Journal of NeuroVirology (1996) 2, 234–239

Review

Glia: the not so innocent bystanders

Chun C Chao, Shuxian Hu and Phillip K Peterson

Minneapolis Medical Research Foundation and the University of Minnesota Medical School, Minneapolis, Minnesota 55404, USA

Activated glial cells (microglia and astrocytes) are a hallmark of a variety of neurodegenerative diseases. Recent *in vitro* studies have suggested that mediators derived from reactive glial cells (eg, cytokines, reactive oxygen intermediates, nitric oxide, glutamate or quinolinic acids, and neurotoxins) contribute to neuronal injury. Several of these mediators have been implicated in the neuropathogenesis of HIV-1. Although the precise role of glial cell-mediated neurotoxicity in viral infections of the central nervous system has not been established, it is hoped that research in this field will yield new therapies for these infections as well as for immune-mediated neurodegenerative diseases.

Keywords: microglia; astrocytes; neurotoxicity; cytokine; nitric oxide; glutamate

Historically, the glia (from Greek, meaning 'glue') were recognized first for their supportive functions within the central nervous system (CNS) where they surround the neurons. Not so well appreciated by the neuroscientist research community is the fact that glia outnumber neurons by about eight to one. Astroglial cells are the predominant cell type (comprising approximately 85% of the glia), where microglia (about 10%) and oligodendrocytes (about 5%) are minority cell populations within the CNS.

Although glia clearly play important supportive functions within the brain, in recent years the pathogenetic potential of these cells has received increased attention. Activated glial cells (microglia and astrocytes) are a hallmark of several neurodegenerative diseases, such as AIDS dementia, Alzheimer's disease, Parkinson's disease, and multiple sclerosis. Microglia, derived from the bone marrow early in fetal development (Perry and Gordon, 1988), are the functional equivalents of macrophages in a variety of other tissues (Dickson *et al*, 1991; Peudenier *et al*, 1991). Histopathologic evidence indicates that microglial cells migrate to, differentiate, and proliferate at sites of inflammation in the CNS (del Rio-Hortega, 1932). In addition to their potential role in host defense (Chao *et al*, 1994a), activated microglia may also be destructive (Piani *et al*, 1991; Boje and Arora, 1992; Chao *et al*, 1992a). Astrocytes are of neuroectodermal origin, and in addition to their critical contribution to neurotransmitter metabolism and biochemical functions, these glial cells may also be involved in host defense against neurotropic fungi and parasites (Lee *et al*, 1994; Peterson *et al*, 1995). Astrocytes may also have a regulatory role in HIV-1 encephalitis by attenuating production of immune mediators by HIV-1-infected monocytes (Nottet *et al*, 1995). Reactive astrocytes (astrogliosis) are a histopathological characteristic of a number of neurodegenerative diseases (Eddleston and Mucke, 1993), while impairments of astroglial function by microbes or host-derived factors have the potential to contribute to neurologic disorders as well (Mucke and Eddleston, 1993).

The potential role of these reactive glial cells at sites of inflammation has received increased attention in the past decade. A growing body of evidence supports the hypothesis that activation of glial cells by immune stimuli or cytokines contributes to neurotoxicity. Although the precise mechanism underlying glia-mediated neurotoxicity remains to be established, it has been proposed that mediators derived from reactive glial cells are responsible for injury to neighbouring neurons. *In vitro*, activated glial cells generate substantial amounts of cytokines (both pro-inflammatory and anti-inflammatory), reactive oxygen intermediates (ROI), nitric oxide (NO), N-methyl-D-aspartate (NMDA) receptor ligands (eg, glutamate and quinolinic acids) and yet to be characterized neurotoxins. This mini review focuses on the potential mechanisms underlying glial cell-mediated neurotoxicity. It is hoped that research in this field will yield insights leading to the development of new therapies for viral infections of the CNS as well as for immune-mediated neurodegenerative diseases.

Correspondence: Chun C Chao
Received 15 February 1996; revised 5 April 1996; accepted 22 April 1996

Cytokine-mediated neurotoxicity

Several studies have indicated that cytokines released by glial cells are neurotoxic. Cytokine-mediated neurotoxicity involves both direct and indirect mechanisms. Tumor necrosis factor (TNF)-α is produced by microglia in response to a variety of activating signals; however, astrocytes also release TNF-α when stimulated with IL-1 (Lee *et al*, 1993a; Chao *et al*, 1995a). It has been shown that TNF-α induces apoptotic death of SK-N-MC human neuroblastoma cells (Talley *et al*, 1995). The mechanism underlying TNF-α-induced apoptosis may involve formation of ROI. In one study, exposure of human cortical neurons to TNF-α for 48 h was found to induce toxicity (Gelbard *et al*, 1993). TNF-α-induced neurotoxicity was reversed by a non-NMDA receptor antagonist but not by MK-801, suggesting the involvement of a non-NMDA receptor mechanism (Gelbard *et al*, 1993). In contrast, in a separate study TNF-α was found to potentiate glutamate receptor-mediated neurotoxicity by interfering with the astrocyte's detoxifying ability (eg, glutamine synthetase and glutamate uptake activities) (Chao and Hu, 1994). TNF-α also induces neurotoxicity indirectly by reducing the supporting activity of astrocytes (Bernton *et al*, 1992). From these *in vitro* studies, it appears that TNF-α-induced neuronal injury is mediated mainly via an indirect mechanism by suppressing the astrocyte's ability to maintain neuronal survival. In murine neuronal cell cultures treated with interferon (IFN)-γ, TNF-α has been shown to induce neuronal loss indirectly via generating NO release from microglia. In this culture system, interleukin (IL)-4 exerts a neuroprotective effect by inhibiting NO production (Chao *et al*, 1993).

In murine brain cell cultures, transforming growth factor (TGF)-β has been demonstrated to potentiate NMDA receptor-mediated neurotoxicity (Chao *et al*, 1992b). The neurotoxic effect of this cytokine also is indirect via inhibiting astrocyte glutamine synthase, an enzyme which plays a key role in metabolizing the 'excitotoxic' neurotransmitter glutamate (Toru-Delbauffe *et al*, 1990). Glial cells are known to produce the latent form of TGF-β (Constam *et al*, 1992; da Cunha and Vitkovic, 1992). More recently, TNF-α-activated microglia were also found to generate the biologically active form of TGF-β (Chao *et al*, 1995b). In contrast to the findings with murine cell cultures, TGF-β protects rat neurons against glutamate neurotoxicity and hypoxia (Prehn *et al*, 1993) by unidentified mechanisms. In human fetal brain cell cultures, TGF-β also prevents neuronal injury triggered by β-amyloid protein (Chao *et al*, 1994b). This neuroprotective effect is probably related to the immunosuppressive properties of TGF-β, ie, by inhibiting β-amyloid protein-induced activation of microglial cells. In a variety of studies of microglial cell cultures, TGF-β has been shown to be a potent immunosuppressive cytokine (Chao *et al*, 1995a,c). The precise mechanism by which TGF-β exerts its neuroprotective effects, however, awaits further investigation.

When stimulated *in vitro*, both the α and β isoforms of IL-1 are mainly produced by microglia and not by astrocytes (Lee *et al*, 1993b). In combination with IFN-γ in human fetal brain cell cultures, IL-1β elicits neuronal injury via a NO-related mechanism (Chao *et al*, 1996). Since MK801 blocked IL-1β plus IFN-γ-induced neurotoxicity, this finding suggests involvement of a NMDA receptor mechanism. In combination, IL-1β plus IFN-γ have been shown to potentiate NMDA receptor-mediated neurotoxicity in murine neuronal cell cultures (Hewett *et al*, 1994). In human glial cell cultures, however, astrocytes rather than microglia are the major cell type capable of producing NO in response to IL-1β (Lee *et al*, 1993b). Human astrocyte NO production is differentially regulated by anti-inflammatory cytokines such as IL-4, IL-10, and TGF-β (Hu *et al*, 1995). Although neither TNF-α nor IL-1β alone are neutoxic, in combination these cytokines cause neuronal injury via eliciting astrocyte NO production. Interestingly, a NMDA receptor mechanism appears to be involved in this NO-related neurotoxicity (Chao *et al*, 1995d). IL-1β plus TNF-α also inhibits astrocyte glutamine synthetase and glutamate uptake activities (Chao *et al*, 1995d), which foster glutamate neurotoxicity (Herz, 1979). Taken together, these findings suggest a final pathway involving NMDA receptors in cytokine-mediated neuronal injury.

NO-mediated neurotoxicity

Cytokine-activated murine microglia generate substantial amounts of NO which kills neurons (Chao

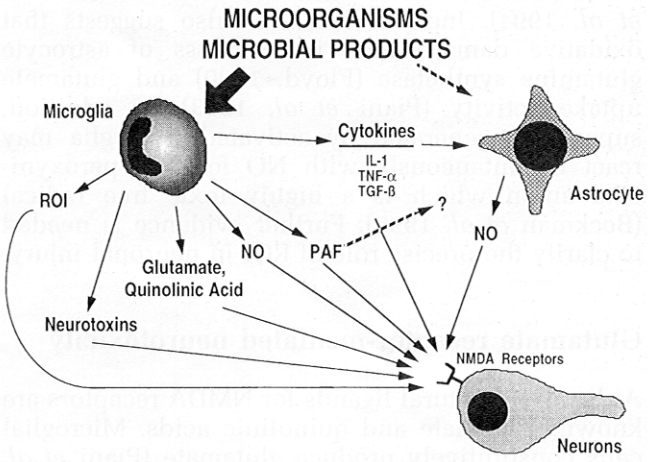

Figure 1 Diagram depicting potential mechanisms underlying glial cell-mediated neurotoxicity.

et al, 1992a; Boje and Arora, 1992). Inhibition of NO generation blocks this microglia-mediated neurotoxicity (Chao *et al*, 1993). The precise mechanism underlying NO-mediated neurotoxicity, however, is unclear. Because NMDA receptor antagonists partially attenuate cytokine-mediated neurotoxicity (and NO-mediated toxicity), involvement of NMDA receptors in NO-mediated neurotoxicity has been suggested (Boje and Arora, 1992). We also have found that NO generated chemically by sodium nitroprusside induces neuronal loss via a NMDA receptor mechanism in human neuronal cell cultures (unpublished data). Human microglia generate relatively small amounts of NO in response to immune stimuli, which is insufficient to induce neuronal injury *in vitro* (Peterson *et al*, 1994). HIV-1-infected macrophages have been reported to generate modest amounts (2–5 μM) of NO (Bukrinsky *et al*, 1994). Whether NO generated following HIV-1-infection impairs neuronal function or survival is unknown. However, in human neuronal cell cultures, IL-1β plus IFN-γ (Chao *et al*, 1996) and IL-1β plus TNF-α (Chao *et al*, 1995d) stimulate astrocyte NO production which mediates neurotoxicity partially (between 60 and 80%) via a NMDA receptor mechanism. Other unidentified mechanisms are also possibly involved in NO-mediated neurotoxicity. In addition, under certain conditions NO could be neuroprotective depending upon its redox state and site of action (Lipton *et al*, 1993).

ROI-mediated neurotoxicity

Cytokine-activated murine (Colton *et al*, 1992; Hu *et al*, 1994) and human (Chao *et al*, 1995c) microglia release ROI as assessed by measuring superoxide production *in vitro*. Direct evidence that ROI are neurotoxic is lacking (Piani *et al*, 1992). A recent study, however, found that microglia-derived ROI are toxic to a neuronal cell line, PC12 cells (Tanaka *et al*, 1994). Indirect evidence also suggests that oxidative damage may induce loss of astrocyte glutamine synthetase (Floyd, 1990) and glutamate uptake activity (Piani *et al*, 1993). In addition, superoxide generated by activated microglia may react instantaneously with NO forming peroxynitrite anion which is a highly toxic free radical (Beckman *et al*, 1990). Further evidence is needed to clarify the precise role of ROI in neuronal injury.

Glutamate receptor-mediated neurotoxicity

At least two natural ligands for NMDA receptors are known: glutamate and quinolinic acids. Microglial cells constitutively produce glutamate (Piani *et al*, 1991), and quinolinic acids can also be generated by human microglia (Espey *et al*, 1995). Binding to NMDA receptors by these ligands opens channels which results in calcium influx and neuronal death. In HIV-related neuronal injury, gp120 has been found to induce neurotoxicity via NMDA receptors (Lipton, 1994). In addition, in the absence of microglial cell activation, NO also has been shown to induce glutamate neurotoxicity in primary murine cortical cultures (Dawson *et al*, 1991). The precise mechanism underlying NO's involvement in glutamate receptor-mediated neurotoxicity awaits further elucidation. A recent study has suggested that depending upon mitochondrial function, glutamate-induced neuronal death can proceed by either a necrotic or an apoptotic pathway (Ankarcrona *et al*, 1995; Bonfoco *et al*, 1995). The precise mechanism of NMDA receptor-mediated neurotoxicity, however, awaits further elucidation.

Platelet-activating factor (PAF)-induced neurotoxicity

PAF is a lipid molecule involved in the inflammatory process and in cell-cell communication. PAF can be detected in HIV-1-infected macrophages shortly after coculturing with astroglial cells (Gelbard *et al*, 1994), suggesting a potential induction of this cytokine following cell-to-cell contact. It has been shown that TNF-α stimulates PAF production in human fetal microglial cell cultures, suggesting a rich source of PAF in the brain (Jaranowska *et al*, 1995). Exposure of human cortical neurons to exogenous PAF decreases neuronal survival in a dose-dependent manner (Gelbert *et al*, 1994). It has been found that PAF can increase neuronal calcium and lead to augmented excitatory neurotransmission through enhanced glutamate release (Bito *et al*, 1992; Clark *et al*, 1992; Shukla, 1992). PAF can also elicit neuronal death by mechanisms involving increased intracellular calcium (Bito *et al*, 1992) and NMDA receptors (Gelbard *et al*, 1994). The precise mechanism whereby PAF injures neurons awaits further determination.

Unknown neurotoxins

Work from several laboratories has provided support for indirect mechanisms of HIV-induced neuronal injury (Gendelman *et al*, 1994; Lipton and Gendelman, 1995). Using brain ball cultures of human fetal neurons, Pulliam *et al* (1991) suggest that neurotoxic factors are released from infected macrophages. Giulian *et al* (1991) demonstrated a similar result from HIV-infected promonocytic cells, and indentified this factor to be a low molecular weight and protease resistant neurotoxic molecule released from these cells. This neurotoxic factor appears to be an amine-like molecule (Giulian,

1995). When cocultured with astroglia, HIV-1-infected macrophages release cytokines (eg, TNF-α and IL-1β) and arachidonic metabolites, which have been implicated in the neuropathogenesis of HIV disease (Genis *et al*, 1992). The mechanism underlying neurotoxicity induced by this factor involves mainly NMDA receptors and partially a NO-related mechanism. However, Bernton *et al* (1992) failed to confirm a neurotoxic effect of HIV-1-infected macrophages. By a cell-to-cell interaction mechanism, HIV-1-infected monocytic cells are neurotoxic (Tardieu *et al*, 1992). Recently, we have found that HIV-1-infected human microglia release a transferable toxin which kills neurons via a NMDA receptor-mediated mechanism (unpublished data). Further characterization of factors contributing to neurotoxicity resulting from HIV-1-infected microglial cell culture supernatants is warranted.

Summary

In the past decade, research on glia-neuronal interactions has accelerated. Activation of glial cells and mediators produced by these cells appear to be involved in the pathogenesis of a variety of brain diseases. Although *in vitro* evidence has suggested that mediators derived from reactive glial cells contribute directly or indirectly to neurotoxicity, the exact mechanism underlying activated glial cell-mediated neurotoxicity is still unclear. We have proposed a model of glial cell-mediated neurotoxicity based on studies in our and many other laboratories (see Figure 1). Although it will be some time before the intracellular mechanisms are deciphered, research in this area of neuroscience holds promise for new therapies for a number of neurodegenerative diseases and potentially for viral infections of the CNS.

Acknowledgements

This study was supported in part by USPHS grants DA-04381, DA-09924, AI-35110, T32-DA-07239 and a grant from the Alzheimer's Association.

References

Ankarcrona M, Bypbukt JM, Bontoco E, Zhivotovsky B, Orrenlus S, Lipton SA, Nicotera P (1995). Glutamate-induced neuronal death: a succession of necrosis or apoptosis depending on mitochondrial function. *Neuron* **15:** 1–20.

Beckman JS, Beckman TW, Chen J, Marshal PA, Freeman BD (1990). Apparent hydroxyl radical production by peroxinitrite: Implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci USA* **87:** 1620–1624.

Bernton EW, Bryant HU, Decoster MA, Orenstein JM, Ribas JL, Meltzer MS, Gendelman HE (1992). No direct neurotoxicity by HIV-1 virons or culture fluids from HIV-1 infected T cells or monocytes. *AIDS Res Human Retroviruses* **8:** 495–503.

Bito H, Nakamura M, Honda Z, Isumi T, Iwatsubo T, Seyama T, Segura A, Kido Y, Shimizu T (1992). Platelet-activating factor (PAF) receptor in rat brain: PAF mobilizes intracellular Ca^{2+} in hippocampal neurons. *Neuron* **9:** 285–294.

Boje KM, Arora PK (1992). Microglia-produced nitric oxide and reactive nitrogen oxides mediate neuronal cell death. *Brain Res* **587:** 250–256.

Bonfoco E, Krainc D, Ankarcrona M, Nicotera P, Lipton SA (1995). Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. *Proc Natl Acad Sci USA* **92:** 7162–7166.

Bukrinsky MI, Nottet HSLM, Schmidtmayerova H, Dubrovsky L, Flanagan CR, Mullins ME, Lipton SA, Gendelman HE (1994). Regulation of nitric oxide synthase activity in human immunodeficiency virus type 1 (HIV-1)-infected monocytes: implications for HIV-associated neurologic disease. *J Exp Med* **181:** 735–745.

Chao CC, Hu S, Molitor TW, Shaskan EG, Peterson PK (1992a). Activated microglia mediate neuronal cell injury via a nitric oxide mechanism. *J Immunol* **149:** 2736–2741.

Chao CC, Hu S, Tsang M, Weatherbee J, Molitor TW, Anderson WR, Peterson PK (1992b). Effects of transforming growth factor-β on murine astrocyte glutamine synthetase activity: Implications in neuronal injury. *J Clin Invest* **90:** 1786–1793.

Chao CC, Molitor TW, Hu S (1993). Neuroprotective role of IL-4 against activated microglia. *J Immunol* **151:** 1473–1481.

Chao CC, Hu S (1994). Tumor necrosis factor-α potentiates glutamate neurotoxicity in human fetal brain cell cultures. *Dev Neurosci* **16:** 172–179.

Chao CC, Gekker G, Hu S, Peterson PK (1994a). Human microglial cell defense against *Toxoplasma gondii*: the role of cytokines. *J Immunol* **152:** 1246–1252.

Chao CC, Hu S, Kravitz FH, Tsang M, Anderson WR, Peterson PK (1994b). Transforming growth factor-β protects human neurons against β-amyloid-induced injury. *Molec Chem Neuropathol* **23:** 159–178.

Chao CC, Hu S, Sheng WS, Peterson PK (1995a). Tumor necrosis factor-alpha production by human fetal microglial cells: regulation by other cytokines. *Dev Neurosci* **17:** 97–105.

Chao CC, Hu S, Sheng WS, Tsang M, Peterson PK (1995b). Tumor necrosis factor-α mediates the release of bioactive transforming growth factor-β in microglial cell cultures. *Clin Immunol Immunopathol* **77:** 358–365.

Chao CC, Hu S, Peterson PK (1995c). Modulation of human microglial cell superoxide production by cytokines. *J Leukocyte Biol* **58:** 65–70.

Chao CC, Hu S, Erhlich L, Peterson PK (1995d). Interleukin-1 and tumor necrosis factor-α synergistically mediate neurotoxicity: involvement of nitric oxide and N-methyl-D-aspartate receptors. *Brain Behav Immun* **9:** 355–365.

Chao CC, Hu S, Sheng WS, Bu D-F, Bukrinsky MI, Peterson PK (1996). Cytokine-stimulated astrocytes damage human neurons via a nitric oxide mechanism. *Glia* **16:** 276–284.

Clark GD, Happel LT, Zorumski GF, Bazan NG (1992). Enhancement of hippocampal excitatory synaptic transmission by platelet-activating factor. *Neuron* **9:** 1211–1216.

Colton CA, Yao J, Keri JE, Gilbert D (1992). Regulation of microglial function by interferons. *J Neuroimmunol* **30:** 89–98.

Constam DB, Phillip J, Malipiero UV, ten Dijke P, Schachner M, Fontana A (1992). Differential expression of transforming growth factor-β1, β2, and β3 by glioblastoma cells, astrocytes, and microglia. *J Immunol* **148:** 1404–1410.

da Cunha A, Vitkovic L (1992). Transforming growth factor beta-1 (TGF-β1) expression regulation in rat cortical astrocytes. *J Neuroimmunol* **36:** 157–169.

Dawson VL, Dawson TM, London ED, Bredt DS, Snyder SH (1991). Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. *Proc Natl Acad Sci USA* **88:** 6368–6370.

del Rio-Hortega P (1932). Microglia. In: *Cytology and Cellular Pathology of the Nervous System*. Penfield W, (ed). vol. 2. Paul P. Hocker: New York, pp 481–584.

Dickson DW, Mattiace LA, Kure K, Hutchins K, Lyman WD, Brosnan CF (1991). Microglia in human disease, with an emphasis on acquired immune deficiency syndrome. *Lab Invest* **64:** 135–156.

Eddleston M, Mucke L (1993). Molecular profile of reactive astrocytes-implications for their role in neurologic disease. *Neuroscience* **54:** 15–36.

Espey MG, Moffett JR, Namboodiri MAA (1995). Temporal and spatial changes of quinolinic acid immunoreactivity in the immune system of lipopolysaccharide-stimulated mice. *J Leukocyte Biol* **57:** 199–206.

Floyd RA (1990). Role of oxygen free radicals in carcinogenesis and brain ischaemia. *FASEB J* **4:** 2587–2597.

Gelbard HA, Dzenko KA, DiLoreto D, del Cerro C, del Cerro M, Epstein LG (1993). Neurotoxic effects of tumor necrosis factor alpha in primary human neuronal cultures are mediated by activation of the glutamate AMPA receptor subtype: implications for AIDS neuropathogenesis. *Dev Neurosci* **15:** 417–422.

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

Gendelman HE, Lipton SA, Tardieu M, Bukrinsky MI, Nottet HSLM (1994). The neuropathogenesis of HIV-1 infection. *J Leukocyte Biol* **56:** 389–398.

Genis P, Jett M, Bernton EW, Boyle T, Gelbard HA, Dzenko K, Keane RW, Resnick L, Mizrachi Y, Volsky RW, 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.

Giulian D, Vaca K, Noonan CA (1991). Secretion of neurotoxins by mononuclear phagocytes infected with HIV-1. *Science* **250:** 1593–1596.

Giulian D (1995). Brain mononuclear phagocytes drive CNS injury during HIV-1 infection. In: *Pathophysiology of Astrocytes and Microglia: Focus on Multiple Sclerosis and HIV-related Brain Damage*. Institute Superiore Sanita: Rome. Abst. 25.

Hertz L (1979). Functional interactions between neurons and astrocytes. I. Turnover and metabolism of putative amino acid transmitters. *Prog Neurobiol* **13:** 277–323.

Hewett SJ, Csernansky CA, Choi DW (1994). Selective potentiation of NMDA-induced neuronal injury following induction of astrocyte iNOS. *Neuron* **13:** 487–494.

Hu S, Sheng WS, Peterson PK, Chao CC (1994). Cytokine modulation of murine microglial cell superoxide production. *Glia* **13:** 45–50.

Hu S, Sheng WS, Peterson PK, Chao CC (1995). Differential regulation by cytokines of production of nitric oxide by human astrocytes. *Glia* **15:** 491–494.

Jaranowska A, Bussolino F, Sogos V, Arese M, Lauro GM, Gremo F (1995). Platelet-activating factor production by human fetal microglia. *Mol Chem Neuropathol* **24:** 95–106.

Lee SC, Liu W, Dickson DW, Brosnan CF, Berman JW (1993a). Cytokine production by human fetal microglia and astrocytes: differential induction by lipopolysaccharide and IL-1β. *J Immunol* **150:** 2659–2667.

Lee SC, Dickson DW, Liu W, Brosnan CF (1993b). Induction of nitric oxide synthase activity in human astrocytes by interleukin-1β and interferon-gamma. *J Neuroimmunol* **46:** 19–24.

Lee SC, Dickson DW, Brosnan CF, Casadevall A (1994). Human astrocytes inhibit *Cryptococcus neoformans* growth by a nitric oxide-mediated mechanism. *J Exp Med* **180:** 365–369.

Lipton SA, Choi Y-B, Pan Z-H, Lei SZ, Chen H-SV, Sucher NJ, Loscalzo J, Singel DJ, Stamler JS (1993). A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. *Nature* **364:** 626–632.

Lipton SA (1994). HIV-related neuronal injury. Potential therapeutic intervention with calcium channel antagonists and NMDA antagonists. *Mol Neurobiol* **8:** 181–196.

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

Mucke L, Eddleston M (1993). Astrocytes in infectious and immune-mediated diseases of the central nervous system. *FASEB J* **7:** 1226–1232.

Nottet HSLM, Jett M, Flanagan CR, Zhai Q-H, Persidsky Y, Rizzino N, Bernton EW, Genis P, Baldwin T, Schwartz J, LaBenz CJ, Gendelman HE (1995). A regulatory role for astrocytes in HIV-1 encephalitis. An overexpression of eicosanoids, platelet-activating factor, and tumor necrosis factor-α by activated HIV-1-infected monocytes is attenuated by primary human astrocytes. *J Immunol* **154:** 3567–3581.

Perry VH, Gordon S (1988). Macrophages and microglia in the nervous system. *TINS* **11:** 273–277.

Peterson PK, Hu S, Anderson WR, Chao CC (1994). Nitric oxide production and neurotoxicity mediated by activated microglia from human versus mouse brain. *J Infect Dis* **170:** 457–460.

Peterson PK, Gekker G, Hu S, Chao CC (1995). Human astrocytes inhibit intracellular multiplication of *Toxoplasma gondii* by a nitric oxide-mediated mechanism. *J Infect Dis* **171:** 516–518.

Peudenier S, Hery C, Montagnier L, Tardieu M (1991). Human microglial cells: characterization in cerebral tissue and in primary culture, and study of their susceptibility to HIV-1 infection. *Ann Neurol* **29:** 152–161.

Piani D, Frei K, Do KQ, Cuenod M, Fontana A (1991). Murine brain macrophages induce NMDA receptor mediated neurotoxicity in vitro by secreting glutamate. *Neurosci Lett* **133:** 159–162.

Piani D, Spranger M, Frei K, Schaff S, Fontana A (1992). Macrophage-induced cytotoxicity of N-methyl-D-aspartate receptor positive neurons involves excitatory amino acids rather than reactive oxygen intermediates and cytokines. *Eur J Immunol* **22:** 2429–2436.

Piani D, Frei K, Pfister H-W, Fontana A (1993). Glutamate uptake by astrocytes is inhibited by reactive oxygen intermediates but not by other macrophage-derived molecules including cytokines, leukotrienes or platelet-activating factor. *J Neuroimmunol* **48:** 99–104.

Prehn JH, Peruche B, Unsicker K, Kreiglstein J (1993). Isoform-specific effects of transforming growth factor-beta on degeneration of primary neuronal cultures induced by cytotoxic hypoxia or glutamate. *J Neurochem* **60:** 1665–1672.

Pulliam L, Herndier BG, Tang NM, McGrath MS (1991). Human immunodeficiency virus-infected macrophages produce soluble factors that cause histological and neurochemical alterations in cultured human brains. *J Clin Invest* **87:** 503–512.

Shukla SD (1992). Platelet activating factor receptors and signal transduction mechanisms. *FASEB J* **6:** 2296–2301.

Talley AK, Dewhurst S, Perry SW, Dollard SC, Gummuluru S, Fine SM, New D, Epstein LG, Gendelman HE, Gelbard HA. (1995). Tumor necrosis factor alpha-induced apoptosis in human neuronal cells: protection by the antioxidant *N*-acetylcysteine and the genes *bcl*-2 and *crm*A. *Mol Cell Biol* **15:** 2359–2366.

Tanaka M, Sotomatsu A, Yoshida T, Hirai S, Nishida A (1994). Detection of superoxide production by activated microglia using a sensitive and specific chemiluminescence assay and microglia-mediated PC12h cell death. *J Neurochem* **63:** 266–270.

Tardieu M, Hery C, Peudenier S, Boespflug O, Montagnier L. (1992). Human immunodeficiency virus type-1 infected monocytic cells can destroy human neural cells after cell-to-cell adhesion. *Ann Neurol* **32:** 11–17.

Toru-Delbauffe D, Baghdassarian-Chalaye D, Gavaret JM, Courtin F, Pomerance M, Pierre M. (1990). Effect of transforming growth factor β1 on astroglial cells in culture. *J Neurochem* **54:** 1056–1061.