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CNS reservoirs for HIV: implications for eradication

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Abstract

Controversy exists as to whether the central nervous system (CNS) serves as a reservoir site for HIV, in part reflecting the varying perspectives on what constitutes a 'reservoir' versus a mere site of latent viral integration. However, if the CNS proves to be a site of HIV persistence capable of replicating and reseeding the periphery, leading to failure of virological control, this privileged anatomical site would need dedicated consideration during the development of HIV cure strategies. In this review we discuss the current literature focused on the question of the CNS as a reservoir for HIV, covering the clinical evidence for continued CNS involvement despite suppressive therapy, the theorised dynamics of HIV integration into the CNS, as well as studies indicating that HIV can replicate independently and compartmentalise in the CNS. The unique cellular and anatomical sites of HIV integration in the CNS are also reviewed, as are the potential implications for HIV cure strategies.

Keywords

HIV reservoirs; CNS compartmentalisation of HIV; HIV cure; viral latency

Introduction

Moving from the era of HIV control with antiretroviral therapy (ART) to the hope-filled possibility of an HIV cure requires that the sites of viral persistence be understood in order for these reservoirs to be immunologically controlled or eradicated. However, there is a debate on whether the central nervous system (CNS) requires consideration when developing HIV cure strategies, joining portions of the gut, bone marrow and lymphoid tissue as an anatomical reservoir of the virus, where replication-competent virus is integrated, autonomous replication can occur, and from which systemic viral reseeding could emerge. This question unearths the disputed use of the term 'reservoir', which carries contextually varying definitions. On the most basic level, a reservoir of HIV is a site of persistent HIV DNA integration that has the potential to reactivate and replicate viral RNA, perhaps even in the context of suppressive antiretroviral therapy. Alternately, an HIV reservoir may be an anatomical site of ongoing, low-level viral replication despite

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appropriate, suppressive therapy. In thinking towards the projected needs of HIV cure strategies, however, the most rigorous and relevant definition of an HIV reservoir may speak to its functional potential: a site where integrated HIV DNA can not only reactivate, but also cause systemic rebound and failure of virological control. As a specialised anatomical and immunological compartment, the CNS is not composed of just brain tissue, but also choroid plexus, meninges, cerebrospinal fluid (CSF), each of which may serve differential roles as reservoir sites for HIV. While further research is needed to fully characterise its involvement, existing literature supports that the CNS requires consideration when contemplating reservoir sites of HIV.

Evidence of continued CNS perturbation despite ART

Evidence from the clinical realm indicates that combination antiretroviral therapy (ART) alone has not eliminated the CNS sequelae of HIV infection, as one may anticipate if the CNS were not a reservoir site. Although the advent of ART has led to reduced prevalence of HIV-associated dementia (HAD), neurological perturbations such as neuropsychological deficits, continued CNS inflammation, and markers of neuronal injury, all can persist despite suppressive treatment. The CHARTER study of 1,555 HIV-infected individuals in the ART era found that 52% met criteria for an HIV-associated neurocognitive disorder (HAND) based on neuropsychological testing, with a greater prevalence of the least clinically severe subtypes [1]. Although 71% of these subjects were on ART, a notable 59% of subjects had not achieved plasma viral suppression and 34% still had detectable HIV RNA in CSF, with some participants having potentially confounding comorbidities including substance use [1]. Eliminating the variables of time on ART, effective plasma viral suppression and substance use, a separate study of 116 men with advanced HIV, all on suppressive ART for an average of 5 years, found 18.1% with neuropsychological impairment [2]. Additionally, among individuals with HIV-associated cognitive impairment started on ART, persistent neuropsychological deficits were observed in 62.8% (59/94) when re-tested a mean of 60 months later [3]. The heterogeneity of ART penetration into the CNS provides an opportunity to examine whether direct drug exposure to this compartment is important for neurological outcomes, which would imply that localised control of viral replication is necessary. In 2,636 HIV-infected subjects with plasma viral suppression below 50 copies/mL for 6 or more weeks, increased CNS-penetration effectiveness (CPE) score of ART regimen was positively associated with subjects' neuropsychological performance for individuals on more than three antiretrovirals (P=0.004) [4]. Whether CPE has clinical relevance for most patients with chronic HIV remains controversial, with two studies failing to demonstrate efficacy [5,6].

The argument for a CNS reservoir for HIV is furthered by the observed persistence of immune activation in the CNS despite effective suppression of viral replication in the periphery. In a longitudinal study of 28 subjects treated with ART for 2 years with resulting plasma and CSF virological suppression, only 55% had normalisation of CSF neopterin, a marker of activated macrophages and dendritic cells [7]. This finding was extended in 15 subjects on suppressive ART for more than 3.5 years, wherein 60% continued to have elevated CSF neopterin [8]. These studies raise the possibility that CNS inflammation could be driven by modest levels of CNS viral replication below the limits of assay detection. A

similar proportion of subjects (57%) had persistently elevated CSF neopterin in the context of undetectable CSF HIV RNA using an assay with a 2.5-copies/mL lower limit of detection [9]. However, this and another study demonstrated that the CSF neopterin level associates with low-level CSF HIV RNA, as patients with greater than 2.5 copies/mL had higher CSF neopterin levels than those with fewer than 2.5 copies/mL [10]. Only one study has preliminarily shown an association between elevated CSF neopterin levels and cognitive performance in treated patients [11], so the significance of CNS immune activation to HAND in suppressed individuals remains unclear.

Neuroimaging data reinforces the findings of CSF studies, demonstrating persistent brain inflammation in patients on ART. Compared to uninfected controls, 124 HIV-infected, neuro-asymptomatic individuals on 12 weeks of stable ART had neuroimaging evidence of inflammation as demonstrated by elevated myoinositol/creatinine in all brain regions and choline/creatinine in the basal ganglia and mid-frontal cortices, even though HIV RNA was undetectable in plasma in 79% of subjects and in CSF of 62% of subjects [12]. Persistent neuroinflammation during ART has also been associated with neurological damage, through assessment of neurofilament light chain (NFL), a component of myelinated axons released into the CSF during neuronal breakdown. In a study of 252 HIV-infected subjects, CSF NFL was elevated compared to age-matched HIV-uninfected controls in 8% of virally suppressed subjects [13]. While ART reduced NFL levels in 63% of subjects started on therapy ($P<0.01$), virally supressed subjects showed higher NFL levels than HIV-negative controls [13]. Collectively, these studies suggest that functional and biological markers of CNS inflammation and injury are reduced, but not necessarily eliminated by ART, even with plasma suppression to limits of standard assay detection. This perturbation of the CNS despite ART may reflect persistent HIV integration or even ongoing low level replication within cells and tissues of the CNS, thus suggesting a CNS reservoir for infection.

Timing and dynamics of CNS reservoir formation

Identification of the pathophysiological events of CNS HIV infection that may underlie establishment of viral reservoirs is challenging, as there are practical limitations to comprehensive CNS assessments of HIV-infected individuals throughout the course of infection. In contrast, animal models of CNS infection allow direct evaluations of both RNA and DNA in CSF and brain tissue over time, in addition to precise timing of intervention with ART. The accelerated SIV model in pig-tail macaques, in which 90% of animals display CNS manifestations at 3 months post infection, parallels the clinical and pathological course of severe CNS disease and HIV encephalitis in humans, though with a more rapid course [14,15]. Although there are limitations to extrapolating findings in this accelerated animal model to humans living with HIV, it allows for essential assessments of early SIV replication in the CNS, as demonstrated by SIV RNA levels, as well as signs of SIV DNA integration into cells.

In a study of 18 accelerated SIV-infected macaques left untreated and sacrificed at intervals of either 10, 21 or 56 days post infection, those in the acute, symptomatic phase (day 10) initially showed detectable SIV RNA in brain tissue indicative of productive viral replication, although SIV RNA was absent in the majority of animals sacrificed at or after

day 21 [16]. In contrast, SIV RNA in the CSF was consistently found across all three time points of the 56-day span of infection, indicating possible replication from infected cells in the meninges or choroid plexus, or a spill over of SIV RNA from replicating virus in the periphery, due to breakdown in the blood–brain barrier [16]. Importantly, viral DNA in macaque brain tissue also persisted across time points in the initial 56 days of infection, implying that SIV integrates in brain cells in the acute phase of the disease and subsequently remains there. More recent work in this macaque model extends these findings by showing that ART started 12 days after infection does not change SIV DNA levels in the basal ganglia or parietal cortex, suggesting that early treatment does not impact integration of SIV DNA into brain tissue, and thus does not limit formation of potential CNS SIV reservoirs [17]. Others have narrowed the window of ART initiation to just 4 days post infection in this animal model, and similarly found no decrease in SIV DNA in brain tissue [18]. However, these studies used ART regimens that are not particularly CNS penetrant, and it is possible that medications with better CNS exposure may exert greater influence on SIV DNA integration into parenchymal cells.

To further examine establishment of SIV within the CNS, Matsuda and colleagues studied brains of four macaques with SIV encephalitis, meningitis, or a combined syndrome, and used laser capture microdissection to isolate viral RNA from brain parenchyma versus the meninges [19]. Sequencing of this viral RNA revealed distinct SIV populations in the parenchyma compared to the meninges among the conventional progressors of disease, suggesting that studies sampling SIV or HIV RNA in CSF may not accurately reflect SIV or HIV populations present in the brain parenchyma. If these studies in macaque SIV can be extrapolated to HIV, they would suggest that HIV RNA may enter the CNS quite early in the course of infection, rapidly integrate as HIV DNA into distinct CNS tissues, and may persist despite ART. However, it is reasonable to question if these findings in primate models, some predisposed to the extreme neurological sequelae of HIV, accurately reflect the neuropathogenesis occurring in humans with HIV.

While we are limited in understanding the early CNS pathophysiology that probably influences the later neurological sequelae of the disease in HIV-infected patients, human studies also indicate that HIV RNA can rapidly enter the CNS within days of infection. Early in the AIDS epidemic, a case report identified a patient with an accidental iatrogenic exposure to HIV, resulting in death 15 days later. On autopsy, HIV nucleic acid was found in brain tissue [20]. Systematic assessment of individuals in acute HIV, the initial days to weeks of infection, has revealed HIV RNA detection in CSF as early as 8 days after estimated infection, as well as initiation of processes of CNS immune activation associated with neuropathogenesis and persistent infection during this period [21]. However, in a study of 42 subjects with acute HIV, 10 were found to not yet have quantifiable CSF HIV RNA and were in the earliest stages of infection, Fiebig I–III [22]. In humans it is unknown what factors modulate transit of HIV RNA from the periphery into the CNS, or the dynamics of HIV DNA integration into various CNS tissues.

Persistent CNS HIV and CSF viral escape during ART

A range of human studies supports the concept that HIV can persist in the CNS despite adequate therapy. In an autopsy study of 196 HIV-infected individuals from the ART era, 57% of whom were on active ART within 1 year of death, subjects with premorbid HAND and brain tissue displaying pathological findings of HIV encephalitis (HIVE) and microglial nodule encephalitis (MGNE) had higher brain HIV RNA and DNA compared to 33/196 subjects without premorbid HAND and no pathological findings of HIVE or MGNE [23]. Interestingly, these 33 subjects without neuroclinical or neuropathological findings of HIV had detectable levels of HIV RNA and DNA in the brain. A recent study examined the brains of 10 HIV-infected subjects on ART prior to death (range: 3 months to 7.9 years) and found HIV DNA in the brain tissue of four of the subjects. Although three of the four were known not to have achieved plasma viral suppression, their plasma HIV RNA levels were relatively low (range: 169–4400 copies/mL) in the months prior to death [24].

While these post-mortem studies are confounded by heterogeneity of subjects in terms of ART exposure, regimen and duration, among other factors, studies in living subjects suggest the possibility of low-level CNS HIV persistence during suppressive ART. In a raltegravir intensification study, blood and CSF samples from HIV-infected subjects with initial plasma and CSF HIV RNA below 50 copies/mL were re-examined with a highly sensitive single copy assay (SCA). Thirteen of 17 individuals had detectable plasma HIV RNA by SCA while one of 16 had detectable CSF HIV RNA by SCA [25], revealing the possibility of low-level CNS viral replication undetectable by standard assays in neurologically stable individuals. Additionally, discordant plasma and CSF HIV RNA levels have been noted in asymptomatic patients. In a retrospective study, seven of 69 individuals on modern ART regimens for greater than 6 months with plasma HIV RNA below 50 copies/mL had detectable CSF HIV RNA (interquartile range: 54–213 copies/mL), a phenomenon termed asymptomatic viral escape [26].

Perhaps the most compelling evidence for a CNS reservoir of independently replicating HIV is the example of symptomatic CNS viral escape in patients on systemically suppressive ART regimens. A recent case series described 14 HIV-infected individuals with ‘satisfactory’ plasma HIV control who developed an HIV-associated encephalitis, of whom 11 were on ART and six had undetectable plasma viral loads within the preceding 6 months [27]. Thirteen of the 14 patients were found to have a CD8+ lymphocytic encephalitis, of whom 10 had pathological specimens from brain biopsy showing perivascular infiltration of these CD8+ cells that also manifested on MRI as multiple linear gadolinium-enhanced perivascular lesions [27]. Canestri and colleagues reported on 11 HIV-infected patients identified over the course of 5 years from a busy ID clinic (∼6000 patients) who were on stable ART for at least 10 months, most of whom had plasma HIV RNA suppression below 50 copies/mL, who developed acute or subacute neurological symptoms and were found to have elevated CSF HIV RNA (range: 558–12,885 copies/mL), frequently in the context of apparently sequestered genotypic resistance in CSF HIV [28]. A more recent paper found subjects with acute or subacute development of neurological symptoms along with elevated markers of CSF markers inflammation and CSF HIV RNA levels consistent with CNS escape (range: 134–9056 copies/mL), including five subjects with plasma HIV RNA

suppressed below 50 copies/mL at the time of neurological presentation [29]. Collectively, these studies imply that HIV can replicate independently in the CNS, despite ART-induced control of systemic viral replication. It is plausible that this CNS-specific viral replication emerges from reactivated DNA reservoirs in the brain, perhaps formed early in the course of the disease.

Identifying CNS compartmentalisation of HIV through viral sequencing

As HIV rapidly mutates, viral sequencing serves as a robust technique to determine the origins, evolution and trafficking dynamics of HIV. Different viral populations in an individual can be mapped by examining phylogenetic relationships of HIV variants between biological compartments. Examinations of CNS compartmentalisation of HIV have revealed that HIV replication and evolution may occur in the CNS independent from that in the periphery. In the macaque model of accelerated CNS SIV, two animals sacrificed at 56 days post infection with posited re-emergence of SIV RNA in brain tissue had RNA genotypes more similar to HIV DNA genotypes in brain parenchyma than to those in peripheral blood mononuclear cells [16]. This suggests that CNS HIV RNA originated from viral reactivation from brain-based cellular reservoirs, rather than from SIV RNA transit from the periphery. In humans, cross-sectional studies have revealed significant CNS compartmentalisation, where sequences recovered from CSF encoding for envelope or other HIV genome regions may be phylogenetically distinct from those in blood, especially in late-stage HIV infection and in the context of HAD [30,31]. Furthermore, longitudinal studies demonstrate that compartmentalised HIV species may locally evolve only within the CNS, providing further evidence of a CNS source of viral replication independent from that in the blood [29]. Although local CNS tissue replication has been presumed to be a late-stage occurrence associated with prolonged infection with HIV or HIV encephalitis, more recent data has indicated that CNS compartmentalisation can be detected during the first year of infection in adults and children with HIV [32,33]. Importantly, CNS compartmentalised HIV variants can persist and even evolve only within the CNS starting during the first year of infection, suggesting that the nervous system may be a site of local HIV replication beginning early in the course of disease [34].

Analytic treatment interruption (ATI) offers the opportunity to examine re-emerging viral populations in distinct compartments, possibly providing insight into the cellular and tissue sources of rebounding HIV. In a study of five patients with CSF sampling following ATI, four of five experienced a steeper rise in CSF HIV RNA than in plasma, possibly due to virus independently replicating in the CNS compartment [35]. Future studies closely examining viral sequences of HIV RNA in CSF and blood pre-ART and post-ATI should help reveal origins of resurgent HIV in these tissues.

Perhaps the most important question that viral sequencing can address is whether HIV originating in the CNS has the capacity to seed the peripheral tissue, and even cause failure of systemic control in otherwise virologically suppressed individuals. While this has not been evidenced in a living subject on therapy, an extensive, multisite viral sequencing study of tissue from deceased HIV-infected patients showed a unique, apparently CNS-derived HIV sequence present in peripheral tissue in one subject, suggesting that HIV can exit the

CNS [36]. Studies are needed that examine the potential compartmentalisation and, in particular, evolution of HIV in the CNS in patients on suppressive ART. Technical limitations, such as difficulty amplifying low quantities of HIV RNA, have made this challenging [37].

Cellular and anatomical site of CNS HIV reservoirs

Understanding the cellular populations that harbour latent HIV DNA in the CNS is relevant to addressing possible cure strategies, as patterns may differ from those in the periphery. While entry assays based on the HIV envelope from the blood of subjects with HAD have revealed plasma HIV genetically most adept at entering T cells, viral variants found in the CSF of these subjects were observed to be both T cell and macrophage-tropic, with macrophage tropism largely confined to virus in the CNS [30,38]. Although T cell populations may be critical sources of CNS HIV in early infection [34], perivascular macrophages and microglia are considered the primary cells that harbour HIV replication in chronic HIV infection [30,38]. In thinking towards cure strategies, both CNS macrophage and microglia cell populations can be long-lived, and microglia are thought not to undergo renewal from peripheral sources during an individual's life-span [39,40]. A number of studies indicate that HIV may also invade and disrupt the function of astrocytes, the most common cell type in the brain [41,42]. In brain samples from deceased patients with CNS manifestations of HIV (HAD and/or HIVE), up to 20% of astrocytes were found to harbour HIV DNA, which is noted to be similar to the proportion of infected lymphocytes found in lymph nodes of AIDS patients [43]. However, other data suggest that, while astrocytes may integrate HIV DNA, they are not capable of replicating and producing infection [44].

It should also be considered that the brain serves as a privileged site of access, with both a blood–brain barrier mediating access into the brain parenchyma and the blood–cerebrospinal fluid barrier serving to referee entry into the CSF via the choroid plexus. As suggested in animal models, there is evidence that the CNS may not comprise one single anatomical reservoir, but instead the tissue compartments within the brain parenchyma, meninges and choroid plexus may form differentially distinct reservoir sites. In a macaque model of CNS HIV, CSF SIV RNA was present at day 21 of infection, although no SIV RNA was detected in the brain parenchyma at this time point, suggesting unique viral replication in the choroid plexus or meninges, or trafficking from blood leading to this CSF-specific finding [16]. Macaque models have also revealed distinct viral RNA populations in the brain parenchyma compared to the meninges in certain clinical subsets of animals [19]. Supporting this notion, a pathological study of human brains found HIV-infected cells in the choroid plexus in eight of 14 subjects with AIDS, including two of seven individuals who were neurologically asymptomatic prior to death [45]. CNS reservoirs of HIV may be varied, composed of particular cell types within specific tissue compartments across the brain parenchyma, choroid plexus, meninges and CSF, and primary sites of HIV replication within the CNS may change throughout the course of infection.

Implications for HIV cure strategies

It is worth reviewing the different approaches made towards HIV cure, considering the plausibility of CNS reservoirs for HIV. Notably, the sterilising HIV cure achieved in the Berlin patient, consisting of haematopoietic stem cell transplant from a CCR5 homozygous delta 32 donor, did not require specific targeted therapy for the CNS, other than a single treatment of whole body irradiation [46,47]. There are several possibilities for the reason behind this: he graft versus host disease he sustained was advantageous in destroying affected host cells in the CNS; the single dose of irradiation impacted putative CNS reservoir cellular turnover; his latent viral reservoir in the CNS was incapable of replication; or that he had never formed specific HIV reservoirs in the CNS prior to his transplantations.

The successful virological control, or a ‘functional cure’ observed in a portion of the Visconti cohort of acutely infected and treated patients who later underwent treatment interruption suggests that reservoirs, including those in the CNS, may not form as rapidly as seen in the accelerated macaque model, or that the reservoirs remain small enough to maintain virological control [48]. This unique group of study participants argues that the initiation of therapy in the acute phase of HIV may dramatically alter reservoir formation, although the CNS was unfortunately not examined in these subjects. The ongoing SEARCH 010 cohort in Bangkok, aimed at identification and treatment of individuals during the earliest stages of acute infection, will offer an unprecedented opportunity to examine potential functional cure and prevention of CNS reservoirs through early treatment, as planned studies include ATI in some individuals coupled with detailed CSF studies and viral sequencing (clinicaltrials.gov: NCT00796146)[21].

When considering the existing approaches to HIV eradication and control through the lens of potential CNS reservoirs, issues emerge as to whether these strategies will adequately penetrate the CNS and impact the appropriate cell populations. Peripherally focused strategies targeting CD4+ T cells may not address the monocyte/macrophage population thought of as the primary substrate for HIV persistence and replication in the CNS. Additionally, it is not known whether strategies employing histone deacetylase (HDAC) inhibitors to reactivate latent viral reservoirs have differential effects in CNS cell populations, or may pose particular risk to the brain through induction of local immune activation and neuronal injury.

To maximally inform the global agenda towards HIV cure, studies are still needed to determine where and when HIV reservoirs are formed in the CNS, as well as what specific strategies are needed to eliminate these sites of HIV persistence, or to maintain their long term immunological control.

Conclusions

The brain serves as a specific site of infection and compartmentalisation in HIV, and evidence from SIV models suggests that HIV DNA integrated in the CNS has the capacity to reactivate and produce viral RNA. Additionally, low levels of continual HIV replication can occur in humans in the CNS despite suppressive antiretroviral therapy, most likely from

local viral reservoirs. What remains to be seen is whether CNS-derived HIV DNA has the capacity to reactivate, replicate and reseed the periphery. As the cellular and anatomical sites of HIV persistence differ in the CNS compared to the rest of the body, it will be critical that emerging HIV cure strategies consider CNS-specific approaches, and there are continued efforts to investigate the tissues and mechanisms supporting the CNS as a reservoir site of HIV.

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