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Compartmentalization, Viral Evolution, and Viral Latency of HIV in the CNS

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Abstract

Human immunodeficiency virus type 1 (HIV-1) infection occurs throughout the body, and can have dramatic physical effects, such as neurocognitive impairment in the central nervous system (CNS). Furthermore, examining the virus that resides in the CNS is challenging due to its location and can only be done using samples collected either at autopsy, indirectly form the cerebral spinal fluid (CSF), or through the use of animal models. The unique milieu of the CNS fosters viral

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compartmentalization as well as evolution of viral sequences, allowing for new cell types, such as macrophages and microglia, to be infected. Treatment must also cross the blood brain barrier adding additional obstacles in eliminating viral populations in the CNS. These long-lived infected cell types and treatment barriers may affect functional cure strategies in people on highly active antiretroviral therapy (HAART).

Keywords

Human immunodeficiency virus; HIV; Compartmentalization; Tropism; Latency; Evolution; Eradication; CNS; CSF; Animal Models

Introduction

Compartmentalization of HIV-1 likely occurs throughout the body in tissues such as the liver, gut, lungs, kidney, and in fluids like breast milk and CSF. It is most well defined in the genital tract and the central nervous system [1]. The brain represents a unique environment not only for viral compartmentalization and evolution of virus but also for pathogenesis and potential harboring of latent virus that could present special challenges for eradication. Neurological dysfunction was recognized shortly after the discovery of HIV-1 infection. However, the mechanisms of basic questions about entry and viral evolution in the CNS are still debated, and insights into the causes of neurological impairment are continually updated and revised [2-4].

Recently, great effort has gone into finding a “cure” for HIV-1 by i) eradicating the CD4+ memory T cell latent reservoir using transcription regulating small molecules, ii) improving our ability to measure the actual size of the reservoirs, iii) trying to identify how and when the reservoir is established, and iv) determining what other cell types may be involved in harboring latent proviruses [5]. To this end, there needs to be a thorough understanding of HIV-1 infection in the brain, including compartmentalization and viral tropism.

Evidence for compartmentalized populations in the CNS and elsewhere

HIV-1 in the CNS has been extensively studied using autopsied brain or cerebrospinal fluid (CSF). Examination of HIV-1 in the CNS using brain tissue collected at autopsy samples demonstrates that people with HIV-associated dementia (HAD) harbor distinct viruses in their brains [6-10]. The CSF, which circulates the brain, can be used to asses viral dynamics in the CSF and at some level in the CNS which permits analysis of CNS viral populations in living subjects. The distinctions between HIV-1 populations in CSF versus blood are well documented, and it has been suggested that viral populations in the CSF and blood can originate from different routes [11-14]. Three states of virus have been described in the CSF when compared to the blood: equilibrated (where virus in the blood and CSF are very similar), compartmentalized (where blood and CSF viral populations are distinct, indicating separately evolving populations in these compartments), and clonal amplification (where a single variant is greatly expanded within a compartment). Clonal amplification and compartmentalization of HIV-1 subtype B and C strains in the CSF have been described using phylogenetic analysis of the single copies of full-length *env* genes in adults [15, 16] as

well as children [17]. Cell tropism studies of the pseudotyped virus, generated using full-length *env* clones, in the CD4-inducible Affinofile cell line [18] revealed two types of viral entry phenotypes, which will be discussed at length later.

The CNS/CSF is not the only anatomical site where compartmentalized viral populations can exist. HIV-1 compartmentalization has been found in both the male and female genital tract (MGT and FGT). Viral load discrepancies between the blood and FGT were observed in 30 to 50% of cases [19, 20]. This discordance between viral loads in the blood and genital tract suggests that the virus is able to replicate independently in these compartments [21-23]. There is also evidence that viral shedding is influenced by local factors of the genital compartment [24]. Even subjects that are fully suppressed on HAART and have undetectable plasma viral loads can experience HIV-1 shedding in the FGT, which suggests that the FGT could serve as a potential reservoir for HIV-1 [25, 19]. Virus that is genetically distinct from that in the blood can also be detected in semen [26-31].

Recent studies analyzing the *env* genes from semen revealed compartmentalization for 30% (4/12) of subjects with chronic HIV-1 subtype C infection [32]. In both subtype C and subtype B infection extensive clonal amplification was present in the MGT, with one study reporting 50% (6/12) of subjects, and another 100% (5/5) ([33], Dukhovlinova et al *in preparation*). These bursts of viral amplification suggest active viral replication in the MGT. Similar observations were found for virus harvested from cervicovaginal lavage (CVL) samples. Even though the FGT does not seem to be as isolated a body compartment as the MGT, HIV-1 compartmentalization has been detected about half of the time in the FGT (7/13 and 5/10) ([34], Dukhovlinova et al *in preparation*). A direct relationship between compartmentalization in the FGT and blood T cell counts suggests that the origin of the compartmentalization in the GT may often be from short-lived and trafficking CD4+ T-cells ([35], Dukhovlinova et al *in preparation*). This assumption is confirmed by the fast turnover (as early as 8 weeks) of both clonally amplified and compartmentalized viral strains in the GT. An exception would be the apparent compartmentalization can be partially due to recombination between clonally amplified variants, making the GT population look more complex and distinct then it actually is ([34, 36] Dukhovlinova et al *in preparation*). In addition to T cell trafficking and viral replication in local CD4+ T cells, long-lived cells may also play a role in maintaining a distinct viral population in the GT. Viruses with an increased ability to enter macrophages (described below) have been observed in the MGT and in a clonally amplified viral population in FGT (Dukhovlinova et al *in preparation*), suggesting a potential for a macrophage reservoir to play a role in the viral dynamics of the GT compartment.

Establishment and early evolution of virus in the CNS

CNS infection by HIV-1 can be significant and can occur early after transmission; however the details of local replication in the CNS can be quite diverse and evolve over time. HIV-1 may persist in the CNS during therapy due to insufficient CNS penetration of some antiretroviral drugs and potentially due to the long-lived nature of resident CNS cells. The extent of the physiological effects of CNS infection can be seen in patients with milder forms of HIV-associated neurocognitive disorders (HAND), and the more extreme HAD.

These physiological effects have been linked to increased levels of neurological injury due to inflammatory signals. These immune system changes were recently reviewed by Price et al. [37].

Virus can be found in the CSF at low levels very early after transmission. In a subset of infected individuals, there is minimal CNS viral burden early [38, 39]; thus, HIV-1 is likely entering the CSF/CNS at low levels via incomplete partitioning of virus at the blood-brain barrier, or background level trafficking of immune cells, including small numbers of infected CD4+ T cells. Often, HIV-1 RNA loads within the CNS are elevated during primary infection and these elevated levels of virus can correspond with higher levels of CSF white blood cell (WBC) counts (i.e. pleocytosis) [39]. Thus, the CNS viral burden early in infection may be the result of the release of virus from an increased number of infiltrating infected CD4+ T cells trafficking from the periphery into the CNS, perhaps in response to HIV-1 replication in the CNS or due to another inflammatory condition.

Compartmentalized viral populations, genetically distinct from viral populations replicating in the periphery, can be detected in the CSF/CNS throughout the course of infection [40]. Two major classifications of compartmentalization have been defined that appear to be similar to the types of compartmentalization seen in the genital tract, as described above. The first is clonal amplification, the rapid expansion of genetically similar variants yielding a CSF viral population of low complexity. These populations require high levels of CD4 for entry, and are thus CCR5 (R5)-using T cell-tropic (discussed below). During primary infection, clonally amplified populations have been detected as early as two to six months post infection [39]. A second more complex form of CSF viral compartmentalization has also been identified, often consisting of macrophage-tropic virus (discussed below). Even during primary infection, these more genetically complex populations most often correspond with a longer time since HIV-1 infection, indicative of a more prolonged period of isolated replication and evolution of the population and entry phenotype [39, 17, 15].

While extensive compartmentalization can be a strong indicator of neuropathogenesis contributing to HIV-associated dementia (HAD) [40-42], compartmentalization has also been observed during primary infection, indicating that independent CNS replication can occur in the absence of overt neurological symptoms [40, 39, 15]. While a longitudinal link has not been made, this raises the possibility that early detection of compartmentalized CSF variants may identify subjects with a higher risk of developing HIV-associated neurological complications. In a recent study of primary infection [39], a compartmentalized CSF/CNS viral lineage established less than six months post infection persisted over a period of at least two years, showing the maintenance and evolution of a compartmentalized viral population within the CNS over a long duration of time starting during early infection. Additionally, in other subjects during primary infection [39], multiple distinct compartmentalized populations were observed within the CNS at different time points post infection, indicating that even when single compartmentalized populations aren't maintained, the CNS may provide a permissive environment for viral replication in which variants are successively and independently amplified within the CNS starting quickly post transmission.

Vertically and horizontally transmitted viruses are often highly homogeneous, representing infection seeded by a single variant and characterized by low diversity [43, 42]. Thus, in most situations, compartmentalization within the CNS during primary infection results from the establishment of independently replicating viral populations that originated from the single transmitted lineage within the periphery. Alternatively, in recent primary infection studies following both vertical and horizontal transmission, a small subset of subjects were identified who were infected with multiple variants, with one of the variants largely sequestered in the CNS/CSF and the second variant replicating within the periphery [39, 17]. Based on these observations, one of the transmitted variants may have had a selective CNS tropism and was able to escape immune surveillance in this compartment, or CNS infection was a low probability event influenced by the chance introduction of a founder virus. Overall, during primary infection there are two distinct pathways to compartmentalization: early sequestration of a transmitted virus in the CNS, or the later establishment of independently replicating virus that originates in the periphery.

Link between compartmentalization and/or viral load in CSF and neurocognitive effects/HAD

HIV-1 can be found in the CNS/CSF within the first months of systemic infection, and the presence of virus can be accompanied with immune activation [44-46]. The majority of cases are asymptomatic [47-49]. In some cases, HIV-1 is clearly replicating in the CNS and this can lead to damage in the CNS and dementia or other neurocognitive disorders. Prior to HAART, these HIV associated neurocognitive disorders resulted in severe cognitive and motor disorders and dementia presenting with the onset of the severe immunosuppression stage of AIDS. This outcome has shifted with the availability of HAART. HAND now presents as more mild disturbances in psychomotor function, processing, and memory in patients on long-term therapy. These more minor symptoms can still disrupt daily life. This is not to say that, severe forms of HAND are not still seen in resource-limited settings, in people who are non-adherent, and in people who enter care late in disease [50, 51].

Many studies have attempted to elucidate mechanisms and correlations of HIV-1 neuropathogenesis as it relates to HAND. As noted above, CSF viral RNA levels are typically lower than those in plasma [52]. One recent study of a small sample of HAND and cognitively normal (NC) subjects in Hawaii and Puerto Rico found HIV-1 DNA levels in CSF monocytes (CD14+) was higher in subjects with HAND than NC subjects, while the NC subjects had higher HIV-1 DNA levels in CSF lymphocytes (CD14-) compared to HAND subjects. This difference did not, however, correlate with HIV-1 RNA viral loads [53]. However, studies have reported CSF RNA viral load levels one log higher than plasma HIV-1 RNA viral load in subjects on drug therapy and with neuropathological HIV-1 disease [54]. These subjects also had markers of CNS inflammation presenting as increased CSF protein or albumin level, which are atypical of neurologically asymptomatic patients on therapy. In addition, iron transport deficiency in the brain, red blood cell count, mean red blood cell volume, mean cell hemoglobin, hemoglobin [55], and increased levels of the light subunit of neurofilament protein (NFL) [56] have been suggested as indicators of neurologic dysfunction in persons infected with HIV-1.

A genetic distinction between viral sequences in the CSF and in the plasma can also be a result of the separation of independent evolution in the two compartments and a potential indicator of neurologic irregularities. Most of the time, in response to drug therapy, virus in the CSF and virus in the plasma decay in parallel. In some cases, virus in the CSF decays at a slower rate than virus in the periphery [16, 57-60]. This may be accounted for by poor drug penetration in the CNS, a compartmentalized evolution of drug resistance, or continued production of virus in the face of drugs due to infection of a long-lived tissue cell such as macrophage or microglia. While genetically distinct, independently evolving populations of HIV envelope genes have been reported [15], compartmentalization is not exclusively specific to the envelope gene. Previous studies found compartmentalized *pol* and *tat* genes, and the LTR, and one recent study found brain-specific *nef* sequences. The *nef* mutations are predicted to be required for activation of tyrosine kinases that may ultimately promote efficient replication in macrophages (reviewed in [2, 61]).

Evidence for CNS Latency and general strategies for eradication and how they might work in the brain

Latent HIV-1, defined as integrated and transcriptionally silent proviruses, is untouched by current antiretroviral therapy, and poses a significant hurdle to eradication and cure of HIV infection. Latency is predominately attributed to the resting CD4+ memory T-cells reservoir [62], leaving the role of myeloid linage cellular reservoirs less clear. The strongest evidence for macrophage infection has been seen in the CNS of HIV infected patients with HAD [63, 6-10], and when using simian models of infection. As one example of the ability of macrophages to be infected, in primates infected with a SHIV, >95% of infected cells were tissue macrophages in late stage disease (reviewed in [64]). Isolated viruses from these experiments had reduced titers, easily neutralized Env proteins, and were immune suppression-dependent to cause productive infection [64]. Similarly, high viral loads in plasma produced by tissue macrophages can be seen after CD4+ T-cell depletion in primary infection [64]. These experiments indicate that macrophage infection is dependent on viral evolution and requires either immunodeficiency to occur at a high level or immunologically privileged compartments like the CNS, where HIV-1 could also cause latency.

The CNS has 4 types of macrophages—microglia, perivascular macrophages, meningeal macrophages, and macrophages of the choroid-plexus—as well as other long-lived cell types such as circulating monocytes and astrocytes [65]. Turnover rates range from months to years for these cells types potentially making the CNS an ideal location for latency; however direct evidence of established latency from macrophages is lacking and largely limited to autopsy specimens [64]. Infection of macrophages in the CNS has been implicated in neurological impairment of HIV- infected patients however (reviewed in [66]). The role of infection of circulating monocytes and astrocytes is even more controversial. Early experiments establishing monocytes as a site of HIV infection were unable to ensure no T-cell contamination, and although new sorting techniques remedy this issue the role of monocytes is still vague [66]. Astrocyte infection has also been widely debated (reviewed in [2]), most recently evidence of prolonged infection (160 days) has been shown but evidence of latency has never been established [67]. Knowing which (if any) of these cells can give

rise to a latent infection will be necessary to be able to target eradication strategies of the future.

Curative strategies for eliminating the latent reservoir are typically grouped into two main categories, functional cure approaches and sterilizing cure approaches. The proof of concept for a cure is the now well-known “Berlin patient”, but this was a highly restricted approach with significant complications [68-70]. The approaches to a functional cure can be divided into three distinct strategies: (1) early intervention where initiation of HAART occurs within days of infections to prevent the reservoir from being established [71, 72], (2) global T-cell activation, which has been shown to have undesirable toxicity effects, and (3) “kick and kill” using small molecules to reactivate HIV synthesis [73]. The “kick and kill” strategy has been the most tested method to date, however recent improved methods of analyzing the latent reservoir shows only a small fraction <0.2% of the reservoir is “kicked” [74, 75]. Additionally, in clinical trials using the histone deacetylase inhibitors (HDACi) vorinostat, romidepsin, and panobiostat, and disulfiram (an alcoholism drug) no reduction in the resting CD4+ memory T-cell reservoir size has been observed, showing the limitation of this strategy at least to date [76-78]. On top of all of the above issues, the CNS offers one more important road block, the blood brain barrier. Only some drugs are accessible to the CNS [79-81] depending on lipophilicity, the blood's protein binding molecules, and molecular weight [82]. Given the numerous challenges that are apparent with trying to activate the latent reservoir in general, a continued need for more sensitive measurements of the latent reservoir and more robust small molecules with good blood brain barrier penetration are areas of research that will need to be explored to attack potential reservoirs in the brain.

Viral tropism and sequence evolution

Most of HIV-1 is adapted to and replicating in CD4+ T memory cells [83, 84] reflected in the need for relatively high levels of the CD4 receptor and requirement for the CCR5 coreceptor on the target cells surface for entry [85, 86], thus we have applied the term CCR5-using T cell-tropic (R5 T-tropic) viruses. In late stage disease, the viral population can evolve alternative receptor and coreceptor requirements to allow expansion into alternative target cell types (reviewed in [3]). It is important to note that viral populations that evolve to use additional cell types do not lose the ability to infect CD4+ T memory cells. The most common change in tropism is coreceptor switching, which occurs in late stage disease in approximately 50% of cases [87]. Coreceptor switching refers to viruses that acquire the ability to use CXCR4 as an alternative coreceptor allowing expansion into naïve CD4+ T cells [88-90], which makes them CXCR4-using (X4) T-tropic viruses. Another tropism shift that emerges during late disease or in distinct compartments (discussed above), but at a much lower frequency, is the ability to use lower levels of CD4 to expand into macrophages [18, 91-93]. Because these macrophage-adapted viruses generally use the original CCR5 coreceptor, [94-96] they are termed R5 macrophage-tropic (M-tropic) viruses.

R5 M-tropic variants are detected infrequently within the total population of HIV-infected humans, but are enriched in compartmentalized viral populations in the CSF/CNS [93, 16]. Some compartmentalized populations represent viruses that have adapted to macrophages by

enhancing their ability to enter using low levels of CD4 on the macrophage surface, which is approximately 20-fold lower in CD4 density compared to T cells [97, 95]. The compartmentalized R5 M-tropic viral populations tend to be complex, often as complex as the systemic R5 T-tropic populations isolated from the blood [16].

The remaining compartmentalized CSF populations represent the typical R5 T-tropic phenotype, but these cases are usually associated with pleocytosis [16], which means there is, temporarily, a supply of activated T cells that have infiltrated the CNS. These infiltrating T cells may undergo expansion of an infected T cell, resulting in clonal amplification of one or a few viral sequences, or may provide target cells for free virus already present in the CNS that would usually be abortive for a lack of T cells found in the CNS under normal conditions. In either case, these R5 T-tropic compartmentalized populations contain little or no diversity [16].

Although R5 T-tropic viruses preferentially target activated CD4+ T cells, it is possible for these viruses to enter macrophages [16, 95], which express both CD4 and CCR5, but the low levels of CD4 on macrophages make the probability of entry very low for viruses adapted to the high levels of CD4 on T cells. It is possible that this very low frequency infection of macrophages by R5 T-tropic cells may provide the seed for the evolution of R5 M-tropic viruses, because even a low probability infection will occur if the low-efficiency target is in the presence of a sufficiently large population of viruses, which may be provided by the burst of viral replication in the CNS observed with pleocytosis.

Exactly how viruses evolve to enhanced low CD4 usage is still less clear. Though several groups have found single or double mutations that modulate R5 M-tropism in specific viral genomes (reviewed in [3]), none of the currently proposed mutations appear in all R5 M-tropic viruses [98, 6, 3]. This could be for several reasons. First, the interaction between HIV-1 Env and CD4 is complex and may require a large constellation of mutations that have not all been identified yet. Alternatively, because R5 M-tropic variants are not transmitted and so must evolve independently anew in each case, each viral population may evolve along a slightly different path and achieve the same phenotype with a different set of mutations.

Compared to the modifications required for coreceptor switching (for X4 T-tropism), which are mostly in the V3 region (the main interface with coreceptor) and somewhat predictable using different algorithums [99, 100], the complexity of enhancing CD4 usage (for R5 M-tropism) appears to be more complex. Until genetic determinants of R5 M-tropism are more fully understood, it will be difficult to compare the evolutionary paths to X4 T-tropism and to R5 M-tropism, but two important differences are notable. First, each of these paths is solving a different problem: switching from CCR5 to CXCR4 means a difference of protein shape or contact, but switching from using a high density of CD4 to a low density of CD4 is a difference of adapting to abundance of the same target. Second, the coreceptor binding site is generally hidden from immune surveillance until the virus has made contact with the target cell and is only exposed for a short time before fusion and so can evolve in relative safety, but the CD4 receptor binding site has to be generally available to make that first contact with the cell.

Animal models of neuropathogenesis

Two animal models for HIV-1 infection are widely used: humanized mice and nonhuman primates. Humanized mice are generated from genetically immunocompromised mice that are engrafted with human lymphoid tissues to reconstitute cells of the human immune system (reviewed in [101]). Studies using NOD/*scid*-IL-2Rγ_c^{null} mice reconstituted with human hematopoietic stem cells obtained from cord blood (hNSG mice) show that human macrophages can be detected in the mouse brain, and HIV-1 proteins are found in a small number of cells [102, 103]. Yet the use of humanized mice for studying HIV-1 neuropathogenesis is an incompletely developed method, in part because human microglia are not reconstituted in humanized mice. Microglia largely originate in early stages of *in utero* development independent of bone marrow hematopoiesis and remain in the brain throughout life with limited cell division (reviewed in [104]). Current humanized mouse models do not permit investigating the relative importance of infected macrophages versus microglia in HIV-1 neuropathogenesis.

Simian immunodeficiency virus (SIV) infection of nonhuman primates is also widely used as an animal model for HIV-1 infection. SIV isolates from old world primates represent a diverse set of viruses. In some cases an isolate from one species will cause AIDS-like pathogenesis in a different species. The most popular model that uses this concept is the pathogenic infection of Rhesus macaques with SIVsm (isolated from sooty mangabeys). These models have been used to study neuropathology in the context of independent (compartmentalized) SIV replication in the CNS or CSF compared to the peripheral blood and other lymphoid tissues.

SIVsmE543-3 is a SIVsm molecular clone that causes encephalitis in a fraction of infected macaques, starting as early as four months post-infection [105]. SIVsmE543-3 infects both CD4+ T cells and, with modest efficiency, monocyte-derived macrophages (MDMs) in culture, and is found in various immune cells within perivascular cuffs in the meninges and the parenchyma of the brain [105]. Macaques inoculated with SIVsmE543-3 or its derivatives display either conventional or rapid progression to AIDS, with encephalitis in some animals [106]. Conventional, but not rapid, progressors can have compartmentalized virus in the CSF that replicates better in MDMs compared to the parent SIVsmE543-3, which supports the idea that these viruses must evolve to replicate efficiently in macrophages in the CNS [106].

A neurovirulent viral swarm, SIVsmH783Br, is a derivative of SIVsmE543-3 that has recently been used to study compartmentalization of SIV in the CSF as compared to the CNS of macaques [107]. SIVsmH783Br-infected macaques, with rapid or conventional disease progression, were shown to have compartmentalized virus in the CSF [107]. Phylogenetic analyses demonstrated that virus was also compartmentalized within different regions of the brain. CSF viral sequences were most closely genetically related to sequences obtained from the meninges, and both compartments contained sequences with a truncated gp41 cytoplasmic domain. These data suggest intermixing of SIV between the CSF and the meninges [107]. Rapid, but not conventional, progressors had at least one CSF viral sequence that clustered with parenchymal viral sequences, whereas conventional progressors

had a distinct cluster of parenchymal viral sequences [107]. Collectively these data suggest a model were virus enters the CSF and replicates in the meninges, and may enter and replicate in the deeper brain tissues of the parenchyma depending on viral replication kinetics. Thus, each respective CNS compartment can harbor distinct viral populations compared to the periphery, with the parenchymal viruses being the most distinct from any other compartment.

Although the CSF-producing choroid plexus is considered a site of HIV-1 production in the CNS [108, 109], it is likely that SIV replicates throughout the brain, and undergoes selective pressure depending upon the environment of a specific brain region. Indeed, macaques infected with a molecular viral clone, SIVmac239, develop encephalitis of varying degrees marked by infiltration of peripheral viral species into different regions of the brain [110]. In another primate model, macaques infected with two viruses (a neurovirulent SIV clone and an uncloned immunosuppressive SIV strain) progress rapidly to disease and exhibit compartmentalization of SIV in the CNS that appears to have undergone selective pressure upon entry to the brain [111].

At least two macaque models of SIV neurovirulence support the theory that compartmentalized virus in the CSF plays a role in neuropathology [107, 112]. Macaques inoculated with SIVsmE660 viral swarm have elevated levels of MCP-1 (CCL2), a CNS inflammation marker, when CSF virus is compartmentalized [112]. The study by Matsuda et al. described above shows that distinct, compartmentalized viral populations are found in the meninges versus parenchyma and are likely responsible for meningitis and encephalitis, respectively [107]. However, compartmentalization is not a prerequisite for neuropathology, as rapid disease progression marked by encephalitis can occur in the absence of compartmentalized CSF virus [107]. Naturally SIV-infected African green monkeys exhibit CSF/CNS virus compartmentalization and robust viral replication without associated neuropathology, although peripheral infection does not cause simian AIDS either [113]. Still, CSF/CNS compartmentalization usually correlates with length of infection, as conventional and long-term progressors consistently demonstrate a higher degree of compartmentalization associated with strong phylogenetic statistics, as compared to rapid progressors [106, 107, 110]. Thus nonhuman primate models demonstrate that SIV compartmentalization occurs in the CSF and CNS of animals with neuropathogenesis, but additional studies are needed to clarify the origin of viral populations within specific brain compartments and the relative contribution of these respective viral populations to CNS disease.

Conclusion

The brain is a unique compartment for viral evolution and latency. In-depth analysis of the virus isolated for the CSF of humans and the use of monkey models has illuminated viral tropism evolution in relation to neurocognitive effects and compartmentalization, as well as illustrated the possibility for a large isolated viral reservoir that could present an obstacle for a functional cure. Limitations in the penetration of small molecules across the blood brain barrier will likely have important consequences in eliminating the latent reservoir. Future efforts to better understand HIV-1 infection of the CNS will need to focus on subjects

receiving therapy. Generating more sensitive measurement techniques for evaluating latent reservoirs as well as evaluating drug penetration into the CNS will be important waypoints on the trip to eradicate viral populations throughout the body.

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