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Is the central nervous system a reservoir of HIV-1?

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Abstract

Purpose of the review—To summarize the evidence in the literature that supports the CNS as a viral reservoir for HIV-1 and to prioritise future research efforts.

Recent findings—HIV-1 DNA has been detected in brain tissue of patients with undetectable viral load or neurocognitive disorders, and is associated with long-lived cells such as astrocytes and microglia. In neurocognitively normal patients, HIV-1 can be found at high frequency in these cells (4% of astrocytes and 20% of macrophages). CNS cells have unique molecular mechanisms to suppress viral replication and induce latency, which include increased expression of dominant negative transcription factors and suppressive epigenetic factors. There is also evidence of continued inflammation in patients lacking a CNS viral load, suggesting the production and activity of viral neurotoxins (for example Tat).

Summary—Together, these findings provide evidence that the CNS can potentially act as a viral reservoir of HIV-1. However, the majority of these studies were performed in historical cohorts (absence of cART or presence of viral load) which do not reflect modern day patients (cART-treated and undetectable viral load). Future studies will need to examine patient samples with these characteristics to conclusively determine if the CNS represents a relevant and important viral reservoir.

Keywords

HIV-1; CNS; reservoirs; latency; cure

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Introduction

The major barrier to the eradication of human immunodeficiency virus type 1 (HIV-1) is the persistence of latently infected cells located in viral reservoirs in different anatomical sites throughout the body [1,2]. While the most significant and well studied reservoir to date resides in resting $CD4^+$ T cells, reservoirs have also been proposed in tissues including the brain, blood, gut-associated lymphoid tissue, bone marrow and genital tract [3]. The advent of combination antiretroviral therapy (cART) has done little to reduce the size of these viral reservoirs, because it mainly targets replicating virus. These viral reservoirs have become the focus of a renewed research effort to directly target these reservoirs in the hope that if we can eliminate them, we will be one step closer to curing HIV-1. However, while the majority of the research effort is directed against the largest viral reservoir contained within resting $CD4^+$ T cells, limited studies have been done on other viral reservoirs, including the brain. There is also contention in the literature about the relevance of the central nervous system (CNS) reservoir and whether it will be important for cure strategies. In this review we will discuss what evidence currently exists to support the CNS as a viral reservoir for HIV-1, and also use this opportunity to propose and prioritize the future work that is required to conclusively determine whether the CNS reservoir needs to be considered when designing HIV-1 eradication strategies.

HIV-1 cure

The introduction of cART resulted in the prevention of HIV-1 disease progression to AIDS in most treated patients, whilst also restoring life expectancy approaching that of the general population [4]. However, cART treatment remains life-long, with cessation eventually leading to viral rebound and disease progression [5,6]. This has sparked a concerted effort to directly address the source of the virus responsible for the viral rebound and constitutes a new area of investigation termed ‘HIV-1 cure research’, which aims to directly target and eliminate viral reservoirs [7]. HIV-1 cure can be divided into two different types, functional and sterilising cure [8]. Functional cure is defined as a reduction in the viral reservoir size to a point where patients can cease cART and live long-term in the absence of viral rebound. Patients would have low to undetectable viral load (<50 copies/ml), the virus is present but their immune system is able to suppress the virus and prevent disease progression, similar to that seen in HIV-1 elite controllers [9,10]. Sterilizing cure is defined as the complete eradication of all cells harbouring HIV-1 DNA, with no detectable viral load, or cell associated HIV-1 DNA (as described in Lewin *et al.* 2011), similar to that seen in the ‘Berlin patient’ [11]. Of these, functional cure is believed to be the more achievable outcome for most patients, with sterilising cure being significantly harder to achieve and demonstrate.

Recent case studies in the field of HIV-1 have identified several patients and patient cohorts that suggest a cure for HIV-1 may be possible. These include the ‘Berlin patient’ [11,12] the ‘Mississippi Baby’ [13] the two ‘Boston patients’ [14,15] and the ‘Visconti cohort’ [16]. The outcomes in each of these cases have been broad, with some patients displaying sterilising cure (Berlin), others functional cure (Visconti) and some patients eventually experiencing relapse with viral rebound requiring cART intervention (Boston/Mississippi). In the case of the Mississippi baby and the Visconti cohort, cART was used to initiate

treatment very early (before seroconversion), which is thought to have reduced the seeding of viral reservoirs, whilst preserving anti-HIV-1 immune function. This observation has been repeated in several other studies and highlights the potential benefits of early cART treatment to HIV-1 cure efforts [17-20]. Nevertheless, each of these cases has provided new information and strengthened the belief that a HIV-1 cure is indeed feasible.

Several challenges remain for HIV-1 cure research, which include defining and locating all potential reservoirs, determining which reservoirs are the most important, measuring the size of each reservoir, and developing ways to measure the effectiveness of eradication strategies. This information will be key to informing future studies and ensuring that any approach takes into consideration all of the variables that may have an impact on treatment outcomes.

Defining a viral reservoir

There are several characteristics which tissues/cells must meet before they can be considered to have a biologically significant role in the establishment of a HIV-1 reservoir, as defined by Blankson *et al.* 2002. First, cells must contain a replication competent integrated provirus. Second, cells must have a mechanism which allows the virus to escape from biochemical decay processes or immune mechanisms and persist for long periods of time, i.e. long-lived cells. Third, cells must have molecular mechanisms in place to suppress viral replication and establish a latent infection. Fourth, cells must be infected in significant numbers so as to contribute to the establishment of a viral reservoir. Finally, cells must have the potential to be activated to produce new viral particles that can reseed the infection [21].

Several HIV-1 reservoirs have been identified in multiple anatomical sites that harbor cells that fulfil some or all of these characteristics. These include resting memory $CD4^+$ T cells in blood [1], lymph node [22], gut-associated lymphoid tissue [23] and genital tract [24]; resting naïve $CD4^+$ T cells in bone marrow [25]; macrophages in lymph nodes [26], gut-associated lymphoid tissue [27], lung [28], kidneys [29], genital tract [30]; and astrocytes [31], microglia [32] and perivascular macrophages [31] in the CNS. Resting memory $CD4^+$ T cells constitute the largest viral reservoir for HIV-1, and these cells demonstrate all the characteristics required to be considered a viral reservoir [1]. However, for the remaining cells and anatomical sites, there is incomplete evidence that these cells/compartments fulfil all the requirements to be considered viral reservoirs. Additional work is required to conclusively show that these other potential reservoirs possess the needed criteria in order to play a biologically significant role. In the case of the CNS, there is still contention in the literature that the viral reservoir found there is relevant and meets all the necessary criteria [33,34]. Below we have summarized the evidence available that supports the CNS as a putative HIV-1 viral reservoir.

Evidence supporting the CNS as a reservoir for HIV-1

HIV-1 invades the CNS during early infection, predominantly targeting perivascular macrophages, microglia and astrocytes [35-38]. CNS cells convincingly satisfy most of the first criterion for harbouring a replication competent integrated provirus. Integrated HIV-1 provirus has been detected in all three of these cell types using fluorescence in situ

hybridisation (FISH) or laser capture micro dissection (LCM) coupled with PCR [31,32,39,40]. However, the nature of the samples used in these studies (formal fixed paraffin embedded tissues with fragmented DNA) has precluded the amplification of full-length intact viral genomes that are required to test for their replication competency. Nevertheless, the small regions that have been amplified suggest they would result in a replication competent virus if introduced into a competent backbone. Additionally, the patients studied were from historical cohorts (absence of cART or presence of viral load in the cerebrospinal fluid, CSF), which do not reflect modern day patients (cART-treated and no CSF viral load).

CNS cells are well known to experience long half-lives and exist in an environment of reduced immune surveillance, satisfying the second criterion. Perivascular macrophages have a half-life of ∼3 months [27,41], microglia have a half-life of months-years to years-lifetime [42] while astrocytes have a half-life of months-years [43,44]. The longevity of these cells enables the virus to persist for long periods of time and promotes the persistence and maintenance of a viral reservoir within these cells.

We and others have shown that CNS cells possess mechanisms to establish viral latency within these cells, satisfying the third criterion. CNS-derived viral promoters (long terminal repeat, LTR) have reduced basal transcriptional activity, which is associated with mutations within the basal/core promoter region [45]. This coupled with increased expression of transcriptional repressors in astrocytes, including Sp3, suggests CNS cells may harbour unique regulatory mechanisms that govern the establishment of latency within the CNS (Gray LR, Churchill MJ, unpublished data). An SIV study showed that IFN-β suppresses SIV LTR activity in the CNS by inducing expression of the dominant negative isoform of C/EBP-β, resulting in the establishment of latency [46]. Another study of post-mortem brain tissue from HIV-1 positive latent cases, observed an increase in CTIP2, HP1, MeCP2 and HDAC1 levels, suggesting they may play a role in the establishment of latency within the CNS [47].

Another important consideration is the frequency of infection within CNS cells, which constitutes the fourth criterion. Several studies have analyzed HIV-1 infection frequency within CNS cells, predominantly in symptomatic neurocognitively impaired patients but there are a few studies that have examined neurocognitively normal patients. HIV-1 can be found at high frequency in astrocytes, ranging from 3-11% of cells in neurocognitively normal patients, and up to 14-19% of cells in neurocognitively impaired patients [32,36]. Infected macrophages are detected to equivalent or much higher levels, ranging from 17% of cells in neurocognitively normal patients, and up to 30% of cells in neurocognitively impaired patients [32]. Infected microglia were also detected, ranging from 14% of cells in neurocognitively normal patients, and only 9% of cells in neurocognitively impaired patients [32].

The final criterion will probably prove the hardest to show experimentally, the ability to reactivate the integrated provirus to produce new progeny virions. At least within an *in vitro* setting, astrocytes can be induced with interferon gamma (IFN-γ), granulocyte-macrophage colony stimulating factor (GM-CSF) and tumor necrosis factor alpha (TNF-α) to artificially

produce virus particles [48,49]. However, researchers are yet to amplify full-length infectious provirus from pure CNS cell populations and demonstrate that this same virus is capable of reverting to a productive infection following stimulation or activation of the latently infected cell.

Obstacles if the CNS is a viral reservoir

Despite the ongoing debate over the possibility of the CNS acting as a viral reservoir and its biological relevance to the HIV-1 cure effort, it presents several formidable barriers to curing HIV-1. These include different modes of cellular entry, altered transcriptional regulation, reduced bioavailability of cART, reduced potency of antiretrovirals, and minimal capacity to replenish cells lost following immune clearance or viral cytolysis.

Recent work by our group has highlighted the potential of HIV-1 to enter astrocytes via CD81-lined vesicles, bypassing the traditional CD4/coreceptor-dependent entry mechanisms [50]. Furthermore, these same compartments may play a role in viral dissemination within the CNS, via cell-to-cell spread, thereby potentially rendering entry inhibitors less effective [50]. The altered transcriptional activity of CNS-derived LTRs suggest that activation strategies designed against T cell reservoirs may be ineffective at inducing CNS reservoirs [45-47]. Work by Letendre *et al.* has clearly shown the differential penetration of cART into the CNS, affecting the bioavailability of antiretrovirals and their ability to control viral infection within the CNS [51,52]. Additionally, we recently reported that antiretrovirals used in Neuro-cART regimens (cART containing antiretrovirals with high CNS penetrating-effectiveness scores) have reduced efficacy in astrocytes, suggesting that even if the drugs penetrate the brain, they may not function as well, as in T cells in the blood [53]. Finally, the brain has a very limited capacity to replenish the cells lost due to immune clearance and viral cytolysis, which presents a major issue when considering the very high frequency of infected cells, and their potential loss during eradication strategies.

The vast majority of transcriptional activators that are being proposed as potential curing agents have unknown efficacy, toxicity and bioavailability profiles when it comes to the CNS [33] (Churchill MJ, unpublished data). It is highly likely that they will either be excluded from the CNS, have altered efficacy or may result in increased levels of toxicity. Their use comes with several caveats. If they are able to penetrate the CNS and act effectively, will we be able to control the emerging virus with cART? Will the size of the viral reservoir reduce or expand in the context of the CNS? Will the reduced immune surveillance in the CNS be sufficient to clear the virus producing cells? Will their clearance lead to a worsening of patient's neurocognitive ability? Will the reactivated virus disseminate from the CNS into the blood and re-seed peripheral reservoirs? These are all important factors that need to be considered when designing HIV-1 cure strategies, so that measurements and monitoring can be put in place to address these concerns.

The critical questions that remain unanswered

Together these findings provide evidence that the CNS can potentially act as a viral reservoir of HIV-1. However, there are several key questions that remain unanswered and need to be addressed as a priority to conclusively determine whether or not the CNS is a

viral reservoir of HIV-1 with biological importance. An additional stumbling block is access to the patient samples that fit the strict criteria that will enable us to answer these questions. There are relatively few patient samples available from well controlled, cART treated individuals who lack a viral load in the CSF and blood and have neurocognitively normal function. This is in part due to the success of cART in restoring life expectancy and preventing disease progression. Patients are now living longer and healthier lives in the modern day cART era, which ultimately means fewer autopsy patient samples for research needs.

While longitudinal sampling of blood is relatively non-invasive, we don't have the same luxury when it comes to the CNS. Samples are often only available following the death of the patient, with not all patients consenting to the use of their tissue in research, or causes of death precluding their use. Additionally there is the processing of the tissue samples that can have a dramatic effect on the future applications. Formalin fixed paraffin embedded tissue storage is normally the default method, but results in shearing of the genetic material and preventing the amplification of full-length proviral genomes. However, fresh frozen tissue is now also being stored which will allow for the extraction of full-length HIV-1 from latently infected CNS cells and will be an important first step in determining the presence of replication competent virus within this reservoir.

Another important area is the use of animal models to recreate HIV-1 infection of the CNS and use these systems to dissect treatment outcomes following administration of HIV-1 eradication strategies [22,54-57]. Studies performed in infected macaques determined that treatment with cART for 6 months resulted in rapid suppression of viral replication in the blood and CSF but the level of SIV DNA in the brain did not change [56]. Other studies in macaques suggest that immune escape variants may become archived in the brain and re-emerge following cessation of cART [57]. These models may be useful to analyze the establishment of latency and the CNS viral reservoir, because tissue samples can be readily accessed. However, these approaches are not without issues. Animal models will always be criticized for producing an artificial system that doesn't truly reflect the nature of HIV-1 infection of the human brain. Additionally, noteworthy differences exist between macaques/mice and humans which also limits the ability to directly translate findings to humans. Nevertheless, they represent an important alternative approach that can generate valuable data that would otherwise not be possible in human patients, or when appropriate human tissue samples are lacking.

It is still unknown what affect the use of epigenetic modifiers and transcriptional activators (histone deacetylase inhibitors, Akt signallers, histone methyltransferase inhibitors, cytokines, PKC activators) will have on cells within the CNS. Several studies have shown that sampling viral load in the CSF often correlates (but not always) to viral replication within the CNS [58,59] (National NeuroAIDS Tissue Consortium, unpublished data). Patients can exhibit neurocognitive impairment in the absence of a CSF viral load, while other neurocognitively impaired patients have pronounced HIV-1 encephalitis upon autopsy examination but had undetectable viral load in the CSF (National NeuroAIDS Tissue Consortium, unpublished data). As mentioned previously, the critical nature of the brain precludes tissue sampling while patients are living, so how are we to measure the impact of

cure strategies on the CNS reservoir? Thorough neurocognitive assessment during clinical trials may be the only safe way to measure treatment outcomes during eradication strategies, but will these be sufficient and measurable over the time span of the trial?

Research priorities moving forward

The discussions above now allow us to formulate the research priorities that need addressing as a matter of urgency so that the field can come to a consensus on the role of the CNS as a viral reservoir and for the HIV-1 eradication effort. First, researchers need to show that modern day cART-treated patients, with undetectable viral load in the CSF and blood and who are neurocognitively normal, have HIV-1 DNA detectable within their CNS cells (microglia, perivascular macrophages, and astrocytes). Ideally these cells would be negative for HIV-1 p24 or viral production but positive for HIV-1 DNA. Further, researchers need to show that these same cells contain a full-length integrated HIV-1 provirus that is replication competent. Second, researchers need to identify the underlying molecular mechanisms that are controlling HIV-1 latency within these cells, and distinguish if this is different to that found in latently infected cells in the blood. Third, researchers need to demonstrate that the virus found in latently infected cells within the CNS can be activated to produce new progeny virus. Finally, research needs to analyze the potential efficacy of eradication strategies using transcriptional activators in latently infected CNS cells, either *ex vivo*, *in vitro* using CNS-derived latent virus or in animal models. Together, these findings will enable us to clarify the CNS as a potential viral reservoir and also to hypothesize potential treatment outcomes within the brain during clinical trials to eradicate HIV-1.

Conclusion

This review has highlighted the current information that is available for consideration of the CNS acting as a viral reservoir for HIV-1. The CNS fits several of the criteria needed to be defined as a viral reservoir. The CNS contains cells with an integrated provirus, the virus exists within long-lived cells, the cells have mechanisms to control latency, and are infected at high frequency. However, there are still some criteria which are yet to be proven, the absence of which continues to fuel debate and controversy. Can latently infected CNS cells be activated to produce virus, is the integrated virus replication competent, and is integrated virus detectable in virally supressed patients? We have identified the prioritized the critical questions that remain unanswered and need to be addressed in order to conclusively determine whether or not the CNS is a viral reservoir for HIV-1 with biological importance for HIV-1 cure efforts. The vast majority of these research efforts will involve the use of very rare patient samples, which is another factor that limits the work that can presently be done. As more patient samples become available, we will eventually have the resources needed to answer these key questions. Ultimately, these samples will enable us to determine whether the CNS is an important compartment that needs to be considered when designing, conducting and evaluating potential HIV-1 cure strategies.

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Key points

- Anatomical sites must meet several criteria before they can be considered a HIV-1 viral reservoir: presence of integrated virus, maintained in a latent state, exists within long-lived cells, found at high frequency, virus can be activated, and virus is replication competent.
- The CNS satisfies several of these criteria, HIV-1 is integrated, maintained in a latent state, exists within long-lived macrophages, microglia and astrocytes, and found at high frequency.
- Modern day cART-treated patient samples are needed to determine whether HIV-1 persists within the CNS, if it is replication competent and if it can be activated.
- Eradication strategies to cure HIV-1 may potentially result in adverse outcomes within the CNS where antiretroviral bioavailability and immune surveillance are less prominent.