



Review

Macrophages and HIV-1-Associated Dementia

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Abstract. One of the strongest predictors for HIV-1-associated dementia is the presence of monocytic infiltration in perivascular areas of the brain. Therefore, macrophages have been suggested to play a major role in the development of this disease. This review focuses on possible mechanisms through which the macrophage may enhance disease progression by mediating neuronal damage.

Key words: HIV-associated dementia; macrophages.

General Introduction

HIV-1-associated dementia (HAD) is a neurodegenerative disease that affects about one-third of all adults and half of the children with AIDS^{61, 71}. Clinical disease manifestations include mental and physical slowing, diminished recognition and memory loss and difficulties with reading or carrying out simple tasks. Eventually, florid dementia with hallucinations, seizures and then coma ensues⁶¹. Major hallmarks of HAD are, among others, massive infiltration of mononuclear cells into the brain parenchyma and the formation of multi-nucleated giant cells (MGCs) in the brain⁵³. MGCs are known to be major virus producers within the brain and are formed when HIV-infected and uninfected macrophages fuse together⁹¹. Although most demented AIDS patients have high levels of virus in the brain⁹⁰, high-level viral gene expression does not always correlate with clinical manifestations or neurological impairment^{21, 90}. In addition, a discrepancy between the localization of HIV-infected cells and the severity of neurological abnormalities has been reported²¹. Furthermore, small numbers of productively HIV-infected brain macrophages have been detected in

individuals with severe clinical and pathological brain deficits. Thus, viral load is not very well correlated with disease and does not appear to be a strong predictor for disease progression. In contrast, the presence of large mononuclear infiltrates in perivascular areas seems to be strongly associated with HAD²⁹. This review focuses on the effects of the macrophage and its secretory products on cellular functions in the neuropathogenesis of HIV-1 infection.

Effect of Viral Proteins on Cellular Functions

Although the viral load does not directly correlate with disease, many mechanisms leading to neurotoxicity have been attributed to HIV-1 or to viral proteins. In addition to macrophages, HIV-1 proteins have been detected in many other cell-types, such as neurons, microvascular endothelial cells, cells of the choroid plexus and oligodendrocytes¹⁰. In contrast to the productive nature of HIV-1 infection in macrophages, viral infection of astrocytes is highly restricted. HIV-infected astrocytes have been shown only to secrete early viral gene products such as tat and nef¹⁰. Still, since astrocytes are the

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most abundant cell type in the CNS, even restricted virus production may have major consequences.

Early viral protein-associated neurotoxic mechanisms

The HIV trans-activating protein, tat, regulates HIV-1 gene expression and is essential for viral replication *in vitro*^{37, 84}. A protein is currently being studied intensively for its role in the neuropathogenesis of HIV infection. That protein is a 86–104 amino acid protein which is essential for HIV replication³². The tat sequence can be divided into different regions which all have unique properties. The cysteine-rich domain (amino acids 20–31), whose function is still mostly unclear, was recently reported to have an effect on chemotaxis⁴. Other regions of tat, all involved in RNA binding, are a core region (amino acids 32–57), a basic region (amino acids 48–57) containing the nuclear localization sequence, and a glutamine-rich region (amino acids 60–67)⁷⁵. Like HIV-1, tat has been shown to affect major signal transduction pathways by activating the transcription factor nuclear factor- κ B (NF- κ B). Various inflammatory genes that are under the control of NF- κ B can thus be switched on by tat and many of these gene products, such as TNF- α ¹² or IL-1 β , can be directly or indirectly neurotoxic^{12, 59}. In addition, tat appears to play a major role in the induction of macrophage infiltration into the brain parenchyma. The tat protein has been shown to have strong chemoattractant properties⁴⁵. A peptide containing the cysteine-rich and core domains of tat have been shown to retain most of the chemotactic potential⁴ and this peptide actually mimics a sequence that is found in many CC chemokines⁵. In fact, the peptide can interact with the CC chemokine receptors CCR2⁵, which is involved in monocyte chemotaxis^{71, 43}. Besides being chemotactic itself, tat is also able to elicit the production of molecules that enhance monocytic migration into the brain. First, tat induces various adhesion molecules such as E-selectin, VCAM-1 and ICAM-1 on endothelial cells¹⁸, which leads to increased adhesion of blood monocytes to the vessel walls. Second, tat can induce MCP-1 in astrocytes which may result in increased migration into the brain parenchyma¹⁵. Third, the synthesis of platelet-activating factor (PAF), a potent mediator of cell motility, was induced by tat protein¹⁷. And finally, tat was shown to induce matrix metalloproteinase-9 (MMP-9), which can facilitate passage through the basement membrane of the blood-brain barrier (BBB)⁴⁴.

Besides having multiple regulatory functions, the tat protein can directly induce neurotoxicity by activation

of N-methyl-D-aspartate (NMDA) receptors⁵¹. Overstimulation of NMDA receptors, a glutamate receptor, will result in an excessive influx of Ca²⁺ into neurons, resulting in so-called excitotoxic injury. Furthermore, tat can induce neuronal apoptosis⁶² and amino acids 31–61 are suggested to be involved in direct neurotoxicity^{60, 76}.

Literature concerning neurotoxic properties of other early HIV proteins, such as rev and nef, is much less extensive and even contradictory^{56, 74}. Thus, the early HIV protein tat may play an important role in HAD, in contrast to other early proteins.

Late viral protein-associated neurotoxic mechanisms

Besides the early HIV protein tat, late viral proteins have also been implicated in neurotoxic mechanisms. Like the tat protein, the major envelope glycoprotein of HIV, glycoprotein 120 (gp120), can increase the production of the neurotoxic cytokines TNF- α and IL-6^{25, 86} and can induce NMDA receptor-mediated neurotoxicity²². Furthermore, gp120 induces neuronal apoptosis⁴⁶, probably through an indirect pathway via activation of chemokine receptors on macrophages/microglia³⁸. Gp120 can also enhance ICAM-1 gene expression in primary rat astrocytes, primary human astrocytes, a human astrogloma cell line CRT, and primary rat microglia⁷⁸. The signal transduction events involved in gp120-mediated enhancement of ICAM-1 appear to involve activation of both protein kinase C (PKC) and tyrosine kinase⁷⁸. In addition, brain tissue from patients with HIV-1 encephalitis and from gp120 transgenic mice also showed increased PKC immunoreactivity⁹². Gp120 can also activate both c-Jun N-terminal kinase (JNK) and p42 extracellular-regulated kinase (ERK) in primary CNS cells⁴⁶. This indicates that gp120-induced dysregulation of signal transduction pathways may represent a general mechanism of HIV-associated pathogenesis.

Another structural HIV protein, glycoprotein 41 (gp41), is also implicated in the neuropathogenesis of HIV infection¹. Recently it has been demonstrated that the carboxy terminus of gp41 impairs the transport of glutamate and aspartate, the major excitatory amino acids, in glial cells, which may contribute to excitotoxic damage to neurons in HIV-1 infection of the CNS⁴¹. ADAMSON et al.³ showed that gp41 induces neurotoxicity in mixed neuronal cultures-glial cultures. Gp41-induced neurotoxicity resulted in the induction of inducible nitric oxide synthase (iNOS), required the presence of glial cells and was mediated via a nitric oxide-dependent mechanism, since NOS inhibitors provided

neuroprotection³. In addition, they showed that the severity and rate of progression of HAD correlates with indices of immune activation as well as with levels of iNOS and gp41².

Besides its capacity to induce neurotoxic mechanisms, gp41 can also indirectly lead to enhancement of HAD. It has been shown that gp41 can increase MMP-2 activity¹³, which may increase the BBB permeability, thus facilitating monocytic infiltration. Thus, in addition to early viral proteins, the structural proteins may also be involved in the pathogenesis of HAD.

HIV-1-Induced Molecules

Like the viral proteins, HIV-1 infection itself can also have profound consequences on cellular functions. As mentioned above, within the CNS, HIV-1 productively replicates only in blood-derived brain macrophages and microglia, the resident brain macrophages. HIV-infected monocytes/macrophages appear to have a selective advantage in entering the brain and an important role has been proposed for adhesion molecules such as E-selectin and VCAM-1⁶⁶. It was shown that HIV-infected macrophages, in contrast to uninfected macrophages, selectively induce high levels of E-selectin and VCAM-1 on brain endothelium⁶³, probably via increased expression of the pro-inflammatory cytokines IL-1 β and TNF- α ^{82, 89}. After migration through the endothelial cell layer, the HIV-infected macrophages encounter the basement membrane that surrounds the abluminal side of the BBB. Since HIV-1 induces gelatinase B activity, the capacity of HIV-infected monocytes to digest and invade the basement membrane gel matrix was significantly greater than that of uninfected control cells^{19, 20}. In addition, HIV-1 will also enhance monocytic infiltration by modulating chemokine production. Chemokines are often upregulated in inflammatory processes and play a major role in the mobilization as well as in the activation of all blood leukocytes^{23, 72}. Their role in the pathogenesis of HIV-1 infection appears to be extensive and was recently reviewed by LEE and MONTANER⁴⁷. Based on their function, sequence and chromosomal location, two major families of chemokines may be distinguished, the CC and the CXC chemokines^{6, 58}. Whereas the CXC chemokines mainly have an effect on neutrophils, the CC chemokines mostly attract lymphocytes and monocytes/macrophages^{11, 83}. CC chemokines have been found to enhance migration of monocytic cells across the BBB⁷³ and into the brain parenchyma⁶⁹. *In*

vivo, CC chemokines are significantly elevated in brains of demented AIDS patients^{15, 77} and *in vitro*, HIV-1 was shown to induce the CC chemokine MCP-1 in macrophages and U937 promonocytic cells⁵⁷. Thus, by increasing chemokine levels and adhesion molecules, HIV-1 infection may lead to enhanced infiltration of monocyte into the brain.

HIV-1 infection of macrophages results in the production of low-molecular weight, heat-stable, proteinase-resistant molecules that are highly neurotoxic and can result in NMDA receptor-mediated neuronal injury^{26, 27}. HIV-1 infection of macrophages results in an increased expression of various molecules such as Ntox²⁸, quinolinic acid^{39, 40} and PAF²⁴. In particular the latter is being studied intensively for its role in HAD. PAF is a phospholipid mediator that can elicit various biological responses relevant for the development of inflammatory reactions⁷⁰. PAF is overexpressed by LPS-stimulated HIV-1-infected monocytes⁶⁵ and has been shown to be toxic to neuronal fetal cultures²⁴, an effect that appears to be mediated by NMDA receptors²⁴, and to induce neuronal apoptosis⁶⁸. Other functions of PAF that may mediate neuronal death in HAD are reviewed by MACLENNAN et al.⁵⁰

Interactions between Macrophages and Astrocytes

Once inside the brain, the immune-activated macrophages stay in close contact with both endothelial cells as well as astrocytes. *In vitro*, interactions between macrophages and astrocytes result in the induction of oxidative stress³⁴. As recently reported, HIV-1 induces superoxide anion in macrophages⁹ and iNOS and nitric oxide are elevated in astrocytes that were cocultured together with HIV-1-infected macrophages³⁴. *In vivo*, in perivascular areas of brain tissue of HAD patients, the presence of various immune activation products, such as pro-inflammatory cytokines, are described^{9, 82} and the presence of these cytokines may have major consequences. IL-1 β is known to be a major mediator of many immunological responses and is able to induce chemokines in astrocytes^{31, 55} and reactive oxygen species (ROS) in macrophages⁴⁸, but also neuroprotective molecules such as neurotrophic factors (NTF's)⁸ in various cell types and these molecules are indeed observed in brain tissue of patients suffering from HAD^{8, 15, 82, 85}. There appear to be strong feedback mechanisms between neurotrophic factors and molecules involved in oxidative stress. Increased oxidative stress induces production of NGF and bFGF⁶⁷ which in turn

can downregulate oxidative stress by inducing ROS scavengers, such as superoxide dismutase^{52, 54}, or down-regulate enzymes that can contribute to oxidative stress, such as iNOS¹⁴. However, these feedback mechanisms do not appear to be functional in patients with HAD, since both NTFs as well as oxidative stress are increased in brain tissue^{8, 9, 79}. In fact, they can both enhance disease progression by increasing chemokine production, which enhances monocyte infiltration into the brain⁸⁸.

Interactions between Macrophages and the BBB

The presence of high numbers of perivascular immune-activated monocytic cells will have major effects on the integrity of the BBB. It correlates very well with loss of tight junction structures and with perivascular oxidative stress, in particular with the presence of peroxynitrite (unpublished observations). *In vitro*, peroxynitrite and oxygen radicals can increase the permeability of brain endothelial cells and may even be toxic^{30, 35}. In addition, it has been demonstrated that phosphorylation of kinases, such as PKC, are essential for the proper assembly of tight junction proteins⁸¹. Interference with this phosphorylation may very well result in the disruption of tight junctions. Peroxynitrite can inhibit kinase phosphorylation by nitrating tyrosine residues³³ and may thereby also modulate subsequent enzymatic reactions, thus affecting tight junction assembly.

In addition, macrophage secretory products will also contribute to an increased migration of monocytic cells into the brain parenchyma. Chemokines are produced locally in response to interactions between endothelial cells and macrophages⁴⁹ and the presence of HIV-1 will even enhance chemokine expression even further (unpublished observations). Furthermore, MMPs have been recently implicated to be involved in HAD. MMP's are proteolytic enzymes that are involved in the degradation of specific components of the extracellular matrix and may be responsible for disruption of the BBB as well as for myelin destruction⁸⁷. MMP's have been suggested to play an important role in various neurodegenerative diseases, such as Alzheimer's disease, multiple sclerosis and also in malignant gliomas⁸⁷. Recently, it was shown that MMP levels are elevated in cerebrospinal fluid of demented AIDS patients as compared to nondemented AIDS patients^{16, 80}. Moreover, TNF- α , which is increased in brain tissue of HAD patients, was suggested to be involved in this MMP induction¹⁶.

Conclusions

Macrophages indeed seem to have a central role in the neuropathogenesis of HIV-1 infection. Over the years, overwhelming evidence has been provided that all demonstrate that the infiltration of macrophages into the brain and the subsequent production of neurotoxins are highly correlated with neuronal damage and death. It has also been shown that HIV-1 infection will immune-activate macrophages and, indeed, that immune activation is also strongly associated with HIV-1-associated dementia. The presence of pro-inflammatory cytokines may start a cascade that leads to massive immune activation in the brain. Subsequently, this may be responsible for the production of more neurotoxins, chemokines, cytokines, oxidative stress, etc., which all probably contribute to disease progression.

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