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HIV-1 and Compromised Adult Neurogenesis: Emerging Evidence for a New Paradigm of HAND Persistence

Raj Putatunda[1,2], Wen-Zhe Ho[1] and Wenhui Hu[1,2]

[1]Department of Pathology and Laboratory Medicine, [2]Center for Metabolic Disease Research, Temple University Lewis Katz School of Medicine, Philadelphia, Pennsylvania, USA

Abstract

The face of the HIV-1/AIDS pandemic has changed significantly thanks to the development of antiretroviral therapy (ART) regimens. Unfortunately, several HIV-associated comorbidities continuously occur in the clinical population, most notably HIV-associated neurocognitive disorders (HAND). While many molecular and cellular mechanisms have been characterized by describing HAND pathology (specifically neuroinflammatory insults and oxidative stress) in the ART era, compromised adult neurogenesis is emerging as a potential new mechanism. Neurogenesis is a dynamic process that generates new neurons and glial cells from neural stem cells (NSCs) and neural progenitor cells (NPCs) in specific areas of the brain. There are increasing observations that HIV-1 can productively and non-productively infect NSCs and NPCs. HIV-1 proteins and/or secondary immune/inflammatory responses impair the initial differentiation process of NSCs to NPCs, restrict neuronal lineage differentiation, and aberrantly promote astrocytic lineage differentiation. Recent studies with HIV-1 transgenic animal models demonstrate varying degrees of adult neurogenic deficits, which correlate with milder to moderate forms of neurocognitive impairments. The neurogenic dysfunction underlying HAND highlights the importance of developing potential therapeutics to restore adult neurogenic homeostasis in HIV-1 patients. **(AIDS Rev. 2019;21:11-22)**

Corresponding author: Wenhui Hu, whu@temple.edu

Key words

HIV-1 latency. HIV-associated neurocognitive disorders. Neurogenesis. Neural stem cell, Antiretroviral therapy.

Introduction

Persistent HIV-associated neurocognitive disorders (HAND) in the antiretroviral therapy (ART) era

In the past three decades, several scientific advances have helped to convert HIV-1 infection from a fatal disease to a chronic, yet manageable disorder. One of the most important advances in HIV-1 research is the development of ART in 1996. Once administered as a monotherapy, new ART regimens have evolved so that several drugs could be administered with a lower pill burden to inhibit HIV-1 infection/replication at several stages of the viral life cycle. For example, nucleoside and non-nucleoside reverse transcriptase inhibitors

Correspondence to:
Wenhui Hu
Center for Metabolic Disease Research
Department of Pathology and Laboratory Medicine,
Temple University Lewis Katz School of Medicine,
3500 N Broad Street, PA 19140, Philadelphia, USA
E-mail: whu@temple.edu

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(NRTIs/NNRTIs) were developed to inhibit the reverse transcription of the proviral RNA into cDNA. Integrase inhibitors were developed to hamper viral cDNA integration into the host genome, while a plethora of proteases and viral entry inhibitors block HIV-1 entry as well as viral protein production. As a result, mortality rates from HIV-1 infection/AIDS progression and its subsequent opportunistic infections have decreased significantly in the ART era.

However, even with the ART, patients began developing varying levels of cognitive dementia, which came to be collectively coined as HAND. Under the Frascati criteria, HAND is a spectrum of neurocognitive impairments that are described as an asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), and HIV-associated dementia (HAD)[1]. Patients are diagnosed with ANI if they have developed slight impairments in more than two neurocognitive domains, with very minimal alterations in daily lifestyles. Patients with MND also show slight impairments in more than two neurocognitive domains. However, these impairments impede with their everyday lifestyles. Patients with HAD have robust impairments in more than two neurocognitive domains and are completely unable to function independently.

Since the start of the ART era, the clinical presentation of HAND has evolved. Before the development of ART, HAD was the most common, and the deadliest form of HAND. During a clinical study conducted by the Multicenter AIDS Cohort Study (MACS), 20% of all HIV-1 patients enrolled before 1991 met the diagnostic criteria for HAD[2]. However, between 2001 and 2003, only 5% of the enrolled HIV-1 patients manifested HAD[3]. During the pre-ART era, an HIV-1 patient with HAD only had a 5-month life expectancy[4]. In contrast, the average life expectancy of a HAD patient diagnosed between 1996 and 2000 was 38.5 months[4]. Extrapyramidal motor abnormalities such as bradykinesia, tremors, and aberrant rigidity, as well as subcortical dysfunction such as psychomotor slowing and robust cognitive disabilities, are rare in ART-controlled HIV-1 patients[5]. Thankfully, these promising epidemiological studies showed that ART remarkably diminished HAD incidence. While this data are optimistic, 15-55% of all HIV-1 patients can still develop milder/moderate forms of HAND[2].

Atrophic brain signature in HAND patients

While ANI or MND are not quite as severe as HAD, patients with these forms of HAND can develop worsened cognitive prognosis. The results from the central nervous system (CNS) HIV-1 Antiretroviral Therapy Effects Research (CHARTER) group showed that patients with ANI at baseline were more likely to develop worsened cognitive prognosis at follow-up evaluations than patients with normal cognitive performance. This suggests that there are microstructural alterations in the brain which can develop even before symptoms manifest. In fact, imaging studies demonstrated that HIV-1 can induce structural changes in the brain almost 100 days after primary infection, before the onset of symptoms[6]. Abnormal white matter volume is significantly associated with the severity of HAND, while patients with HAD or MND also have smaller gray and white matter volumes than neurocognitively unimpaired HIV-1 patients[7]. A recent study showed that specific frontal white matter atrophy contributes to ANI, while more widespread subcortical atrophy is associated with MND[8]. These specific types of brain atrophy could possibly predict neurocognitive morbidity in HIV-1 patients[9].

Integrative biomarkers for biological aging and HAND

Risk factors for HAND have also evolved[5,10,11]. In the pre-ART era, high levels of plasma and cerebrospinal fluid (CSF) HIV-1 RNA, low $CD4^+$ T-cell counts, and robust immunosuppression due to infection all correlated with an increased risk for dementia. However, in the post-ART era, HIV-1 related immunosuppression and high viral loads are no longer relevant in determining the progression of HAND. Instead, older age, illicit substance abuse, low CD4 nadir counts, depression, hypertension, diabetes, and high cholesterol levels all correlate with worsened HAND diagnoses, especially in older HIV-1 patients[5,12,13]. To assess all of these current risk factors, several biomarkers are currently being evaluated for their reliability in accurately diagnosing the stages of HAND[13]. Biomarkers for immune activation include elevated plasma and CSF levels of sCD163, sCD14, interleukin (IL)-1α, tumor necrosis factor α (TNFα), MMP2, MCP1, osteopontin, and fractalkine[14,15]. Additional CSF biomarkers for HAND include molecules involved in cellular metabolisms, such as CSF TCA cycle substrates, fatty acids, and triglycerides[5,10,16].

With the optimization and wider availability of ART drugs, it is imperative to acknowledge the contribution of premature aging to the evolution of HAND[17]. In fact, approximately 73% of HIV-1 patients will be over 50 years old by 2030[18,19]. Preliminary data from the

CHARTER study demonstrate that HIV-1 infection and older age may synergistically lead to worsened neuropsychiatric performance than either factor alone[20]. In addition, several clinical studies have also shown that HIV-1 patients with pre-existing cardiovascular complications have an approximately six-fold higher chance of developing cognitive impairment than HIV-1 patients with no cardiovascular abnormalities[21,22]. Therefore, therapeutics for HIV-1 and HAND need to take aging into account during administration[23].

Molecular and cellular pathogenesis underlying HAND

The development of ART has significantly changed the histopathological progression of HAND. For example, patients with HAD exhibited varying degrees of cerebral encephalitis, as assessed postmortem. After the start of the ART era, the incidence of encephalitis in HIV-1 patients decreased from 54% to 15%[24]. While overt neuronal loss was a histological characteristic of HAND patients in the pre-ART era, the veracity of this assumption is waning. While neurons themselves are not completely lost, their functionality is significantly compromised[25,26]. The mechanisms by which HIV-1 causes neuronal damage, as well as systemic neurophysiological alterations, are still not completely understood. At present, the general consensus is that HIV-1 infects the brain through a Trojan Horse-type mechanism. HIV-1 infects $CD4^+$ T-lymphocytes and monocytes in the peripheral circulatory system. This infection is directly dependent on the CD4 receptor in addition to viral coreceptors CXCR4 and CCR5[27]. Once peripheral HIV-1 infection is established, it takes some time for the virus to invade the CNS. This CNS infiltration is attributed to infected monocytes crossing the blood–brain barrier and then actively releasing viruses and viral particles into the CNS. This secondary infection leads to the activation of resident glial cells, such as microglia and astrocytes. These activated cells generate an inflammatory environment through the release of several viral proteins (such as Tat, gp120, and Rev) and pro-inflammatory cytokines. These factors may damage neurons through the production of reactive oxygen species, membrane depolarization, and disruption in neurotransmitter homeostasis[27].

While HIV-1 infection and subsequent inflammatory insults can contribute to the downstream CNS damage, there is an increasing set of literature implicating ART drugs in HAND progression. In fact, ART drugs that are capable of penetrating the CNS at a higher efficiency have been correlated with worsened cognitive status[28-30]. One *in vitro* study demonstrated that 15 different ART drugs had varying degrees of neuronal toxicity, as assessed by cell viability assays, neuronal arborization analyses, and mitochondrial membrane potential measurements[31]. The findings of aberrant mitochondrial membrane potential were notable, as they raised the possibility that ART drugs induced oxidative stress in neurons. Another study demonstrated that one NRTI (AZT) and two protease inhibitors (Ritonavir and Saquinavir), both individually and synergistically, induced varying degrees of oxidative stress and neuronal damage in the CNS, while astrocytes remained spared from damage[32]. This was supported by histological evidence that SIV-infected pigtail macaques treated with ART had more synaptodendritic damage than ART-naïve SIV-infected macaques[32]. Further investigation using Ritonavir and Saquinavir demonstrated that these two front-line protease inhibitors induced the integrated stress response in neurons, which could be pharmacologically reversed with a drug that inhibited the phosphorylation of $eIF2\alpha$[33]. Another study demonstrated that Ritonavir and Lopinavir hampered oligodendrocyte survival and maturation at the *in vitro* and *in vivo* levels[34]. Interestingly, AZT had no effects on oligodendrocyte integrity. Further Western blotting studies on prefrontal cortical brain lysates revealed that HIV-1 patients on ART had significant decreases in myelin basic protein expression than ART-naïve HIV-1 patients and uninfected patients[34]. While this body of literature is relatively small, these studies demonstrate that front-line ART therapies damage the CNS through mechanisms such as oxidative stress, activating the integrated stress response, and inducing varying degrees of white matter damage, thus possibly contributing to HAND progression.

Given these interesting findings, strategies involving the switching and simplification of ART regimens (one or less NRTIs, totaling <3 drugs) have been extensively explored to mitigate drug-induced toxicity, viral resistance, and adherence interruption[35-39]. However, the benefits of simplified ART regimens remain controversial because they may be less effective in suppressing CNS viral loads and improving neurological function[40-42]. A long-term three-decade retrospective study using a large cohort of 4992 HIV-positive patients with regimen switching protocols demonstrated that neurocognitive deficits do not necessarily improve in most cases, even if systemic viral suppression is successfully achieved[43]. Therefore, further prospective long-term studies are warranted to optimize a "better" ART regimen.

While many studies support the theory of indirect Trojan-Horse mechanism for HIV-1 induced neuronal damage and ART-induced CNS dysfunction, the notion of productive neuronal infection by HIV-1 remains controversial. This could be explained by two reasons. One might be the lack of CD4 expression on neurons, even though they express the HIV-1 coreceptors CXCR4 and CCR5[27]. While CD4-independent mechanisms of infection may exist, they are not as commonly reported in literature[44]. Another explanation could be the higher levels of HIV-1 specific host restriction factors in neurons. Several well-characterized HIV-1 host restriction factors include the tripartite motif 5 (TRIM5) family of proteins, the Apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3 family of proteins, the myxovirus resistance protein, Bone Marrow Stromal Cell Antigen 2 (BST2, or Tetherin), and SAMHD1[45]. While expression of these host restriction factors is highly restricted to immune-lineage cells, the Human Protein Atlas revealed higher levels of expression of TRIM5 and SAMHD1 in neurons than those in glial cells, which may explain why HIV-1 infection is undetectable in neurons[27].

HIV-1 latent infection in the CNS

The formation of a latent reservoir allows HIV-1 to persist throughout a patient's lifetime, even while adhering to ART. Several cellular reservoirs have been characterized to harbor low-level chronic HIV-1 infection, which contributes to a barrier for a sterile HIV-1 cure. Examples of these reservoirs include: resting memory $CD4^+$ T-lymphocytes, microglia, monocytes, macrophages, and astrocytes. Out of all these reservoirs, the resting memory $CD4^+$ T-lymphocytes contain the highest HIV-1 load.

During the initial stages of CNS infiltration, HIV-1 predominantly infects perivascular macrophages, microglia, and astrocytes. These CNS cells satisfy most of the criteria for an HIV-1 reservoir, as described by Blankson et al.[46]. For example, these cells have been shown to harbor integrated HIV-1 provirus, as demonstrated through methods such as *in situ* hybridization and laser capture microdissection coupled with PCR[47]. The perivascular macrophages have a half-life of about 3 months[48], while microglial cells and astrocytes have a half-life ranging from several months to years, potentially upward of an entire lifetime[49]. Furthermore, these cells harbor the specific molecular machinery to facilitate the HIV-1 provirus into a latent state. For example, the CNS-derived HIV-1 long terminal repeat (LTR) promoter has lower basal transcriptional activity due to mutations in the core promoter region[50]. Robust suppression of SIV LTR activity has also been corroborated in SIV infection models, as IFN-β induced the expression of a dominant-negative form of C/EBP-β in the CNS[51]. In HIV-1 postmortem brain specimens, CTIP2, HP1, MeCP2, and HDAC1 levels were all elevated, giving rise to the possibility that they may play a role in HIV-1 transcriptional silence in the CNS[52].

Since astrocytes are the most abundant cell type in the CNS, their roles in HAND have been extensively studied. It remains controversial whether astrocytes can be productively and/or latently infected by HIV-1. Early immunohistochemical studies using postmortem brain tissue from HAD patients demonstrated the presence of HIV-1 proteins in hippocampal astrocytes[53]. The highly sensitive PCR analysis of laser-captured single astrocytes revealed HIV-1 DNA in up to 19% of astrocytes from HAD patients[54]. Even in asymptomatic individuals, HIV-1 DNA was detected in laser-captured astrocytes[47]. However, most *in vivo* studies only demonstrated the detection of early HIV-1 transcripts, such as Nef[55]. *In vitro* studies showed contradicting conclusions. For example, while primary astrocytes or astrocytic cell lines demonstrated either productive[56] or non-productive[57] infection, Boutet et al. reported that astrocytes remained completely resistant to HIV-1 infection[58]. Nevertheless, persistent HIV-1 infection in astrocytic cell lines[59] or primary human astrocytes[60] may contribute to the pathogenesis of HAND in the ART era[60]. Endocytosis[56] and cell-to-cell interactions[44] have been identified as possible mechanisms of HIV-1 entry into astrocytes. The concept of latent HIV-1 infection in astrocytes has been also controversial. Early studies in stable cell lines after HIV-1 cDNA transfection demonstrated latency and reactivation-like properties[61]. Subsequent studies with latency-reversing agents showed the epigenetic regulation of HIV-1 latency in primary astrocytes[57] and human neural stem cells (NSC)-derived astrocytes[62]. Recent reports demonstrated that human astrocytes sustain long-term productive HIV-1 infection without the establishment of reversible viral latency[60]. Although the frequency of HIV-infected astrocytes is very low, the large number of total astrocytes in the brain, cell-to-cell transmission, and long-term HIV-1 infection may justify the important contribution of long-lived astrocytes to HAND persistence.

The inflammatory and immunological regulation of adult neurogenesis

Adult neurogenesis describes the generation of new neurons and glial cells mainly in two specific locations

in the adult CNS: the subventricular zone (SVZ) lining the lateral ventricles and the subgranular zone (SGZ) lining the inner dentate gyrus of the hippocampus. In the SVZ neurogenic niche, slowly proliferating radial glial cells (GFAP expressing B cells) differentiate into rapidly proliferating transit-amplifying cells (Mash1 expressing C cells), which then differentiate into neuroblasts (doublecortin-expressing A cells)[63]. These neuroblasts then tangentially migrate to the olfactory bulb using a tunnel-like astrocytic network termed the rostral migratory stream. After migration, these neuroblasts detach from the stream, and terminally differentiate into either periglomerular neurons or olfactory granule cells, which radially integrate into the olfactory bulb to modulate olfactory stimuli processing[63]. In the SGZ, the neurogenic process is similar, except the migration distance of NSC/neural progenitor cells (NPC) is much shorter than that for SVZ neurogenesis. As the NSCs terminally differentiate into mature dentate granule neurons, they radially migrate into the granule cell layer of the dentate gyrus and extend mossy fibers toward the CA3 region of the hippocampus. In both neurogenic niches, NSCs represent only a small population of quiescent and slowly dividing cells, whereas NPCs are a larger population of amplifying, rapidly dividing cells[63].

NSCs/NPCs are always mixed in culture conditions because NSCs continuously differentiate into NPCs. *In vivo* studies have identified the presence of quiescent (type Ia) and actively proliferating NSCs (type Ib)[64]. The types of cells in each stage can be identified with cell-specific markers (Fig. 1). For example, GFAP expression with Sox2 and/or nestin expression is characteristic for NSCs ($GFAP^+$), while NPCs only express Sox2 and/or nestin. Type 1b NSCs are $Nestin^+/GFAP^+/Sox2^+/Ki67^+$ immunoreactive, while type 1a NSCs are $Nestin^+/GFAP^+/Sox2^+/Ki67^-$. Mature astrocytes express GFAP and $S100\beta^+$, while oligodendrocytes express PLP, MBP, and O4.

The adult neurogenic process is regulated by many intrinsic and extrinsic factors, up to but not including mood states, exercise, and injury. One of the most important modulators of adult neurogenesis is neuroinflammatory stimuli[65]. In physiological conditions, microglia are immune cells that surveil the CNS for external pathogens as well as endogenous damage. Interestingly, there is a high concentration of microglia in the SVZ and SGZ niches, indicating that these cells tightly regulate the neurogenic process[66,67]. During CNS injury, microglia secrete a variety of cytokines and chemokines that can modulate adult neurogenesis.

IL-1α has been shown to promote the differentiation of mesencephalic NSCs into dopaminergic neurons while having an inhibitory effect on postnatal hippocampal neurogenesis[68]. IL-1β decreased NSC proliferation and neuronal differentiation, which was reversed by treatment with IL-1 receptor antagonist[69]. However, IL-1β treatment promotes astrocytic lineage differentiation[70]. Most studies involving TNF-α showed increased glial lineage differentiation and decreased neuronal lineage differentiation. For example, treating human fetal cortical NSCs/NPCs with TNF-α induced higher levels of apoptosis promoted glial lineage differentiation, and hampered neuronal differentiation[71]. However, in mouse SVZ NSCs, treatment with TNFα resulted in NFκB-dependent neuronal lineage differentiation, which could be reversed by an NFκB inhibitor[72]. These conflicting reports could be attributed to differences in animal species where the NSCs/NPCs were derived from, the type of NSCs/NPCs (embryonic vs. adult) used, as well as the concentrations of TNF-α tested. Human hippocampal NSC treatment with IFN-γ did not alter neuronal lineage differentiation but increased astrocytic lineage differentiation[73]. However, in rat striatal NPCs, IFN-γ treatment reduced NSC/NPC proliferation and enhanced apoptosis[74].

These inflammatory mediators play an important role in modulating the spread of HIV-1 infection. IL-1α and IL-1β have been shown to reactivate latent HIV-1 in U1 cells in a dose-dependent manner[75]. In addition, EcoHIV infection levels were higher in IL-1 receptor knockout mice than wild-type control mice[76]. TNFα has the ability to reactivate HIV-1 expression in latently infected cells through an NFκB dependent mechanism[77], while IFN-γ has been shown to enhance natural killer cell and $CD8^+$ T cell responses to combat HIV-1 infection[78]. Finally, IL-4 has been shown to increase HIV-1 replication in $CD4^+$ T-cells[79]. These studies suggest that inflammatory mediators not only directly impair neurogenesis but also facilitate the spread of HIV-1 in the CNS. Therefore, inflammatory modulation of adult neurogenesis is likely to be a contributor to the progression of HAND.

Productive and latent HIV-1 infection in NSCs

There is an increasing body of literature demonstrating active and latent HIV-1 infection in NSCs that may serve as a new reservoir for HIV-1[80-82]. Several prerequisites are needed for a target cell to serve as an HIV-1 reservoir. The first is that the cells must contain a replication-competent integrated provirus. Second, the

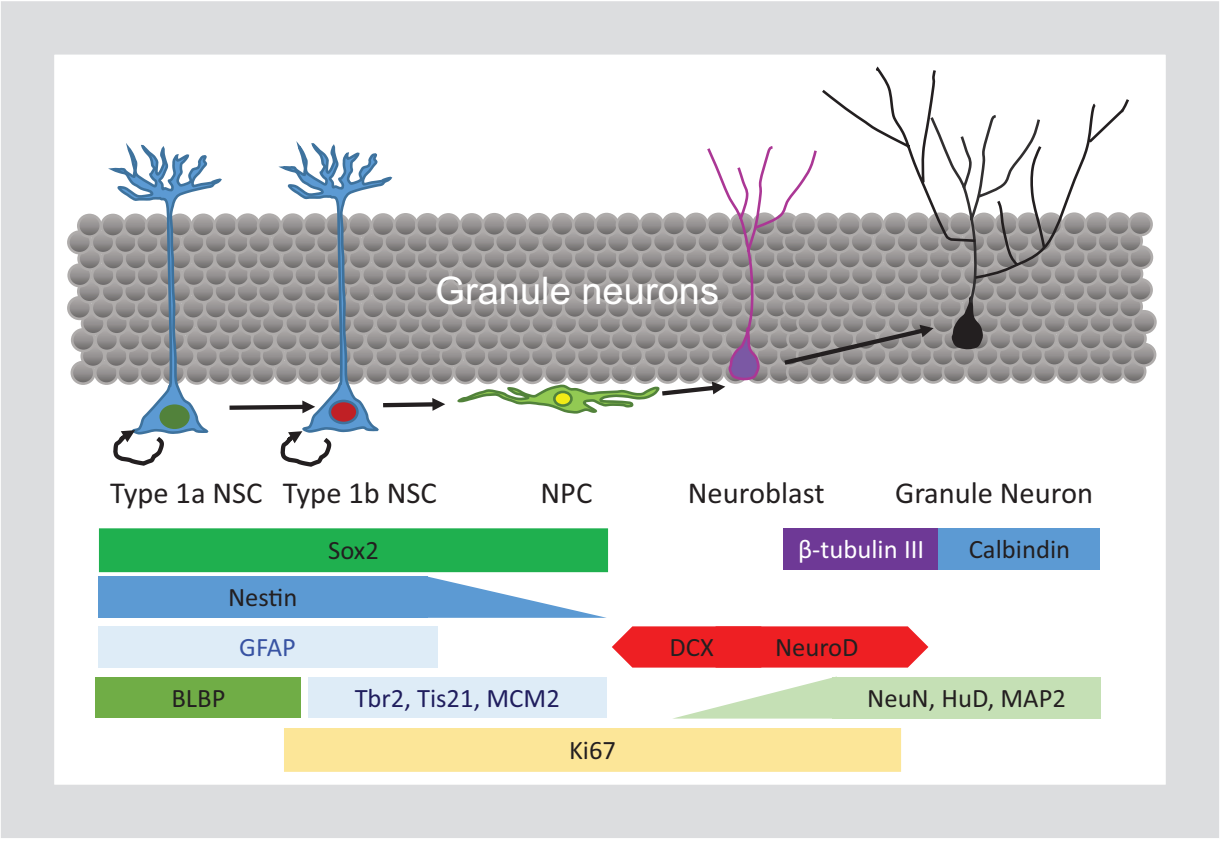

Figure 1. *Diagram of Hippocampal Neurogenesis Progression. Neural stem cells (NSC) and their progeny cells express stage-specific markers along the differentiative process. Specifically, Type 1a (quiescent) and 1b (active) NSCs express Nestin, GFAP, and Sox2. Additional transcription factors that are expressed are BLBP, Tbr2, Tis21, and MCM2. Type 1b NSCs, neural progenitor cells (NPC), and neuroblasts express Ki67, as they are proliferative in nature. NPCs lose GFAP expression, but retain Nestin, Sox2, Tbr2, Tis21, and MCM2 expression. Neuroblasts express doublecortin and lower levels of NeuroD, while granule neurons express β-tubulin III, Calbindin, NeuN, HuD, and MAP2.*

virus must have a mechanism for escaping biochemical decay and establish long-term persistence. The third requirement is that the cells must have the molecular machinery to help the HIV-1 provirus to enter a transcriptionally silent (aka. latent) state. The fourth requirement is that the cells must be capable of reactivating the latent provirus to re-seed new rounds of infection. These sum up the active infection and reversible latency requirements that are characteristic of most HIV-1 cellular reservoirs[46].

Early studies using both CXCR4 and CCR5-tropic strains of HIV-1 showed that primary human neuroblasts derived from fetal olfactory tissue and a neuroblastoma cell line are susceptible to HIV-1 infection[83]. Another study utilizing a human NSC line (HNSC.100) demonstrated effective HIV-1 infection[84]. Viral release lasted for over 2 months, and the cell-associated HIV-1 markers (proviral DNA and early HIV-1 transcripts) persisted during the entire observation period (115 days), suggesting the presence of a long-term productive and latent infection[84]. These previous findings were further validated by a recent study utilizing an immortalized human embryonic ReNcell VM NSC line and a mouse NE-4C NSC line infected with HIV-1 and EcoHIV, respectively[85]. While these findings were interesting, these studies were performed in the immortalized cell lines, which are not as clinically relevant as human primary NSCs.

One early study[86] that utilized primary NSCs to assess productive and latent HIV-1 infection was conducted by Lawrence et al. in 2004. They showed that two CXCR4-tropic HIV-1 strains (HIV_{NL4-3} and HIV_{IIIB}) were capable of infecting human fetal brain-derived primary NSCs/NPCs *in vitro*. This infection was productive, as a viral release from these cells peaked at 3-6 days post-infection. Even though viral production was undetectable after 10 days post-infection, treatment with TNF-α could reactivate the virus in the HIV-1-infected NSCs/NPCs[86]. These findings were significant, as they implicated NSCs for the first time as a

possible new latent reservoir in the CNS, through which HIV-1 latent infection can persist, and be reversed through latency reversing agents such as TNFα. Interestingly, this latency formation was independent of the HIV-1 entry receptors because transfection of an HIV-1 pNL4-3 vector induced similar latency-like infection[86].

A recent study[87] with human primary NSCs expanded on these initial *in vitro* findings to assess whether a CCR5 tropic viral strain (HIV-1_{BaL}) could productively infect human fetal NSCs/NPCs. This study revealed that HIV-1 can not only productively infect NSCs, but also transmit from the infected NSCs to non-infected NSCs[87]. This observation bolstered the notion that NSCs are the host for HIV-1 propagation, although the types of the propagating cells after initial HIV-1 infection of NSCs remain to be determined. A new study demonstrated that EcoHIV was capable of infecting mouse primary NSCs/NPCs[85]. Interestingly, both neurons and glial cells differentiated from EcoHIV-infected mouse primary NSCs/NPCs retained high copies of integrated and latent EcoHIV proviral DNA at 2 weeks post-infection. This latent reservoir (integrated proviral DNA) remained responsive to treatment with the latency-reversing agent SAHA by expressing HIV-1 RNA transcripts and p24 protein[85]. Although the differentiated cell types (neurons and astrocytes or oligodendrocytes) on reactivation to retain HIV-1 infection need further characterization, to delineate whether NSC-derived astrocytes are HIV-1 latent reservoir would be important in understanding the pathogenesis of HAND persistence. Latently HIV-1-infected astrocytes have been shown to transmit the virus to other cell populations, such as microglia/macrophages and T cells to induce a full productive infection[88]. Astrocytes are capable of retaining long-term productive infection[60]. In addition, the presence of the integrated HIV-1 DNA in fully differentiated neurons is also an interesting finding, supporting earlier reports of integrated HIV-1 DNA in the neurons of pediatric HIV-1 patients[27,89].

While many of these pioneering *in vitro* studies have provided evidence that HIV-1 can productively and latently infect human NSCs[85-87], only one clinical study demonstrated the presence of the HIV-1 proviral genome in both the SVZs and SGZs of four pediatric AIDS patients[90]. Therefore, more solid *in vivo* evidence is urgently needed to corroborate these initial findings. The recent development of RNA/DNAScope technology[91] and digital droplet PCR analysis[92] may allow for more sensitive detection of HIV-1 DNA and RNA in the CNS cellular reservoirs in ART-controlled HIV-1 patients. Alternatively, human brain organoids derived the induced pluripotent stem cells may serve as a novel *ex vivo* model to validate HIV-1 infection in human primary NSCs[93]. Since only a small number of GFAP$^+$ cells are susceptible to HIV-1 infection, and NSCs express GFAP, a better understanding of the similarity and diversity for HIV-1 infection between NSCs and astrocytes should provide optimized therapeutics for the HIV-1 patients with HAND.

Neurogenic deficits by HIV-1 infection and viral proteins

Persistent HIV-1 infection in NSCs may alter their functional features such as self-renewal, tripotential differentiation, and survival. Early studies in human primary NSCs and hippocampal slices demonstrated that HIV-1 coat proteins or the CSF from HIV-1 patients inhibited the differentiation of NSCs into NPCs by inducing NSC quiescence[94]. Early studies in an HNSC.100 cell line showed that HIV-1 infection inhibited neuronal differentiation and promoted astrocytic lineage differentiation[84]. A recent study showed that HIV-1 infection not only decreased neuronal lineage differentiation but also compromised the morphological arborization of differentiated neurons[85]. However, a recent *in vitro* study has shown that active HIV-1 infection of human NSCs promotes both neuronal and astroglial lineage differentiation[87]. This discrepancy may result from different viral strains and experimental conditions utilized.

The first study to assess the effects of HIV-1 proteins on neurogenesis was conducted in 2007 by Okamoto et al.[95]. While rat NSCs treated with HIV-1 gp120 did not have apoptosis, their proliferation was significantly reduced at the *in vitro* and *in vivo* levels due to stimulation of the p38-MAPK signaling cascade[95]. However, gp120 had little effect on the neuronal or astrocytic lineage differentiation. Further studies examining the role of gp120 on neurogenesis were conducted with a GFAP-gp120 transgenic mouse[96], in which the gp120 is constitutively expressed in GFAP$^+$ astrocytes and NSCs. Initial analysis revealed significant astrogliosis and synaptodendritic damage in GFAP-gp120 transgenic mouse when compared to non-transgenic control mice[96]. While this study was essential to understanding the role of gp120 in HIV-1-induced neuropathology, only mature astrocytes were examined. Since NSCs also express GFAP, adult neurogenesis could also be potentially impaired in

these mice. A more recent study validated the expression of gp120 in NSCs using immunohistochemical analysis[97]. GFAP-gp120 mice had significant decreases in NSC proliferation and neuroblast/neuron production in the SGZ, which were rescued by exercise or paroxetine treatment. Interestingly, using retroviral labeling technology, they showed that newborn dentate granule neurons in GFAP-gp120 transgenic mice exhibited increased dendritic arborization and length when compared to newborn dentate granule neurons in the non-transgenic littermates. This aberrant initial dendritic outgrowth was also rescued by exercise and paroxetine treatment[97]. These deficits in adult neurogenesis could be rescued by modulation of the endocannabinoid system. Treating GFAP-gp120 mice with the CB2 agonist AM1241 enhanced NSC/NPC proliferation, decreased apoptosis, and promoted higher levels of neuronal differentiation when compared to untreated GFAP-gp120 mice[98]. In addition, the genetic deletion of the fatty acid amide hydrolase in GFAP-gp120 mice rescued gp120-induced neurogenic deficits in a similar manner as AM1241 treatment[99]. These findings are consistent with previous reports implicating the neuroprotective role of endocannabinoid signaling in the CNS[100].

HIV-1 Tat protein has been reported to impair adult neurogenesis. The first *in vitro* study implicating Tat in neurogenesis showed that increasing doses of Tat decreased secondary neurosphere size, decreased NSC proliferation, and decreased neuronal lineage differentiation without inducing any changes in NSC viability[101]. The deficits in NSC proliferation are attributed to the decreased cyclin D1 expression and ERK1/2 pathway activation[101]. Another *in vitro* study demonstrated that Tat impaired the proliferation of human embryonic NSCs through disruption of the p38/JNK MAPK signaling pathways, which was attenuated by pre-treatment with platelet-derived growth factor-BB[102]. To understand the role Tat plays in modulating neurogenesis at the *in vivo* levels, the doxycycline-inducible GFAP-Tat transgenic mice were generated, wherein the tetracycline responsive element is placed ahead of the mouse GFAP promoter, allowing for Tat expression in $GFAP^+$ cells on doxycycline induction[103]. On treatment with doxycycline, the inducible GFAP-Tat mice exhibited a smaller NSC pool, decreased NSC/NPC proliferation, decreased neuronal lineage differentiation, and increased astrocytic lineage differentiation, when compared to untreated GFAP-Tat mice[81]. These deficits are consistent with the neurogenic studies conducted in GFAP-gp120 mice[97-99]. These mice were also used to assess the mechanism behind the increased preference for astrocytic lineage differentiation of NSCs. The authors found that increased STAT3 activation in GFAP-Tat mice leads to an increased GFAP expression in NSCs[104], which further confirmed an *in vitro* study elucidating the role of Tat in inducing astrocytic lineage differentiation in cortical NSCs/NPCs[71].

Correlation of neurogenic deficits with cognitive abnormalities

While the GFAP-gp120 mouse and doxycycline-inducible GFAP-Tat mouse were integral in their roles for elucidating the effects of gp120 and Tat on neurogenesis, virtually no behavioral studies have been conducted in parallel with the *in vivo* neurogenesis experiments[81,97-99]. However, some studies have shown varying degrees of neurocognitive deficits in these mice. For example, while GFAP-gp120 mice had no deficits in novel object recognition or novel object location tasks, they showed deficits in an object-in-place recognition task[105]. This same study also showed that GFAP-gp120 mice had no defects in spatial learning and memory in a Barnes maze behavioral task, as assessed by the delayed time to enter the target hole and number of reference errors made[105]. However, the doxycycline-inducible GFAP-Tat mice showed different results as these animals had robust deficits in contextual fear conditioning, Morris water maze escape tasks, and Barnes maze latency to escape[106-109]. These spatial memory deficits correlate with selective loss of the vulnerable CA1 hippocampal interneurons[109]. Collectively, these observations indicate that Tat may have more of a neurotoxic effect on the cognitive outcome than gp120. However, the correlation of adult neurogenic impairment with the neurocognitive dysfunctions in HIV-1 transgenic animals or HIV-1 infected patients has not yet been evaluated.

Outstanding questions and future directions

Collectively, the literature shows that adult neurogenesis is compromised in HIV-1 infected patients in both the pre- and post-ART eras[90,110]. NSCs are capable of being productively and latently infected by HIV-1. It is likely that the integrated proviral DNA may persist as the NSCs differentiate into neurons, astrocytes, or oligodendrocytes. Or, the latent provirus may be reactivated as the NSCs differentiate into rapidly amplifying NPCs, spreading the infection to neighboring infiltrat-

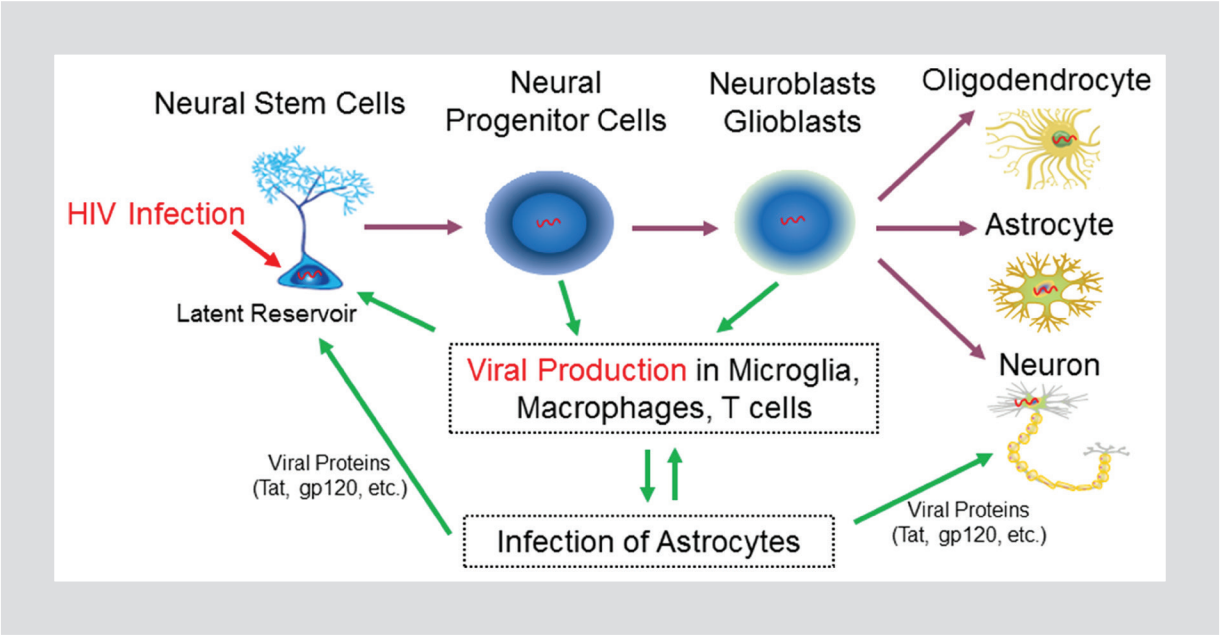

Figure 2. *Working Model for Neural Stem Cells as an HIV-1 Reservoir. Productive infection of neural stem cells by HIV-1 results in integration of the proviral genome in these cells. As these neural stem cells differentiate into neural progenitor cells, the integrated provirus robustly reactivates and generates new virions. Neuroblasts and/or glioblasts could potentially harbor the integrated proviral genome, which could lead to viral integration in neurons, astrocytes, and oligodendrocytes. This new HIV-1 virus further infects microglia and infiltrating immune cells in the brain, which subsequently infects astrocytes. Astrocytic infection could result from direct cell-to-cell contact with infected immune cells or from stimulation from inflammatory cytokines, which could upregulate the receptors needed for HIV-1 infection. These astrocytes would release viral proteins that damage neuron, astrocyte, and oligodendrocyte function. In addition, these secreted virions could subsequently infect other neural stem cells, and re-seed that specific reservoir for further HIV-1 latency establishment.*

ing immune cells and astrocytes. This secondary infection could re-seed the latent reservoir in NSCs or damage the NSCs and their progeny cells (Fig. 2).

The HIV-1 proteins gp120 and Tat exert differential effects on adult neurogenesis at the molecular, cellular, and neurobehavioral levels. However, most of the reported studies focused on single viral proteins (Tat or gp120) in a specific cellular type ($GFAP^+$ astrocytes and/or NSCs). HIV-1 patients in the ART era have the entire HIV-1 proviral genome integrated into their cells. To mimic the persistent and latent HIV-1 infection during the ART era, two rodent models have been generated: the HIV-1 Tg26 mouse[111] and the HIV-1 transgenic rat[112]. Both models contain multiple copies of a replication-deficient HIV_{NL4-3} strain integrated into their cells and express various degrees of HIV-1 transcripts in different organs/tissues[111,112], particularly in the neurogenic zones[80]. Even though spatial learning and memory deficits have been demonstrated in the HIV-1 transgenic rat[113], they have not been directly correlated with alterations of adult neurogenesis. Our recent *in vitro* and *in vivo* study demonstrated that HIV-1 Tg26 mice exhibited significant deficits in the early- and late-stage neurogenesis[80]. It would be interesting to determine how neurogenesis is modulated in rodent models with acute or chronic infection (i.e., direct infection with Eco-HIV in wild-type mice or HIV-1 infection in humanized mouse models with engrafted human brain organoids). Finally, it would be of importance to assess any gender-specific differences in adult neurogenesis in the context of HIV-1 infection. Given that women make up a majority of the HIV-1-infected population worldwide and clinical studies evaluating the incidence of HIV-1/HAND in male and female patients have been inconsistent[114], modeling any possible gender-specific neurogenesis deficits in animal models of HIV-1 disease would significantly benefit the HAND research. Finally, the effects of neurogenesis in the aging HAND population should be evaluated. Since the prevalence of HIV-1 infection in patients aged 50 and older is increasing, and hippocampal neurogenesis depletes with aging, understanding the role of aging in the development of HAND is of clinical relevance and significance.

Conflicts of interest

None.

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Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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