

THE NEUROPATHOGENESIS OF AIDS

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Abstract | HIV-associated dementia (HAD) is an important complication of the central nervous system in patients who are infected with HIV-1. Although the incidence of HAD has markedly decreased since it has become possible to effectively control viral replication in the blood by administering highly active antiretroviral therapy, a less severe form of HAD, comprising a milder cognitive and motor disorder, is now potentially a serious problem. Brain macrophages and microglia are the key cell types that are infected by HIV-1 in the central nervous system, and they are likely to mediate the neurodegeneration seen in patients with HAD; however, the precise pathogenesis of this neurodegeneration is still unclear. Here, we discuss the studies that are being carried out to determine the respective contributions of infection, and monocyte and macrophage activation, to disease progression.

HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART). Aggressive anti-HIV combination therapy that includes three or more protease and reverse-transcriptase inhibitors.

INCIDENCE
Number of new cases of a particular disease per year per group of population.

PREVALENCE
Percentage or proportion of a population that is affected by a particular disease at a given time.

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Before the widespread use of **HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART)** in the developed world, ~20–30% of individuals infected with HIV-1 (referred to as HIV from this point) developed a range of cognitive and motor symptoms, including impaired short-term memory, reduced concentration and leg weakness, that are collectively known as HIV-associated dementia (HAD; also known as the **AIDS dementia complex**). These symptoms often occurred together with behavioural symptoms, such as personality changes, apathy and social withdrawal, and HAD could, in its more severe forms, lead to a nearly vegetative and mute state. The onset of HAD is correlated with high plasma viral loads, which accounts for the strong effects of HAART on reducing its **INCIDENCE**¹, although with the longer lifespan of patients with HIV, there is increasing **PREVALENCE** of HAD. With the use of HAART, a more subtle form of central nervous system (CNS) dysfunction — minor cognitive motor disorder (MCMD) — has either become more common or is more obvious because it does not progress to HAD². In this syndrome, loss of memory and decreases in computational and other higher cortical functions are much less pronounced. It has recently been estimated that ~10% of HIV-infected adults have HAD but that MCMD might be several times more common, involving perhaps as many as 30% of the HIV-infected population. Furthermore, the clinical presence of MCMD has been associated with

pathological changes in the CNS that are characteristic of HIV invasion (known as HIV encephalitis), and MCMD is associated with a worse overall prognosis for HIV-infected individuals^{3,4}. One potential explanation for the development of MCMD is that low-level viral replication, as occurs with all but the most successful HAART regimens, leads to slow progressive neurodegeneration. This is consistent with the much longer lifespan of treated patients⁵ and possibly with the insufficient penetration of certain antiretroviral drugs into the brain⁶.

The CNS of children seems to be more vulnerable to the effects of HIV than that of adults, perhaps because the paediatric CNS is still developing and contains less differentiated cell types. Accordingly, the prevalence of CNS dysfunction in untreated children is ~50% (and is known as HIV encephalopathy rather than dementia). Because the progression of the disease and the cell types that are involved, but not the response to HAART, differ in children compared with adults, we focus on adult disease in this review.

Here, we describe our current knowledge of the mechanisms that are implicated in dysregulation of normal cell physiology and in initiation of the neurodegeneration that is associated with HIV infection, and we discuss potential avenues for neuroprotection that could improve the quality of life for some HIV-positive patients. Furthermore, because macrophage-mediated neurodegeneration might also have a role in diseases

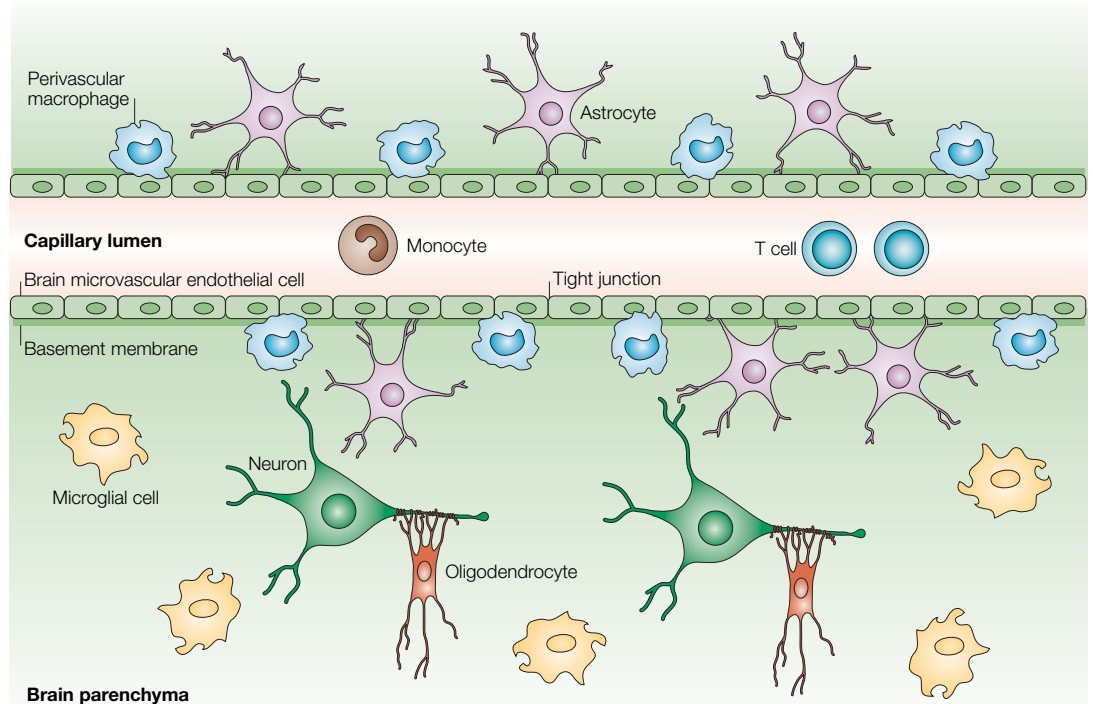


Figure 1 | The location of different cell types in the brain. The brain parenchyma is separated from the rest of the body by the blood–brain barrier, which is a selectively permeable, continuous cellular layer that comprises brain microvascular endothelial cells linked to each other by tight junctions. It provides anatomical and physiological protection for the central nervous system, and it strictly regulates the traffic of substances and blood cells into the brain. In the region surrounding the endothelium of brain capillaries, astrocytes and perivascular macrophages are found. Astrocytes have a crucial role in brain homeostasis, because they establish contacts with endothelial cells and regulate the permeability of the blood–brain barrier through the release of soluble factors. Perivascular macrophages are located around blood vessels, and they are replenished through the migration of circulating monocytes. By contrast, microglia are located in the brain parenchyma and have a much slower turnover than macrophages. In the brain parenchyma, oligodendrocytes extend processes that wrap around segments of neuronal axons and produce the myelin sheath, which consists of lipid- and protein-rich membranes that isolate axons and speed the conduction of nerve impulses.

MULTIPLE SCLEROSIS
Neurodegenerative disorder that is characterized by demyelination of bundles of nerve fibres in the central nervous system. Symptoms depend on the site of the lesion but include sensory loss, weakness in leg muscles, speech difficulties, loss of coordination and dizziness.

ALZHEIMER'S DISEASE
Degenerative mental disease that is characterized by progressive brain deterioration and dementia, and by the presence of senile plaques, neurofibrillary tangles and neuropil threads. Disease onset can occur at any age, and women seem to be affected more frequently than men.

BLOOD–BRAIN BARRIER
Selectively permeable cellular layer formed by brain microvascular endothelial cells, which are linked by tight junctions. It is crucial for the maintenance of homeostasis in the brain environment.

CHOROID PLEXUS
Site of production of cerebrospinal fluid in the adult brain. It is formed by invagination of ependymal cells into the ventricles, which become highly vascularized.

such as MULTIPLE SCLEROSIS and ALZHEIMER'S DISEASE, the therapeutic strategies discussed might have more general applications.

Neuroinvasion by HIV

The CNS is susceptible to infection by retroviruses of various species and by members of the lentivirus family in particular⁷. The specific requirements for entry to the brain and the many cell types in the CNS increase the complexity of virus–cell interactions in the brain. The CNS is separated from the rest of the body by the BLOOD–BRAIN BARRIER (BBB); the cerebrospinal fluid (CSF) is also separated from the periphery by the blood–CSF barrier of the CHOROID-PLEXUS epithelium. The structure of the brain tissue and the BBB are schematically shown in FIG. 1. In theory, five main cell types — astrocytes, oligodendrocytes, neurons, perivascular macrophages and microglia — are susceptible to retroviral infection, but of these five, the latter two are the most commonly infected by HIV (TABLE 1). Perivascular macrophages and microglia arise from bone-marrow-derived cells that settle in the CNS at various times during development and throughout adult life; this has implications for the expression of HIV receptors by these cells and for the turnover of different subpopulations in the CNS.

HIV enters the brain early after systemic infection^{8,9}, and to do this, it must cross the BBB. The BBB is a selectively permeable, rather than impermeable, continuous cellular layer that consists of brain microvascular endothelial cells; these cells are linked to each other by tight junctions, which regulate the traffic of cells and substances from the bloodstream to the CNS. Although the ‘invasion’ of immune cells across the BBB occurs as part of immune surveillance¹⁰, it is carefully regulated; therefore, entry of HIV has been the subject of many studies using animal models and tissue-culture preparations that mimic the BBB. Using the sheep-specific lentivirus visna virus as a model of HIV infection, Haase and colleagues^{11,12} first proposed that HIV and other lentiviruses enter the CNS as passengers in cells that are trafficking to the brain (the ‘Trojan horse’ hypothesis). For HIV — which infects several types of CD4⁺ cell that circulate in the blood, such as T cells and monocytes — this model not only is the most intuitively appealing but also has the most compelling supporting evidence (FIG. 2). *In situ* hybridization and immunohistochemical studies have shown virus accumulation in the perivascular regions, mainly in CD14⁺ cells^{13–19}. There is limited pathological evidence of infection of endothelial cells, which is consistent with the absence

Table 1 | Susceptibility of cells of the central nervous system to infection with HIV

Cell type	CD4 antigen	Chemokine receptors	HIV susceptibility	Productive infection
Perivascular macrophages	Yes	Yes	Yes	Yes
Microglia	Yes	Yes	Yes	Yes
Astrocytes	No	Yes	Yes	No
Oligodendrocytes	No	Yes	<i>In vitro</i>	No
Neurons	No	Yes	No	No
Brain microvascular endothelial cells	No	Yes	<i>In vitro</i>	No

of expression of conventional HIV receptors by these cells, although BBB abnormalities due to HIV infection have been shown¹⁹. In addition, pathological changes in the brains of patients with HAD correlate with the presence of activated bone-marrow-derived cells, such as macrophages^{20,21}. Alternatively, on the basis of experimental support from *in vitro* models, some investigators have suggested that HIV might enter the CNS by TRANSCYTOSIS of endothelial cells^{22–24} or by direct infection^{25–27}. However, these mechanisms are unlikely to account for most of the virus entering the brain.

Microglia and brain macrophages

In the perivascular region of the brain, several cell types — mainly astrocytes, perivascular macrophages and microglia — come into direct contact with virus-infected cells that have entered the CNS from the blood (TABLE 1). Of these, perivascular macrophages and microglia are the most important in HIV infection in the CNS. They constitute the resident immunocompetent cells of the brain and respond to all types of insult, ranging from vascular problems to the protein accumulation that is associated with some neurodegenerative diseases, such as Alzheimer's disease^{28–31}.

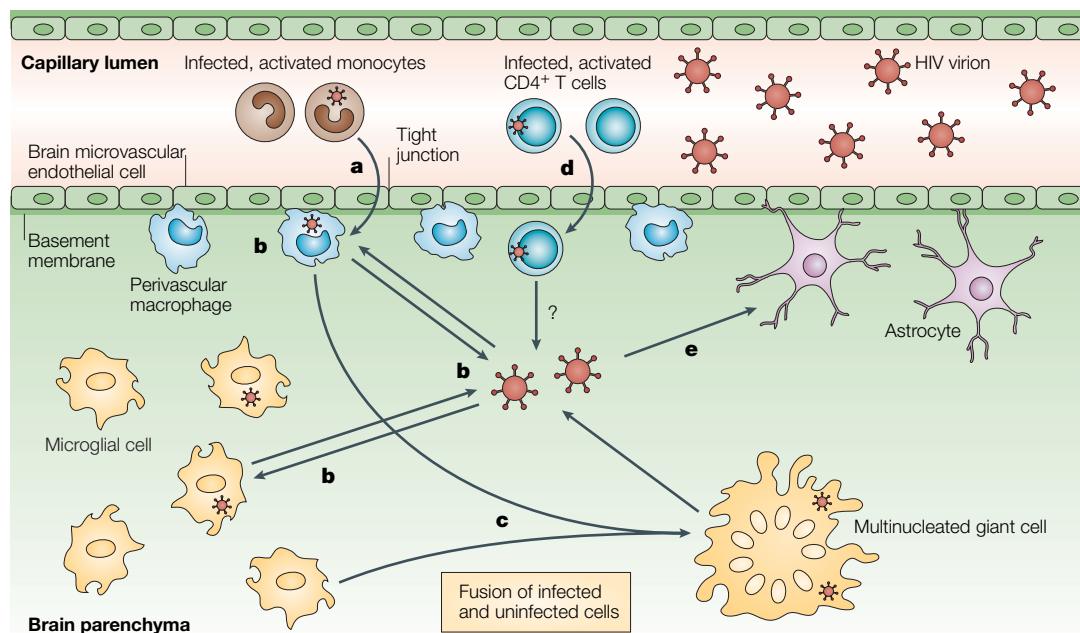


Figure 2 | HIV neuroinvasion and multinucleated giant-cell formation. **a** | It has been proposed that HIV enters the brain by a 'Trojan horse' mechanism, through its presence in infected monocytes that migrate across the blood–brain barrier to replenish the population of perivascular macrophages. Infected monocytes have an activated phenotype that renders them more prone to migrate into tissues than uninfected, activated monocytes. **b** | All of the virus that is produced in the brain is probably initially derived from monocytes that have differentiated into perivascular macrophages. Subsequently, microglia might become infected and contribute to the production of virus. In addition, activation of microglia might have a role in the mechanisms of neuropathogenesis. **c** | The HIV-envelope glycoproteins that are expressed at the surface of infected cells mediate cell-to-cell fusion with cells that express both CD4 and HIV co-receptor. In the brain, cell-to-cell fusion involves macrophages and microglia, and it results in the formation of large multinucleated giant cells, or syncytia, that also produce virus before they eventually die. **d** | HIV might also enter the brain in infected CD4⁺ T cells; the contribution of these cells to the pool of replicating virus in the brain is still unclear, because genotypic and phenotypic analyses seem to show that viruses from the brain are more similar to those from monocytes and/or macrophages than to those from T cells. **e** | Although several mechanisms for HIV infection of astrocytes have been described, it is generally accepted that infection of these cells is not productive and that they do not contribute to viral replication. However, both limited expression of HIV genes (by astrocytes) and dysregulation of cytokine and chemokine signals (resulting from infection of macrophages or microglia) might contribute to astrocytic malfunction and neuropathogenesis.

TRANSCYTOSIS

Process of transport of material across an epithelial layer by uptake on one side of the epithelial cell into a coated vesicle that might then be sorted through the *trans*-Golgi network and transported to the opposite side of the cell.

Subtypes of brain macrophage and microglia are defined by their location and morphology, and by the presence of specific cell-surface markers. The term microglia refers mainly to glial cells that are derived from the MESODERM, and these are clearly distinguishable from neurons, astrocytes and oligodendrocytes, all of which are derived from the ECTODERM³². Microglia have a similar antigenic cell-surface phenotype (that is, express similar cell-surface molecules) to other cells of the MONONUCLEAR PHAGOCYtic SYSTEM³³; morphologically, they appear as highly branched cells when in a resting state (known as ramified microglia) and more-rounded cells when activated (known as amoeboid microglia). Amoeboid microglia are absent in the normal adult brain but can be detected in the developing brain, as well as in inflammatory or demyelinating conditions in the fully developed CNS. Perivascular macrophages are flat and elongated, and are located adjacent to the brain microvasculature; other macrophage populations are often identified by their locations in the choroid plexus or the MENINGES. Although defining an individual cell as a macrophage or a microglial cell is difficult, perivascular macrophages are CD14⁺CD45⁺, and by contrast, parenchymal microglia are CD14⁺CD45^{low} (REFS 34,35).

All of these cell populations are replenished but with considerably different lifespans. Perivascular macrophages and other macrophages that are close to the interface with the periphery have a faster turnover than microglia, perhaps because of this proximity^{36,37}. Tissue macrophages are terminally differentiated cells, with some, although limited, mitotic potential. Parenchymal microglia are much more quiescent, and in mice, it has been calculated that no more than 20% of the ramified parenchymal microglia are repopulated throughout the lifetime of the animal³⁸. Although it is difficult to extrapolate these numbers with precision to the longer human lifespan, the lifespans of perivascular macrophages and microglia do differ considerably³⁹.

Replenishment of the perivascular-macrophage population by circulating monocytes that migrate into the brain has the side-effect of 'opening the door' to intracellular pathogens. As the monocytes take up residence in the CNS, they differentiate into macrophages, as they do in other tissues. Immunohistochemistry and *in situ* hybridization studies have conclusively shown that, in the CNS, the cell population that is most infected by HIV-1 or HIV-2 is the perivascular-macrophage population; this has also been shown using the rhesus macaque simian immunodeficiency virus (SIV) model, by examining for active virus replication and the production of new virions^{13–19}. Furthermore, MULTINUCLEATED GIANT CELLS (MNGCs), which are the hallmark of HIV neuropathology, express CD14 and CD45, as shown in both HIV infection and the SIV model^{17,40}. Although these markers could be expressed after cell fusion, it is reasonable to assume that some of these infected cells are monocytes that were infected peripherally and have differentiated into macrophages in the CNS compartment.

Infection of parenchymal microglia is more controversial, because some investigators do not think that these cells are ever infected, whereas others do. If CD14 and

CD45 are considered markers for perivascular macrophages, then expression of viral antigens by parenchymal microglia (which are CD14⁺CD45^{low}) is detected much less frequently than by other brain macrophages — although parenchymal microglia might harbour latent infections. The differences might reflect the difficulties in defining an active disease on the basis of static 'pictures' that are obtained at the end-point of infection. *In vitro*, our own experiments, and those of other research groups, have clearly indicated that microglia isolated from the non-inflamed CNS can support marked replication of HIV^{41–43} and, as terminally differentiated cells, have a high level of expression of the HIV co-receptor CC-chemokine receptor 5 (CCR5)^{44–46}.

The expression of viral-envelope glycoproteins at the surface of infected cells leads to the formation of MNGCs or syncytia, which occurs after the fusion of infected and uninfected cells (probably of a macrophage and a microglial cell)^{13,47,48}. In tissue culture, the formation of syncytia is a common manifestation of HIV infection and is mediated by the interaction between the cell-surface-expressed viral glycoproteins and the cellular receptors, but *in vivo*, it is observed in only a few tissues, such as the dermis and in the CNS (FIG. 2). The presence of MNGCs in the brain tissue of HIV-positive patients is the main neuropathological finding in HAD and is considered a hallmark of HIV encephalitis. Only cells that express both CD4 and an HIV co-receptor can be fused by the viral glycoproteins; perivascular macrophages and microglia express CD4, CCR5 and CXC-chemokine receptor 4 (CXCR4), with CCR5 being the most important HIV co-receptor for adult microglia and brain macrophages^{41,45}. However, similar to other tissue macrophages, brain perivascular macrophages and microglia express lower levels of CD4 than activated lymphocytes, and the viral phenotypes that promote fusion between CNS macrophages and microglia might therefore be different from those that promote fusion of lymphocytes^{49–52}. Lymphocytes can also enter the brain as part of the surveillance mechanism of the CNS^{10,37,53}, but their contribution to syncytia formation and to the virus pool in the CNS is unclear. Other cell types in the CNS do not participate in syncytia formation because they do not express the appropriate receptors.

Compartmentalization of HIV in the CNS

HIV enters the CNS soon after peripheral infection of circulating T cells and monocytes^{8,9}. It is unclear whether it remains in the CNS or is later cleared, only to re-enter late in infection; various studies support different points of view. Overall, we favour the hypothesis that HIV penetrates the CNS at various times during infection, thereby establishing genetically distinct variants in the brain and perhaps even between various regions of the brain (REF. 54, and M. Chen and F.G.S., unpublished observations). Phylogenetic reconstructions have shown clustering of sequences according to the tissue of origin, indicating that viruses from the CNS are more closely related to each other than to viruses originating from peripheral tissues (that is, the blood, lymph nodes or spleen) of the same individuals^{55–59}. However, studies

MESODERM

Middle of the three germ layers of the embryo. It gives rise to the blood, to the musculoskeletal, circulatory and urogenital systems, and to the connective tissue (including that of dermis), and it contributes to some glands.

ECTODERM

Outer of the three germ layers of the embryo. It gives rise to the epidermis and most of the neural tissue.

MONONUCLEAR PHAGOCYtic SYSTEM

Group of bone-marrow-derived cells with different morphologies (monocytes, macrophages and dendritic cells), which are mainly responsible for phagocytosis, cytokine secretion and antigen presentation.

MENINGES

Surrounding membranes of the brain and spinal cord. There are three layers of meninges: the dura mater (outer), the arachnoid membrane (middle) and the pia mater (inner).

MULTINUCLEATED GIANT CELLS

(MNGCs). Conglomerates of cells that form through the fusion of infected and uninfected macrophages and microglia. The fusion is mediated by HIV-envelope glycoproteins present at the surface of infected cells and CD4 and chemokine receptors at the surface of uninfected cells. MNGCs are the pathological hallmark of HIV neuropathology.

have not yet conclusively proven whether this genetic differentiation results from a 'founder effect' and reflects independent entry events or whether it results from specific adaptation to replication in brain cells, which is facilitated by the much lower neutralization titres of antibody in the CNS and possibly by a reduced cytolytic T-cell response. Furthermore, if replication in the CNS is more limited, then genetic drift of the viral population in the CNS is likely to be less marked than in the periphery, and this has been shown, to some extent, in studies of the SIV model of CNS disease⁶⁰.

To test this idea, Ryzhova and co-workers⁶⁰ amplified short SIV DNA sequences from single MNGCs from the brain and compared the sequences obtained with the sequence of the molecularly cloned inoculum. Remarkably, even two years after infection, there were sequences that were close or even identical to the initial inoculum, indicating the persistence of the original genome in long-lived cells. Although limited in scope, this study supports the idea that infection of the parenchymal microglia is possible, because it is unlikely that virus would persist for such a long time in a population of cells with a faster turnover, such as perivascular macrophages. After studying infection with a chimeric HIV–SIV, Miyake and co-workers⁶¹ also argue that the virus genome persists in the brain throughout infection, but they suggest that there is little production of infectious virions.

Recently, Gabuzda and colleagues⁶² have shown that the envelope glycoprotein of a highly fusogenic, neuron-apoptosis-inducing, CCR5-using virus required lower levels of expression of CCR5 and CD4 to mediate cell-to-cell fusion and infection than the envelope of another CCR5-using virus that was less pathogenic but similarly neurotropic. This indicates that a more efficient receptor interaction might be characteristic of a pathogenic viral phenotype that contributes to the neurodegenerative manifestations of AIDS. These results agree with our observations that indicate that the gp120 (glycoprotein 120) of a virus that has been adapted *in vitro* to grow in microglia can more efficiently mediate the infection of cells that express low levels of CD4 (REF. 63) and has an increased affinity for CD4 (J.M.G. and F.G.S., unpublished observations). Both the *in vitro* adaptation of virus that enables it to grow in microglia and the generation of neuropathogenic variants have also been reported in the SIV model⁶⁴.

Peters *et al.*⁶⁵ have also analysed HIV-envelope proteins that have been isolated from both brains (after autopsy) and lymph-node tissues that were obtained from HIV-positive individuals with neurological complications. In general, compared with lymph-node-derived envelope proteins, brain-derived envelope proteins showed higher fusogenicity, a tropism for macrophages, increased ability to infect cells that express only low levels of CD4 and/or CCR5, and lower sensitivity to a CD4-specific antibody. These experiments indicate that specific viral phenotypes might have resulted *in vivo* from the adaptation of HIV to replication in the brain environment, either by selection of variants that arise from replication in brain macrophages or by selection of

variants that comprise a small minority in the periphery but are amplified in the absence of strong immunological pressure.

There is little evidence that the other main CNS cell types (that is, astrocytes, oligodendrocytes and neurons) directly participate in virus production, although astrocytes support limited infection (BOX 1). Nevertheless, they participate in HIV neuropathology in more indirect ways, such as by decreasing their ability to remove toxins; these roles are discussed later.

Chemokines and their receptors in the brain

Because of their role in HIV entry and in many physiological and inflammatory processes, chemokines and their receptors have received much attention in studies of the pathogenesis of HAD⁶⁶. In the CNS, chemokines and their receptors are involved in the migration, differentiation and activation of some cells and in the proliferation of glia and neurons. Being essential components of neuronal physiology, they might have a crucial role in the balance between protection of the CNS (neuroprotection) and the deleterious neuronal effects (neurodegeneration) that are characteristic of HAD.

TABLE 2 summarizes the effects of selected chemokines and chemokine receptors with respect to HAD and neurodegeneration or neuroprotection. All members of the CXCR family are expressed in the brain, mainly by neurons, which show marked increases in intracellular Ca²⁺ levels after exposure to α -chemokines (which bind CXCR-family members), such as CX₃C-chemokine ligand 1 (CXCL1; also known as GRO- α) or CXCL12 (also known as SDF1 α and SDF1 β)^{67–72}. CXCR4 and its only ligand CXCL12 are both widely expressed in the CNS^{46,68,69,71–75}. Members of the CCR family have also been detected in the brain in both physiological conditions and during inflammatory and neurodegenerative diseases; they are mainly expressed by neuronal-cell subsets, microglia and astrocytes^{44,46,68,69,74–79}. However, β -chemokines (which bind CCR-family members) are only weakly expressed or not expressed in the normal brain.

Neurons themselves release chemokines, such as CX₃C-chemokine ligand 1 (CX₃CL1; also known as fractalkine), that might ultimately affect cellular function in the CNS^{80–83}. However, the most important relationship between HAD and chemokines occurs in the macrophage, microglia and lymphocyte populations. For example, the CD14⁺CD16⁺ monocyte subpopulation, which releases high levels of pro-inflammatory cytokines, is increased in HIV infection⁴⁰; in contrast to CD14⁺CD16[−] monocytes, these cells express high levels of CX₃C-chemokine receptor 1 (CX₃CR1) and CXCR4, and they undergo transendothelial migration in response to CX₃CL1 and CXCL12 (REF. 84), indicating a potential contribution to vascular and tissue injury in conditions in which cell numbers are increased.

Altered expression of chemokines and chemokine receptors in HIV-infected brains. Altered expression of α - and β -chemokines in the brain is also a common finding in HIV encephalitis and could contribute to neuronal

Box 1 | Involvement of CNS cells (other than macrophages and microglia) in HIV infection**Astrocytes**

Astrocytes are responsible for maintaining homeostasis in the central nervous system (CNS). They express receptors that enable them to respond to almost all known neuroactive compounds, including neurotransmitters. Through these receptors, astrocytes also function as CNS sentinels and regulate the levels of neurotransmitters, such as glutamate. In addition, astrocytes help to establish and maintain CNS boundaries, such as the blood–brain barrier, through their interactions with endothelial cells. Tissue damage, as seen in the brains of HIV-infected patients, results in a strong proliferative response by astrocytes^{166,167}.

Astrocytes might be infected by HIV to some extent, although any infection of astrocytes is unlikely to result in robust viral replication, because few astrocytes are found to express viral antigens. The mechanism of viral entry to astrocytes is unclear, because they do not express detectable levels of cell-surface CD4 or the main HIV co-receptors, and the process of infection seems to be insensitive to inhibition by either chemokines or antibodies specific for CD4 or the chemokine-receptor-binding site of gp120 (REF. 168). In patients with HIV-associated dementia (HAD), astrocytosis that is induced by viral proteins and macrophage products might have a role in the loss of astrocytic function and brain homeostasis, leading to neuropathogenesis^{13,14,169}.

Oligodendrocytes

Oligodendrocytes produce the myelin sheath, a lipid- and protein-rich membrane that promotes fast axonal conduction. There is no evidence of oligodendrocyte infection *in vivo*, although the binding of gp120 to galactosylceramide or other proteoglycans might reduce myelin synthesis and increase intracellular Ca²⁺ levels and apoptotic-cell numbers, as has been shown *in vitro*¹⁷⁰.

Neurons

As the main effectors of cognitive and motor function, neurons must be involved in HIV neuropathogenesis, and indeed, there is significant neuronal cell death in HIV-infected brains^{171–173}. However, there are no traces of neuronal infection in patients with HAD, although there are other effects (for details, see the main text). Accordingly, neurons do not express CD4, although different subsets express some of the chemokine receptors that are co-receptors for HIV infection^{46,66,73}. These seem to be important for neuronal migration and development, and for neuronal responses to survival and apoptotic signals, but they have not been shown to mediate HIV entry.

degeneration and dysfunction^{73,85,86}. Increased expression of receptors, such as CCR3 and CCR5, has also been reported in the CNS of macaques infected with SIV and in the brains of children and adults with AIDS, mostly by perivascular macrophages and microglia. In adult brain-tissue samples, microvascular endothelial cells also expressed CCR3 (REFS 76,78). These observations, together with the increased expression of β -chemokines in the brains of monkeys with SIV encephalitis^{87,88} and in the CNS of patients with HIV encephalitis^{86,89,90}, might support a role for chemokines in the development of neuropathogenesis. However, the effects of selected chemokines on neuronal function might be either positive or negative. No neurotoxic effects of β -chemokines have been shown so far *in vivo*, whereas several *in vitro* studies have shown that β -chemokines, such as CC-chemokine ligand 4 (CCL4; also known as MIP1 β) and CCL5 (also known as RANTES), can protect cultured neurons from apoptosis that is induced by gp120 (REFS 69,83,91).

The α -chemokines CXCL10 (also known as IP10) and CXCL12 also seem to be present at increased levels in the CSF and brain of HIV-infected individuals and might be implicated in neuropathogenesis^{75,85,92,93}. However, their effects are unclear, because they seem to depend on *in vitro* culture conditions^{85,91–93}, and modulation of the expression levels of their receptors might also influence neuronal responses. So, neurons cultured in the presence of fibroblast growth factor showed a dose-dependent decrease in expression of CXCR4, which might result in decreased neurotoxicity of HIV gp120 (REF. 94). In contrast to its chemokine ligand

CXCL12, the interaction of both soluble and virion-bound gp120 with neuronal CXCR4 elicits marked increases in intracellular Ca²⁺ levels and substantial neurotoxicity^{92,95,96}. However, it is important to note that such effects have not yet been shown for envelope glycoproteins derived from primary HIV isolates or, more importantly, from isolates obtained from the CNS.

Therefore, although there are clear abnormalities in the expression of chemokines and their receptors in the lentivirus-infected CNS, the net effect of these (positive or negative) has not been conclusively elucidated.

Mechanism of neurodegeneration

Because there is not marked neuronal infection in HAD (as neurons do not express CD4), other mechanisms must be implicated in the neuropathological damage of AIDS (FIG. 3). The expression of chemokines and their receptors seems to be important for neuronal migration and development, and for mediating neuronal responses to survival and apoptotic signals, but chemokines and their receptors have not been shown to mediate entry of primary HIV isolates independently of CD4. However, infection of perivascular macrophages and microglia (and possibly astrocytes) can cause disruption of normal neurological functions, either by a direct mechanism (production of viral proteins such as gp120, Tat (transcriptional transactivator) or Vpr (viral protein R) by infected cells) or by an indirect or 'bystander' effect, in which neurons are damaged mainly as a consequence of the inflammatory processes. In addition, it has been proposed that, after the inflammatory process has 'taken hold', the viral infection itself might have a more

Table 2 | Role of selected chemokines and chemokine receptors in HIV-associated dementia

Chemokine	Cellular sources	Effects in the brain	Chemokine receptor(s)	Location of receptor expression in brain
CXCL8 (IL-8)	Activated astrocytes and microglia (induced by HIV Tat)	Modulation of synaptic transmission and plasticity; and inhibition of long-term potentiation* in hippocampus	CXCR1 CXCR2	Microglia, subsets of neurons, astrocytes and oligodendrocytes Microglia, neurons, astrocytes, and oligodendrocyte precursors
CXCL10 (IP10)	Astrocytes (induced by HIV Tat), microglia and brain endothelial cells; increased in SHIV encephalitis	Alteration of synaptic plasticity in hippocampus; and induction of leukocyte infiltration	CXCR3	Microglia, subsets of neurons, and astrocytes
CXCL12 (SDF1 α , SDF1 β)	Astrocytes, microglia, specific neurons from select anatomical regions (cortical neurons and cerebellar granule cells), injured neurons; overexpressed in gliomas	Promotion of neuronal migration during cerebellar development, microglial chemotaxis and mesenchymal stem-cell migration to site of injury; promotion of survival or apoptosis of hippocampal neurons; regulation of cholinergic and dopaminergic systems; promotion of astrocyte proliferation; and promotion of cytokine and glutamate release	CXCR4	Microglia, neurons, astrocytes and endothelial cells
CCL2 (MCP1)	Astrocytes and microglia in patients with AIDS and in SHIV-induced encephalitis (induced by Tat); increased levels in CSF of patients with HAD	Protection of neurons and astrocytes from NMDA- or HIV Tat-induced apoptosis, through release of astrocyte growth factors	CCR2	Human fetal glia and neurons, astrocytes and NT2.N cells
CCL3 (MIP1 α)	Microglia, neurons, astrocytes and oligodendrocytes (in Alzheimer's disease), glia and macrophages (in multiple sclerosis); increased levels in patients with HAD and in SIV encephalitis	Development of CNS; migration of astrocytes and microglia; recruitment of monocytes to brain parenchyma in patients with HAD or other neurological disorders	CCR1 CCR5	Subsets of neurons, astrocytes and oligodendrocyte precursors Microglia, neurons and astrocytes
CCL4 (MIP1 β)	Increased levels in patients with HAD and macaques with SIV encephalitis; might decrease infection of macrophages and microglia	Recruitment of monocytes to brain parenchyma; involvement in migration of macrophages, microglia and astrocytes	CCR5	Microglia, neurons and astrocytes
CCL5 (RANTES)	Astrocytes, macrophages and microglia; higher levels in HIV infection and SIV encephalitis	Recruitment of monocytes to brain parenchyma; involvement in migration of macrophages, microglia and astrocytes	CCR1, CCR3 and CCR5	Microglia, neurons and astrocytes
CCL7 (MCP3)	Astrocytes in patients with HAD and macaques with SIV encephalitis; absent in normal human brain	Recruitment of monocytes to brain parenchyma	CCR1, CCR2 and CCR3	Microglia, neurons and astrocytes
CX ₃ CL1 (Fractalkine)	Neurons (upregulated during injury) and endothelial cells; for astrocytes and microglia, can be upregulated by TNF and IL-1 β	Recruitment of receptive cells (mainly microglia), when in soluble form; polymorphisms affect the development of AIDS	CX ₃ CR1	Microglia, subsets of neurons, astrocytes and endothelial cells

*Long-term potentiation is a persistent increase in the size of the synaptic response that is induced by several mechanisms; in the hippocampus, it is thought to be the synaptic basis of learning and memory in vertebrates. CCL, CC-chemokine ligand; CCR, CC-chemokine receptor; CNS, central nervous system; CSF, cerebrospinal fluid; CXCL, CXC-chemokine ligand; CX₃CL1, CX₃C-chemokine ligand 1; CXCR, CXC-chemokine receptor; CX₃CR1, CX₃C-chemokine receptor 1; HAD, HIV-associated dementia; IL, interleukin; IP10, interferon- γ -induced protein of 10 kDa; MCP, monocyte-chemotactic protein; MIP1, macrophage inflammatory protein 1; NMDA, *N*-methyl-*D*-aspartate; RANTES, regulated upon activation, normally T-cell expressed and presumably secreted; SDF1, stromal-cell-derived factor 1; SHIV, simian-human immunodeficiency virus; SIV, simian immunodeficiency virus; Tat, transcriptional transactivator; TNF, tumour-necrosis factor.

N-METHYL-*D*-ASPARTATE (NMDA). Amino-acid derivative that functions as a specific agonist of the NMDA receptor and therefore mimics the action of the neurotransmitter glutamate on that receptor. In contrast to glutamate, it binds and opens only the NMDA receptor and not other glutamate receptors.

limited role in the degenerative process, having established a self-sustaining 'chain reaction'. Although these two mechanisms are not mutually exclusive and might coexist, in our opinion the bystander theory is more consistent with most of the evidence.

Direct injury hypothesis. In *in vitro* experiments, HIV gp120 interacts with chemokine receptors, which leads to neuronal injury; this might indicate that it is the release of this viral protein that mediates neuronal cell death^{69,83,85,91,92,97}. Although this hypothesis is appealing, most studies that show this interaction have used gp120 proteins derived from isolates that use CXCR4

as a co-receptor; by contrast, most of the isolates from the CNS that have been described so far use CCR5 as a co-receptor. In addition, most studies have used monomeric gp120 and not the physiological form of the protein, which is a trimer. Furthermore, previous interaction with CD4 is required for gp120 to bind the cellular co-receptor. Therefore, the *in vivo* relevance of a direct interaction between gp120 and chemokine receptors at the cell surface of neurons and the subsequent neuronal apoptosis remains to be proven. gp120 can also induce neuronal cell death through direct interaction with the NMDA (N-METHYL-*D*-ASPARTATE) receptor⁹⁸⁻¹⁰¹ or by a mechanism that involves the production of

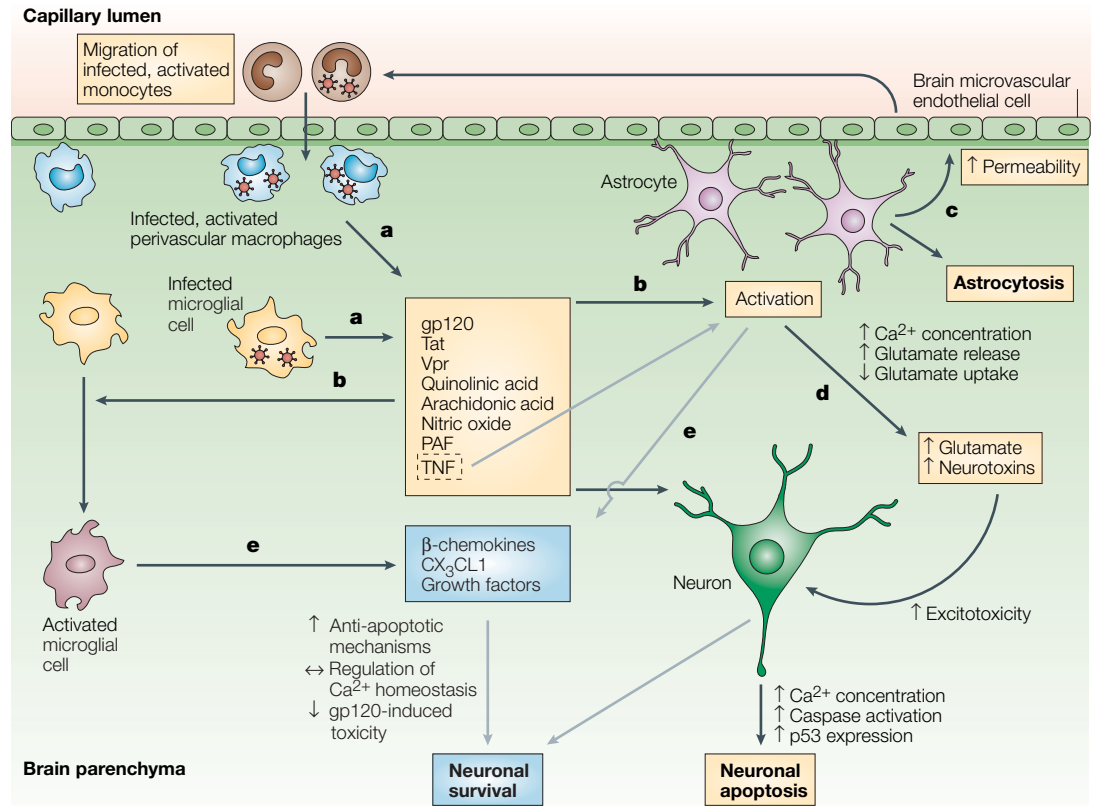


Figure 3 | **Mechanisms of neurodegeneration and neuroprotection in AIDS.** **a** | Infected perivascular macrophages and microglia are responsible for producing HIV but might also release viral proteins that can be deleterious to the central nervous system. The HIV-envelope protein gp120 (glycoprotein 120), Tat (transcriptional transactivator) and Vpr (viral protein R) have all been shown to be toxic *in vitro* to neurons and/or astrocytes, although their relevance *in vivo* is unknown. Infected and activated cells also produce other factors — such as cytokines (including tumour-necrosis factor, TNF), quinolinic and arachidonic acid, platelet-activating factor (PAF) and nitric oxide — that are known to have neurotoxic effects. **b** | Importantly, they promote the further activation (and to some extent, proliferation) of macrophages and/or microglia, as well as the proliferation and activation of astrocytes. **c** | Activated astrocytes modify the permeability of the blood–brain barrier and promote the migration of more monocytes into the brain. **d** | In addition, through increases in release of intracellular Ca²⁺ and glutamate and through decreases in glutamate uptake, the brain concentration of glutamate and other neurotoxins increases and results in excitotoxic death of neurons. **e** | However, activation of macrophages and/or microglia, and TNF-mediated activation of astrocytes, also results in the release of β-chemokines, CX₃C-chemokine ligand 1 (CX₃CL1) and growth factors, all of which are known to regulate Ca²⁺ homeostasis in neurons, to promote anti-apoptotic signalling pathways and to decrease gp120-mediated and excitotoxic neuronal cell death, thereby promoting neuronal survival. Grey arrows indicate neuroprotective pathways.

tumour-necrosis factor (TNF) by non-neuronal cells, with subsequent activation of caspase-mediated cell death of neurons through interaction with the TNF receptors (TNFRs)^{102,103}. Therefore, gp120 might have a role in further stimulation of macrophages and microglia, rather than in directly injuring neurons, thereby contributing to the general increase in cellular activation that is characteristic of the neuropathology of HAD. However, an important unanswered question is whether the concentrations of gp120 that are present in the CNS are high enough to mediate the effects observed in cultured cells¹⁰⁴.

Two other viral proteins, Tat and Vpr, have also been suggested as potential effectors in HAD. Tat exerts its main activity in the nucleus and is effective at low concentrations, although it can be secreted at high levels *in vitro*¹⁰⁵. Secreted Tat might cause direct injury to neurons; however, its presence as a free protein in body fluids or serum has not been shown and is subject to the

same caveats as gp120, with the additional concerns that viral replication in the CNS is limited and that a protein that mediates its effect intracellularly might not be abundantly secreted — despite *in vitro* or systemic evidence to the contrary. Tat has also been shown to alter tight-junction protein expression and BBB function and to upregulate expression of many inflammatory mediators that promote monocyte infiltration into the brain, such as CCL2 (also known as MCP1); it has also been shown to be neurotoxic through mechanisms that involve multiple intracellular-signalling pathways^{106–109}. So, if Tat is present as free protein in high quantities *in vivo*, it could contribute to the neuronal injury seen in HAD.

The function of Vpr is more difficult to summarize: it induces cell-cycle arrest, promotes transcription, facilitates nuclear import of the PRE-INTEGRATION COMPLEX, and when secreted, it might have a role in pathogenesis, because it has been shown to activate replication in latently infected cells and to induce the

PRE-INTEGRATION COMPLEX
Ensemble of the viral RNA genome that is present in the virion (which consists of the nucleocapsid protein, the structural protein p6, the accessory protein Vpr, the integrase protein and several copies of the matrix protein), where the synthesis of viral DNA occurs. By engaging cellular proteins, the viral DNA can then be transported to the nucleus, where it can be integrated into the genome of the host cell.

killing of bystander cells¹¹⁰. In the CNS, both intracellular and extracellular Vpr have been shown to induce apoptosis of human neuronal-precursor cells (the NT2 cell line) and of mature, differentiated neurons, through a caspase-8-dependent mechanism^{111,112}. In contrast to Tat, Vpr has been detected in the CSF of patients with HIV¹¹³, but the caveats regarding appropriate concentrations also apply in this case.

Bystander effect hypothesis. Despite these findings, it is possible that HIV infection is only part of the picture; HIV encephalitis is associated with immune activation that seems to be out of proportion to the amount of virus that is present, and inflammation itself results in modification of the extracellular secretory functions of microglia and brain macrophages (FIG. 3). Chemokines, pro-inflammatory cytokines — such as TNF and interleukin-1 β (IL-1 β) — and other soluble products lead to the activation of uninfected cells^{20,21,91} and to the migration of HIV-specific, activated T cells into the CNS^{114,115}, thereby contributing to the amplification of HIV-induced neurotoxicity. Accordingly, progression to HAD has been correlated with the following: increased expression of macrophage activation markers¹¹⁶, synthesis by macrophages and microglia of quinolinic and arachidonic acids and related metabolites¹¹⁷, increased production of TNF¹¹⁸, increased expression of mRNA encoding inducible nitric-oxide synthase, increased production of free radicals^{119–122}, and proliferation and apoptosis of astrocytes¹²³. In addition, some of these macrophage products can also modify the permeability of the BBB and induce the expression of adhesion molecules by endothelial cells, thereby promoting further monocyte migration into the brain. Moreover, the overexpression of MATRIX METALLOPROTEINASES in HIV-infected individuals and in HIV and SIV models^{124–126}, as well as the overexpression of intercellular adhesion molecule 1 (ICAM1) and vascular cell-adhesion molecule 1 (VCAM1) in the brains of patients with HAD^{127–129}, have been described.

Although astrocytes are secondary components in this complex scenario (because they are not productively infected by HIV), the interaction between infected macrophages and astrocytes inhibits important protective astrocytic functions, such as the uptake of excitatory amino acids (for example, glutamate). As previously discussed, increased expression of both pro-inflammatory and non-inflammatory cytokines (including transforming growth factor- β (TGF- β), IL-1 α , IL-1 β , IL-6 and TNF) has been documented in the brain and/or CSF of patients with AIDS, particularly of those with HAD^{117,118,130–132}. However, it has also been reported that the co-culture of astrocytes with HIV-infected macrophages might decrease the levels of neurotoxins that are released by these macrophages¹³³. This argues against the long-standing hypothesis that reactive astrocytes respond to insults only by producing glial scars and by affecting neuronal survival, and it implies that the process is exceedingly complex and that the final equilibrium depends on as-yet-undefined factors.

For example, although TNF is a macrophage-secretion product that is involved in HAD, its activity

as a neurotoxin is still under debate, because *in vitro* studies show that it can also be neurotropic^{134,135}. As a toxin, TNF functions in an autocrine manner to promote the activation of the mitogen-activated protein kinase (MAPK) p38, resulting in the production of more TNF¹³⁶. Astrocytes also produce TNF and other inflammatory cytokines after activation¹³⁷, resulting in a feedback mechanism that amplifies excitotoxic glutamate release and decreases glutamate uptake by the astrocytes themselves¹⁰². The presence of high levels of glutamate or other excitatory molecules in the vicinity of neurons causes uncontrolled Ca²⁺ influx, loss of cellular homeostasis and neuronal-cell death through apoptosis¹³⁸. This Ca²⁺-dependent killing seems to be common to many neurodegenerative disorders.

Christopher Power's research group¹³⁹ recently proposed an interesting model that involves many of these toxic molecules. They identified a neurotoxic cleavage product of CXCL12 (amino acids 5–67) and propose that it is produced by the activity of matrix metalloproteinase 2. This protease is secreted by macrophages or microglia that are infected by HIV^{124,125}. This model takes into account the presence of chemokine receptors, in particular CXCR4, at the cell surface of neurons, but in contrast to other proposed mechanisms, it depends on viruses that use CCR5 as a receptor (which, in the CNS, are the most abundant).

In contrast to these studies, there is a growing body of work that indicates that microglial activation and specific T-cell subpopulations in the brain can have neuroprotective effects^{30,31}. TNF might also have a neuroprotective role against toxicity and prevent or attenuate Ca²⁺ accumulation in the cytosol of neurons^{140–144}. In addition, TNFR activation of signalling that involves the anti-apoptotic kinase AKT (also known as PKB; an important component of pro-survival signalling pathways) and nuclear factor- κ B (NF- κ B)^{141,142,145} results in alterations in gene expression that are crucial for the response of neurons to insults^{142,146}. Furthermore, β -chemokines and CX₃CL1 have also been shown to protect neurons from the toxicity induced by the interaction of gp120 with its receptor; this protection is mediated by phosphatidylinositol-3-kinase-dependent activation of AKT and nuclear translocation of downstream NF- κ B^{83,91}. These data indicate that neuronal chemokine receptors mediate the neurotropic effects of β -chemokines and CX₃CL1. Because TNF induces the production of β -chemokines by activated glia and of CX₃CL1 by astrocytes^{147,148}, it is probable that these cytokines and chemokines are involved in a complex network of both paracrine and autocrine interactions between neurons and glia. However, it is also probable that the various effects of TNF *in vivo* depend on the length and the intensity of the exposure of the cells to TNF. And they are also likely to depend on the ratio of TNFR1 to TNFR2 expression by specific cells, because it has been shown that, depending on the cell type and the levels of expression of these two types of TNFR (which are highly regulated), TNF can either mediate similar effects through different intracellular-signalling pathways or mediate completely different functional consequences¹⁴⁹.

MATRIX METALLOPROTEINASES
Peptide hydrolases that use a metal for their catalytic mechanism and degrade the extracellular matrix. They have an important role in several neurodegenerative processes.

Whereas the effects mediated by TNF might be either beneficial or deleterious, TGF- β 1 has been shown to be neuroprotective^{101,150}. Therefore, the increased levels of TGF- β 1 in the brain of patients with HAD^{131,151} are likely to be neuroprotective.

Potential avenues for therapeutic intervention

The need for specific therapies for CNS complications of HIV infection largely depends on two unknown factors: whether the brain is a long-term reservoir for HIV; and whether MCMD, and other less severe forms of encephalopathy, will progress despite effective systemic HAART. Only further study of the HIV epidemic will resolve these questions. At present, we know that, although the probable latent infection of microglia and other long-lived cells supports the concept that HIV might reside in the CNS in a semi-protected state, viral re-entry from the CNS into the systemic circulation has not been shown. MCMD is indeed a chronic manifestation of HIV in the CNS, and it could progress to a more severe dementia. However, further studies are necessary to determine this, because in the short term, the incidence of dementia has remained low in patients being treated with HAART.

The penetration of the currently used antiretroviral drugs into the CNS is variable, and protease inhibitors are specifically excluded from the CNS by the BBB⁶. Newly developed chemokine-receptor antagonists and entry inhibitors could help reduce the viral load in the brain, provided that they can cross into the CNS; this also applies to new reagents that are being developed, such as inhibitors of the viral integrase protein^{152–154}. However, because neuronal apoptosis might be more dependent on cellular activation and secondary products than on the total amount of virus or viral proteins in the brain, if a specific therapy is developed, it will probably need to be directed to the ‘amplification’ mechanisms of the virus, rather than to the virus itself.

The use of NMDA-receptor antagonists and anti-oxidants are good examples of this approach. The NMDA-receptor antagonist memantine, an open-channel blocker, has been shown to prevent excessive NMDA-receptor activity and to attenuate the neuronal damage induced by HIV-infected macrophages and gp120 (REFS 155–157); it is now approved for therapy for Alzheimer’s disease^{158,159}. Anti-oxidants could be useful to reduce direct damage by nitric oxide and to reduce the effect of nitric oxide on promoting HIV replication in monocytoid cells¹²².

Another approach might be to directly target the mechanisms involved in neuronal apoptosis. Caspases

(molecules that carry out the apoptotic programme) and the MAPK p38 have been targeted by specific inhibitors, and these have been shown to prevent neuronal loss that can be induced by excitotoxicity, gp120 or the α -chemokine CXCL12 (REFS 91,160–163). Overexpression of the anti-apoptotic protein BCL-2 (B-cell lymphoma 2), which suppresses p38 and NF- κ B activation, has been found to reduce neuronal β -amyloid-induced cell death; indeed, p38- and NF- κ B-specific inhibitors also have been shown to attenuate neuronal apoptosis by upregulating the expression of BCL-2 (REF 164). The anti-apoptotic effects of BCL-2 were also shown in relation to the neurotoxic effects induced by HIV, as upregulation of BCL-2 gene expression has been found to confer neuroprotection and to counteract the neurotoxicity that is induced by supernatants from HIV-infected macrophages¹⁶⁵. Other similar approaches might be useful in future.

Concluding remarks

In developed countries, the impressive effects of HAART have converted AIDS from a fatal disease to a more manageable chronic infection, and they have led to the hope that, with aggressive therapy, the virus could be completely eradicated. Unfortunately, regions that remain relatively isolated from the systemic circulation, such as the CNS, might provide a ‘sanctuary’ for latent or slowly replicating virus. Although the incidence of HAD has diminished considerably, there is still a population of patients that, despite therapy, are more prone to developing MCMD, together with its potentially negative consequences. So, MCMD raises the possibility that HIV is replicating at low levels in the CNS and providing a barrier to the complete eradication of the virus. In addition, with time, this troublesome, but not ‘lifestyle-defining’, problem could evolve into a ‘full-blown’ dementia-inducing process, particularly in a population that is now older, having survived the opportunistic infections that characterized the early periods of the AIDS epidemic. Both HAD and MCMD therefore require therapies that are specifically tailored to protect the brain from the effects of viral infection and, in particular, from the consequences of the inflammatory process.

In this review, we have emphasized the importance of bone-marrow-derived cells and their potentially toxic products in the development of CNS dysfunction during infection with HIV. Furthermore, given that some mediators might be neuroprotective, such as TGF- β , future therapeutic efforts should also target these novel neuroprotective mechanisms to attempt to reverse some of the negative effects of immune activation.

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