imaging the HIV reservoirs, extrapolations of viral entry into the central nervous system (CNS) have been made by viral detection in the CSF. A recent study shows that HIV RNA could be detected in the CSF in 15 of 18 patients as early as 8 days after estimated HIV transmission. On average, the CSF HIV RNA level was 2.42 log(10) copies/ml lower than that in plasma. There were no cases in which the CSF HIV RNA level exceeded that in plasma [7]. However, the presence of virus in the CSF does not necessarily mean that it has established reservoir in the brain. In fact in some patients even at autopsy no evidence of any productive viral infection of other neuropathological changes could be found even in the preantiretroviral era. This situation suggests that timing of viral invasion into the brain may be variable and there might be a window of opportunity to clear the virus from the periphery before it enters the brain at least in some individuals.

Infection of macrophages and microglia

Our knowledge of brain infection by HIV *in vivo* is limited to the study of terminal stages of the disease since the availability of brain tissues is almost exclusively limited to autopsy specimens. It is well established that macrophages and microglial cells are a critical reservoir of HIV in the brain. Most productive infection occurs in these cells in the brain. As there are no resident lymphocytes in the brain, it is not surprising that the virus adapts and evolves in the brain to efficiently infect these cell types. HIV-infected macrophages show regional preferences within the brain. In individuals with HIV-associated dementia (HAD) the HIV-infected macrophages are predominantly along the midline structures of the brain (thalamus, medulla, hippocampus, cerebellum, basal ganglia, and pons) and the mesial temporal lobe [8]. Further, in cases where HIV-infected macrophages can be detected, the putamen and thalamus are involved in every case [9]. The reasons for this regional

preference are not known. A recent study shows that brain-derived HIV envelopes have enhanced ability to interact with CD4 compared with lymph node-derived envelopes, thereby permitting HIV entry into cells such as macrophages that express low levels of CD4 [9,10■■]. These brain-derived envelopes adopt specific conformations that interact with CD4 and the N-terminus of CCR5 in tandem allowing efficient entry of the virus. It has only recently been recognized that there is a rich collection of macrophages in the meninges within the subarachnoid space. These meningeal macrophages also get infected with HIV and this may be a yet important reservoir for the virus [10■■].

Infection of astrocytes

Whereas it is well established that macrophages and microglia are the major sites of HIV-1 replication in the CNS, with HIV-1-specific antigens co-localizing with microglial, multinucleated giant cells and perivascular macrophages in HIV-1-specific CNS lesions [11–13], the role of astrocytes in HIV-1-associated neurocognitive disorders and as a possible HIV-1 reservoir is more controversial.

Astrocytes are neuroglial cells that arise from the neuroectoderm during development and are the most abundant cell population within the CNS. They have multiple biological functions, including biochemical support of brain endothelial cells essential to maintaining the blood–brain barrier, production of neurotrophic immune-modulatory factors, neuronal homeostasis, and repair and scarring following CNS trauma [14].

The presence of reactive astrocytosis is a significant pathological feature in HAD. However, due to the restricted nonproductive infection of astrocytes, in-vitro [15,16] current models of the neuropathogenesis of HAD do not favor a major role for astrocytes [17]. Stimulation of astrocytes with cytokines that may be found in increased levels in late stage disease can result in activation of virus from infected astrocytes *in vivo* with the potential to initiate new rounds of infection [18]. Additionally, a subpopulation of latently infected astrocytes undergoes apoptosis *in vivo* which correlates with the severity of HAD [19].

The mode and extent of astrocyte infection remains controversial with only trace amounts of CD4 receptor being demonstrated on the cell surface. The endocytic uptake of HIV virions and subsequent ‘pass on’ of virus to susceptible cells by astrocytes has been reported [20]. Alternatively astrocytes may be efficiently infected by cell-to-cell contact with HIV-infected lymphocytes [21]. This may occur in the cerebral vasculature where the astrocytic foot processes may be in contact with circulating lymphocytes or in the context of immune reconstitution inflammatory syndrome when there is infiltration of lymphocytes within the parenchyma of the brain. Astrocytes have been demonstrated to contain integrated HIV *in vivo* [22]; however, the low frequency of astrocyte infection *in vivo* in early studies utilizing in-situ PCR [12,23] questioned their importance for infection of the CNS. Recent studies using sensitive molecular techniques have demonstrated the frequency of astrocyte infection to be up to 20%, with the frequency of infection correlating with both the severity of HIV encephalitis and proximity to perivascular macrophages and multinucleated giant cells [24]. Additionally, persistent infection of astrocytes with low level virus production has been demonstrated *in vivo* [25,26].

Whether cells/tissues can contribute to a viral reservoir ultimately depends on their capacity to harbor integrated viral genomes that can be reactivated to produce infectious virus capable of reinitiating disease [27]. Whereas studies have shown astrocytes, in significant numbers, do contain integrated genomes [23,25], reactivation and virus production from these cells remain unclear. However, it has been determined that these cells can 'pass on' infectious virus possibly produced from susceptible cells in the CNS including macrophages and microglia, ultimately contributing to the spread of CNS infection. Additionally, despite evidence supporting the production of virus by astrocytes, it is well established that astrocyte are capable of producing the HIV regulatory protein Tat. Tat-encoding mRNA has been detected in the CNS of HAD and non-demented HIV-infected individuals [28,29]. Tat protein has been detected in perivascular brain macrophages in AIDS patients [29] and latently infected astrocytes have been shown to secrete HIV-1 Tat [30] and accumulate Tat mRNA transcripts [31]. It remains unknown as to what cellular factors in astrocytes prevent viral replication despite the production of Tat. However, Tat is also a potent neurotoxin and has been demonstrated to cause neuronal damage in both in-vitro and in-vivo experimental systems (reviewed in [32]). Thus strategies aimed at activating latent virus from latently infected cells may have the potential to activate transcription of HIV-1 in astrocytes, and whereas little evidence supports the production of infectious virions, the production of Tat could potentiate neurological complications for the patient.

Viral evolution in the brain

Studies demonstrating that HIV sequences in the brain cluster separately to those obtained from other organs strongly support the brain as a unique reservoir of HIV. The observed compartmentalization suggests that the virus evolves in the brain over time and thus may acquire unique properties. As discussed above, the envelope protein evolves to become more macrophage tropic [33], have a low positive charge [34], and unique brain-specific mutations have been identified [35]. Some of these properties show that the 'macrophage tropic' strains of HIV in the brain are functionally different compared to 'macrophage tropic' strains derived from the immune system [34]. Similarly, the Tat protein of HIV also evolves in the brain but maintains its HIV activation properties [36], but Tat sequence differences from the brain may vary in their neurotoxic potential [37]. Another recent study examined brain-derived HIV nef sequences and found that there were unique sequences in patients with dementia compared with those without [38]. Another study showed that normalized nonsynonymous substitutions in the *nef* gene were more frequent at the divergence of lymphoid and brain sequences, suggesting a stronger adaptive selection in brain compared to lymphoid tissue [39]. The brain-specific nonsynonymous substitutions were found in regions of functional importance which included a cytotoxic T-cell epitope, the PACS1-binding motif, or regions involved in activation of tyrosine kinase Hck. These results reflect altered requirements for efficient replication in macrophages [39]. Further, viral sequences derived from brain macrophages and astrocytes show compartmentalization, suggesting that cell-specific evolution occurs in the brain [40]. Viral sequencing shows that the major tissue harboring virus from both the brain and peripheral tissues is the meninges which suggest that HIV-1 is capable of migrating out of the brain, and the meninges are the most likely primary transport tissues [10■■]. A publicly available database (http://www.HIVBrainSeqDB.org) of envelope sequences has recently been created which contains

2517 envelope sequences from 90 patients, obtained from 22 published studies. 1272 sequences are from brain; the remaining 1245 are from nonbrain tissues [41]. This resource can be useful for studying HIV neuropathogenesis.

The effect of ART on evolution of HIV in the brain is not well understood. Since the penetration of antiretrovirals is restricted by the blood–brain barrier, one might expect that antiretroviral-resistant sequences would be less frequent in the brain and hence these drugs might drive the compartmentalization of HIV in the brain. Combined ART puts evolutionary pressure on the virus such that it evolves from using co-receptor CCR5 to co-receptor CXCR4. This switch may appear later in the CNS compartment compared to the periphery [42]. Further even in patients on antiretrovirals analysis of HIV-1 nef, gp120, and gp41 sequences showed maximal viral evolution within brain tissues of individuals with HAD compared with non-HAD cases [43].

CONCLUSION

The brain is an important reservoir for HIV. The virus resides in brain macrophages or microglial cells and astrocytes which may be some of the longest living cells in the body. Here the virus can escape the immune system and antiretrovirals. The persistence of HIV in the brain has important consequences. A concern is that under the influence of antiretrovirals, HIV in the brain could evolve to become more adaptable to the brain and more neurovirulent. The evolved virus can be released to the circulation and thus spread to other tissues. This is particularly important today since multiple strategies are being considered to eradicate the virus from the periphery. If these efforts are successful in riding the virus from immune reservoirs but not the brain not only would the neurotropic virus reseed the periphery but the refurbished immune system may target the virus in the brain leading to a devastating encephalitis called CNS-immune reconstitution inflammatory syndrome (reviewed in [44]. Hence future efforts need to focus on either prevention of viral entry into the brain or development of strategies that would keep the virus in a latent state in the brain as purging the virus from brain cells may not be possible with available strategies.

Acknowledgments

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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 250).

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KEY POINTS

- In the brain, HIV infects macrophages and astrocytes where it may reside for extended periods of time.
- The virus evolves in these cells to acquire the ability to infect cells with low CD4 expression and becomes more neurovirulent.
- Eradication of peripheral lymphoid reservoirs, if successful, may result in lymphocyte-mediated encephalitis if a viral reservoir has been established in the brain.
- Therapeutic strategies are necessary that would maintain the virus in a latent state within the brain.