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REVIEW

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Unravelling the triad of neuroinvasion, neurodissemination, and neuroinflammation of human immunodeficiency virus type 1 in the central nervous system

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Abstract

Since the identification of human immunodeficiency virus type 1 (HIV-1) in 1983, many improvements have been made to control viral replication in the peripheral blood and to treat opportunistic infections. This has increased life expectancy but also the incidence of age-related central nervous system (CNS) disorders and HIV-associated neurodegeneration/neurocognitive impairment and depression collectively referred to as HIV-associated neurocognitive disorders (HAND). HAND encompasses a spectrum of different clinical presentations ranging from milder forms such as asymptomatic neurocognitive impairment or mild neurocognitive disorder to a severe HIV-associated dementia (HAD). Although control of viral replication and suppression of plasma viral load with combination antiretroviral therapy has reduced the incidence of HAD, it has not reversed milder forms of HAND. The objective of this review, is to describe the mechanisms by which HIV-1 invades and disseminates in the CNS, a crucial event leading to HAND. The review will present the evidence that underlies the relationship between HIV infection and HAND. Additionally, recent findings explaining the role of neuroinflammation in the pathogenesis of HAND will be discussed, along with prospects for treatment and control.

Abbreviations: ANG-1, angiopoietin-1; ANI, asymptomatic neurocognitive impairment; APP, β-amyloid precursor protein; ART, antiretroviral therapy; BBB, blood–brain barrier; bFGF, basic fibroblast growth factor; cART, combined antiretroviral therapy; CCL2, chemokine (C-C Motif) Ligand 2; CCR2, C-C chemokine receptor type 2; CCR3, C-C chemokine receptor type 3; CCR5, C-C chemokine receptor type 5; CCR8, C-C chemokine receptor type 8; CPE, CNS penetration effectiveness; CX3CR1, C-X3-C chemokine receptor type 1; CXCR4, C-X-C chemokine receptor type 4; CXCR6, C-X-C chemokine receptor type 6; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; dsDNA, double-strand DNA; Env, envelope; Gag, group specific antigen; GDNF, glial cell-derived neurotrophic factor; Gp120, glycoprotein 120; surface envelope glycoprotein of HIV-1 encoded by the *env* gene; Gp41, glycoprotein 41; transmembrane envelope glycoprotein of HIV-1 encoded by the *env* gene; HAD, HIV-associated dementia; HAND, HIV-associated neurocognitive disorders; IL-1β, interleukin 1β; IL-6, interleukin 6; IL-8, interleukin 8; IP-10, Interferon gamma-induced protein 10; also known as C-X-C motif chemokine ligand 10 (CXCL10); IRIS, immune reconstitution inflammatory syndrome; L-SIGN, Liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin; MND, mild neurocognitive disorder; Mø, macrophages; ND, neurological disorders; Nef, negative factor; p17, protein 17; matrix protein of the HIV-1 encoded by the *gag* gene; p24, protein 24; major core protein of the HIV-1 encoded by the *gag* gene; PNS, peripheral nervous system; PRR, pattern recognition receptor; R5, CCR5-using strain; ssRNA, single-strand RNA; Tat, trans-activator of transcription; TJ, tight junction; VEGF, vascular endothelial growth factor; Vpr, viral protein R; X4, CXCR4-using strain.

KEYWORDS
central nervous system, HIV-1, HIV-associated neurocognitive disorders, HIV-cell interactions

1 | INTRODUCTION

The human immunodeficiency virus type 1 (HIV-1) and HIV type 2 (HIV-2) are the causative agents of acquired immunodeficiency syndrome (AIDS). This syndrome was first recognized in 1981 and has since then become one of the world's major public health problems, particularly in developing countries. The virus is transmitted through unprotected sexual intercourse, exposure to infected blood, and by mother-to-child transmission (i.e., during pregnancy, childbirth, or breastfeeding). Since the beginning of the pandemic, 85.6 million people have been infected and approximately 40.1 million people have died from HIV-1. By the end of 2022, approximately 40.4 million people worldwide are living with HIV-1 and 1.3 million people become newly infected.[1]

As well as affecting the immune system and leading to AIDS, HIV-1 can also cause neurological disease, culminating in varying degrees of dementia.

HIV-1 entry into the central nervous system (CNS) occurs as early as 8 days after transmission,[2] mainly carried by infected CD4+ T lymphocytes and monocytes from the bloodstream, and the resulting structural changes in the brain are detectable within the first year.[3] In individuals who have never been treated with combined antiretroviral therapy (cART-naïve individuals), increased glial cell activation and neuronal injury are observed,[4] and HIV-1 has been detected in microglia in *post-mortem* brain samples from asymptomatic HIV-infected individuals.[5]

Between 20% and 50% of the patients exhibit neurological impairment collectively known as HIV-associated neurocognitive disorders (HAND).[6] Remarkably, although the virus infects the brain early in HIV-1 infection, the neurological disease or complications defined by HAND, tend to occur during chronic infection as a consequence of long-term HIV-1 replication within the brain and persistent neuroinflammation directly or indirectly induced by HIV-1 infection.

HIV-1 can affect the CNS in three different ways: (i) as primary HIV-1 neuropathology, in which the virus is both necessary and sufficient to cause the disease; (ii) as secondary or opportunistic neurological disease, in which HIV-1 interacts with other pathogens that take advantage of the progressive immunodeficiency caused by HIV-1 infection; and (iii) treatment-related neurological disease, such as immune reconstitution inflammatory syndrome, a disease- or pathogen-specific immune response similar to an opportunistic infection.[7–9]

The objective of this review is to describe the mechanisms by which HIV-1 invades and disseminates in the CNS, a crucial event leading to HAND. The review will present the evidence that underlies the relationship between HIV infection and HAND. Additionally, recent findings explaining the role of neuroinflammation in the pathogenesis of HAND will be discussed, along with prospects for treatment and control.

2 | NEUROLOGICAL DISORDERS

Neurological disorders (NDs) are diseases that affect the CNS and the peripheral nervous system, in diverse and heterogeneous ways, including epilepsy, Alzheimer's disease and other dementias, multiple sclerosis, Parkinson's disease, neuroinfections, brain tumours and traumatic disorders. These diseases can be divided into several categories, including neurodegenerative, neuroinflammatory, and neoplastic diseases.[10–13]

The first report of a CNS infection leading to NDs was in 1992 by Bowery et al.[14] Today, NDs affect hundreds of millions of people worldwide, representing the second leading cause of death.[10,12] In Europe alone, approximately 60% of the population suffers from a neurological disease.[15] The population is currently ageing and NDs are becoming more common.[16,17]

The chronic dysfunction of neuronal cells, a characteristic of NDs, may be caused by irreversible damage and cell death. Many studies have linked NDs to viral infections, which may cause neurological symptoms or lead to immune responses that trigger these pathological signs.[10,18,19]

NDs in individuals infected with HIV-1, caused by either direct HIV-1 infection or opportunistic CNS infections, are a prevalent cause of morbidity and mortality.[10,18] However, the majority of neurological pathologies, including dementia, opportunistic infections, meningitis, and neuropathy, manifest only during the advanced stages of HIV-1 infection, subsequent to the onset of AIDS.[19] The development of these cognitive syndromes has been associated with poor adherence to antiretroviral therapy (ART), which compromises the control of HIV-1 replication and the survival of infected individuals. In fact, some studies have linked less severe neurological and cognitive problems in people on ART with a controlled viral load,[19,20] suggesting that HIV-1 replication and viral proteins expression are a major cause of the neurocognitive impairment.

HAND is a clinical condition resulting from the direct effects of HIV-1 infection, which are not related to opportunistic infections linked with immunodeficiency.[18,19] According to the Frascati criteria, HAND is currently classified as asymptomatic neurocognitive impairment, mild neurocognitive disorder, and HIV-associated dementia (HAD).[20] HAND is closely related to several aspects of HIV infection, including adherence to ART therapy, plasma viral load, level of immune deficiency, late initiation of ART, comorbidities that may contribute to the prevalence of HAND and even the health literacy.[21] The prevalence of HAND varies depending on the population enroled in the study, reflecting these multifactorial inputs. For instance, a recent systematic review and meta-analysis reported a range of HAND prevalence between 14% and 88% in African countries.[22] The high prevalence of HAND in Africa may be attributed to several factors. These include delayed initiation of ART and inadequate

health system coverage leading to poor ART adherence, and reduced surveillance and diagnosis of comorbidities or coinfections affecting the CNS. This is particularly relevant in sub-Saharan African countries, where gender and level of education are additional statistically significant factors that determine the development of neurocognitive impairment. Females are three times more affected, and individuals with low education levels are twice as likely to experience neurocognitive impairment compared to those with higher education.[23] Measures included in the World Health Organization's HIV 2030 initiative may improve preventive measures to protect HIV patients from developing neurological complications.[19]

3 | NEUROINVASION

HIV-1 invades the CNS within the first week(s) of infection and persists despite potent cART.[2,24–29] HIV-1 neuroinvasion leads to inflammatory responses that result in the manifestation of HAND, but also to the establishment of the CNS as a site of persistent viral infection and HIV-1 compartmentalisation, as evidenced by the presence of distinct HIV-1 genetic sequences in the CNS compared to plasma and lymphoid tissues.

However, the mechanisms of HIV-1 entry into the CNS are still not fully understood. One theory, the 'Trojan Horse' hypothesis, proposes that the virus can enter the CNS from the bloodstream via infected monocytes or CD4+ T lymphocytes.[30] Alternatively, HIV-1 can increase the permeability of the blood–brain barrier (BBB) by altering the expression of proteins that maintain BBB tight junctions (TJs), namely through the action of the viral protein Tat.[31–33] Other studies have also shown that free viral particles can cross the BBB by transcytosis[34] and that endothelial cells can be infected, facilitating direct entry of HIV-1 into the CNS.[35]

3.1 | Structure of the blood–brain barrier

The BBB is a highly selective and semipermeable barrier that prevents most substances, including toxins and pathogens, from entering the brain from the bloodstream.[36] It is formed by a continuous cellular layer of brain microvascular endothelial cells connected by TJ proteins, located between the bloodstream and the basement membrane (Figure 1). The basement membrane is an extracellular membrane, composed mainly of collagen IV and laminin, that anchors endothelial cells to the underlying tissue. In close contact with the basement membrane are perivascular pericytes and glial cells.[37,38] Pericytes appear to contribute to structural support and also play a role in angiogenesis and TJ formation.[39] Astrocyte end-feet and pericytes surround the vessel wall, which appears to be critical for the induction and maintenance of TJs. However, astrocytes do not appear to have a barrier function. These cells interface with neurons to form a functional neurovascular unit that protects the brain from pathogens, maintains homoeostasis and regulates the trafficking of cells and substances between the brain parenchyma and the bloodstream.[40,41] In the perivascular space, surrounding arterioles and venules, are located the perivascular macrophages which maintain the brain homoeostasis and are directly involved in immune surveillance.[42]

3.1.1 | Brain microvascular endothelial cells

The morphology, biochemistry and function of brain endothelial cells renders them phenotypically distinctive from other endothelial cells situated elsewhere in the body. Adjacent endothelial cells are held together by specialised TJs that maintain vascular integrity and permeability, thereby preventing the paracellular flux of molecules across the BBB endothelium.[43–45] Another characteristic of CNS endothelial cells is their abnormally low vesicle trafficking rate, which restricts transcytosis and enhances their barrier properties.[46]

They also contain higher levels of mitochondria, which are thought to be critical for generating adenosine triphosphate to drive the ion gradients critical for transport functions,[47] and have an extremely low expression of leucocyte adhesion molecules, which limit the entry of immune cells into the CNS.[48] Notably, while endothelial cells form the actual barrier, interactions with neighbouring cells appear to be a prerequisite for efficient barrier function.

3.1.2 | Pericytes

Pericytes are perivascular cells situated around microvessels in the basement membrane and are closely associated with endothelial cells. They play an essential role in the development and maintenance of the BBB integrity and have a variety of important functions in the brain. Pericytes have similar characteristics to smooth muscle cells and can regulate the capillary diameter and the cerebral blood flow.[49,50] They can also perform immune cell-like functions such as phagocytosis, removing toxic metabolites.[51] In vitro studies have demonstrated that pericyte-associated endothelial cells exhibit greater resistance to apoptosis than isolated endothelial cells. This finding supports the role of pericytes in maintaining the structural integrity and genesis of the BBB.[52] The loss of pericytes in a mouse model with subsequent microaneurysm formation suggests that pericytes are also involved in maintaining the stability of the microvascular wall.[53]

3.1.3 | Astrocytes

Astrocytes have multiple and important roles in the CNS. For example, astrocytes serve as support cells that contribute to the structural and regulatory properties of the BBB by secreting growth factors such as vascular endothelial growth factor, glial cell-derived neurotrophic factor, basic fibroblast growth factor and angiopoietin-1.[54,55] Astrocyte-secreted growth factors are important in the formation of TJs,[54] the promotion of enzymatic activities and

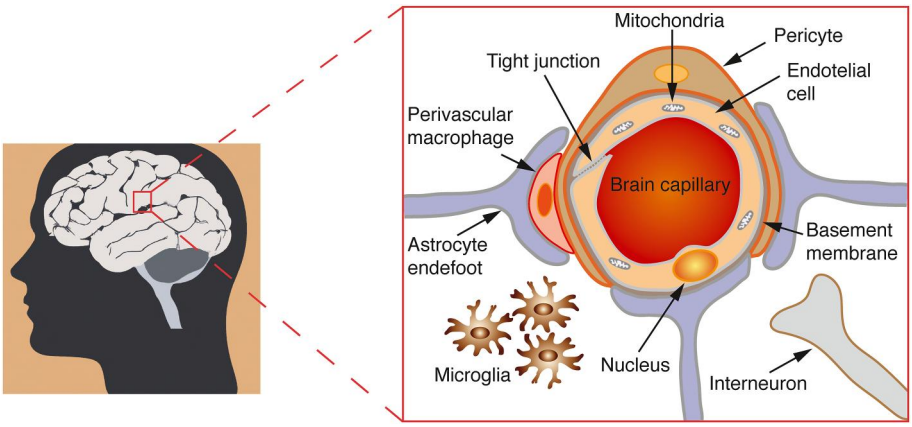

FIGURE 1 Schematic representation of the blood–brain barrier structure and neurovascular unit (transverse section).

the polarisation of transporters.[55] These growth factors are also involved in neuronal growth and survival during brain injury.[56]

In addition, astrocytes terminal structures, known as end-feet, provide the link between the brain vasculature and the glial cells in the neurovascular unit, allowing modulation of both neuronal activity and cerebral blood flow.[57,58] One study demonstrated that cultured astrocytes can induce endothelial cells in the CNS to form the BBB when introduced into areas of leaky capillaries and venules.[59] Another study showed that endothelial cells can be primed to form a TJ monolayer when in direct contact with astrocytes.[60]

3.1.4 | Brain perivascular macrophages

Brain perivascular macrophages are brain-resident myeloid cells located within the perivascular space surrounding arterioles and venules. They participate in several processes in normal and disease condition. In physiological conditions, perivascular macrophages contribute to BBB integrity, phagocytosis and antigen presentation. In disease process, such as encephalitis associated with HIV-1 infection, there has been observed an increased number of perivascular macrophages coincident with viral neuroinvasion suggesting that these cells facilitate BBB disruption.[61,62] Several studies using SIV-infected models reported the existence of RNA and proteins in perivascular macrophages suggesting that SIV-infected perivascular macrophages participate in viral production.[63–66] Furthermore, HIV-1 DNA was also detected in HIV-infected individuals perivascular macrophages suggesting that these cells may be reservoirs of HIV-1.[67]

3.2 | How HIV-1 crosses the BBB

To establish a local brain infection, HIV-1 must cross the BBB and migrate into the CNS. The mechanisms of neuroinvasion are not fully understood, but certain theories have been proposed to explain how HIV-1 crosses the BBB: from free viral entry to cell-mediated invasion (Figure 2).

3.2.1 | 'Trojan horse' hypothesis

This is the most widely accepted theory, which suggests that the virus (as proviral DNA) enters as a 'passenger' in cells that travel to the brain, such as infected CD4+ T lymphocytes and monocytes, and then spreads the infection to resident cells, helping to establish a persistent infection in the brain.[30,68] This mechanism has been described for other retroviruses and lentiviruses and it is likely to be the main mechanism by which HIV-1 enters the brain.[69–71]

Several observations support this theory. For example, proviral DNA has been found in some bone marrow-derived monocytes obtained from HIV-infected individuals. Since these cells do not express viral proteins, they can evade immune surveillance, facilitating the spread of HIV-1 to different body compartments.[72,73] Interestingly, a minor monocyte subset, the $CD14^{low}/CD16^{high}$, expands during HIV-1 infection. These cells show an altered expression profile of cytokines and cellular markers normally associated with resident tissue macrophages.[74] These characteristics allow these cells to potentially dysregulate the transport mechanisms of the BBB and allow them to cross the BBB more easily. In addition, $CD14^{low}/CD16^{high}$ monocytes are more susceptible to HIV-1 infection and replication, providing a continuous source of viral replication during cART.[75,76]

3.2.2 | Alteration of BBB permeability

Another route of viral entry is mediated by the loss of BBB integrity, which increases permeability and allows migration of cell-free viral particles, with significant and direct implications for HIV-1 neuroinvasion and pathogenesis.[31,32,77] The structural changes leading to permeability alteration may be due to a reduction in pericyte coverage of the endothelium in HIV-infected patients, with or without cART, leading to monocyte neuroinfiltration, including migration of HIV-infected cells.[78] It has also been described that increased release of proinflammatory cytokines during chronic neuroinflammation may induce pericyte depletion, leading to pericyte detachment.[78–80] Reduction in brain pericyte density and

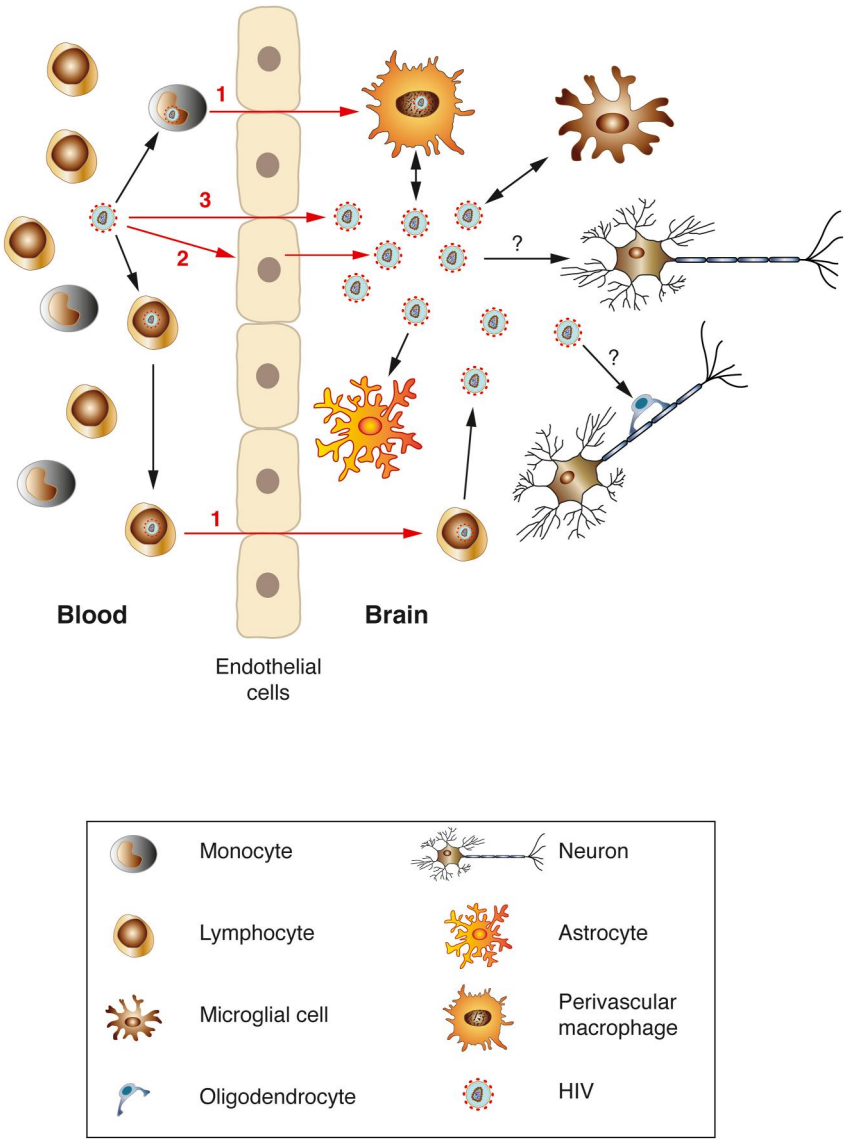

FIGURE 2 The various routes by which HIV enters the central nervous system (red arrows) and the interaction of HIV with neural cells (black arrows). (1) The 'Trojan horse' mechanism, occurs when monocytes or CD4+ T lymphocytes infected with HIV cross the BBB; (2) transcytosis across brain capillary endothelial cells; (3) direct entry, which may occur in the presence of increased permeability of the BBB resulting from the damage or dysfunction of its cells. BBB, blood–brain barrier; HIV, human immunodeficiency virus.

detachment from the brain endothelium leads to BBB dysfunction, which may accelerate neurodegeneration during HIV-1 infection.[81]

3.2.3 | Transcytosis

Another hypothesis proposes that viral neuroinvasion may also occur by transcytosis across brain endothelial cells.[34,82] An in vivo study was performed to determine the role of the gp120-gp41 complex in the transport across the BBB using radioactive virions with (Env+) or without (Env–) envelope glycoproteins. They show that free HIV-1 can be taken up by brain endothelial cells and cross the BBB, proving that envelope proteins are essential for this mechanism as they appear to mediate the process of transcytosis.[34]

Transcytosis can also occur and even be facilitated when the BBB structure is altered by an increase in proinflammatory cytokines. In cases where tumour necrosis factor alpha levels were upregulated, disruption of the BBB and an increase in HIV-1 p24 antigen levels in the brain were observed.[83]

3.2.4 | Infection of endothelial cells

For HIV-1 to enter a cell, it must bind to CD4 and subsequently to a chemokine receptor that acts as a coreceptor.[84] Endothelial cells lack CD4 expression, but do show significant expression of multiple chemokine receptors, specifically CCR5, CXCR4, CCR3, and C-type lectins including dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin and L-SIGN. This indicates a potential role in HIV-1 entry.[85] It has been reported that HIV-1 can directly infect endothelial cells, and although there is no convincing evidence that endothelial cells are a major target of HIV-1 infection in humans,[86] residual endothelial cell infection may play an important role in compromising the BBB and facilitating HIV-1 neuroinvasion.[85,87]

4 | NEURODISSEMINATION

Theoretically all the major cell types in the CNS compartment can be infected with HIV-1 since they possess the receptors and/or co-receptors for HIV-1 entry. The first cells to be infected are microglia and brain perivascular macrophages at the neurovascular unit,[88] which further disseminate HIV-1 to parenchymal microglia and astrocytes.[63] Synaptic damage and loss associated with HIV-1 infection is thought to be mediated by infected cells (macrophages, microglia and astrocytes), which release viral proteins and soluble neurotoxins, causing excessive calcium influx and disruption of homoeostatic balance.[7,89]

4.1 | CNS-resident immunocompetent cells

4.1.1 | Macrophages and microglia

The initial cells that come into contact with infected cells in the perivascular region are microglia, brain perivascular macrophages, and astrocytes. Microglia and brain perivascular macrophages are the resident immunocompetent cells of the brain, and their primary role is to respond to all types of neurological injury.

Microglia and brain perivascular macrophages are considered to be the primary targets of productive HIV-1 infection in the brain.[90] They express the CD4 receptor and the CCR5 chemokine receptor (major receptor and coreceptor, respectively), which can be used by HIV-1 to infect these cells. Other chemokine receptors, such as CCR3, CCR2b, CCR8, CXCR6, and CX3CR1, are also expressed by these cells, but are less efficiently used by HIV-1.[91]

Brain perivascular macrophages are resistant to the cytopathic effects of HIV-1 and can therefore maintain viral infection for long periods of time.[90,92,93] On the other hand, microglia can develop a productive infection that can lead to syncytia formation and cell death[94]; however, the possibility of latent infection, as observed in brain perivascular macrophages, also exists.

Productive infection of primary cultures of human microglia has been shown to be more readily established by CCR5-using (R5) strains of HIV-1 than by a CXCR4-using (X4) strains.[95] In vitro studies indicates that long-lived mixed glial cultures extracted from the human brain have the capability of generating infectious viral progeny for as long as 2.5 months when infected with R5 HIV-1, even at low levels of viral replication.[96] This slowly productive infection provides an immune-activated environment that can damage the immune system, for example, by recruiting new target cells for HIV-1 replication and promote non-AIDS related morbidities.[96,97] Microglia are thought to be the major cellular reservoir of latent HIV-1[98] and once activated, these cells and brain perivascular macrophages release viral proteins, inflammatory cytokines, and neurotoxins, causing astrocyte differentiation, apoptosis, and the alteration of the normal neurogenesis, leading to neuroinflammation.[40,99–101] Recently, HIV-1 replication in macrophages and microglia has been associated with multivesicular bodies and the β-amyloid precursor protein amyloidogenic pathway has been implicated as a suppressor of HIV-1 replication in such cells.[102]

4.1.2 | Astrocytes

Astrocytes are important components of the BBB and are the most abundant cells in the CNS. They are involved in neuronal function and metabolism, ion homoeostasis in the CNS, scar formation, control and pruning of neuronal synapses, and tissue repair. They also regulate the immune response in the brain.[103,104] Astrocytes exhibit regional diversity[105] and, when reactive, also show heterogeneity in gene expression profiles.[106]

Astrocytes have been shown not to express the CD4 receptor on their membrane, but some authors suggest that CXCR4 may be expressed upon astrocyte activation, and possibly other HIV-1 co-receptors including CCR5.[107,108] In the absence of CD4 and given the important role of this receptor during HIV-1 infection, the mechanism of HIV-1 entry into astrocytes remains unclear.

Nevertheless, astrocytes are capable of sustaining minimal levels of HIV-1 replication, leading to viral persistence within the CNS[87] and the establishment of an infected cell population. Moreover, HIV-1 infection does not induce a cytopathic effect on astrocytes, allowing the virus to remain latent in these cells, making them a long-lived HIV-1 reservoir.[109] Infected cells may occasionally be activated by inflammatory cytokines, leading to productive viral replication and the possibility of spreading infection to other cells within the CNS.[110,111] Indeed, reactivation of astrocytes results in efficient cell-to-cell viral transfer, providing a mechanism for viral spread within the brain.[109] It also highlights that latently HIV-infected astrocytes exhibit increased levels of inositol trisphosphate, which can diffuse through gap junctions to adjacent uninfected astrocytes inducing apoptosis and ultimately contributes to neurodegeneration.[112]

4.1.3 | Neurons

One of the most controversial aspects of HIV neurobiology is whether HIV-1 can infect neurons, even at low levels.[113,114]

Although many studies have suggested that there is no in vivo infection of neurons, a few have demonstrated the presence of HIV-1 genetic material and antigens within neurons.[115] In vitro data have reported limited infection of primary neurons and neuronal cell lines by R5 and X4 viruses.[116,117] In addition, due to subtle changes in envelope glycoproteins some HIV-1 strains have shown the ability to enter target cells in the absence of CD4.[118–123] This CD4-independent mechanism may provide an alternative pathway for selected HIV-1 variants to enter CD4-negative cells, such as neurons.

Irrespective of the susceptibility of neurons to HIV-1 infection, neurocognitive impairment is due to indirect mechanisms mediated by increased toxicity that damages selected neuronal populations. HIV-1 induces the production of inflammatory factors by infected or activated macrophages and microglia, as well as viral proteins with

proven neurotoxic activity (see Section 5), causing indirect injury to neurons.[124] Indeed, HAND are attributed to axonal loss, synaptic degeneration and white matter damage, and patients with AIDS show a significant loss of neocortical neurons.[89,125]

4.1.4 | Oligodendrocytes

As with neurons, the in vivo infection of oligodendrocytes by HIV-1 remains unclear because they do not express the CD4 receptor. Some studies have found viral nucleic acids present in the nucleus, while others have observed no HIV-1 markers in oligodendrocytes.[126] In vitro experiments, using human oligodendrocytes, suggest limited infection by R5 and X4 strains of HIV-1.[127] Recent findings points to oligodendrocyte loss and HIV-induced demyelination in addition to axonal dysfunction, and suggest that remyelination treatment strategies may be indicated in patients with HAND.[125] An interesting recent article raises the possibility that HIV-1 infection impairs oligodendrocyte maturation, function, and even survival based on in vitro studies using oligodendrocyte progenitor cells.[128] The data emphasise the involvement of neurons and glial cells in cognitive impairment in HIV-1 patients and the need for further studies to clarify this issue and develop therapeutic strategies.

5 | NEUROINFLAMMATION

HIV-1 infection is characterised by persistent and pathological levels of pro-inflammatory soluble factors (e.g., IL-6, IL-8, CCL2, IP-10, TNF-α), which are responsible for the systemic chronic inflammation that is considered a hallmark of HIV-1 infection of the human host, ultimately leading to tissue damage and several non-AIDS comorbidities, such as neurocognitive disorders.[129] The induction of chronic inflammation is multifactorial and is the result of direct mechanisms triggered by HIV-1 infection, such as the effects of viral proteins like Nef and viral protein R (Vpr),[130,131] or driven by pattern recognition receptors that recognise viral genomic material (either ssRNA or dsDNA) during the HIV-1 replication cycle.[132–134]

As mentioned above, the mechanisms involved in HIV-1 neuropathogenesis include: (i) infection of brain parenchymal cells (e.g., astrocytes, microglia) following infiltration of either cell-free virus or HIV-infected cells (such as monocytes or CD4+ T lymphocytes) from the peripheral blood into the CNS[2,135–138]; (ii) the production of soluble inflammatory mediators by HIV-infected cells (macrophages, astrocytes and microglia)[139–141]; (iii) the neurotoxic effects of viral proteins (e.g., envelope glycoprotein gp120, Tat, Vpr, and Nef) on neuronal viability and function[142–152]; and (iv) a cascade of inflammatory responses leading to astrocytosis, microglial activation, and neuroinflammation, a hallmark of HAND.[90,153–159] Neuroinflammation may also be sustained by microbial priming resulting from gut dysbiosis and microbial translocation. This can lead to their dissemination through the blood[160] causing chronic inflammation both systemically and in the brain. The persistent immune activation and viral proteins lead to a neurotoxic environment resulting in microgliosis and astrocytosis in the brain tissue of HIV-infected individuals. Autopsy confirms these findings even in cases where cART was used.[161–164]

The inflammatory response involves the activation of inflammasomes in various cells. These large multiprotein complexes assemble as a consequence of pathogen- or stress-induced signals. As a result, cytokines such as IL-1β and IL-18 are activated by caspase 1, and these proinflammatory factors are secreted out the cell following eventual cell death by pyroptosis (reviewed in Ref. 165). The consequence of this necrotic type of cell death is the amplification of tissue damage with increased levels of stress signals, which in turn activate the inflammasome, in neighbouring cells, creating a positive feedback loop of inflammatory response.

In CNS, the assembly of inflammasome complex occurs in the cytosol of various cells, including microglia, astrocytes, neurons, and macrophages.[166] In HIV infection of microglia, Vpr alone is able to induce the release of IL-1β through a NLRP3 inflammasome-dependent mechanism.[167,168] Furthermore, the HIV Tat protein has been shown to activate the NLRP3 inflammasome, leading to increased levels of mature caspase-1 and IL-1β in microglia.[169] Since both Vpr and Tat are released by infected cells, they may affect non-infected neighbouring cells, thereby exacerbating inflammation within the brain parenchyma.

In addition, other HIV-1 proteins secreted by infected cells, even those released by latently infected cells with defective HIV-1 proviruses,[170] have remarkable neurotoxic effects that are directly related to the neuroinflammation observed in HAND. The best characterised of these proteins is the envelope glycoprotein gp120. Studies have shown that gp120 has deleterious effects on neuronal function and viability mediated by the release of inflammatory factors, glutamate and quinolinic acid from brain endothelial cells, macrophages, microglia, and astrocytes.[90,148,171] Furthermore, two other viral proteins, Tat and Nef, have been implicated in neuronal injury and dysfunction via microRNA dysregulation and increased IP-10 secretion, respectively.[142,145–147,149,150,172] Additionally, Tat protein promotes the disruption of the BBB by two major pro-permeability mechanisms: activation of cyclooxygenase 2, which suppresses TJ formation,[173] and induction of matrix metalloproteinase-9 secretion by astrocytes and microglia.[174] Both mechanisms facilitate the entry of more viral particles and infected cells, ultimately increasing neuroinflammation.

Moreover, it has been described that viral Gag proteins, namely p17, are able to penetrate the BBB[175] and form toxic amyloidogenic aggregates that are directly involved in neurocognitive disorders in vivo.[176]

Besides being a source of viral particles and neurotoxic proteins, the infiltration of HIV-infected monocytes and CD4+ T lymphocytes has another consequence: they are directly or indirectly the source of proinflammatory cytokines and chemokines (such as TNFα, IL-1β, CCL2 and IL-6), secondary messengers (nitric oxide and prostaglandins), and reactive oxygen species.[30,177–180] All these mediators exert further deleterious effects on neuronal and glial cells function and survival, helping to explain the neurological impairment observed in HAND.

The study of biomarkers of HAND, has uncovered a direct correlation between Lipocalin-2 (LCN2) and the neurotoxicity caused by HIV infection. LCN2 is a secreted protein with multiple physiological functions, including iron metabolism, innate immune response, and organogenesis, as well as pathological mechanisms linked to cancer and neurodegenerative disorders.[181] In the CNS, LCN2 was found to be upregulated in the neocortex of patients with HIV encephalitis. Its neurotoxicity requires microglia and can be counteracted by the chemokine CCL4, which is a ligand of CCR5.[182]

Osteopontin is another mediator of neuroinflammation implicated in the pathogenesis of HAND and is secreted by monocytes as they mature and differentiate into macrophages. Osteopontin levels are increased in individuals with HIV neurocognitive impairments,[183,184] and it interferes with the selective passage of monocytes highly susceptible to HIV infection through the BBB.[185] Furthermore, it facilitates the infection of monocytes and macrophages by HIV, and protects them from apoptosis, contributing to the establishment and maintenance of long-lived cellular reservoirs of HIV within the CNS.[185]

6 | TREATMENT OF HAND

In the last decade, several approaches have been proposed for the treatment and control of HAND. Although the main focus of this review is not on the treatment of HAND, we will summarise its most important aspects. It is important to note that several reviews have extensively covered this subject.[166,186–189]

HIV-1 replication could be controlled by lifelong cART, which uses drugs that target multiple steps in the HIV-1 replication cycle to achieve long-term suppression of HIV-1 viral load in the peripheral blood. Although the use of cART and the resulting reduction in viraemia levels has decreased the prevalence of more severe forms of HAND such as HAD, cART has a limited effect on reversing milder forms of HAND.[190,191]

This unchanged status of HAND suggests that the level of viraemia is not the only factor driving neurocognitive impairment and must be a consequence of alternative mechanisms. As discussed above, these include (i) the maintenance of low levels of viral replication and the expression of neurotoxic viral proteins from latently infected cell reservoirs in the brain parenchyma (e.g., microglial cells, astrocytes, and perivascular macrophages); and (ii) persistent immune activation and inflammation within the brain. Besides the influence of direct and indirect mechanisms induced by HIV-1 itself, lifelong exposure to drugs included in cART adds significant toxic effects[192] with serious neurological adverse outcomes that need to be reversed or at least controlled.

Thus, it is reasonable to anticipate that HAND could be prevented and treated if: (i) the drugs included in cART could efficiently cross the BBB and reduce HIV-1 replication and the reservoir of latently infected cells in the brain; (ii) the neurotoxicity of the drugs used is reduced; and (iii) the neuroprotective and anti-neuroinflammatory drugs are included to avoid the toxic effects on neuronal and glial cells.

The ability of drugs to cross the BBB is measured by the CNS penetration effectiveness (CPE) score,[192] and the higher the score, the lower the HIV-1 viral load in the CNS.[193] However, this correlation is less well defined when the outcome measured is the neurocognitive performance. Studies have reported better neurocognitive function and lower inflammatory markers when cART included drugs with higher CPE,[194–197] while others failed to observe any positive effect.[198,199] Irrespective of their efficacy in controlling HIV-1 replication and cell reservoirs, it is also expected that drugs with a higher CPE may inherently have more toxic effects on neuronal and glial cells. Hopefully, in the next few years we will see the development of antiretroviral drugs with high CPE and reduced neurotoxicity.

Recent data profiling the transcriptome of microglial cells obtained from SIV-infected rhesus macaques with different patterns of viral replication, showed that the transcriptome of microglia from virally suppressed animals (due to cART) is very similar to that of uninfected controls.[200] These findings emphasise the notion that active viral replication drives changes in microglial phenotype increasing the likelihood of neurotoxic effects.

In addition to inhibiting HIV-1 replication, avoiding and controlling the hyperinflammatory environment that characterises HAND is essential to the prevention and treatment of HAND. Accordingly, adjunctive pharmacological neuroprotection is needed, especially as the life expectancy of virally suppressed HIV-infected individuals has increased, adding age-related comorbidities.

These neuroprotective therapies include anti-inflammatory/antioxidant drugs to reduce cell damage caused by inflammation and oxidative stress, neuropsychiatric drugs, and trophic agents (recently reviewed in Ref. 189). A major goal of these therapies is to reduce the traffic of immune cells from the peripheral blood into the CNS, either by blocking transmigration with monoclonal antibodies targeting α4 integrin, such as natalizumab,[201] or by interfering with chemokine signalling, such as those involving CCR2 and CCR5.[186,202,203]

Furthermore, due to the role of the inflammasome in neuroinflammation, therapies targeting its activation could provide new alternatives to reduce the incidence of HAND and to prevent more severe forms of neurocognitive dysfunction. In this regard, drugs that upregulate the expression of antioxidant enzymes, such as dimethyl fumarate, have been described as capable of reducing oxidative injury and inflammation in the brain.[204]

Notably, HIV-1 infection has a profound effect on mucosal-associated lymphoid tissues, particularly in the gut, where depletion of CD4+ T lymphocytes early after HIV-1 transmission is associated with translocation of microbial products.[205,206] This, together with dysbiosis of the gut microbiota, is directly linked to systemic inflammation, which also affects the CNS and the course of HAND.[207] These effects can be partially reduced by the use of probiotics, which aim to restore a healthy gut microbiota.[208]

Regardless of the therapeutic approach, it is important to emphasise that the majority of clinical trials have failed to clearly demonstrate a significant benefit for patients with HAND. This could be to the small sample sizes used in those studies, but it also suggests that HAND is a multifactorial disease and that the use of combined

therapies, targeting multiple pathological pathways should be considered.

7 | CONCLUSIONS

Soon after transmission, HIV-1 migrates to different body compartments, such as the brain, where the main cells that can be infected by HIV-1 are microglia, perivascular macrophages, astrocytes, and monocyte-derived macrophages. In these cells, the presence of the major viral receptors (CD4, CCR5 or CXCR4) allows HIV-1 entry and productive infection with the release of new virions that amplify and potentiate the direct effects of HIV-1 on the development of HAND. Additionally, viral proteins (e.g., Tat and gp120) released by infected cells exert neurotoxic effects that, together with chronic neuroinflammation and oxidative stress induced by microglial cells, contribute to neuronal injury and dysfunction. The integrity of the BBB is also compromised by HIV-1 replication and neuroinflammation, which trigger the infiltration of either cell-free virus or HIV-infected cells (such as monocytes or CD4+ T lymphocytes) from the peripheral blood into the CNS, fuelling the cascade of HIV-induced effects. In addition, HIV-1 entry into glial cells can lead to latent infection on these cells, allowing the establishment of a viral reservoir within the CNS. These latently infected cells not only impact on brain cell injury through reactivation of HIV-1 replication, but also present a barrier to HIV-1 eradication.

HIV-1 replication can be controlled with lifelong cART, which provides long-term suppression of HIV-1 viral load in the peripheral blood. The use of cART and the resulting reduction in viraemia levels has decreased the prevalence of HAD, but cART has a limited effect in reversing milder forms of HAND. Improved BBB permeability of antiretroviral drugs,[68] allowing more efficient suppression of HIV-1 replication in the CNS, and control of the hyperinflammatory environment that characterises HAND are cornerstones in the prevention and treatment of cognitive impairment induced by chronic HIV-1 infection.

Several aspects of the relationship between HIV infection and HAND remain unknown due to the heterogeneity of factors underlying the neuropathogenesis of HAND. These factors vary between patients, which limits the correct interpretation of the results obtained. For instance, the cognitive dysfunction observed in HIV-infected patients may also be attributed to other infectious agents such as herpes zoster virus, cytomegalovirus, Epstein-Barr virus, systemic syphilis infection, and *Mycobacterium tuberculosis*. The conclusions drawn from studies aiming to address neuropathogenesis of HAND could be further improved if patients enroled in the studies are more accurately stratified based on an improved diagnosis through the detection of specific biomarkers. Additionally, it is important to take into account additional determinants such as sociodemographic and psychological factors, pre-existing morbidities, and therapeutics used.

Clinical trials using multiple therapeutic classes targeting HIV replication, as well as neuroprotective and neuromodulating adjuvant drugs, will help prevent or mitigate HIV-induced neurodegeneration. Nonetheless, recent years have seen significant advances in our understanding of the virological and biochemical mechanisms that contribute to neurocognitive disorders associated with HIV infection. These findings have the potential to enhance the quality of life of affected patients.

AUTHOR CONTRIBUTIONS

Marta Calado: writing (original draft, review and editing), figures. **Rita Ferreira**: editing; **David Pires**: editing; **Quirina Santos-Costa**: editing; **Elsa Anes**: conceptualization, writing (review and editing); **Dora Brites**: writing (review and editing); **José Miguel Azevedo-Pereira**: supervision, conceptualization, writing (original draft, review and editing), figures. All authors have read and agreed to the submitted version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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