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HIV-1 infected mononuclear phagocyte secretory products affect neuronal physiology leading to cellular demise: relevance for HIV-1-associated dementia

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Viral and cellular products from HIV-1-infected and/or immune competent mononuclear phagocytes (MP) (brain macrophages and microglia) affect neuronal function during HIV-1-associated dementia (HAD). Neurotoxic MP factors include, but are not limited to, pro-inflammatory cytokines, chemokines, platelet activating factor, arachidonic acid and its metabolites, nitric oxide, progeny virions and viral structural and regulatory proteins. The mechanisms for immune-mediated neural injury in HAD, only now, are being unraveled. In this regard, we reviewed the current knowledge of how postmitotic neurons, which can neither divide nor be replaced, are damaged by MP secretory activities. Linking neuronal function with brain MP activation was made possible by placing viral and/or immune products onto neurons and measuring cell signaling events or through *ex vivo* electrophysiological tests on MP-treated brain slices. Such linkages are shown, in this report, by select demonstrations of MP factors which cause neuronal dysfunction in HAD. *Journal of NeuroVirology* (2000) **6,** S14–S23.

Keywords: HIV-1; cytokines; chemokines; viral structural and regulatory proteins; neurotoxicity; HIV-1-associated dementia

Introduction

Individuals with progressive human immunodeficiency virus type 1 (HIV-1) disease often suffer from cognitive, behavior, and neurological disturbances commonly known as HIV-1-associated dementia (HAD) (McArthur and Grant, 1988). The neuropathological features, that are linked to disease, include brain infiltration of monocyte-derived macrophages (MDM), the formation of multinucleated giant cells and microglial nodules, astrogliosis, the disruption of the blood-brain barrier (BBB) and neuronal drop-out (Michaels *et al*, 1988; Price *et al*, 1988). Infected mononuclear phagocytes (MPs) (perivascular macrophages, parenchymal brain macrophages and microglia), induce the neuronal injury that underlies cognitive dysfunction. These brain MPs are the natural target cells for HIV-1 in its human host (Epstein and Gendelman, 1993; Everall *et al*, 1991; Ketzler *et al*, 1990; Masliah *et al*, 1992; Wiley *et al*, 1991; Gendelman *et al*, 1997). There is mounting evidence indicating that the MPs are the first cells infected and function as a major tissue reservoir for HIV-1 during all stages of viral infection (reviewed by Meltzer *et al*, 1990; Meltzer and Gendelman, 1992).

Interestingly, the levels of HIV-1 gene products, in infected brain tissue, do not always predict cognitive impairment (Glass *et al*, 1995). This suggests that both HIV-1-infection and MP activation are necessary for neurotoxin production. The

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MP neurotoxins found in spinal fluid and/or brain tissue of subjects with HAD include, but are not limited to, pro-inflammatory cytokines (for example, TNF-α, IL-1β), chemokines (for example stromal-derived factor-one alpha (SDF-1α)), quinolinate, arachidonic acid and its metabolites, free radicals, platelet activating factor, NTox and nitric oxide (reviewed by Zheng and Gendelman, 1997). Apart from the brain cytotoxins, accumulating evidence indicates that viral products also play a role in central nervous system (CNS) injury (Lipton, 1991; Lipton *et al*, 1991). These viral factors include HIV-1 gp120, *tat, rev* and *nef* which mediate their actions directly onto neurons or through stimulating MP secretory activities (Giulian *et al*, 1993; Wahl *et al*, 1989). The recognition that chemokine receptors are expressed on neural cells and macrophages (Horuk *et al*, 1997; Lavi *et al*, 1997) (Figure 1) raises multiple new possible mechanisms for how the CNS might be damaged by MP viral products. In this paper, we provide recent data explaining how cellular and viral products produced from infected and/or immune competent MPs induce alterations in neuronal physiology. Importantly, we provide representative experimental results from other laboratories that bolster the importance of MP secretory products in HAD pathogenesis. It is our thought that these observations strongly support a prominent role for innate immunity in the neuropathogenesis of HIV-1 infection.

HIV-1-infected MP secretions and neuronal demise

The brain's MPs secrete neurotoxins during HAD. Interestingly, these MPs evolve from primary neurotrophin-secreting cells to ones that produce cytotoxins. Such immune events follow viral infection and MP immune activation. It is widely accepted that neuronal cell death found in the brains of AIDS patients is mediated, in large measure, by indirect mechanisms. This occurs through alterations in MP secretory function following viral infection. Both viral and cellular products

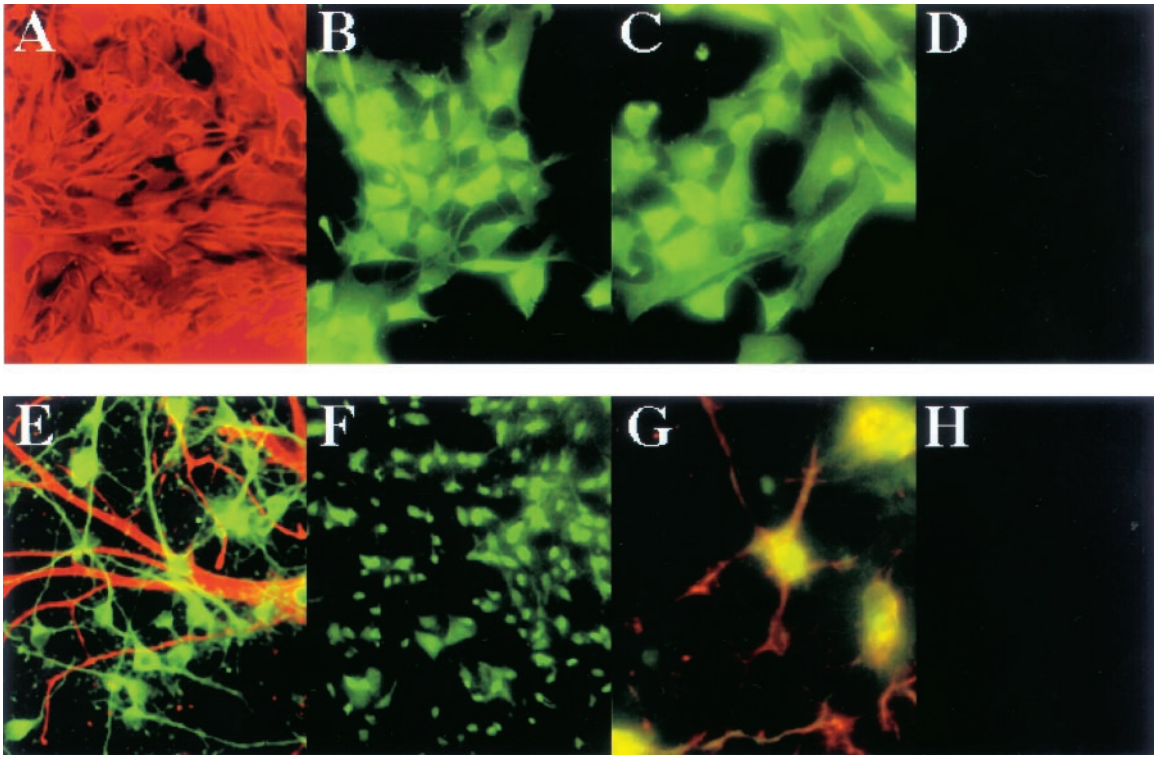

Figure 1 CCR3 and CXCR4 antigen expression in human neurons and astrocytes. Human neurons and astrocytes were propagated onto chamberslides as adherent monolayers, then immunostained with monoclonal antibodies to CCR3 (7B11) and CXCR4 (12G5). Immunoreactivity was visualized by indirect immunofluorescence using conjugated secondary antibodies to IgG F(ab′)$_2$-FITC or rabbit IgG F(ab′)$_2$-rhodamine. Astrocytes (>95% pure cultures) expressed GFAP (**A**), CRCX4 (**B**), and CCR3 (**C**). Neuronal cultures (>70% pure neurons) expressed MAP-2 (**E**, green). Less than 30% of cells in the neuronal cultures were identified as astrocytes by antibody to GFAP (**E**, red). CXCR4 antigens were expressed on neurons (**F**). To confirm these observations replicate neuronal cultures were double-stained with 12G5 (**G**, green cell bodies) and neurofilament (**G**, red dendritic processes). Replicate neuronal-enriched (**H**) and astrocyte (**D**) cell cultures were immunostained with mouse or rabbit IgG as the primary antibody. This served as a negative control for the immunocytochemical assays. The experiments illustrated are representative of three replicate assays. Original magnification, **A–D**, **F** and **H** (200×); **E** (400×); **G** (600×).

of brain macrophages and microglia are released during disease, which can affect neuronal function by stimulating glial immune responses. Alternatively, soluble and/or virion-associated glycoproteins (for example, gp120) may affect neuronal function directly by engaging specific receptors present on neural cells (Lipton, 1998; Zheng *et al*, 1999b). Although the secretory repertoire of HIV-1-infected MPs comprises a number of candidate neurotoxins (Esser *et al*, 1991; Gendelman *et al*, 1994), there remains some uncertainty as to which of the toxins is relevant to HIV-1 neuropathogenesis [Gendelman *et al*, 1994]. Nonetheless, an emerging body of evidence strongly suggests that chemokines and inflammatory cytokines, as well as viral glycoproteins, play a major role in HIV-1-induced neuronal injury (see below).

Chemokines and their receptors

Recently, chemotactic peptides have been found expressed in various cells within the CNS (Horuk *et al*, 1997; Lavi *et al*, 1997). These have been implicated in the pathogenesis of HAD (Hesselgesser and Horuk, 1999; Meucci *et al*, 1998; Kaul and Lipton, 1999) and other neurodegenerative disorders (Glabinski and Ransohoff, 1999). Chemokine receptors pivotal for HIV-1 infection in lymphocytes and macrophages (CCR3, CCR5, and CXCR4) are expressed on neural cells (microglia, astrocytes and/or neurons). Recently, Meucci *et al* (1998) found that chemokines, including SDF-1α, RANTES, and fractalkine affect neuronal Ca^{2+} signaling by using Fura-2-based Ca^{2+} imaging. This supported the theory that neurons possess a wide variety of chemokine receptors. In whole-cell patch clamp experiments, chemokines inhibited spontaneous excitatory postsynaptic currents (EPSC) at nanomolar concentrations. Voltage-dependent Ca^{2+} currents, in neurons, were reduced. RT–PCR revealed that CCR1, CCR4, CCR5, CCR9/10, CXCR2, CXCR4, CX3CR1 and fractalkine were expressed within neurons confirming the signaling studies.

There is compelling evidence that chemokines are upregulated in HIVE and HAD. Schmidtmayerova *et al* (1996) showed significant chemokine expression in macrophages/microglia during HIVE. In a subsequent study, monocyte chemotactic protein 1 (MCP-1) was detected in brains and cerebrospinal fluid (CSF) of patients with HAD (Conant *et al*, 1998). Sanders *et al* (1998) demonstrated MCP-1 expression in microglia/macrophages, astrocytes and endothelium in and around microglial nodules in HIV-1 encephalitis (HIVE) brain tissue. In order to extend these observations, work from our laboratories confirmed the production of chemokines from various neural cell types during HAD (Persidsky *et al*, 1999). In these works, we performed sets of cross-validating laboratory, animal model and human brain tissue analyses. First, we demonstrated that microglia and MDM secrete a variety of chemokines that affect monocyte migration through an artificial BBB system. This BBB system was composed of human brain endothelial cells and astrocytes seeded on the opposite sides of porous membrane in tissue culture inserts (Persidsky *et al*, 1997). Fully differentiated MDM or microglia (infected or uninfected) were placed in the bottom of wells with inserts covered by endothelium/astrocytes. Microglia-containing constructs (HIV-1-infected in particular) induced the most prominent monocyte migratory responses (2.5–3 times) than those recorded with MDM. The beta-chemokines (MCP-1, MIP-1α, MIP-1β, RANTES) were secreted up to 20 times more in microglia than in MDM (Persidsky *et al*, 1999). When supernatants derived from HIV-1 activated microglia or MDM were applied on primary human astrocytes, the cells produced significant quantities of beta-chemokines (most notably, MCP-1). These laboratory results were substantiated in an animal model system of HIVE. Here equal numbers of infected or uninfected MDM and microglia were inoculated into the basal ganglia of severe combined immunodeficiency disease (SCID) mice. The salient features of HIVE were reproduced in the mice and included neuropathologic changes, neurotoxin production and behavioral abnormalities (Persidsky *et al*, 1996). SCID mice which received infected microglia showed prominent neuropathologic changes (including astrogliosis and mouse microglia reaction) and elicited BBB transendothelial migration of mouse macrophages. Most importantly, the severity of HIVE (level of macrophage brain infiltration, formation of microglial nodules and astrogliosis) correlated with microglia activation. These findings demonstrated the importance of chemokines produced by glial cells in regulating monocyte migration into the brain. The expanded number of MDM recruited into the brain serve both as a reservoir for persistent viral infection and as a cell source of toxins that affect neuronal function and survival during progressive disease (Zheng *et al*, 1999a, b).

Cytokines

Pro-inflammatory cytokines, for example, interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α), are produced in abundance following MP activation. These have been implicated in HAD pathogenesis and are markers for advanced HIV-1 disease (Gendelman *et al*, 1997). Such cytokines, while mediating host responses to infection and injury, also have a myriad of nervous system activities (Haas and Schauenstein, 1997). These include alterations in the hypothalamic-pituitary-adrenal axis (Rothwell and Hopkins, 1995), in age-related cognitive function (Lynch, 1998); in neurodegenerative processes (Rothwell and Relton, 1993; Mrak *et al*, 1995; Rothwell, 1999); in regulating neuronal gene

expression, in glial proliferation (Mehler *et al*, 1993); and in synaptic transmission and plasticity (Patterson and Nawa, 1993). Cytokine influences on neuronal physiology appear interesting from the standpoint of immune regulation of neural function. IL-1β produced by glial cells inhibits long-term potentiation (LTP) in the CA1 and CA3 areas of the hippocampus (Bellinger *et al*, 1995; Katsuki *et al*, 1990) and the dentate gyrus (Cunningham *et al*, 1996). Inhibition of synaptic transmission mediated by the enhancement of inhibitory postsynaptic potential (IPSP) follows an excitatory postsynaptic potential (EPSP). This occurs as the membrane conductance, during IPSP, is increased by IL-1β (Zeise *et al*, 1992). IL-1β-induced inhibition of synaptic transmission occurs in a variety of neuronal subpopulations (Yu and Shinnick-Gallagher, 1994). Electrophysiological studies demonstrate that IL-1 enhances GABA-mediated increases in chloride permeability. Indeed, pharmacological studies showed an augmentation of GABA receptor function by IL-1 in cortical synaptic neural preparations (Miller *et al*, 1991). IL-1β may mediate synaptic inhibition through an adenosine-dependent mechanism (Luk *et al*, 1999). Moreover, the IL-1β-induced synaptic inhibition could attribute to inhibition of glutamate release (Murray *et al*, 1997) and/or the blockade of Ca^{2+} channels (Plate-Salaman and Ffrench-Mullen, 1992). IL-2 (Tancredi *et al*, 1990), IL-6 (Li *et al*, 1997) and TNF-α (Tancredi *et al*, 1992) also inhibit LTP, but at variable levels. In contrast, platelet-activating factor (PAF) enhances LTP (Wieraszko *et al*, 1993). Results from our laboratories confirm these published observations. In attempts to make this relevant for HIVE, we tested levels of such products found in HIV-1 infected and immune activated MDM culture fluids. In addition, we ascertained the relative role of several of these factors in altering neuronal electrophysiological functions. It was our thought that not all MP products are neurotoxic. Thus, the effects of IL-1β, PAF and TNF-α (at levels produced by activated MPs) on LTP activities in the CA1 region of the rat hippocampus were explored. IL-1β inhibited induction of LTP when applied by bath perfusion (Xiong *et al*, 1999b). The role of IL-1β in the HIV-1-infected and activated MP synaptic responses was revealed by IL-1β receptor antagonist (IL-1βra) (Figure 2). In contrast, TNF-α had no significant effects on LTP, though it inhibited synaptic function (Figure 3). Interestingly, we observed that PAF inhibited LTP at low concentrations (0.5–1.0 μM). However, at higher concentration (20 μM), it enhanced synaptic transmission (Figure 3). These results support the notion that HIV-1-infected, immune-activated MP products can affect neuronal function. Most importantly, the observations bolster the claim that some, but not all, of MP secretory factors can affect neuronal function during disease. The delineation of the composition of factors that cause neural injury may prove critical in providing future adjunctive therapies for HAD.

HIV-1 gp120 and tat

Neurotoxicity induced by HIV-1 gp120 has been demonstrated on cultured rat cortical (Muller *et al*, 1992) and hippocampal neurons (Meucci and Miller, 1996). HIV-1 gp120 is shed by virally infected cells and can affect neuronal function (directly or indirectly) through MP activation (Lipton, 1994). Inoculation of HIV-1 gp120 (both native and recombinant) into brains of adult rats leads to impaired learning and memory (Glowa *et al*, 1992). In neonatal rats, it results in dystrophic changes in pyramidal neurons of the cerebral cortex (Hill *et al*, 1993). Transgenic mice constitutively expressing glial fibrillary acidic protein-driven HIV-1 gp120 (from brain astrocytes) demonstrate neuronal and glial changes resembling abnormalities in HAD (Toggas *et al*, 1994). The severity of damage in these mice correlated positively with brain levels of HIV-1 gp120 mRNA, with the highest levels in the

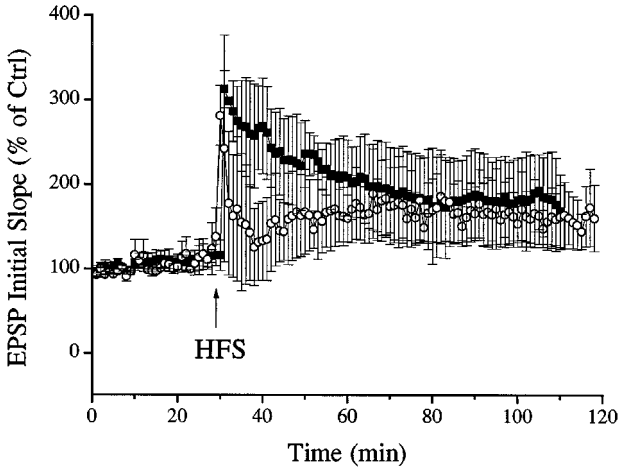

Figure 2 HIV-1-infected and activated MP culture fluids mediate alterations in synaptic potentiation which is partially blocked by the IL-1β receptor antagonist (IL-1βra). Monocytes were recovered from PBMCs of HIV-1-seronegative donors and purified by centrifugal elutriation. The cells, $<98\%$ monocytes, were cultured for 7 days as adherent monolayers then exposed to HIV-1_{ADA} at an MOI of 0.1 for an additional 7 days. Select cultures were treated 8 h with LPS (1 μg/ml). The crude culture fluids were applied to the brain slices by superfusion. Bath application of culture fluids collected from HIV-1-infected and activated MPs containing progeny virus inhibited both short-term potentiation and long-term potentiation in the CA1 region of rat hippocampal slices (open circle, n=4). However when IL-1βra was added to the culture fluids 5 min prior and during bath perfusion of HIV-1-infected and activated MP culture fluids, STP was at near control levels, indicating involvement of IL-1β in inhibition of synaptic potentiation (filled circle, n=3). Arrow indicates the time when high frequency stimulation (100Hz) was delivered. Data are presented as mean $\pm$ s.e.m.

neocortex and hippocampus. In an attempt to understand the neurophysiology of the transgenic animals, Siggins and coworkers (Krucker *et al*, 1998) studied excitatory post-synaptic potentials in the CA1 region of the hippocampus in these animals. Slices from the HIV-1 gp120 transgenic mice showed an increase in mean slopes of normalized population excitatory post-synaptic potentials; a larger paired-pulse facilitation after induction of LTP at 50 ms interpulse intervals; a marked increase in short-term potentiation (STP); and a significant reduction in the magnitude of LTP as compared to controls (nontransgenic animals). Importantly, HIV-1 gp120 was shown to elevate intracellular calcium through either voltage-gated calcium and/or *N*-methyl-D-aspartate (NMDA) receptor-operated channels.

Excessive influx of Ca^{2+} after the release of neurotoxic substances may underlie neuronal injury in HAD. Lipton and coworkers found that HIV-1 coat protein gp120 increased intracellular Ca^{2+} and acted synergistically with NMDA receptors to affect rat retinal ganglion cells (Lipton *et al*, 1991; Lipton, 1994). The HIV-1 gp120-increased intracellular Ca^{2+} was abrogated by lowering extracellular Ca^{2+} or by adding the dihydropyridine Ca^{2+} channel blocker, nimodipine. HIV-1 gp120-induced Ca^{2+} oscillations were also observed on single cultured rat hippocampal neurons and were inhibited by a Ca^{2+} channel antagonist nitrendipine, a NMDA receptor antagonist CGS19755 and a sodium channel blocker tetrodotoxin (Lo *et al*, 1992). Apart from increasing intracellular Ca^{2+} by activating voltage-gated Ca channels and NMDA receptor-operated channels, HIV-1 gp120 could elevate intracellular Ca^{2+} through mobilization of intracellular Ca^{2+} stores. Using patch-clamp recordings and Ca^{2+} imaging techniques, (Medina *et al*, 1999) examined

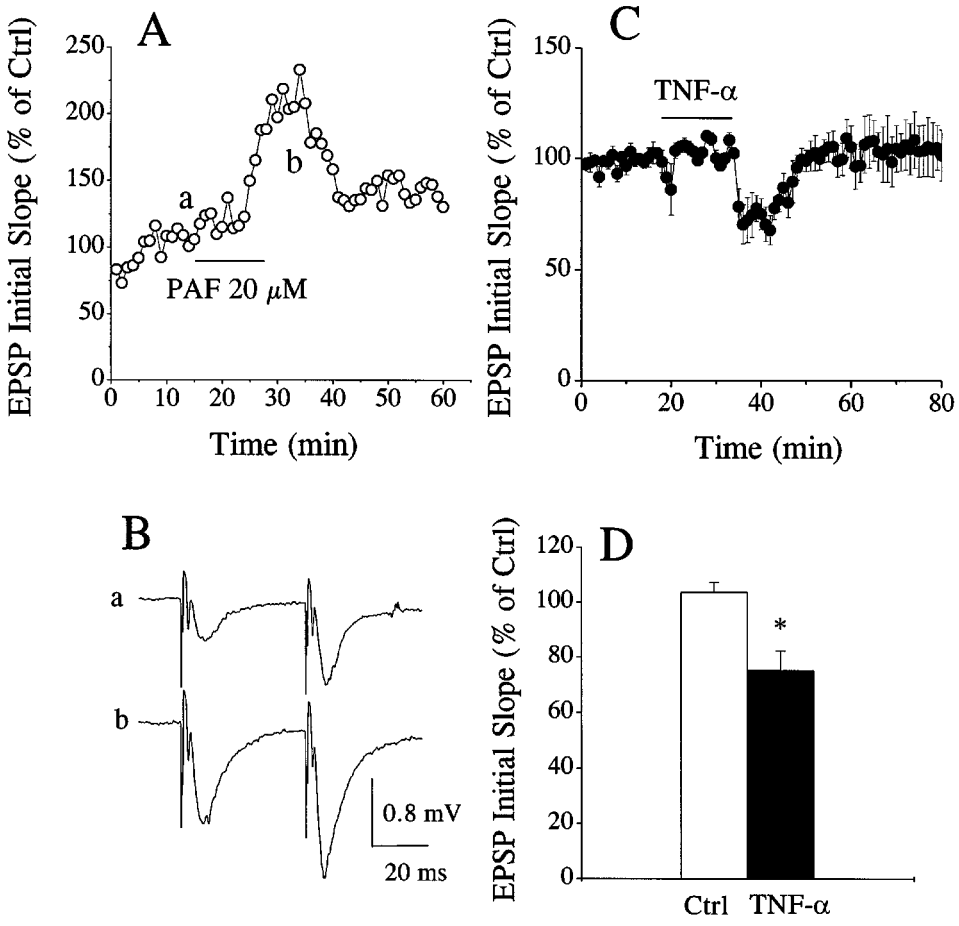

Figure 3 PAF and TNF-α alter synaptic transmission in the CA1 area of rat hippocampal slices. Bath application of PAF (20 μM) administered to the CA1 region of rat hippocampal slices enhanced synaptic transmission. This lasted for ~20 min (**A**). (**B**) shows individual EPSPs recorded from different time periods indicated by letters (a) and (b). Note the increase in field EPSPs following bath application of PAF. (**C**) illustrates the time course and magnitude of the inhibition of synaptic transmission induced by bath application of TNF-α (10 ng/ml). TNF-α produced a reversible inhibition of synaptic responses shortly after bath application (n=6). The bar graph in (**D**) shows that the synaptic responses, as indicated by initial slope of field EPSPs, was reduced to 75.3±6.9% (filled bar, n=6) after application of TNF-α. In comparison to synaptic responses prior to TNF-α application (white bar, n=6), the difference was statistically significant ($P<0.01$).

the contribution of intracellular stores of Ca^{2+} in the effects of HIV-1 gp120. They reported that in rat hippocampal neuronal cultures, HIV-1 gp120 mediated a dramatic and persistent increase in intracellular Ca^{2+}, which was prevented by drugs that either depleted or blocked Ca^{2+} release from intracellular stores. In contrast, the increase in intracellular Ca^{2+} was not affected by either NMDA receptor(s), a receptor implicated in a variety of neurodegenerative disorders (Seisjo, 1988; Young *et al*, 1988) an antagonist or Ca^{2+} channel blockers. However, the hypothesis that HIV-1 gp120 may cause NMDA receptor activation and lead to elevation of intracellular free Ca^{2+} and consequent neuronal dysfunction/injury is supported by findings that HIV-1 gp120-induced neurotoxicity or elevation of intracellular Ca^{2+} is prevented by NMDA receptor antagonists (Lo *et al*, 1992; Dreyer *et al*, 1990; Lipton 1991, 1992; Muller *et al*, 1992; Savio and Levi, 1993; Bennett *et al*, 1995; Wu *et al*, 1996). Whole-cell patch-clamp recording revealed that HIV-1 gp120 does not directly evoke an NMDA-like response or enhance glutamate/NMDA-activated currents in rat retinal ganglion cells (Lipton *et al*, 1991). The rise of intracellular Ca^{2+} is, therefore, not the consequence of direct action of HIV-1 gp120 on NMDA receptors and resultant opening of NMDA receptor-operated Ca^{2+} channels. HIV-1 gp120-stimulates MDM and microglia release NMDA receptor agonists, cystine and quinolinate, which open NMDA receptor-operated Ca^{2+} channels, leading to Ca^{2+} influx. HIV-1 gp120 may directly inhibit glutamate re-uptake into astrocytes (Lipton, 1998). In addition, production of arachidonic acid from HIV-1 gp120-activated macrophages/microglia (Wahl *et al*, 1989; Dreyer and Lipton, 1995; Genis *et al*, 1992) might contribute indirectly to the elevation of intracellular Ca^{2+} concentration, as arachidonic acid had previously been shown to inhibit glutamate re-uptake into astrocytes and synaptosomes (Barbour *et al*, 1989; Volterra *et al*, 1992). It is conceivable that the arachidonic acid released from HIV-1-infected and/or HIV-1 gp120-stimulated MP might affect the re-uptake of excitatory amino acids, such as glutamate. In fact, HIV-1 gp120 inhibits excitatory amino acid uptake in cultured astrocytes at picomolar concentrations (Dreyer and Lipton, 1995). Depletion of monocytic cells from purified neonatal rat astrocyte culture abolishes HIV-1 gp120-induced inhibition of excitatory amino acid uptake into astrocytes (Dreyer and Lipton, 1995). These data indicate the complexities of neuronal effects by HIV-1 gp120.

Limited studies have investigated the effects of HIV-1 gp120 on membrane ion currents. Whole-cell patch clamp studies on cultured rat retinal ganglion cells showed that HIV-1 gp120 did not directly evoke an NMDA-like response or enhance glutamate/NMDA-activated currents. However, synergistic effects of HIV-1 gp120- and NMDA receptor-mediated neurotoxicity were observed (Lipton *et al*, 1991). Similar effects of HIV-1 gp120 on membrane currents were described on cultured human embryonic cerebral and spinal neurons maintained in culture for 10–30 days. HIV-1 gp120 or gp160 (a precursor of HIV-1 gp120) had no effect on voltage-gated sodium, calcium and NMDA-induced currents after exposure to 250 pM HIV-1 gp120 or gp160. HIV-1 gp120 and gp160 potentiated the large rises in intracellular Ca^{2+} induced by NMDA. The potentiation of NMDA-induced Ca^{2+} responses required the presence of Ca^{2+} in the medium and was abolished by the NMDA receptor antagonist and voltage-gated Ca^{2+} channel blocker nifedipine (Lannuzel *et al*, 1995). Failure to affect membrane currents by HIV-1 gp120 has also been demonstrated on cultured rat hippocampal neurons (Medina *et al*, 1999). However, HIV-1 gp120 did inhibit NMDA-induced currents in *Xenopus* oocytes and attenuated NMDA-stimulated Ca^{2+} flux and cytotoxicity in cultured cerebellar granule cells (Sweetnam *et al*, 1993). In rat astrocytes, HIV-1 gp120 can activate large-conductance apamin-sensitive potassium channels (Bubien *et al*, 1995). The activation of these K^+ channels by HIV-1 gp120 is referable to cellular alkalinization subsequent to Na^+/H^+ exchange stimulation. HIV-1 gp120 fails to activate K^+ channels in the absence of external Na^+ or by amiloride, an inhibitor of Na^+/H^+ exchange. Subsequent loss of K^+ from astrocytes into the restricted extracellular space surrounding neurons lead to neuronal depolarization, activation of voltage-sensitive Ca^{2+} channels, and, eventually, cell death. Thus, abnormal activation of astrocyte K^+ channels by HIV-1 gp120 may contribute to the neuropathogenesis of HAD. HIV-1 gp160, decreased voltage-gated Kv1.3 current from Jurkat E6.1 T cells. The reduction of Kv1.3 current by gp160 was likely mediated by increased the PKC-dependent phosphorylation of Kv channel proteins (Dellis *et al*, 1999). Since Kv1.3 channel regulates resting membrane potential and controls cell volume, inhibition of Kv1.3 channel depolarizes the cell and increases the cell volume, which may cause cell death.

HIV-1 *tat* may also be implicated in neuronal demise during HAD. Sufficient quantities of Tat have been detected in cerebrospinal fluid of HIV-1-infected patients by Western blot analysis (Cheng *et al*, 1998). *In vitro* studies have shown that Tat is released extracellularly by HIV-1 infected lymphocytes (Ensoli *et al*, 1993). Tat has been shown to interfere with electrophysiological properties of neurons. Sabatier *et al* (1991) reported that Tat induced depolarization in cockroach giant interneurons, accompanied by a decrease of membrane input resistance. The depolarization was not blocked by a voltage-dependent Na^+ channel blocker (tetrodotoxin), or a Ca^{2+} channel blocker (Cd^{2+}). This indicated that Tat acts on non-selective ion

channels. In another study, very low concentrations of Tat depolarized rat CA1 hippocampal and human cortical neurons (Magnuson *et al*, 1995). Tat-induced neuronal cell depolarization was conformational dependent. The active site of this protein resides within the first exon of Tat between residues 31–61 (Nath *et al*, 1996). Tat 31–61, but not 48–65, increased intracellular Ca^{2+} levels and cytotoxicity in cultured human fetal brain cells (Nath *et al*, 1996). Tat-induced depolarization, elevations of intracellular Ca^{2+} and neuronal death were prevented by non-NMDA receptor antagonists (Magnuson *et al*, 1995; Nath *et al*, 1996). The specific NMDA receptor antagonist D-2-amino-5-phosphonovalerate (APV) was ineffective, indicating that the Tat-induced responses did not involve NMDA receptors. However, Tat-induced neurotoxicity was reduced by NMDA receptor antagonists (Starling *et al*, 1999; Srijbos *et al*, 1995). Since Tat can directly excite neurons in the absence of accessory cells, investigators have speculated that Tat may be an important pathogenic agent in HAD (Cheng *et al*, 1998).

HIV-1-infected MP secretions and neuronal physiology

The mechanism(s) for MP-induced neuronal dysfunction has recently been uncovered through electrophysiological tests. Such techniques provide quantitative measures that help pinpoint how MPs mediate neuronal dysfunction. In this regard, our laboratories have begun testing the influence of macrophage secretory factors on neuronal physiology. Our works showed that virus-infected and immune-activated MPs inhibit excitatory synaptic transmission in hippocampal CA1 neurons (Xiong *et al*, 1999a). Interestingly, the inhibition of synaptic transmission by MP fluids appears potent and reversible. The site of action for the changes in synaptic transmission was localized to pre-synaptic terminals by paired-pulse facilitation tests. To further explore how HIV-1-infected and immune-activated MPs alter neuronal function, we analyzed neural induction of LTP. LTP is an indicator of learning and memory and an electrophysiological manifestation of long-lasting increases in synaptic strength. The electrophysiological experiments, performed in the CA1 region of the rat hippocampus (Xiong *et al*, 1999b), demonstrated that LTP was inhibited by HIV-1-infected and immune-activated culture fluids. Although progeny virus enhanced this LTP effect, when HIV-1 was applied alone, it had limited action on synaptic activities. Importantly, HIV-1 infection or immune activation of MPs alone produced only modest LTP changes. The results highlight the importance of both viral infection and MP immune activation as inducers of changes in synaptic plasticity. As neuronal and synaptic loss can occur in the hippocampus, as well as in other brain regions of AIDS patients (Everall *et al*, 1991,1994; Ketzler *et al*, 1990; Masliah *et al*, 1992; Reyes *et al*, 1994), these results may have biological relevance for HAD.

The mechanisms for the alterations in synaptic function by MP products may be gleaned, in part, from previously published works. These demonstrate that the induction of LTP, at Schaffer-collaterals to CA1 synapses, in the hippocampus is dependent on the activation of post-synaptic NMDA receptors (Collingridge *et al*, 1983). This involves a rise in intracellular Ca^{2+} in post-synaptic neurons. LTP can be blocked with NMDA receptor antagonists (Coan *et al*, 1987) or by injection of Ca^{2+} chelators in postsynaptic neurons (Lynch *et al*, 1983; Malenka *et al*, 1988). However, a quick rise in intracellular Ca^{2+} is unlikely to be sufficient for LTP stabilization, as application of NMDA onto CA1 neurons induces STP, but not LTP (Kauer *et al*, 1998). Activation of metabotropic glutamate receptors and its resultant Ca^{2+} mobilization from intracellular stores contribute to LTP stabilization (Bashir *et al*, 1993). In works performed in our laboratories, HIV-1-infected, immune-activated MP culture fluids inhibited both STP and LTP (Xiong *et al*, 1999b). We assumed that both cellular and viral factors from MP supernatants could interact with NMDA and metabotropic glutamate receptors to mediate the LTP suppression observed in the CA1 area. This may be relevant for HAD as interactions between the neuronal NMDA and metabotropic glutamate receptors may retard disease progression.

In summary, it is likely that a host of cellular and viral products produced by infected and immune competent MPs effect neuronal demise and thus influence the cognitive symptoms of HAD. The use of integrative studies of MP function coupled with the most sensitive means for analyzing neuronal injury should prove invaluable in deciphering the biologically active composition of MP secretions that effect HIV-1-associated neuronal demise.

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