Journal of NeuroVirology (1999) 5, 519–528

http://www.jneurovirol.com

Soluble HIV-1 infected macrophage secretory products mediate blockade of long-term potentiation: a mechanism for cognitive dysfunction in HIV-1-associated dementia

Huangui Xiong[1,2], Yong Chun Zeng[1,2], Jialin Zheng[1,2], Michael Thylin[1,2] and Howard E Gendelman*[,1,2]

[1]*The Center for Neurovirology and Neurodegenerative Disorders, University of Nebraska Medical Center, Omaha, Nebraska, NE 68198-5215, USA* [2]*Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska, NE 68198-5215, USA*

It is generally accepted that viral and cellular products from immune competent mononuclear phagocytes (MP) (brain macrophages and microglia) underlie the neuropathogenesis of HIV-1-associated dementia (HAD). What remains unanswered, however, is the composition of and mechanisms for such MP-induced neurological dysfunctions. In attempts to address these issues culture fluids from HIV-1_{ADA}-infected monocyte-derived macrophages (MDMs) (depleted or enriched with progeny virus) were placed onto the CA1 area of rat hippocampal brain slices (the site of mammalian learning and memory) and neuronal long-term potentiation (LTP) assayed. LTP was induced by high frequency stimulation (HFS). Lipopolysaccharide (LPS) served as a surrogate macrophage activator. Synaptic strength was assayed by the initial slope of evoked field excitatory postsynaptic potentials (EPSPs). Synaptic potentiation following HFS was observed in slices incubated with uninfected (control) MDM culture fluids. The magnitude of the LTP response was 150.2 $\pm$ 21.10% compared to basal levels (*n*=6). Synaptic strength was enhanced in virus-infected (135.7 $\pm$ 28.9%, *n*=8) and LPS-activated MDM (123.3 $\pm$ 5.1%, *n*=7) but at lower levels than controls. The lowest levels of LTP were in brain slices incubated with virus-infected and LPS-activated MDM fluids at (109.5 $\pm$ 9.9% *n*=12). Interestingly, bath application of progeny HIV-1 virions showed minimal LTP effects. Virus-infected, LPS-activated MDM fluids, with progeny virus, reduced synaptic strength but were not statistically different than replicate culture fluids depleted of virus. In contrast, IL-1β and quinolinic acid, significantly diminished synaptic strength. These results, taken together, suggest that soluble HIV-1-infected MDM secretory products, but not virus *per se*, significantly affect LTP. This electrophysiological system, which monitors neuronal function following cell exposure to HIV-1 infected materials could provide a novel testing ground for therapeutics designed to protect brain function in HAD.

Keywords: human immunodeficiency virus; monocyte-derived macrophages; long-term potentiation; hippocampal slices; neurotoxicity; HIV-1-associated dementia

Introduction

HIV-1-associated dementia (HAD) is a complication of progressive viral infection usually occurring during significant immunosuppression (Glass and Johnson, 1996; Lipton and Gendelman, 1995; Spencer and Price, 1992). Neurological deficits consist of a spectrum of cognitive, motor and/or behavior impairments which may lead to death in the absence of opportunistic infections. Common pathological features, associated with cognitive dysfunction include monocyte-macrophage brain infiltration, formation of multinucleated giant cells,

*Correspondence: HE Gendelman, Center for Neurovirology and Neurodegenerative Disorders, University of Nebraska Medical Center, 985215 Nebraska Medical Center, Omaha, NE 68198-5215, USA
Received 1 December 1998; revised 13 May 1999; accepted 20 May 1999

astrogliosis, microglial nodules, myelin pallor, disruption of the blood-brain barrier and neuronal drop-out (Michaels, 1988; Price *et al*, 1988). Interestingly, HIV-1 does not infect neurons. Disease is likely caused through macrophages/microglia immune products disrupting neural homeo-#stasis (Epstein and Gendelman, 1993; Everall *et al*, 1991; Ketzler *et al*, 1990; Masliah *et al*, 1992; Wiley *et al*, 1991). It is now well established that the major target cell for virus is brain macrophages and microglia. Surprisingly, the levels of HIV-1 in brain produced by productive macrophage infection does not always predict cognitive decline (Glass *et al*, 1995). Indeed, virus appears necessary, but not sufficient, to cause HAD. An observed discordance between neuropathology and neurological deficits, taken together with the ability of virus-infected macrophages to elicit neuronal loss has led to an hypothesis that HAD is a metabolic encephalopathy (Zheng and Gendelman, 1997). In support of this is the findings that HIV-1-infected macrophages secrete a variety of neurotoxins including, but not limited to, pro-inflammatory cytokines (for example, TNF-α, IL-1β), quinolinic acid (QUIN), arachidonic acid and its metabolites, platelet activating factor (PAF), NTox and nitric oxide.

Among these neurotoxic substances pro-inflammatory cytokines appear to be involved in many neuropathological and neuroendocrine processes. This may occur through cytokine activation of the hypothalamic-pituitary-adrenal axis (Rothwell and Hopkins, 1995); by modulation of neuronal activity within the limbic system (Haas and Schauenstein, 1997); by age-related impairments in cognitive function (Lynch, 1998); by effecting acute and chronic neurodegenerative disorders (Rothwell and Relton, 1993, Mrak *et al*, 1995); through regulation of neuronal gene expression and cell proliferation (Mehler *et al*, 1993); and by cytokine's influence on synaptic plasticity (Patterson and Nawa, 1993). Importantly, cytokines have been reported to inhibit long-term potentiation (LTP) in rodent hippocampal cells (Bellinger *et al*, 1993; Cunningham *et al*, 1996; Katsuki *et al*, 1990; Li *et al*, 1997; Tancredi *et al*, 1990, 1992). It is quite probable, therefore, that macrophage secretory products, which include cytokines, may play a pivotal role in the neuropathogenesis of HAD by altering the induction of neuronal function which includes LTP.

Mammalian-based memory function involves modifications of synaptic connections between neurons. LTP is a ubiquitous form of use-dependent synaptic plasticity that is present at most excitatory synapses in the central nervous system (CNS). Importantly, LTP is thought to be an indicator of learning and memory function and an electrophysiological manifestation of a long-lasting increase in the strength of stimulated synapses. LTP was first described in the hippocampus (Bliss and Lømo, 1973), and it has now been observed at numerous synapses in both the invertebrate and vertebrate CNS. It is induced by brief repetitive high frequency stimulation (HFS) and may last for hours or days. The prominence of LTP is in structures involved in learning and memory, such as hippocampus and amygdala. This has led to the idea that LTP may underlie mnemonic functions. Indeed, lesions of the hippocampus produce severe amnesia in mammals (Squire, 1992). Because LTP shares certain properties with associative learning it has been intensively studied as a primary experimental model for memory functions in the mammalian CNS (Bliss and Collingridge, 1993). Moreover, it has been used to investigate effects on functions of neuronal networks, such investigations might not be assessable during studies of single cell function.

Can LTP be used as an experimental system to study the neuropathogenic processes of HAD? Several lines of evidence suggest that it can. First, recent reports have shown neuronal killing following neural cell exposure to virus-infected macrophage secretions (Xiong *et al*, 1999; Zheng *et al*, 1999). Second, a physiologic assay to dynamically monitor the neurotoxic effects of virus-infected macrophage products has remained elusive. Since a primary manifestation of HAD consists of deficits in learning and memory electrophysiological techniques to investigate the effects of macrophage secretory products on LTP appears both reasonable and well founded. In this regard, our current works utilized the CA1 area of rat hippocampus as a neuronal target and lipopolysaccharide (LPS) as the inducer of macrophage secretory products to study LTP.

Enhancement of synaptic strength, induced by HFS was assayed by the initial slope of field excitatory postsynaptic potentials (EPSPs). Cell culture fluids prepared from HIV-1_{ADA}-infected monocyte-derived macrophages (MDM) with/without LPS activation were applied to hippocampal brain slices. A prolonged enhancement of synaptic strength was observed following hippocampal incubation of control MDM fluids. Increased synaptic strength, but at reduced levels, was observed by fluids from virus-infected and LPS-activated MDM. This reduction of synaptic potentiation was not significantly affected by addition of progeny virus in the culture fluids. Taken together, these data highlight the importance of both viral infection and macrophage immune activation in altering neuronal synaptic plasticity.

Results

Experiments were performed to explore one potential mechanism underlying neuronal dysfunction in HAD namely the effects that immune activated and virus-infected macrophage secretory products have

on neuronal LTP. Fluids were prepared from control (uninfected), HIV-1-infected, LPS-activated/uninfected and LPS-activated/HIV-1-infected MDMs. Replicate samples were obtained from virion-depleted MDM fluids. At the time of maximal viral production (7 days after HIV-1_{ADA} inoculation at a multiplicity of infection (MOI) of 0.1 culture fluids were recovered. The maximal RT activity in the infected MDM fluids was 17.82 ± 0.82 (c.p.m./ml $\times 10^5$ cells); a level 87-fold higher than in replicate uninfected MDM (Xiong *et al*, 1999). MDM (virus-infected and control uninfected) were treated with LPS at 1 μg/ml after 7 days of virus or mock infection. The maximal TNF-α level at peak viral replication following LPS treatment was 0.3 ng/10^5 cells.

Effects of MDM products on LTP

To study the effects of MDM secretory products on the induction of LTP, virus-depleted MDM fluids were placed onto the hippocampal slices in an oxygenated (95% O_2 and 5% CO_2) holding chamber for 1 h before electrophysiological recordings. Control experiments were done on both untreated and basal media-treated slices. Since there was no significant difference between untreated and basal media-treated slices in LTP magnitude they were counted together as assay controls. In control slices, HFS induced a significant enhancement of synaptic strength (LTP) lasting for hours. An example of the HFS-induced LTP in untreated hippocampal slices is shown in Figure 1A. Individual sample EPSPs recorded before (*a*) and after (*b*) HFS are illustrated in Figure 1B. The magnitude of LTP recorded in untreated slices, when measured 50–60 min after HFS, was 148.6 ± 9.6% of basal levels. The difference in synaptic strength recorded before and after HFS was statistically significant (Figure 1C, mean $\pm$ s.e.m, n=18, $P<0.05$). In contrast, after incubation of brain slices with HIV-1-infected and LPS-activated MDM fluids, the synaptic strength following HFS was significantly reduced. The average synaptic strength was 109.5 ± 9.9% of basal levels (Figure 2, n=12). In comparison to the LTP recorded in controls, the differences were statistically significant ($P<0.05$). Thus, the induction of LTP was blocked by HIV-1-infected and LPS-activated MDM. In addition to alterations in LTP induction, HIV-1-infected and LPS-activated MDM fluids also significantly inhibited short-term potentiation (STP), but had no apparent effects on post-tetanic potentiation (PTP), as shown in Figure 3. The magnitude of PTP and STP in control slices was 183.8 ± 16.9 and 164.9 ± 11.5% of basal levels, respectively. While the slices were incubated with HIV-1 infected and LPS-activated MDM fluids, the magnitude of PTP and STP was reduced to 147.4 ± 15.9, and 121.4 ± 11.2% of basal levels, respectively. Importantly, the LTP magnitude was 135.7 ± 28.9% of basal levels (n=8) when slices were treated with

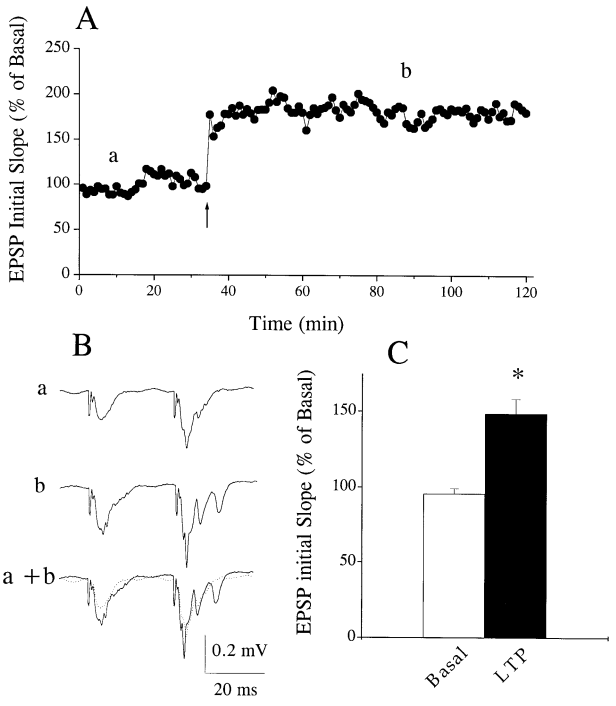

Figure 1 Time course and magnitude of LTP in the Schaffer-collateral to CA1 synapses recorded from rat hippocampal slices (**A**). The graph plots the initial slope of falling phase of the evoked EPSPs recorded from CA1 dendrite field (stratum radium) in response to constant test stimuli, for 30 min before and 90 min following high frequency stimulation (HFS, 100 Hz, 500 ms $\times$ 2). The HFS was delivered at the time indicated by the arrow. Each point in this graph is an average of six consecutive sweeps. Representative traces before and after the induction of LTP, taken at the times indicated by letters *a* and *b* respectively, are illustrated in (**B**). Note the increase in slope of evoked EPSPs following HFS (HFS induced LTP). An average of 18 slices tested is shown in (**C**). The LTP magnitude was 148.6 ± 9.6% (n=18) of basal level when measured at 55–60 min after HFS. The difference is statistically significant ($P<0.05$) in comparison with the magnitude before HFS. *Denotes statistical significance between basal and LTP EPSP levels.

culture fluids from HIV-1-infected MDM (Table 1), and the LTP height was 123.3 ± 5.1% of basal levels when slices were incubated with uninfected LPS-activated MDM (Table 1, n=7). In comparison to the LTP height in control slices, there was no significant difference ($P>0.05$). Neither PTPs nor STPs in these cases were significantly affected (Figure 3). One-way ANOVA indicated that only HIV-1-infected and LPS-activated MDM fluids significantly inhibited STP and LTP. A suppressive effect of HIV-1-infected and LPS-activated MDM culture fluids on the maintenance of LTP was also demonstrated. Bath application of HIV-1-infected, LPS-activated MDM culture fluids reversibly blocked LTP (n=2, data not shown). In contrast, MDM culture fluids obtained from uninfected cells had no effect on LTP. The magnitude of LTP was 150.2 ± 21.1% of

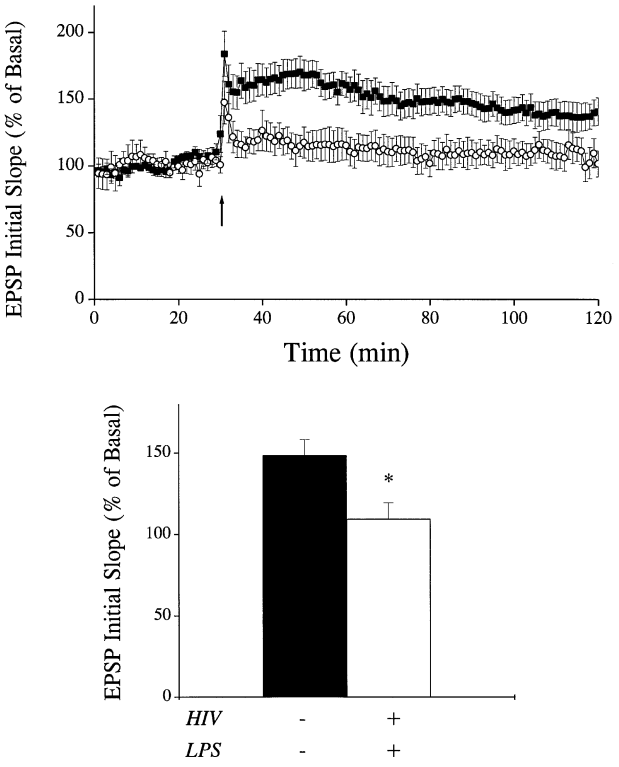

Figure 2 Incubation of hippocampal slices with culture fluids from LPS-activated, HIV-1-infected MDMs blocked LTP induced by HFS in CA1 area (n=12). Monocytes were recovered from PBMCs of HIV-seronegative donors and purified by centrifugal elutriation. The cell, >98% monocytes, were cultured for 7 days 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 (depleted of progeny viruses) were applied to the brain slices for 60 and 30 min prior to the start of recordings, respectively. Upper panel illustrates the time course and magnitude of LTP after incubation of slices with the culture fluids collected from HIV-1-infected, LPS-activated MDMs (open circle) and the time course and magnitude of LTP from normal slices (solid square). Each point (mean $\pm$ s.e.m.) in the graphs represents an average of 12 (open circle) and 18 (solid square) independent experiments. Note that LTP was affected by LPS-activated, HIV-1-infected MDM fluids. Arrow indicates the time when HFS was delivered. The bar graph in the lower panel shows that the averaged initial slope measured at 55–60 min after HFS (LTP) was 109.5 $\pm$ 9.9% of basal level in the slices treated with HIV-1-infected, LPS-activated MDM culture fluids (open bar). In comparison with the averaged initial slope from control slices (black bar), the difference is statistically significant suggesting a blockade of LTP by HIV-1-infected, LPS-activated MDM culture fluids. *Denotes statistical significance between control (black bar) and HIV-1-infected LPS activated MDM fluids (open bar), P<0.05.

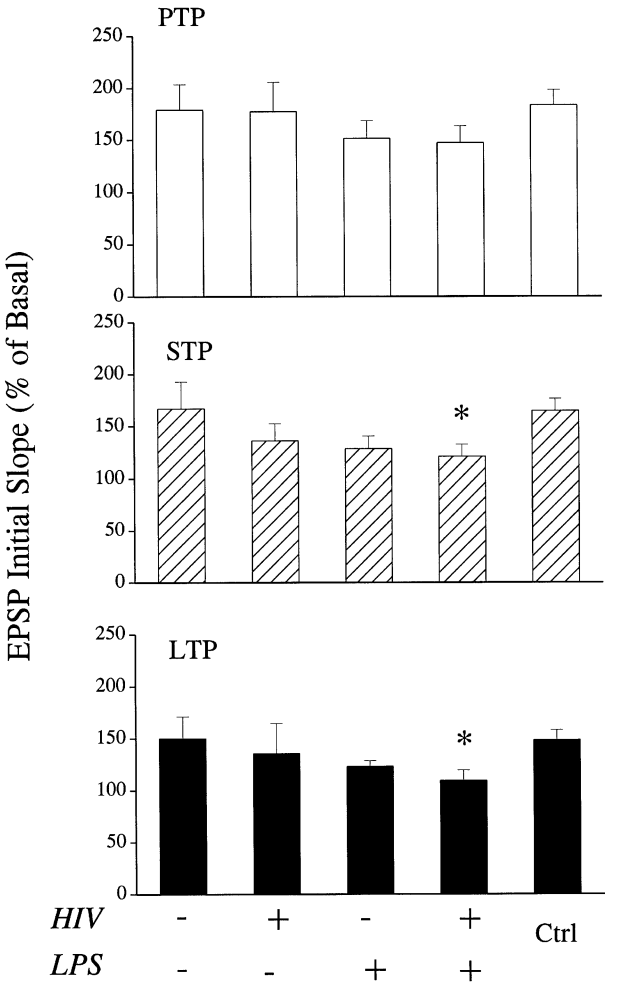

Figure 3 Summary of the effects of MDM culture fluids on neuronal PTP (top), STP (middle) and LTP (bottom). PTP, STP and LTP were expressed as an average of percent change in field EPSP slope at 1, 10–15, and 55–60 min after HFS. Data are expressed as mean $\pm$ s.e.m. Note that HIV-1-infected, LPS-activated MDM suppressed both STP and LTP. *Denotes statistical significance between control group and HIV-1-infected LPS activated MDM fluids, P<0.05, compared with control group.

basal levels (n=5, Table 1) when the slices were treated with culture fluids from uninfected (control) MDM. This enhancement of synaptic strength was statistically significant (P<0.05) in comparison to basal levels. This indicates that the culture fluids from uninfected MDMs without LPS-activation had no effect on the blockade of LTP.

Effects of MDM culture fluids containing progeny virus on LTP

To compare the effects of MDM culture fluids with/without progeny virus (HIV-1_{ADA}), we conducted experiments in a modified BL-3 containment laboratory. Here we tested MDM culture fluids containing progeny virus on LTP. Incubation of the slices with virus-containing MDM culture fluids with LPS-activation completely blocked STP and LTP induction (Figure 4). The PTP was also attenuated. The synaptic strength was below basal levels. The synaptic strength for PTP, STP and LTP was 112.2 $\pm$ 21.8, 93.6 $\pm$ 12.5 and 82.1 $\pm$ 13.9% of basal levels, respectively (n=3). The effect of virus-containing MDM culture fluids without LPS-activa-

Table 1 MDM culture fluids affect neuronal LTP

HIV-1	–	–	+	+	*ctrl*
LPS	–	+	–	+	*ctrl*
LTP (% of basal)	150.2 ±21.1	123.3 ±5.1	135.7 ±28.9	109.5 ±9.9	148.6 ±9.6
Slices tested	6	7	8	12	18
P values:	0.94	0.12	0.59	0.01	–

MDM fluids were placed onto hippocampal brain slices and LTP recorded thereafter. The initial slope of evoked EPSPs was measured as an indicator of LTP following HFS. Unpaired two-tailed *t*-test, compared with control (ctrl) group.

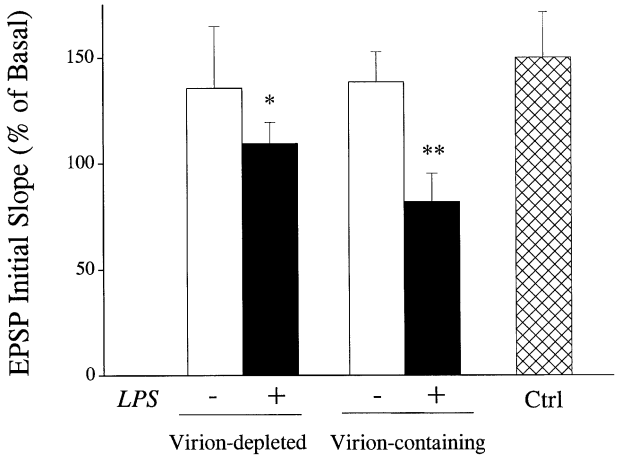

Figure 4 Effects of HIV-1-infected MDM culture fluids depleted or containing progeny virions on neuronal LTP. Incubation of hippocampal brain slices with HIV-1-infected, LPS-activated, virion-depleted MDM culture fluids showed a significant inhibition of LTP as compared to controls ($P<0.05$). However, no statistical differences were observed between LPS-stimulated and control (unstimulated) MDM. In contrast, incubation of slices with virion-containing, HIV-1-infected, LPS-activated MDM culture fluids showed a marked blockade of LTP. The enhancement of LTP inhibition by progeny HIV-1 virions was not statistically significant when compared to replicate controls depleted of virus ($P>0.05$). Importantly, HIV-1-infected, virion-containing MDM fluids showed a modest LTP induction. $^{*}P<0.05$, $^{**}P<0.01$.

tion on LTP was also examined. The LTP magnitude was 138.6 ± 14.2% of basal levels (Figure 4, n=3). The differences were statistically significant in comparison with the LTP magnitude from those slices incubated with virus-containing MDM culture fluids with LPS-activation ($P<0.05$). However, the differences were not significantly different from those slices incubated with virion-free HIV-1-infected MDM culture fluids without LPS-activation ($P>0.05$). Incubation of slices with virions (amount of virus used was based on the RT activity detected on virus-containing MDM culture fluids) alone had no significant effect on LTP. The magnitude of LTP was 141.1 ± 16.4% of basal level (n=4), indicating that virus *per se* was not crucial in the inhibition of LTP induction.

While incubation is a widely used means to test the effect of any viral or cellular product in tissue slices, we observed a 'wash-out' effect in some slices. To overcome this problem, we used bath superfusion in our subsequent experiments. Brief bath perfusion of virion-containing MDM culture fluids (1 : 6 dilution, 3 – 6 min) had same inhibitory effect on both STP and LTP (n=4, data not shown). In contrast, bath perfusion of virus (HIV-1_{ADA}) alone had no significant reduction on LTP (n=2, data not shown). This is consistent with the data obtained from our incubation experiments.

Effects of individual MDM secretory products on LTP

It is well known that HIV-infection and immune activation of brain macrophages and microglia cause release of neurotoxins. Several of these neurotoxic secretory factors have been identified by our laboratory and those of others (Gallo *et al*, 1989; Nottet *et al*, 1996). In attempts to uncover how individual macrophages secretions could affect LTP we tested two of the most widely researched cytokines (TNF-α, IL-1β) and quinolinic acid (QUIN) for their ability to alter LTP. The concentrations of these putative neurotoxins were administered at the exact concentrations observed in virus-infected and activated MDM fluids. This was done to discern whether the blockade of LTP was mediated by the actions of such factors contained in MDM culture fluids. TNF-α, IL-1β and/or QUIN were administered either separately or in combination. When applied by bath, TNF-α had no effect on LTP at concentrations 5 – 10 ng/ml, the concentrations produced by HIV-1-infected, LPS-activated macrophages (Persidsky, unpublished observations). The average LTP height was 153.6 ± 35.1% of basal levels (Figure 5, n=3). In contrast, bath application of IL-1β (5 – 10 ng/ml) inhibited LTP. The average LTP height was 102.9 ± 11.9% of basal levels (Figure 5, n=3). This reduction in LTP was statistically significant ($P<0.05$) when compared to control slices. In addition to cytokines, HIV-1-infected, immune-activated brain macrophage and microglia produce other neurotoxic factors, such as QUIN. As a weak NMDA receptor agonist, QUIN is a neurotoxin but only at high levels (Kim and Choi, 1987). It has been demonstrated that HIV-infected and LPS-activated monocytes produce high levels of QUIN (Nottet *et al*, 1996). To examine the effect of QUIN on LTP, we infused the slices with QUIN at concentrations of 50 nM (n=3), 100 nM (n=3), 200 nM (n=2) and 600 nM (n=3). QUIN inhibited LTP in concentration dependent manner (data not shown). At a concentration of 200 nM, levels achieved in HIV-1-infected LPS-activated MDM (Nottet *et al*, 1996), the magnitude of LTP was

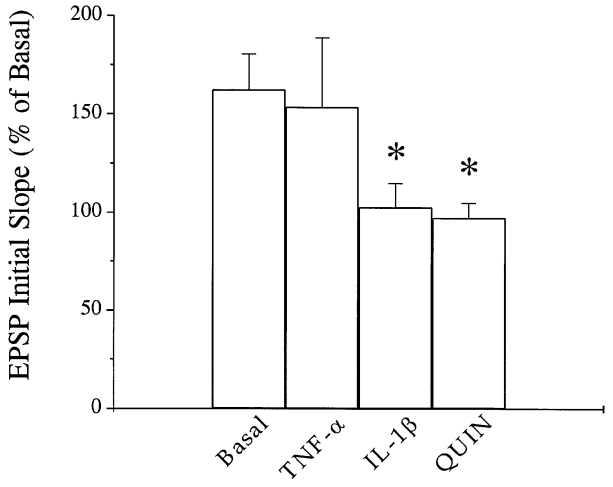

Figure 5 Effects of macrophage secretory products on LTP blockade. TNF-α, IL-1β and QUIN were tested for their abilities to affect LTP at concentrations found in culture fluids of LPS-activated MDM. TNF-α administered to hippocampal slices at 5–10 ng/ml had no effect on LTP. In contrast, IL-1β inhibited LTP in a concentration dependent manner. At 200 nM, IL-1β inhibited synaptic strength when measured 55–60 min after HFS. The height of stimulation was $102.9 \pm 11.9\%$ of basal levels. *This reduction in synaptic strength was statistically significant as compared to control ($P<0.05$). *QUIN administered to the brain slices at 200 ng exhibited the strongest blockade of LTP.

$97.5 \pm 7.5\%$ of basal levels (Figure 5). Combined bath application of QUIN (100 nM) with TNF-α (5 ng/ml) attenuated the effects of QUIN on LTP (n=2, data not shown). Synergistic effects were seen in 1/3 slices tested when QUIN (50 ng/ml) was administered with IL-1β (5 ng/ml) (data not shown).

Discussion

The present study demonstrates that the secretory factors produced by HIV-1_{ADA}-infected and immune-activated MDMs inhibit the induction of LTP in the CA1 region of rat hippocampus. This inhibition of LTP induction was enhanced by HIV-1_{ADA}. To perform these works we employed BL-3 electrophysiological techniques to investigate the effects of MDM culture fluids with/without progeny virus on LTP.

Since neuronal injury or loss has been reported in the hippocampus of AIDS patients (Reyes *et al*, 1994), it is highly relevant to investigate the effects of viral and cellular factors from HIV-1-infected MDM on LTP in the hippocampus. The CA1 area of rat hippocampus served as the neuronal target and LPS as the activator of the macrophage secretory function. Induction of LTP induced by HFS was measured by electrophysiological recording of electrically evoked field EPSPs. MDM culture fluids prepared from HIV-1_{ADA}-infected MDM with or without progeny virus and with or without LPS activation were applied onto brain slices. A prolonged potentiation of synaptic transmission was observed following hippocampal incubation of MDM culture media. However, the synaptic potentiation was significantly reduced following incubation of the brain slices by fluids from virus-infected and LPS-activated MDMs (Figure 2). This inhibitory effect was enhanced with the existence of progeny virus in the culture fluids. Other conditioned culture fluids had no significant inhibitory actions on synaptic potentiation. These results clearly demonstrated the blockade of LTP by secretory products from virus-infected and LPS-activated MDMs. Similar inhibitory effects were observed when bath perfusion of hippocampal slice occurred with culture fluids from virus-infected and LPS-activated MDMs. HIV-1_{ADA} virus *per se* had no significant actions in the blockade of LTP induction when applied by bath, but showed an enhancement on the effect the culture fluids on the inhibition of LTP when the virus was present in the culture fluids. Such data highlight the importance of both HIV-1-infection and immune activation for induction of alterations in synaptic plasticity.

A major question underlying the neuropathogenesis of HAD is how neuronal dysfunction can occur when viral replication is nearly exclusively within brain macrophages and microglia. However, the existence of virus-infected brain macrophages alone is necessary but not sufficient to induce disease. A paradox does exist between the small numbers of productively infected brain macrophages and severity of clinical and pathological deficits. This suggests that some sort of cellular amplification or immune activation is necessary for the generation of cellular and viral toxins leading to neuronal dysfunction (Gendelman *et al*, 1997). Our electrophysiological experimental results fully support this hypothesis. These data indicate that viral infection and immune activation produce high levels of macrophage toxins as reflected by significant suppression of LTP by HIV-1-infected and activated MDM culture fluids. Our data also indicate that virus *per se* is insufficient in the inhibition of LTP induction.

An emerging body of evidence strongly supports the idea that HIV-1-infected brain macrophages secrete neurotoxins (Bukrinsky *et al*, 1995; Gelbard *et al*, 1994; Genis *et al*, 1992; Lipton and Gendelman, 1995; Nottet *et al*, 1995), including but not limited to HIV-1 protein (gp120, tat, nef and others) and cell encoded toxins including cytokines (TNF-α, IL-1β and others), eicosanoids, nitric oxide, glutamate, QUIN, arachidonic acid and its metabolites. It is hypothesized that the secretory neurotoxins mediate neuronal dysfunction or injury (Gendelman *et al*, 1997). It remains to be determined at present which cytokine(s) or other factor(s) within the culture media is (are) the

mediator(s) responsible for the blockade of LTP observed in this study. It was reported that IL-1β inhibits LTP in the areas of CA1 (Bellinger *et al*, 1993) and dentate gyrus (Cunningham *et al*, 1996) of rat hippocampus and in the CA3 area of mouse hippocampus (Katsuki *et al*, 1990). IL-2 (Tancredi *et al*, 1990) and IL-6 (Li *et al*, 1997) have been reported to suppress LTP in the CA1 area of rat hippocampal slices. The induction of LTP was also inhibited by TNF-α in rat hippocampus (Tancredi *et al*, 1992). In contrast to cytokines IL-1β and TNF-α, platelet activating factor (PAF) enhanced LTP (Wieraszko *et al*, 1993). In an attempt to dissect out which secretory product(s) is(are) responsible for the inhibition of LTP induction observed in our experiments, we tested the effects of IL-1β and TNF-α on LTP. We found that IL-1β had inhibitory effects on the induction of LTP, while TNF-α had no apparent effect. Based on our present findings and the aforementioned observations, we could postulate that cytokines secreted from HIV-1-infected, immune-activated MDMs are involved in the inhibition of LTP induction. Another individual factor we tested was QUIN. QUIN is produced at tenfold higher levels from HIV-infected, LPS-activated monocytes than that produced from HIV-infected monocytes. It is also thought to underlie cognitive and motor dysfunction for a variety of neurological disorders (Nottet *et al*, 1996). Specifically, in HIV-associated dementia, QUIN levels correlate with the degree of neurological dysfunction observed in infected individuals. In the present study, we found that QUIN suppressed the induction of LTP. This may be, at least in part, the mechanisms underlying neurological impairments seen in AIDS patients with HAD.

The induction of LTP in the CA1 area of the hippocampus requires an activation of postsynaptic *N*-methyl-D-aspartate (NMDA) receptors involving a rise in intracellular Ca^{2+} concentration in postsynaptic neurons. LTP could be prevented in the presence of NMDA receptor antagonists (Coan *et al*, 1987) or by injection of Ca^{2+} chelators into the postsynaptic neurons (Lynch *et al*, 1983; Malenka *et al*, 1988). However, this quick rise in intracellular Ca^{2+} is unlikely to be sufficient for the stabilization of LTP, because application of NMDA onto CA1 neurons induces only STP, but not LTP (Kauer *et al*, 1988). It was shown that activation of glutamate metabotropic receptors and its resultant elevation of Ca^{2+} concentration from intracellular stores contributes to the stabilization of LTP (Bashir *et al*, 1993). In the present study, we found that HIV-1-infected, immune-activated MDM culture fluids inhibited both STP and LTP. This suggests that at least some of the secretory products within the MDM culture fluids interact with NMDA and glutamate metabotropic receptors, leading to a decrease in intracellular Ca^{2+} concentrations and blockade of LTP. This may be extremely important in seeking potential therapeutic agents interacting with neuronal membrane NMDA receptors to block individual factor(s)-mediated suppression of LTP during HAD.

Neurotoxicity induced by HIV-1 coat proteins, for example gp120, has been demonstrated on cultured rat cortical (Muller *et al*, 1992) and hippocampal neurons (Meucci and Miller, 1996). The neurotoxic effect of HIV-1 coat proteins may be a consequence of direct or indirect actions on neurons, by stimulating neurotoxin release in microglia (Dawson *et al*, 1993). We found that HIV-1 virions *per se*, when applied by bath or by incubation, had no apparent effect on the induction of LTP. This may be due to the application time of virus in our experiments which was too short to produce neuronal injury. Alternatively, the mechanism by which HIV-1 coat proteins induce neurotoxicity is through neurotoxin release by macrophages.

Materials and methods

Isolation and culture of primary human monocytes
Human monocytes were recovered from PBMCs of HIV and hepatitis B seronegative donors after leukopheresis, and purified by counter current centrifugal elutriation (Gendelman *et al*, 1988). Monocytes were cultured as adherent monolayers (3.3×10^6 cells/well in 48 mm plastic culture plate) in DMEM (Sigma, St. Louis, MO, USA) with 10% heat-inactivated pooled human serum, 50 μg/ml gentamicin and/or 10 μg/ml ciprofloxacin (Sigma), and 1000 U/ml highly purified recombinant human macrophage colony stimulating factor (MCSF) (a generous gift from Genetics Institute, Inc., Cambridge, MA, USA). All tissue reagents were screened and found negative for endotoxin (<10 pg/ml; Associates of Cape Cod, Inc., Woods Hole, MA, USA) and Mycoplasma contamination (Gen-probe II; Gen-probe Inc., San Diego, CA, USA). Lipopolysaccharide (LPS), from *E. coli* serotype, was purchased from Sigma.

HIV-1 infection of monocytes and collection of MDM
Seven days after plating, MDM were infected with HIV-1_{ADA} at a multiplicity of infection (MOI) of 0.1 infectious virus/target cell. Culture fluids were half exchanged every 2–3 days. Reverse transcriptase (RT) activity was determined in triplicate samples of culture fluids as previously described (Kalter *et al*, 1991). For RT assays culture fluids were added to a reaction mixture of 0.05% Nonidet P-40 (Sigma Chemical Co.), 10 μg/ml poly(A), 0.25 mg/ml oligo (dT) (Pharmacia Fine Chemicals, Piscataway, NJ, USA), 5 mM DTT (Pharmacia Fine Chemicals), 150 mM KCl, 15 mM $MgCl_2$, and [^{3}H]-dTTP (2 Ci/mmol; Amersham Corp., Arlington Heights, IL, USA) in pH 7.9 Tris-HCl buffer for 24 h at 37°C. Radiolabeled nucleotides were precipitated with cold 10% TCA and 95% ethanol in an automatic

cell harvester (Skatron, Inc., Sterling, VA, USA) on paper filters. Radioactivity was estimated by liquid scintillation spectroscopy. All experiments were performed in triplicate. Seven days after infection, select HIV-1_{ADA} infected and replicate uninfected MDM, were treated with/without lipopolysaccharide (LPS) (1 μg/ml) for 1 h. The cells were washed vigorously and media removed. The MDM conditioned media were filtered through 0.04-μm nylon membrane filter to remove HIV-1 by methods previously described (Oshima *et al*, 1995). The presence of virus in MDM conditioned media before and after filtration was determined by RT and by MDM infectivity assays.

Measurement of TNF-α activity

TNFα bioactivity was measured according to standard laboratory procedures (Genis *et al*, 1992). Briefly, the murine L929 cell line was propagated in DMEM (Sigma Chemical Co.), 5% FCS (Life Technologies, Inc., Grand Island, NY, USA), 2 mM glutamine, and 20 μg/mL gentamicin. Cells were retrieved in log phase and placed (0.7×10^6/well) in 96-well plates (Costar Corp.) with 5 μg/ml actinomycin D (Sigma Chemical Co.). Culture fluids were inoculated into cell monolayers, and degree of cell lysis was determined by crystal violet staining after a 24 h incubation (Hogan and Vogel, 1988).

Preparation of hippocampal slices and recordings of field potentials

The transverse hippocampal brain slices were prepared as previously described (Xiong *et al*, 1996). Briefly, 19–31-day-old male Sprague-Dawley rats were anesthetized with metofane, decapitated and their brains were quickly removed from the cranial cavity. The brains were then placed into an ice-cold (4°C) oxygenated artificial cerebrospinal fluid (ACSF) environment. The hippocampi were dissected free and 400-μm-thick transverse slices were cut using a tissue chopper. The slices were kept in a humidified/oxygenated holding chamber at room temperature for at least 1 h before being transferred into a recording chamber. In the recording chamber single hippocampal slices were fully submerged in a continuously perfused ACSF solution at a constant flow rate of 2 ml/min with the use of a peristaltic pump (Rainin Instrument Co., Woburn, MA, USA). The ACSF contained (in mM): NaCl (124), KCl (3), $CaCl_2$ (2), $MgCl_2$ (2), NaH_2PO_3 (1), $NaCO_3$ (26), and Glucose (10). ACSF was equilibrated with 95% O_2 and 5% CO_2, the resulting pH was 7.3–7.5. The temperature of the perfusate was maintained at 30°C with an automatic temperature controller (Warner Instrument Corp., Hamden, CT, USA). A total of 10 μl (5 μl $\times$ 2) MDM culture fluids prepared from both HIV-1-infected (with/without progeny viruses) and uninfected MDM, with/without LPS stimulation, were applied onto hippocampal slices at 60 and 30 min respectively prior to the slices being transferred to the recording chamber. In some experiments, crude MDM culture fluids were applied onto slices via bath perfusion at 1 : 6 dilution. All MDM fluids were stored at -70°C and thawed before application. One treatment was done on each slice. The slices from each rat were used randomly for different treatment.

EPSPs were recorded from the stratum radiatum of the CA1 subfield by a glass microelectrode. The recording microelectrode was made from borosilicated glass capillaries with inner filaments that enabled quick back-filling. The tip diameter of the microelectrode was $\sim$5.0 μl and had a resistance of 1–5 MΩ when filled with ACSF. Orthodromic constant current stimuli were delivered through a bipolar tungsten electrode (insulated except for the tip) placed in the Schaffer collateral-commissural pathway. The intensity (50–400 μA) and duration (0.01–0.05 ms) of stimulation were adjusted to generate approximately 30–40% of a maximal response. The evoked EPSPs were recorded with an Axopatch-1D amplifier (Axon Instruments, Inc., Foster City, CA, USA) interfaced with a Dell Pentium II PC computer (Dell Inc.), and acquired digitized (at a frequency of 2.5 kHz), stored and analyzed using pCLAMP software (Axon Instruments, Inc.). All experiments were conducted in the presence of 20–50 μM of picrotoxin (in the perfusate) unless otherwise indicated. The chemicals used in this study were purchased from Sigma.

In each experiment, a 30 min control recording was conducted in most cases (in some cases, a 20 min control was recorded) once the stimulation parameters were achieved. Each trace was an average of three consecutive evoked EPSPs. High frequency stimulation (HFS, 100 Hz, 0.5 S) was delivered twice at 20 s intervals at the same intensity as that used at low frequency stimulation. After a tetanic (high frequency), PTP was followed by STP (Kauer *et al*, 1998) which decayed within about 20 min, and by a more persistent and significant enhancement in the synaptic transmission, the LTP, that lasted hours (Bliss and Collingridge, 1993). The initial slopes of field EPSPs were measured and expressed in percentage of control (the average of initial slopes from the first 30 min was treated as 100%, i.e. the basal level). To analyze data of individual sets on synaptic potentiation, the mean percentages of the initial slope of the field EPSP at 1 min, from 10–15 min and from 55–60 min after tetanus were used as PTP, STP and LTP. Results from several slices with large fluctuation ($> \pm 2$ s.d.) in basal activity were rejected. Values reflect actual voltage amplitude. Statistical analysis of data was performed using Student's *t*-tests or one-way analysis of variance. Differences were considered significant if $P<0.05$. All data were expressed as the mean $\pm$ standard error of the mean (s.e.m.).

Acknowledgments

This work was supported in part by NIH grants P01NS31492-01, R01NS34239-01, R01NS34239-02, R01NS36126-01, P01MH57556-01, University of Nebraska Biotechnology start up funds, and Carter-Wallace, Inc., Cranbury, NJ, USA.

References

Bashir ZI, Bortolotto ZA, Davies CH, Berretta N, Irving AJ, Seal AJ, Henley JM, Jane DE, Watkins JC, Collingridge GL (1993). Induction of LTP in the hippocampus needs synaptic activation of glutamate metabotropic receptors. *Nature* **363:** 347–350.

Bellinger FP, Madamba S, Siggins GR (1993). Interleukin 1β inhibits synaptic strength and long-term potentiation in the rat CA1 hippocampus. *Brain Res* **628:** 227–234.

Bliss TVP, Lømo T (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol* **232:** 331–356.

Bliss TVP, Collingridge GL (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361:** 31–39.

Bukrinsky MI, Nottet HSLM, Schmidtmayerova H, Dubrovsky L, Mullins ME, Lipton SA, Gendelman HE (1995). Regulation of nitric oxide synthase activity in human immunodeficiency virus type-1 (HIV-1)-infected monocytes: implications for HIV-associated neurological disease. *J Exp Med* **181:** 735–745.

Coan EJ, Saywood W, Collingridge GL (1987). MK-801 blocks NMDA receptor-mediated synaptic transmission and long-term potentiation in rat hippocampal slices. *Neurosci Lett* **80:** 111–114.

Cunningham AJ, Murray CA, O'Neill LAJ, Lynch MA, O'Connor JJ (1996). Interleukin-1β (IL-1β) and tumor necrosis factor (TNF) inhibit long-term potentiation in the rat dentate gyrus in vitro. *Neurosci Lett* **203:** 17–20.

Dawson VL, Dawson TM, Uhl GR, Snyder SH (1993). Human immunodeficiency virus type 1 coat protein neurotoxicity mediated by nitric oxide in primary cortical cultures. *Proc Natl Acad Sci USA* **90:** 3256–3259.

Epstein LG, Gendelman HE (1993). Human immunodeficiency virus type 1 infection of the nervous system: pathogeneic mechanisms. *Ann Neurol* **33:** 277–287.

Everall IP, Luthbert PJ, Lantos PL (1991). Neuronal loss in the frontal cortex in HIV infection. *Lancet* **337:** 1119–1121.

Gallo P, Frei K, Rordorf C, Lazdins J, Tavolato B, Fontana A (1989). Human immunodeficiency type 1 (HIV-1) infection of central nervous system: an evaluation of cytokines in cerebrospinal fluids. *J Neuroimmunol* **23:** 109–116.

Gelbard HA, Nottet HSLM, Dzenko KA, Jett M, Genis P, White R, Wang L, Choi YB, Zhang D, Lipton SA, Swindells S, Epstein LG, Gendelman HE (1994). Platelet-activating factor: A candidate human immunodeficiency virus type 1-induced neurotoxin. *J Virol* **68:** 4628–4635.

Gendelman HE, Persidsky Y, Ghorpade A, Limoges J, Stins M, Fiala M, Morrisett R (1997). The neuropathogenesis of HIV-1 dementia. *AIDS 97* **11:** S35–S45.

Gendelman HE, Orenstein J, Martin MA, Ferrua C, Mitra R, Phipps T, Wahl LA, Lane HC, Fauci AS, Burke D, Skillman D, Meltzer MS (1988). Efficient isolation and propagation of human immunodeficiency virus on recombinant colony stimulating factor 1-treated monocytes. *J Exp Med* **167:** 1428–1441.

Genis P, Jett M, Bernton EW, Boyle T, Gelbard HA, Dzenko K, Keane RW, Resnick L, Volsky DJ, Epstein LG, Gendelman HE (1992). Cytokines and arachidonic metabolites produced during human immunodeficiency virus (HIV)-infected macrophage-astroglia interactions: implications for the neuropathogenesis of HIV disease. *J Exp Med* **176:** 1703–1718.

Glass JD, Johnson RT (1996). Human immunodeficiency virus and the brain. *Ann Rev Neurosci* **19:** 1–26.

Glass JD, Fedor H, Wesselingh SL, McArthur JC (1995). Immunocytochemical quantitation of human immunodeficiency virus in the brain: correlation with dementia. *Ann Neurol* **38:** 755–762.

Haas HS, Schauenstein K (1997). Neuroimmunomodulation via limbic structure – the neuroanatomy of psychoimmunology. *Prog Neurobiol* **51:** 195–222.

Hogan MM, Vogel SN (1988). Production of TNF by γ-IFN-primed C3H/HeJ (Lps^d) macrophages requires the presence of lipid-A-associated proteins. *J Immunol* **121:** 4196–4202.

Kalter DK, Nakamura M, Turpin JA, Baca LM, Dieffenbach C, Ralph P, Gendelman HE, Meltzer MS (1991). Enhanced HIV replication in MCSF-treated monocytes. *J Immunol* **146:** 298–306.

Katsuki H, Nakai S, Hirai Y, Akaji K, Kiso Y, Satoh M (1990). Interleukin-1β inhibits long-term potentiation in the CA3 region of mouse hippocampal slices. *Eur J Pharmacol* **181:** 323–326.

Kauer JA, Malenka RC, Nicoll RA (1988). NMDA application potentiates synaptic transmission in the hippocampus. *Nature* **334:** 250–252.

Ketzler S, Weis S, Haug H, Budka H (1990). Loss of neurons in the frontal cortex in AIDS brains. *Acta Neuropath, Berlin* **80:** 92–94.

Kim JP, Choi DW (1987). Quinolinate neurotoxicity in cortical cell culture. *Neurosci* **25:** 423–432.

Li AJ, Katafuchi T, Oda S, Hori T, Oomura Y (1997). Interleukin-6 inhibits long-term potentiation in rat hippocampal slices. *Brain Res* **748:** 30–38.

Lipton SA, Gendelman HE (1995). Dementia associated with the acquired immunodeficiency syndrome. *N Engl J Med* **332:** 934–940.

Lynch G, Larson J, Kelso S, Barrionuevo G, Schottler F (1983). Intracellular injection of EGTA block induction of hippocampal long-term potentiation. *Nature* **305:** 719–721.

Lynch MA (1998). Age-related impairment in long-term potentiation in hippocampus: a role for the cytokine, interleukin-1 beta? *Prog Neurobiol* **56:** 571–589.

Malenka RC, Kauer JA, Zucker RS, Nicoll RA (1988). Postsynaptic calcium is sufficient for potentiation of hippocampal synaptic transmission. *Science* **242:** 81–84.

Masliah E, Achim CL, Ge N, DeTheresa R, Terry RD, Wiley CA (1992). Spectrum of human immunodeficiency virus-associated neocortical damage. *Ann Neurol* **32:** 321–329.

Meucci O, Miller RJ (1996). gp120-induced neurotoxicity in hippocampal pyramidal neuron culture: protective action of TGF-β1. *J Neurosci* **16:** 4080–4088.

Mehler MF, Rozental R, Dougherty M, Spray DC, Kessler J (1993). Cytokine regulation of neuronal differentiation of hippocampal progenitor cells. *Nature* **362:** 62–65.

Michaels J (1988). Human immunodeficiency virus type 1 (HIV-1) infection of the nervous system: a review. *Immunodeficiency Review* **1:** 71–104.

Mrak RE, Sheng JG, Griffin WST (1995). Glial cytokines in Alzheimer's disease: review and pathogenic implications. *Hum Pathol* **26:** 816–823.

Muller WEG, Schroder HC, Ushijima H, Dapper J, Bormann J (1992). gp120 of HIV-1 induces apoptosis in rat cortical cell culture: prevention by memantine. *Eur J Pharmacol* **226:** 209–214.

Nottet HSLM, Flanagan EM, Flanagan C, Gelbard HA, Gendelman HE, Reinhard Jr JF (1996). The regulation of quinolinic acid in human immunodeficiency virus-infected monocytes. *J NeuroViol* **2:** 111–117.

Nottet HSLM, Jett M, Qu-Hui Z, Flanagan C, Rizzino A, Genis P, Baldwin T, Schwartz J, Gendelman HE (1995). A regulatory role for astrocytes in HIV-1 encephalitis: an over-expression of eicosanoids, platelet activating factor and tumor necrosis factor-α by activated virus-infected monocytes is attenuated by primary human astrocytes. *J Immunol* **154:** 3567–3581.

Oshima KH, Comans TW, Highsmith AK, Ades EW (1995). Removal of human immunodeficiency virus by an 0.04-μm membrane filter. *J AIDS Human Retrovirol* **8:** 64–65.

Patterson PH, Nawa H (1993). Neuronal differentiation factors/cytokines and synaptic plasiticity. *Cell 72: Neuron* **10:** 123–137.

Price RW, Sidtis J, Rosenblum M, Scheck AC, Cleary P (1988). The brain and AIDS: central nervous system HIV-1 infection and AIDS dementia complex. *Science* **239:** 586–592.

Reyes E, Mohar A, Mallory M, Miller A, Masliah E (1994). Hippocampal involvement associated with human immunodeficiency virus encephalitis in Mexico. *Arch Pathol Lab Med* **118:** 1130–1134.

Rothwell NJ, Hopkins SJ (1995). Cytokines and the nervous system II: actions and mechanisms of action. *Trends Neurosci* **18:** 130–136.

Rothwell NJ, Relton JK (1993). Involvement of cytokines in acute neurodegeneration in the CNS. *Neurosci. Biobehav. Rev.* **17:** 217–227.

Spencer DC, Price RW (1992). Human immunodeficiency virus and the central nervous system. *Ann Rev Microbiol* **46:** 655–693.

Squire LR (1992). Memory and hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychol Rev* **99:** 195–231.

Tancredi V, D'Arcangelo G, Grassi F, Tarroni P, Palmieri G, Santoni A, Eusebi F (1992). Tumor necrosis factor alters synaptic transmission in the rat hippocampal slices. *Neurosci Lett* **146:** 176–178.

Tancredi V, Zona C, Velloti F, Eusebi F, Santoni A (1990). Interleukin-2 suppresses established long-term potentiation and inhibits its induction in the rat hippocampus. *Brain Res* **525:** 149–151.

Wieraszko A, Li G, Kornecki E, Hohan MV, Ehrlich YH (1993). Long-term potentiation in the hippocampus induced by platelet-activating factor. *Neuron* **10:** 553–557.

Wiley CA, Masliah E, Morey M, Lemere C, DeTheresa R, Grafe M, Hansen L, Terry R (1991). Neocortical damage during HIV infection. *Ann Neurol* **29:** 651–657.

Xiong H, Baskys A, Wojtowicz JM (1996). Brain-derived peptides inhibit synaptic transmission via presynaptic $GABA_B$ receptors in CA1 area of rat hippocampal slices. *Brain Res* **737:** 188–194.

Xiong H, Zheng J, Thylin M, Gendelman HE (1999). Unraveling the mechanisms for neurotoxicity in HIV-1-associated dementia: Inhibition of neuronal synaptic transmission by macrophage secretory products. *AIDS Res Hum Retrov* **15:** 57–63.

Zheng J, Gendelman HE (1997). The HIV-1 associated dementia complex: a metabolic encephalopathy fueled by viral replication in mononuclear phagocytes. *Curr Opin Neurol* **10:** 319–325.

Zheng J, Thylin MR, Persidsky Y, Xiong H, Ghorpade A, Che M, Leisman GB, Cotter R, Gendelman HE (1999). Neuronal destruction in HIV-1-associated dementia: secretory products from HIV-1 infected macrophages affect neuronal function, differentiation, long term potentiation, and viability. *J Virol* (revised for publication).