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Review

Role of Immune-Derived Diffusible Mediators in AIDS-Associated Neurological Disorders

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Abstract. Neurologic abnormalities are common in HIV-1 infected patients and often represent the dominant clinical manifestation of pediatric AIDS. Although the neurological dysfunction has been directly related to CNS invasion by HIV-1, the pathogenesis of neurologic disorders remains unclear. This review will first discuss the spectrum of potential interactions between HIV-1 and neural (neuronal and glial) cells, in the face of experimental data. Next, we will focus on the role of immune-derived cytokines and other soluble compounds which have been proposed to act as neurotoxic mediators and appear to play a role in the pathogenesis of AIDS-associated neurodegeneration.

Key words: AIDS patients; neurological disorders; neural cells.

Introduction

After the initial description of the acquired immune deficiency syndrome (AIDS), a slowly progressive, dementing illness was recognized in association with HIV-1 infection. Epidemiological studies have estimated that approximately one-third of patients with AIDS-related complex or Kaposi's sarcoma-defined AIDS have evidence of HIV-1 encephalopathy and another one-fourth have subclinical involvement[36, 44]. HIV-1 encephalopathy is often characterized by an insidious onset of a disturbance in intellect. Fatigue and malaise, headaches and increasing social isolation are commonly observed. Job performance declines and self-care may become problematic[1, 44]. HIV-1 encephalopathy generally occurs in the context of advanced immune suppression and coexistent systemic disease though it may represent the presenting and even sole manifestation of the disease, particularly in children[4, 23, 24] where it is accompanied by developmental delay or the loss of motor and intellectual milestones[10]. The gross pathology of HIV-1 encephalopathy is characterized by brain atrophy with sulcal widening and ventricular dilatation. Histologically, the presence of microglial nodules with multinucleated giant cells, diffuse astrocytosis, perivascular mononuclear inflammation, rarefaction of the white matter with microvacuolation, and neuronal loss[9] characterize the lesions. Although the neurological dysfunction has been directly related to CNS invasion by HIV-1[16, 35, 37, 40, 46, 50, 59], the pathogenesis of neurologic disorders remains unclear.

Schematically, two mechanisms, not mutually exclusive, can be hypothesized which involve either direct neurocytopathic effects (depending upon HIV-1 infection of neuronal and/or glial cells) or indirect effects (mediated by HIV-1 infected and/or functionally acti-

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vated accessory and/or inflammatory cells). At present, the potential involvement of infectious agents other than HIV-1 acting as predisposing or inducing co-factors in the pathogenesis of AIDS-associated neurodegeneration remains to be clarified.

HIV-1 Neurotropic Properties: Direct Interactions with Specific Cell Types in the Brain

HIV-1 strain-specific properties may play a role in AIDS-associated neurodegeneration[1]. In fact, HIV-1 neurotropism may, at least in part, depend upon the ability of the virus to enter and replicate in specific target cells (Fig. 1). These events depend upon cellular and molecular interactions that require the expression of specific surface receptors by metabolically and transcriptionally active cells. Recent studies indicated that, in addition to the CD4 molecule, different chemokine receptors such as CXCR4, CCR5 and CCR3 could mediate virus entry in target cells [13–15] (Fig. 1). These molecules are expressed in a broad range of tissues and cell types, including the brain and T cells. In particular, CCR3 is expressed by microglia in primary brain cultures[32], while CXCR4 can be expressed by neurons, possibly playing a part in neurodevelopment[72]. Thus, M-tropic HIV-1 strains that use CCR5 or CCR3 may preferentially infect microglial cells, whereas T-tropic strains that use CXCR4 are unable to enter these cells and may potentially interact with neuronal cells[33]. The CCR3 ligand, eotaxin, anti-CCR3 antibodies as well as

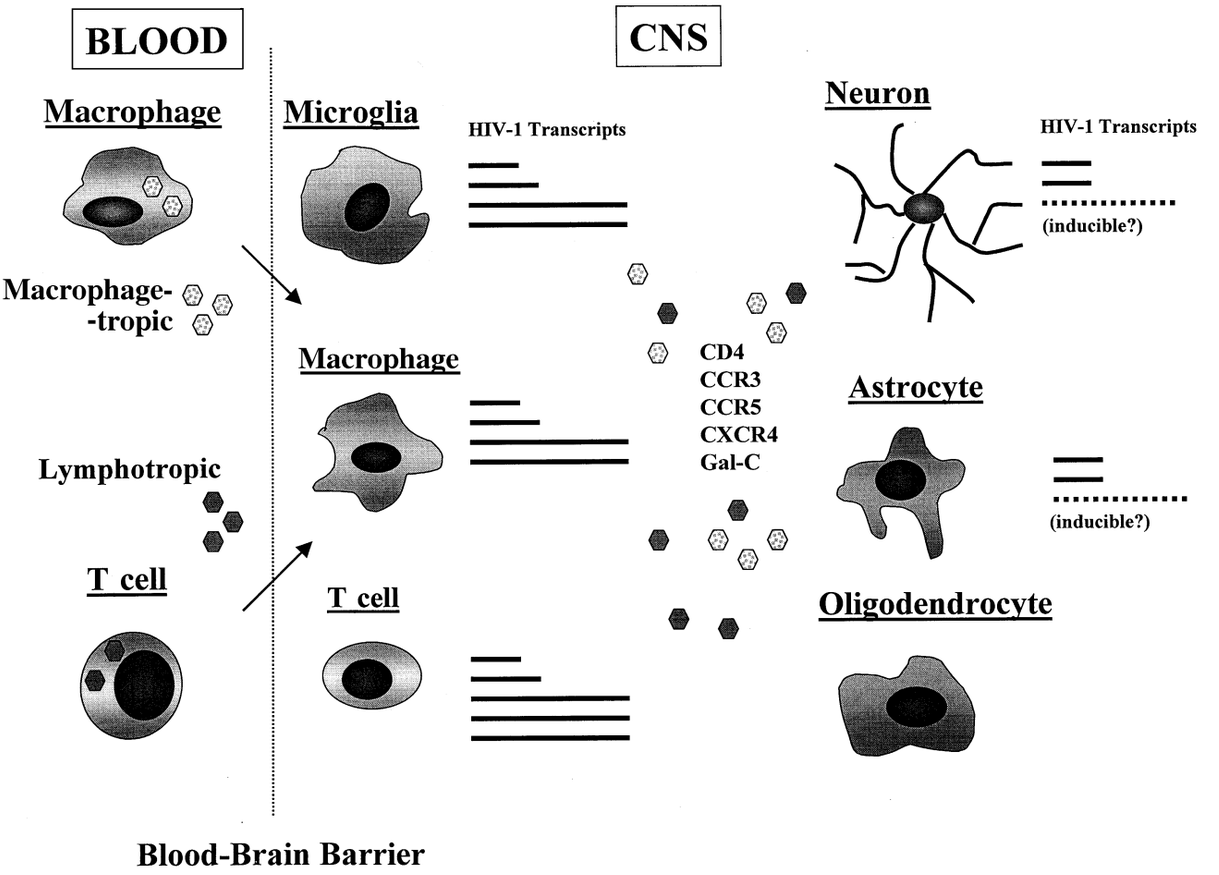

Fig. 1. Mechanisms controlling HIV-1 infection and replication in the brain. The distribution of molecules that can mediate HIV-1 binding and/or viral entry in target cells represents a first level of restriction of HIV-1 infection in the brain. It should be noted that in addition to chemokine receptors, additional molecules, such as a galactosyl-cerebroside, may play a role in mediating HIV-1 entry in neural cells. A second level of restriction is exerted at the intracellular level and corresponds to the degree of permissivity to viral gene expression and replication of specific cell types in the CNS. This is based, at least in part, upon the different utilization of regulatory elements within the viral promoter by distinct cell types, thus determining preferential environments for viral replication in individual neuronal and glial cells. Soluble signals can further modulate such permissivity and contribute in supporting virus latency or virus replication in the CNS[64] (see text for further explanation)

the CCR5 ligand, MIP-1β, can inhibit HIV-1 infection of microglia, suggesting that these molecules may represent a potential target for new therapeutic strategies directed at reducing virus spreading in different tissues, including the brain.

HIV-1 infected neuronal and glial cells, however, have been rarely detected *in vivo* by conventional immunohistochemical techniques[50, 44, 45]. Recently, the use of highly sensitive techniques such as the *in situ*, polymerase chain reaction (PCR), provided evidence for HIV-1 infection of these cells, in both adults and children, though accompanied by a low-level viral gene expression[2, 46, 62]. *In vitro*, many different neuronal and glial cell lines as well as primary neurons and astrocytes appear susceptible to HIV-1 infection through a CD4-independent pathway of virus entry which appears to involve a galactosyl-cerebroside molecule[17, 18, 32, 39, 45, 58, 65] (Fig. 1). Interestingly, the susceptibility to HIV-1 infection and the control of viral gene expression and replication in neural cells appear to depend upon the state of cellular differentiation being higher with more immature precursors than with differentiated cells[17, 18, 22, 70, 71]. This may have important implications for pediatric HIV-1 infection and suggests that variations in the cellular microenvironment, either lineage-or differentiation-dependent, may contribute to influencing the ability of HIV-1 to infect and replicate in the nervous system.

The molecular features of such restricted HIV-1 infection of neurons and glial cells have been studied *in vitro* with cells transfected with the HIV-1 LTR, which contains the cis-acting regions that control virus expression in infected cells [52], or with infectious HIV-1 proviral DNA, thus bypassing restriction posed by cell binding and entry. The predominant species of mRNA in these cells are the multiply spliced 2 kb species associated with regulatory gene expression (*tat*, *rev* and *nef*)[63, 65]. Larger molecular weight RNA species associated with structural gene expression are virtually absent (Fig. 1). It should be noted that such regulatory gene products are generally released by target cells as biologically active extracellular soluble forms and may thus participate in HIV-1 neuropathogenesis by directly altering neuronal and glial viability and function or by contributing to inflammatory cell recruitment in the brain[38, 41, 53]. In addition, recent reports indicate that regulatory elements of the viral LTR are differentially utilized according to the specific neuronal or glial cell types[18, 22, 45, 58]. This could account for the spectrum of post-entry, strain-associated differences in the levels of virus replication which is generally observed with neural (neuronal and glial) cells of different origin[18, 22, 45, 63]. With these cell types HIV-1 replicates at very low, if not undetectable, levels (Fig. 1) and does not appear to induce evident cytopathic effects[17, 18, 32, 62–65]. Taken together, these observations suggest that: 1) HIV-1 infection of neural cells may contribute to establishing a virus reservoir in the CNS whose extent and susceptibility to conventional antiretroviral therapy (including the more recent highly active regimens) are, at present, unknown, and 2) mechanism(s) other than direct virus--target interaction and, possibly, additional components of the brain microenvironment are required to induce the conspicuous pathology and functional damage observed in these patients.

Role of Persistent Immune Activation and Local Production of Diffusible Factors with Neurotoxic Properties

HIV-1 infection in the brain parenchyma is not abundant and does not seem to correlate with the clinical severity of the disease[7, 30, 31]. Such a limited extent of viral burden suggests that the dementia in AIDS patients might be due to indirect effects, such as those induced by soluble factors produced by resident and blood-derived mononuclear cells (microglia, monocyte--macrophages, lymphocytes), which compelling evidence indicates as major targets and principal reservoirs of the virus in the brain[2, 37, 50, 67–69]. Consistent with this hypothesis, recent studies indicated that the number of macrophages in the CNS of patients with AIDS is a better correlate to the dementia than viral burden[7, 30, 31]. In addition to the production of viral structural and regulatory proteins (i.e. gp120, Tat), which, as previously mentioned, have been shown to possess neurotoxic properties *in vitro* and in animal models[6, 38, 53, 61], HIV-1 infected and/or functionally activated mononuclear cells can produce a number of inflammatory cytokines and bioactive substances which may both concur in regulating HIV-1 gene expression and replication in the CNS[64] and in altering neural cell function and survival[29, 49] (Fig. 2). A recent set of studies determined that soluble mediators with neurotoxic properties are spontaneously produced by lymphomonocytes from HIV-1 infected individuals[19, 20, 48]. Similar neurotoxic potentials are also expressed by normal PBMC or purified monocyte/macrophage (M/M) preparation upon functional activation. In fact, cell culture supernatants from both HIV-1 infected and functionally activated PBMC or M/M appear capable of altering the growth and viability of immature neurons as well as the survival of mitotically quiescent neuronal cells[19, 20]. These effects

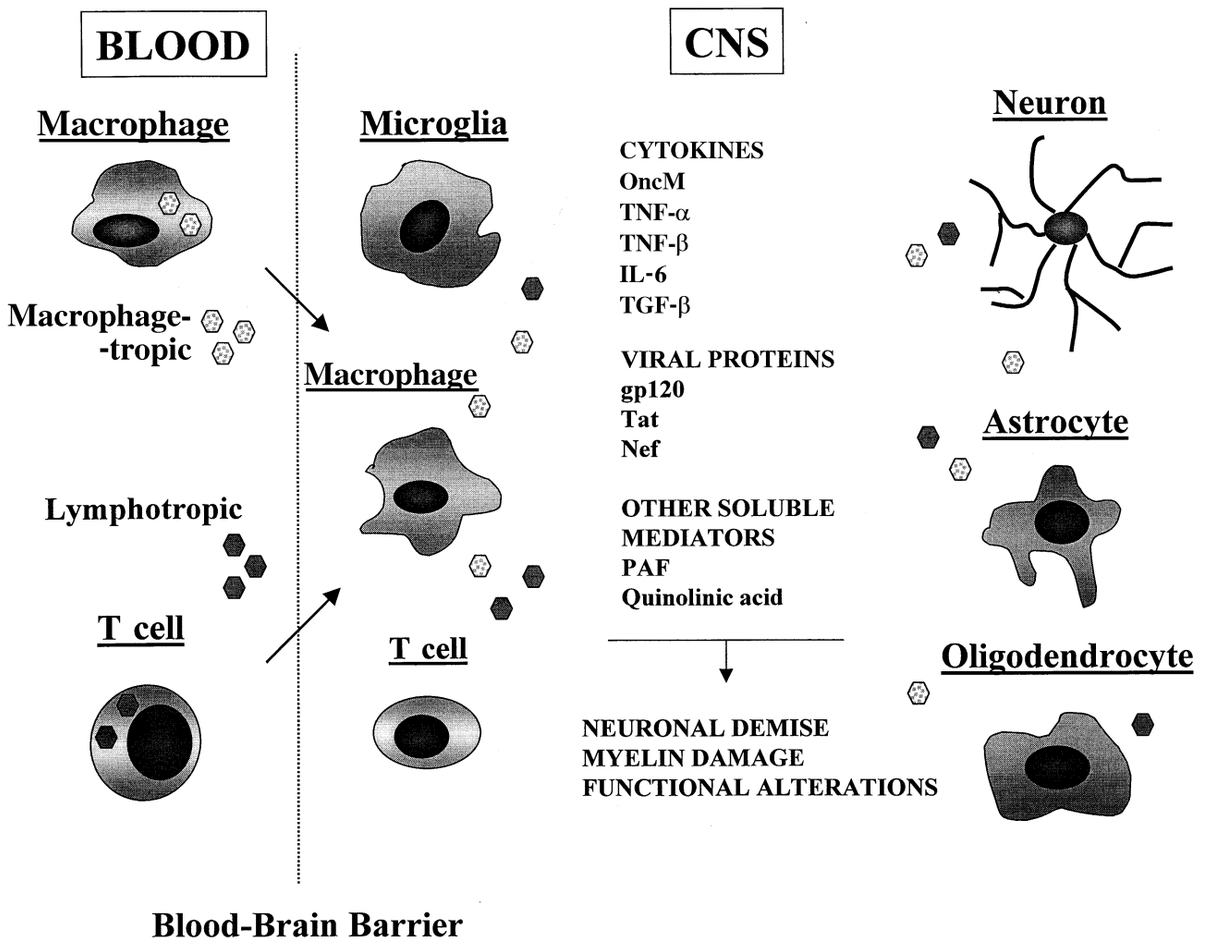

Fig. 2. Neuropathogenesis of HIV-1 infection. Potential contribution of viral proteins, inflammatory cytokines and neurotoxic metabolites in the pathophysiology of AIDS-associated neurodegeneration. Following CNS invasion by HIV-1 and immune cell activation and recruitment, multiple mechanisms, non-mutually exclusive, may participate in inducing neural damage and functional impairment (see text for further explanation)

are accompanied by both morphological and biochemical alterations of the cells consistent with the induction of apoptosis. Similar observations, obtained by distinct studies on different neural model systems[28, 29, 48, 49], suggest that both HIV-1 infection and functional activation can induce lymphomononuclear cells to express neurotoxic potentials which relay upon the production of bioactive substances that, in turn, induce neuronal growth perturbations and apoptotic cell death.

Several diffusible factors released by immune cells possess documented damaging potentials on neural tissues (Fig. 2). Among them, oncostatin M (oncM), a recently characterized proinflammatory mediator[43, 51], has been identified as one cytokine that directly causes a profound inhibition of neuronal proliferation and viability. OncM belongs to a family of structurally and functionally related pleiotropic factors that utilize the gp130 or gp130-related receptor subunits on target cells[43, 51]. These cytokines exhibit differential effects on a variety of cell types, including cells of neural, hematopoietic, lymphopoietic and vascular origin. Indeed, the same protein can act differentially on target cells, depending upon the responsive cell population[21]. Interestingly, expression of oncM in transgenic mice is detrimental to normal mouse development and death is associated with expression in neurons[42], suggesting that oncM production in the brain may exert lethal effects on neuronal cells.

In the presence of oncM, the decrease in neuronal cell viability, documented by the altered membrane per-

meability to propidium iodide staining, is preceded and accompanied by extensive DNA cleavage which indicates that oncM induces apoptotic cell death. Moreover, antibodies neutralizing anti-oncM have been capable of preventing the growth inhibition induced by either HIV-1 infected or functionally activated lymphomonocytes as well as the effects of the recombinant protein[20].

Taken together, these data indicate that native oncM can play a part in HIV-1-associated neurodegeneration, though other factors are likely to be involved in the process, acting either in concert or independently from oncM. For instance, TGF-β_1, but not TNF-α, IFN-γ, IL-6 or virus components such as gp120, can act in concert with oncM, enhancing the inhibitory effects of the cytokine on primary neurons and neuronal cell lines of different origins. In the presence of both cytokines, neuronal growth and survival alterations can be elicited at very low oncM concentrations, consistent with those spontaneously released by PBMC from HIV-1 infected subjects in their supernatants[20]. Thus, an increased oncM production from HIV-1 infected or functionally--activated lymphocytes as well as resident or blood--derived mononuclear phagocytes can directly contribute to neuronal apoptosis in AIDS-associated neurodegeneration, which generally occurs in the absence of direct HIV-1 infection of these cells, and appears to depend upon the induction of diffusible factors[47, 60]. These data also suggest that the total neurotoxic activity potentially exerted by HIV-1 infected or functionally activated lymphomononuclear cells depends upon multiple parameters that include the class of cytokine production, its concentration, potency and interaction(s) with other mediators.

In fact, several inflammatory mediators produced upon HIV-1 infection or functional activation of lymphomononuclear cells can play a part in the pathogenesis of HIV-1 induced neurodegeneration (Fig. 2). Altered levels of proinflammatory cytokines such as IL-1, IL-6, TNF-α and IFN-γ and the presence of neurotoxic metabolites such as quinolinic acid (a monocyte/microglial byproduct of tryptophan with neurotoxic properties) have been documented in sera and CSF of AIDS patients[7, 25, 27, 28, 34, 48, 49]. Production of the same cytokines as well as TGF-β and arachidonic acid metabolites have also been documented in the brain parenchyma, in association with HIV-1 infected and/or activated mononuclear cells and astrocytes[28, 46, 66]. Such soluble factors possess documented damaging potentials on neural tissues. In addition to mediating microglia and astrocytic activation, which in turn lead to increased proinflammatory cytokines production[21], many of them can exert either a positive or a negative regulation of oligodendrocytes proliferation and survival[3, 54]. TNF-α has been shown to mediate myelin and oligodendrocytes damage both *in vivo* and *in vitro*[55, 56] and to exert cytotoxic activity against rat oligodendrocytes, which results in cell death. TNF-β, which is genetically and functionally related to TNF-α, has also been shown to possess potent cytotoxicity against oligodendrocytes. The effect is much more potent than TNF-α and is mediated by apoptotic mechanisms[57]. In addition to the previously mentioned effects of IL-1, TNF-α and TNF-β on oligodendrocyte survival and myelination, IL-6 has been shown to mediate potent neurotoxic effects when expressed in the brain of transgenic mice[11]. Other soluble mediators such as the platelet-activating factor (PAF) and leukotriens/prostaglandins, which are produced upon microglia/macrophages and astrocytes functional interaction, have been reported to exert damaging effects on primary neural cells and neural cell lines[27, 28]. Thus, in the event of lymphoid/monocytic cell infiltration into the CNS and the activation of resident microglia and astrocytes, the complex circuitry of molecular interactions mediated by cytokines appears altered both spatially and temporally and capable of mediating neurotoxic effects. In addition, the pleiotropic properties of such soluble mediators are responsible for multiple and apparently contrasting effects exerted upon different cell types in the brain. For example, TNF-α can either exert direct inhibitory or stimulatory effects depending upon the responsive neural cell type[5, 21, 26]. In fact, TNF-α can even protect primary neurons of different origins from metabolic-excitotoxic insults[12]. This is not surprising, since cytokines such as TNF-α, TGF-β, oncM and IL-6, key regulatory factors in the host response to injury or immunological challenge, can also act as instructive/permissive signals during nervous system development or in events which involve functional or structural tissue remodeling[21]. In addition to direct interaction with responsive neuronal cell targets, the contribution of these cytokines to neuronal demise may involve multiple interactions with all the different non-neuronal cell types in the brain. This notion further emphasizes the concept that the inappropriate, chronic production of different inflammatory cytokines such as TNF-α, TGF-β, oncM and IL-6, which have partially overlapping effects and the potential to exert both direct and indirect CNS injury, can play a major role in the pathogenesis of neurologic AIDS-associated disorders by acting through different mechanisms on different target cells and potentially cooperate by either additive or synergistic modality, thus amplifying their effects on responsive cell types.

Thus, AIDS-associated neurodegeneration appears a complex, multifactorial disease where the combined action of HIV-1 infection, production of viral proteins and soluble inflammatory mediators are implicated in the pathophysiology (Fig. 2).

Concluding Remarks

Several lines of evidence suggest that a cascade of events triggered by HIV-1 infection and involving a chronic dysregulation of cytokine expression are implicated in the setting of neuronal demise in both the immature and adult brain. These findings contribute to identifying novel pathways of immunologically-mediated neuronal injury and may provide a model for the interpretation of neurological disorders depending upon CNS inflammatory conditions from varied insults. This evidence should be taken into account in the development of therapeutic intervention, which should be directed not only toward reducing the viral load in the brain, but also in controlling the deleterious effects of cytokines and protecting neural cells from toxic metabolites.

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