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 (e.g., 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.

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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; Paudenier 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 (Pian 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 neuropathic 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 (astroglisis) 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 (e.g., 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
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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 (e.g., 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 is also 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, i.e., 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 MK-801 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 neurotoxic, 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 et al., 1995a). The diagram (Figure 1) depicts 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,
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.

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