9 The Role of Glia in Excitotoxicity and Stroke

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Abstract: Neurons are highly integrated both anatomically and metabolically with glial cells, and thus glial cells have a major influence on neuronal survival in ischemia and excitotoxicity. Of the three types of glia in the central nervous system—astrocytes, oligodendrocytes, and microglia—the role of astrocytes in excitotoxicity and ischemia has been best characterized. Under different settings, astrocytes can both limit or contribute to excitotoxic neuronal death. Astrocytes also influence oxidative neuronal injury and contribute to neuronal demise through secretion of nitric oxide and cytokines. Microglia, the resident macrophages of the CNS, can also have both deleterious and salutary effects on neuronal survival. Activated microglia can kill neurons, but on the other hand normal microglial function is probably required for brain remodeling after injury. Interactions between microglia and astrocytes engender an additional layer of complexity to these post-ischemic processes.

List of Abbreviations: AA, arachidonic acid; Ala, alanine; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AMPA/KAR, AMPA/kainate receptor; APP, amyloid precursor protein; ATP, adenosine triphosphate; BCAA, branched amino acid; BDNF, brain-derived neurotrophic factor ; bFGF, basic fibroblast growth factor; CDNF, ciliary neurotrophic factor; COX-2, cyclooxygenase; cPLA2, cytoplasmic phospholipase A; DNA, deoxyribonucleic acid; iNOS, inducible nitric oxide synthase; GDNF, glial cell-derived neurotrophic factor; Glu, glutamate; Gln, glutamine; CNS, central nervous system; EAAT, excitatory amino acid transporter; GABA, gamma-aminobutyric acid; GLAST, glial glutamate and aspartate transporter; GLT-1, glial glutamate transporter; GlyT, glycine transporter; GM-CSF, granulocyte-magrophage colony-stimulating factor; GSH, glutathione; HIF-1, hypoxia-inducible factor-1; HO-1, heme oxygenase -1; ICAM-1, intracellular adhesion molecule-1; ICE, interleukin-1 converting enzyme; IGF-I, insulin growth factor I; IL, interleukin; JAK-2, janus tyrosine kinase-2; αKG, α-ketoglutarate; KIC, α-ketoisocaproate; MAPK, mitogen-activated protein kinase; M-CSF, macrophage colony-stimulating factor; MHC, major histocompatibility complex; MIP-1, macrophage inflammatory protein-1; MMP, matrix metalloproteases; NF-κB, nuclear factor-kappaB; NGF, nerve growth factor; NMDA, N-methyl-D-aspartate; NT-3, neurotrophin-3; PA, plasminogen activator; PARP-1, poly(ADP-ribose) polymerase-1; PDGF, platelet derived growth factor; PG, prostaglandin; PKC, protein kinase C; PYK2, proline-rich protein tyrosine kinase; Pyr, puryvate; RANTES, regulation on activation normal T-cell expressed and secreted; SAT, sodium-coupled amino acid transporter; TCA, tricarboxylic acid cycle; TGF, transforming growth factor; TIMP, tissue-specific inhibitor of metalloproteinase; TNF, tumor necrosis factor; t-PA, tissue-specific plasminogen activator; u-PA, urokinase-plasminogen activator; VEGF, vascular endothelial growth factor; VSOAC, volume-sensitive organic anion channel

1 Introduction

Excitotoxic neuronal death is triggered by the sustained activation of glutamate receptors. During brain ischemia, glutamate receptor activation results from massive, sustained elevation in extracellular glutamate concentrations. Neurons are highly integrated both anatomically and metabolically with glial cells, and accordingly the glia have a major impact on neuronal survival in ischemia and excitotoxicity. Of the three types of glia in the central nervous system (CNS)—astrocytes, oligodendrocytes, and microglia—the role of astrocytes in excitotoxicity and ischemia has been best characterized. Under different settings, astrocytes can both limit or contribute to excitotoxic neuronal death. Microglia, the resident macrophages of the CNS, can also contribute to injury resulting from excitotoxicity and stroke, particularly in the postischemic period. The mechanisms by which these glial cell types influence ischemic neuronal survival will be discussed in turn.

2 Excitotoxicity in Stroke

Reductions in blood flow to less than roughly 20% of normal for more than a few minutes leads to a massive increase in extracellular glutamate (Benveniste et al., 1984). The extent and duration of this increase depend

on the severity and duration of the ischemic insult. With ischemia of 10-min duration, there is a transient rise of glutamate followed by a second higher peak of increase during reperfusion (Caragine et al., 1998). Studies with glutamate receptor antagonists have conclusively shown the importance of this cell death pathway in animal models of stroke (Simon et al., 1984; Meldrum et al., 1987; Choi, 1988; Ozyurt et al., 1988; Swan and Meldrum, 1990; Le Peillet et al., 1992). As might be expected, however, the efficacy of these agents is much decreased when administered at time points after glutamate elevations have occurred.

2.1 Mechanisms of Glutamate-Induced Cell Death

Glutamate binding to neuronal *N*-methyl-D-aspartate (NMDA) type glutamate receptors triggers an influx of Na^+, K^+, and Ca^{2+}, which, if sustained, can lead to neuronal death. Key events in the excitotoxic cell death pathway are dysregulation of intracellular calcium homeostasis and the generation of reactive oxygen species. Reactive oxygen species are formed especially during reperfusion after ischemia and include superoxide, nitric oxide, peroxynitrite, and hydrogen peroxide. These agents can damage many critical cell components, but in particular they damage DNA. DNA damage, in turn, can lead to either apoptotic or necrotic cell death.

Glutamate is not the only factor determining NMDA receptor activity. NMDA receptor activity is positively modulated by glycine and D-serine (White et al., 2000) and neuronal membrane depolarization (Novelli et al., 1988; Greene and Greenamyre, 1996). Conversely, NMDA receptor activity is attenuated by coactivation of neuronal GABA and purinergic receptors (Muir et al., 1996; Ortinau et al., 2003).

3 Astrocyte Modulation of Glutamate Neurotoxicity

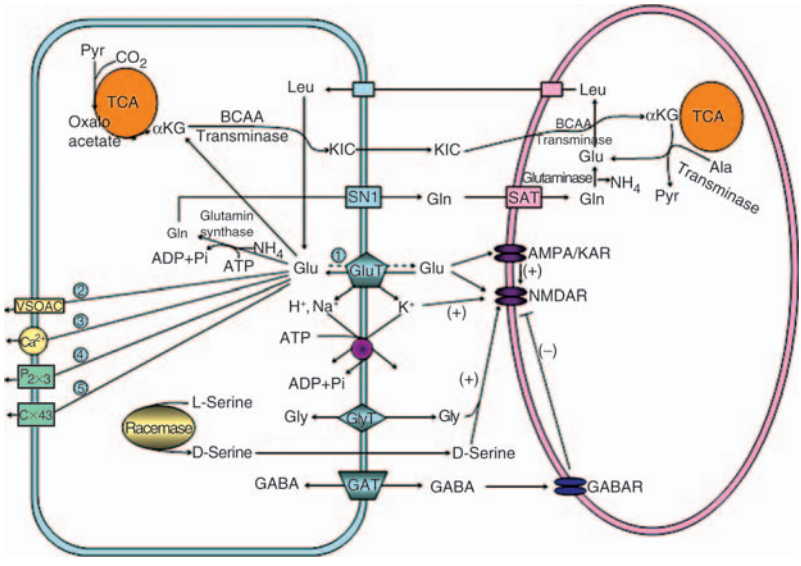
3.1 Astrocyte Glutamate Uptake

Glutamate uptake is the most well-characterized mechanism by which astrocytes influence neuronal survival during ischemia, and this topic has been the subject of recent thorough reviews (Schousboe et al., 1997; Robinson, 1998; Vandenberg, 1998; Anderson and Swanson, 2000; Danbolt, 2001). Clearance of glutamate from the extracellular space is accomplished primarily by Na^+-dependent transporters localized on astrocytes (*Figure 9-1*). There are two main subtypes of glutamate transporters expressed by astrocytes. These were originally cloned from the rat brain and termed GLAST; glial glutamate and aspartate transporter and GLT-1; glial glutamate transporter. Human homologs of these transporters are termed EAAT1 and EAAT2; excitatory amino and transporter 1 and 2 respectively. In cortical cultures, neuronal vulnerability to glutamate is 100-fold greater in astrocyte-poor cultures than in cultures with abundant astrocytes (Rosenberg and Aizenman, 1989), suggesting that uptake by astrocytes can be a limiting factor in glutamate neurotoxicity. The dominant role of astrocyte transporters is further supported by studies using genetic downregulation of transporter subtypes. Antisense "knockdown" of GLAST or GLT-1, but not the neuronal subtype EAAC1, produces excitotoxic neurodegeneration and increased susceptibility to seizures and injury (Rothstein et al., 1996; Tanaka et al., 1997; Watase et al., 1998). Likewise, genetic downregulation of the glial glutamate transporter GLT-1, but not the neuronal glutamate transporter EAAC1, was shown to exacerbate ischemic neuronal damage in the rat brain (Rao et al., 2001), thereby establishing the importance of astrocyte uptake for neuronal survival during ischemia.

Glutamate uptake is energetically costly, requiring roughly 1 ATP per glutamate molecule transported. Given the high energetic cost of glutamate uptake, it is not surprising that complete ischemia leads to abrupt cessation of glutamate uptake and a massive increase in extracellular glutamate concentrations (Benveniste et al., 1984; Choi, 1988; Swanson et al., 1994). During incomplete ischemia, however, continued supply of glucose can fuel continued ATP production by glycolysis, even in the absence of oxygen. Glycolytic ATP production appears sufficient to fuel glutamate uptake in astrocytes (Hakim, 1987; Swanson, 1992; Swanson et al., 1994). However, acidosis is a significant factor determining the ability of astrocytes to maintain ATP levels in the absence of oxygen delivery, and the ability of astrocytes to maintain glutamate uptake during

Figure 9-1

Astrocyte modulation of glutamate excitotoxicity. Neuronal glutamate (Glu) is formed chiefly from astrocyte-derived glutamine (Gln). Gln is formed in astrocytes from glutamate by glutamine synthase and then released from astrocytes via system N transporters (SN1). Gln is taken up by neurons through Na^+-coupled amino acid transporter (SAT). In neurons, Gln is converted back to Glu by glutaminase. Neuronal Glu is also formed from astrocyte-derived α-ketoisocaproate (KIC). KIC is formed in astrocytes by the action of branched amino acid (BCAA) transaminase, whereby leucine (Leu) and α-ketoglutarate (αKG) are converted to KIC and glutamate. KIC is used by neurons for the reverse reaction as an anaplerotic reaction to replenish tricarboxylic acid (TCA) cycle intermediates that are expended for amino acid neurotransmitter release. Astrocyte uptake of glutamate (Glu) by glutamate transporters (GluT) limits glutamate access to neuronal NMDA receptors (NMDAR). The transporters link glutamate transport to the cotransport of 3 Na^+ and 1 H^+ and the counter-transport of 1 K^+ ion. Uptake is thus metabolically coupled to the astrocyte plasma membrane Na^+/K^+-ATPase. NMDA receptor activity is positively modulated by D-serine produced by L-serine racemase in astrocytes, and by glycine, which is actively transported by astrocytes (GlyT). Glutamate binding to NMDA receptor is also positively modulated by neuronal membrane depolarization induced by glutamate activation of AMPA/kainite receptors (AMPA/KAR) and by increased extracellular K^+. Astrocyte uptake of glycine, GABA, and glutamate (1) can also proceed in the reversal direction during astrocyte energy failure. Astrocytes can also release glutamate through volume-sensitive organic anion channels (VSOACs) (2), by Ca^{2+}-dependent, vesicular release in response to various signaling molecules (3), by ATP-gated P2x7 receptor channels (4), and by unpaired connexin-43 hemichannels (Cx-43) (5). Puryvate (Pyr); alanine (Ala); phosphate (Pi)



hypoxia is markedly impaired by acidosis over the pH range resulting from ischemia (Swanson et al., 1995). Oxidative stress-induced activation of poly(ADP-ribose) polymerase-1 (PARP-1) in astrocytes may additionally compromise energy supply, in part, by limiting glycolysis (Ying et al., 2002). PARP-1 activation has been shown to impair astrocyte glutamate uptake in vitro (Swanson et al., 2002), suggesting that this mechanism may also contribute to impaired glutamate uptake during and after ischemia.

Glutamate uptake capacity is dynamically regulated. Astrocytes in culture can rapidly increase glutamate transport capacity in response to elevated extracellular glutamate concentrations by a process mediated by glutamate-induced translocation of glutamate transporters from intracellular space to plasma membranes (Duan et al., 1999; Robinson, 2002); however, this process is unlikely to be an important determinant of

uptake capacity during ischemia in which energy production is the rate-limiting factor. On the other hand, several factors can downregulate astrocyte glutamate transport, and these may further compromise glutamate uptake under ischemic conditions. Reactive oxygen species, particularly peroxynitrite, can reduce glutamate transporter function (Pogun et al., 1994; Volterra et al., 1994; Trotti et al., 1996; Trotti et al., 1998; Chen et al., 2000), possibly through interactions with specific protein sulfhydryl groups. Arachidonic acid (Trotti et al., 1995; Zerangue et al., 1995), endothelin-1 (Leonova et al., 2001), and zinc (Vandenberg et al., 1998) are also released during ischemia and can downregulate or inhibit astrocyte glutamate uptake.

3.2 Astrocyte Glutamate Release

Glutamate transporters, like other transporters, are capable of moving substrates in both inward and outward directions (Szatkowski et al., 1990). In the setting of ATP depletion, as occurs during severe ischemia, the ATP-supported membrane gradients collapse. This results not only in cessation of uptake but also efflux of glutamate via uptake reversal. ATP depletion has been shown to cause uptake reversal in primary astrocyte cultures (Gemba et al., 1994; Longuemare and Swanson, 1995), retina (Zeevalk et al., 1998), spinal cord and brain slice preparations (Li et al., 1999), and in the intact brain (Seki et al., 1999). These studies suggest that the rise in extracellular glutamate in the brain that occurs during energy failure may be due not only to failure of astrocyte glutamate uptake but also to glutamate efflux from astrocytes via reversal of the astrocyte glutamate transporters. However, there is controversy as to whether it is astrocytes or neurons that are the primary source of glutamate released by uptake reversal during ischemia. Studies in brain slice preparations suggest that glutamate efflux from neurons may be quantitatively more important, particularly at early time points (Rossi et al., 2000; Danbolt, 2001; Hamann et al., 2002). On the other hand, in vivo microdialysis studies have shown that dihydrokainate, which preferentially blocks the astrocyte transporter GLT-1, significantly attenuates the rise of extracellular glutamate that occurs during brain ischemia (Seki et al., 1999; Dawson et al., 2000).

Glutamate efflux from astrocytes can also occur by several other routes. Astrocyte swelling induced by elevated extracellular potassium or other factors can induce glutamate efflux via volume-sensitive anion channels (Kimelberg et al., 1990; Rutledge et al., 1998; Longuemare et al., 1999). Astrocytes can also release glutamate in response to bradykinin (Jeftinija et al., 1996) and prostaglandin E2 (Bezzi et al., 1998) in a manner that is sensitive to botulinum B neurotoxin. Glutamate release from astrocytes can be induced by extracellular ATP activation of astrocyte $P2X_7$ receptor channels (Duan et al., 2003). A recent study also reports glutamate release through unpaired connexin-43 hemichannels (Ye et al., 2003). However, the significance of glutamate release during ischemia by routes other than uptake reversal and volume-sensitive anion channels remains to be established.

3.3 Astrocyte Support of Neuronal Glutamate Release

Glutamine released by astrocytes is the major precursor for neurotransmitter glutamate synthesis. Glutamate released at neuronal synapses is taken up by surrounding astrocytes (McLennan, 1976; Rothstein et al., 1996) and converted by glutamine synthetase to glutamine. Glutamine is in turn released from astrocytes for uptake by neurons (Bradford et al., 1978; Waniewski and Martin, 1986; Broer and Brookes, 2001; Chaudhry et al., 2002), where it is metabolized back to glutamate and packaged into synaptic vesicles. Pharmacological inhibition of glutamine synthetase reduces brain glutamine levels, reduces K^+-evoked glutamate release (Paulsen and Fonnum, 1989), and has been reported to reduce infarct size in a focal ischemia model of stroke (Swanson et al., 1990). The cycle of glutamate carbon through neurons and astrocytes is supplemented by a similar flux of branched-chain amino acids in which transamination of leucine and α-ketoglutarate in astrocytes produces glutamate and α-ketoisocaproic acid (Yudkoff et al., 1996). α-Ketoisocaproic acid is released to the extracellular fluid and may be taken up by neurons as a substrate for the reverse reaction.

3.4 Astrocyte Regulation of Extracellular GABA, Glycine, and D-Serine

GABA is an inhibitory neurotransmitter that can attenuate the effects of glutamate receptor stimulation (Muir et al., 1996). GABA transporters are expressed by astrocytes, and pharmacological inhibitors that target astrocyte GABA uptake exhibit anticonvulsant activity (Schousboe, 2000). However, GABA transporters are also widely expressed by neurons (Palacin et al., 1998; Gadea and Lopez-Colome, 2001b), and the specific contribution of astrocyte GABA uptake under normal or pathological conditions remains uncertain (Schousboe, 2000).

Glycine and D-serine act as positive modulators of signal transduction at NMDA-type glutamate receptors (Wroblewski et al., 1989), and these compounds can significantly influence glutamate excitotoxicity during ischemia (Foster et al., 1990). Astrocytes can both release and take up glycine from the extracellular space through the GlyT1 glycine transporter (Gadea and Lopez-Colome, 2001a; Supplisson and Roux, 2002). Interestingly, the kinetic properties of GlyT1 differ from those of the dominant neuronal transporter, GlyT2 (Supplisson and Roux, 2002). Glycine uptake by GlyT2 is coupled to the cotransport of 3 Na^+ and 1 Cl^-, whereas uptake by astrocyte GlyT1 is coupled to 2 Na^+ and 1 Cl^-. This allows neurons to maintain a higher intracellular glycine concentration, a factor that may facilitate neuronal loading of glycine into synaptic vesicles. Higher neuronal intracellular glycine concentrations are also favored by a kinetic restraint on reverse operation of the GlyT2 transporters. By contrast, astrocyte GlyT1 transporters readily function in "reverse" mode, and because these transporters operate near equilibrium, one function of astrocyte GlyT1 transporters may be to allow efflux of glycine into the extracellular space. D-Serine is generated from L-serine by racemase that is exclusively expressed in the protoplasmic astrocytes that typically ensheath synapses (Wolosker et al., 1999; Snyder and Ferris, 2000). Activation of glutamate receptors on astrocytes leads to activation of L-serine racemase and astrocyte release of D-serine. Because D-serine, like glycine, is a positive modulator of neuronal NMDA receptors, this process may contribute to excitotoxic neuronal death (Foster et al., 1990; Snyder and Ferris, 2000).

4 Astrocyte Influences on Downstream Events in Excitotoxic Cell Death

4.1 Astrocyte Influences on Oxidative Neuronal Injury

Reactive oxygen species are generated by several mechanisms during ischemia and ischemia-reperfusion, and the resulting oxidative stress constitutes a major mechanism of ischemic neuronal injury (Chan, 2001). Glutathione (GSH) is the principal antioxidant in brain (Dringen, 2000), and brains depleted of glutathione are sensitized to ischemic injury (Mizui et al., 1992). Evidence suggests that astrocytes contain greater concentrations of glutathione and enzymes involved in glutathione metabolism than neurons (Slivka et al., 1987; Yudkoff et al., 1990; Makar et al., 1994; Wilson, 1997). Similarly, glucose flux through the pentose phosphate pathway in cultured astrocytes is twice that of cultured neurons and increases three times as much as in neurons during H_2O_2 exposure (Ben-Yoseph et al., 1996). These factors suggest that astrocytes are more capable of scavenging reactive oxygen species than neurons and suggest that oxidant-scavenging mechanisms in astrocytes may function to support neuronal survival. In support of this idea, neurons cultured in the presence of astrocytes are more resistant than neurons cultured alone to injury induced by nitric oxide, hydrogen peroxide, or superoxide (Desagher et al., 1996; Lucius and Sievers, 1996; Tanaka et al., 1999b; Xu et al., 1999). Moreover, astrocytes depleted of glutathione show reduced ability to protect neurons from oxidant injury (Drukarch et al., 1997; McNaught and Jenner, 1999; Chen et al., 2001). The effects of astrocyte glutathione on neuronal resistance to oxidative stress may be mediated, in part, by maintaining neuronal glutathione levels. Astrocytes contribute to neuronal glutathione content by an indirect route: glutathione is released by astrocytes and cleaved to the dipeptide CysGly (Dringen et al., 1999), which in turn is cleaved to free cysteine for uptake into neurons as a substrate for neuronal GSH synthesis (Dringen et al., 2001).

Like glutathione, ascorbate is an important antioxidant that is present in the brain at millimolar concentrations (Rice, 2000). Ascorbate can directly react with oxidants and can also serve as a cofactor for reducing

(recycling) oxidized glutathione and α-tocopherol. Evidence suggests an ascorbate cycle between neurons and astrocytes. Neurons release oxidized ascorbate (dehydroascorbate) for uptake by astrocytes, which then convert it to ascorbate and, in turn, release ascorbate for neuronal uptake (Siushansian and Wilson, 1995; Siushansian et al., 1997; Wilson, 1997; Daskalopoulos et al., 2002). Dehydroascorbate passes across the blood–brain barrier readily and is converted to ascorbate, presumably in astrocytes. Treatment with dehydroascorbate has been shown to attenuate ischemic brain injury, suggesting that this cycle is a significant aspect of astrocyte–neuron interaction during ischemia (Huang et al., 2001).

4.2 Erythropoietin Released by Astrocytes Blocks Ischemic Neuronal Death

The glycoprotein hormone erythropoietin is produced in the CNS as well as in the periphery and has substantial effects on neuronal survival after ischemia (Buemi et al., 2003). Evidence suggests that erythropoietin production in the CNS is localized primarily to astrocytes (Masuda et al., 1994; Nagai et al., 2001; Ruscher et al., 2002), although some studies also report expression by neurons (Bernaudin et al., 2000). Erythropoietin activation of neuronal erythropoietin receptors blocks cell death pathways triggered by excitotoxicity and by combined oxygen/glucose deprivation in neuronal cultures (Morishita et al., 1997; Sakanaka et al., 1998; Ruscher et al., 2002; Wen et al., 2002) and reduces neuronal death after ischemia in vivo (Sakanaka et al., 1998; Siren et al., 2001; Wen et al., 2002). Neurons stimulated by erythropoietin show inhibition of caspase-9, increased expression of Bcl-2 family antiapoptotic factors, increased antioxidant capacity, and several other changes that may favor survival during and after ischemia (Calapai et al., 2000; Kawakami et al., 2001; Ruscher et al., 2002; Wen et al., 2002). These effects are mediated by activation of Janus tyrosine kinase-2 (JAK-2) pathway, which in turn activates Akt (protein kinase B) and the transcription factors nuclear factor-kappa B (NF-κB) and STAT5 (Digicaylioglu and Lipton, 2001; Kawakami et al., 2001; Siren et al., 2001; Ruscher et al., 2002). Astrocyte production of erythropoietin is increased after ischemic stress as a result of hypoxia-inducible factor-1 (HIF-1) activation, and postischemic erythropoietin production has been identified as a mediator of ischemic tolerance induced by sublethal cerebral ischemia (Ruscher et al., 2002; Prass et al., 2003).

5 The Astrocyte Inflammatory Response

The reaction of astrocytes to ischemia and other brain insults is similar to the inflammatory response of peripheral tissues. Within a few hours of virtually any type of brain injury, surviving astrocytes in the affected region begin to exhibit hypertrophy and proliferation (Ridet et al., 1997). This response, termed reactive astrogliosis, is fortified by migration of microglia and macrophages to the damaged area. Reactive astrocytes increase the expression of their structural proteins, GFAP and vimentin (Eng et al., 2000), as well as many other proteins. Cu/Zn superoxide dismutase, glutathione peroxidase, and metallothionein are increased in reactive astrocytes after ischemia (Liu et al., 1993; Takizawa et al., 1994; Neal et al., 1996; Campagne et al., 2000), indicating an enhanced capacity to neutralize reactive oxygen species. Similarly, astrocytes express the inducible form of heme oxygenase-1 (HO-1) in response to ischemia and other brain insults (Geddes et al., 1996; Takeda et al., 1996). HO-1 is the first step of heme metabolism and may be important in preventing heme iron participation in metal-catalyzed free radical production, particularly after conditions such as trauma or hemorrhagic stroke that liberate hemoglobin into the brain parenchyma. Other aspects of the astrocyte inflammatory response, such as nitric oxide and matrix metalloproteinase (MMP) expression, may be adaptive in settings such as infection but contribute to delayed neuronal death in settings such as stroke. (R)-(-)-2-propyloctanoic acid, an agent that suppresses the astrocyte inflammatory response, has been shown to reduce infarct size expansion when administered after cerebral ischemia (Matsui et al., 2002; Tateishi et al., 2002), although the detailed mechanism of this effect is not yet established.

5.1 Astrocyte Expression of iNOS and Cytokines

Expression of inducible nitric oxide synthase (iNOS) is a salient aspect of the astrocyte inflammatory response (Endoh et al., 1993). Astrocyte iNOS expression is detectable within a few hours of ischemia and is maximal within 2–3 days (Iadecola et al., 1995; Nakashima et al., 1995). Nitric oxide is a reactive oxygen species that can contribute to neuronal cell death by potentiating glutamate excitotoxicity (Hewett et al., 1994) and by several other mechanisms (Dawson and Dawson, 1998). Mice that are genetically deficient in iNOS exhibited smaller infarct size than control, wild-type mice (Iadecola et al., 1997), although this effect may also be due to reduced iNOS expression in microglia and invading macrophages.

Astrocytes stimulated by ischemic injury also produce many cytokines, including tumor necrosis factors (TNF-α, -β), interleukins (IL-1, -6, -10), and interferons (IFN-α, -β) (Feuerstein et al., 1998; Dong and Benveniste, 2001). The net effect of individual cytokines can be difficult to establish because the effects of many cytokines are strongly influenced by one another and because most cytokines have pleiotropic and cell-type specific effects. For example, IL-6 and TNF-α have been shown to promote demyelination, thrombosis, leukocyte infiltration, and blood–brain barrier disruption (Feuerstein et al., 1998; Dong and Benveniste, 2001). On the other hand, IL-6 has been shown to protect against ischemic and excitotoxic injury (Maeda et al., 1994; Ali et al., 2000), and hippocampal neurons treated with TNF-α are less vulnerable to substrate deprivation and excitotoxicity (Cheng et al., 1994). The specific contribution of astrocyte cytokine release to these processes in vivo remains to be established.

A recently identified regulator of inflammatory processes is PARP-1. PARP-1 is known to function in DNA repair (Ha and Snyder, 2000) but also functions as a coactivator of NF-κB (Kameoka et al., 2000; Chiarugi and Moskowitz, 2003), a transcription factor that plays an important role in the expression of inflammatory mediators. The formation of nuclear PARP-1/NF-κB complex has been shown to enhance DNA binding of NF-κB and transcription of NF-κB-regulated genes. Whether the enzymatic activity of PARP-1 is essential in this process remains unsettled (Chang and Alvarez-Gonzalez, 2001; Ullrich et al., 2001; Chiarugi and Moskowitz, 2003). Downregulation of PARP-1 causes a large reduction in brain infarct size after ischemia (Eliasson et al., 1997). It is likely that this large effect is due, in part, to an attenuated astrocyte inflammatory response, but there has been little study on this point.

5.2 Astrocyte Release of Matrix Metalloproteases

Cytokine and other proinflammatory stimuli of astrocytes in culture have been shown to induce activation of MMPs. MMPs are endopeptidases that are able to cleave protein components of extracellular matrix and are thus associated with tissue remodeling during developmental and pathological processes (Gottschall and Deb, 1996; Lee et al., 2003). Cytokines also modulate components of the plasminogen activator system, urokinase type plasminogen activator (u-PA) and tissue-specific plasminogen activator (t-PA), which are released by astrocytes and other cell types. These agents promote formation of (active) plasmin from (inactive) plasminogen and thus mediate cleavage of pro-MMPs into their active form (Faber-Elman et al., 1995; Tang et al., 2000). MMPs have been shown to not only cleave cytokines like TNF-α and IL-1β from their immature pro forms into their active mature forms (Gearing et al., 1995; Schonbeck et al., 1998; English et al., 2000) but also counterbalance the IL-1β activity by degrading the mature cytokine (Ito et al., 1996). Astrocytes release both MMP-2 and -9 (Rosenberg et al., 2001). Cerebral ischemia has been shown to increase MMP-2 activity in endfeet of rat brain astrocytes at time points (3 h and 5–21 days) relevant to changes in blood–brain barrier permeability, suggesting that astrocyte MMP-2 contributes both to blood–brain barrier opening and to later repair processes (Rosenberg et al., 2001). Astrocytes also express a tissue-specific inhibitor of metalloproteinase-1 (TIMP-1) and inhibitors of plasminogen activators (Tang et al., 2000).

6 Astrocyte Trophic Factor Release

Astrocytes release a variety of trophic factors under normal conditions, and these are likely to influence neuronal survival and plasticity after brain injury. These trophic factors include nerve growth factor (NGF),

basic fibroblast growth factor (bFGF), transforming growth factor beta (TGF-β), platelet-derived growth factor (PDGF), brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), and others (Ridet et al., 1997). Reactive astrocytes increase the expression of several of these, notably NGF, bFGF, BDNF, and neuregulins, which can stimulate neurite outgrowth (Schwartz and Nishiyama, 1994; Strauss et al., 1994; Mocchetti and Wrathall, 1995; Tokita et al., 2001). Reactive astrocytes also overexpress neuropilin-1 and vascular endothelial growth factor (VEGF), which act in concert to promote angiogenesis after cerebral ischemia (Zhang and Chopp, 2002). Although these findings suggest that astrocytes may play an important role in functional recovery after stroke, there has not yet been direct confirmation of this.

The demonstration of accelerated neurogenesis after cerebral ischemia has led to the proposal that newly formed neurons may contribute to functional recovery after stroke (Liu et al., 1998; Jiang et al., 2001; Jin et al., 2001). Factors regulating neurogenesis remain poorly understood, but the essential role of astrocytes in neuronal differentiation during development suggests that astrocytes may similarly be important in regulating neurogenesis. In support of this idea, it has recently been shown that astrocytes induce neurogenesis from adult neural stem cells in culture (Song et al., 2002). Astrocytes are also likely to play a central role in angiogenesis after stroke. Astrocytes promote angiogenesis through the release of VEGF, angiopoietin-1, and epoxyeicosatrienoic acids (Salhia et al., 2000; Acker et al., 2001; Chow et al., 2001; Zhang and Harder, 2002). Studies of brain development show a close relationship between astrocyte expression of angiogenic factors and microvessel development (Acker et al., 2001), and studies of brain injury confirm a temporal and spatial correlation between reactive astrocytosis, VEGF immunoreactivity, and microvascular density.

7 Microglia Effects on Ischemic Injury

Microglia are the resident macrophages of the CNS. They are derived from bone marrow precursors and have a slow interchange with the circulating macrophage pool (Perry and Gordon, 1988; Kaur et al., 2001). Activated microglia express surface markers similar to macrophages, but microglia have faster reaction time, better mobility, and greater proliferation capacity than macrophages (Giulian et al., 1995; Lyons et al., 2000). Microglia in the normal brain have a highly branched "ramified" morphology that can rapidly transform into activated, amoeboid morphology in response to stressors such as ischemia (Morioka et al., 1991; Kreutzberg, 1996; Raivich et al., 1998). Microglia undergo massive activation after stroke that remains detectable for several weeks (Jorgensen et al., 1993). Ischemia-induced changes in extracellular ion composition are thought to be a trigger of microglial activation (Boucsein et al., 2000; Kato and Walz, 2000; Schipke et al., 2002). Microglial activation is also induced and modulated by glutamate binding to glutamate receptors (Gottlieb and Matute, 1997; Biber et al., 1999; Noda et al., 2000; Tikka and Koistinaho, 2001; Tikka et al., 2001; Taylor et al., 2003).

7.1 Neurotoxic Effects of Activated Microglia

In vivo and in vitro studies show that microglial activation precedes neuronal death (Morioka et al., 1991; Gehrmann et al., 1992; Tikka and Koistinaho, 2001), and the timing and location of microglial activation correlates with neuronal death (Morioka et al., 1991, 1992; Jorgensen et al., 1993; Zhang and Fedoroff, 1996). Inhibition of microglial activation improves neuronal survival after excitotoxic and ischemic insults (Rogove and Tsirka, 1998; Yrjanheikki et al., 1999). Activated microglia can promote neuronal death by releasing glutamate (Piani et al., 1991) and the NMDA receptor modulator D-serine (Wu et al., 2004). Microglia also release cytokines, free radicals, and proteases that can affect neuronal viability directly or by modulating function of other brain cells (*Table 9-1*). For example, microglia show early expression of IL-1β after excitotoxic or ischemic insults (Davies et al., 1999; Pearson et al., 1999). Inhibition of IL-1β reduces and the administration of IL-1β increases neuronal death in these settings (Loddick et al., 1996; Lawrence et al., 1998; Davies et al., 1999; Pearson et al., 1999). Activated microglia release nitric oxide through expression of iNOS and may also promote neuron death by release of MMP-9 (Kauppinen and

Table 9-1
Molecules expressed and produced by microglia

Class	Name	References
Surface antigens	MHC I and II, CD4, CD8, CD11b, CD68, ICAM-1	Perry and Gordon (1987), Morioka et al. (1992), Schroeter et al. (2001)
Cytokines and chemokines	ILs (IL-1α and β, -3, -5, -6, -8, -10, -12, -16), TNF-α, M-CSF, GM-CSF, MIP-1, PGs (D_2, E_2, F_2), RANTES	Giulian and Ingeman (1988), Gebicke-Haerter et al. (1989), Thery et al. (1990), Hetier et al. (1991), Sawada et al. (1993), Chao et al. (1995b), Gottschall et al. (1995), Murphy et al. (1995), Sheng et al. (1995), Walker et al. (1995), Aloisi et al. (1997), Hu et al. (1999), Janabi et al. (1999), Schwab et al. (2001)
Enzymes	COX-2, cPLA2, iNOS, ICE, MAPKs (p38, p44/42, JNK), PYK2, PKCs (βI, βII, θ, δ, η, ζ, ι), t-PA, u-PA, PA, MMPs (MMP-1, -2, -3, -9)	Nakajima et al. (1992a), Nakajima et al. (1992b), Gottschall et al. (1995), Paakkari and Lindsberg (1995), Bhat et al. (1996), Clemens et al. (1996), Fiebich et al. (1996), Maeda and Sobel (1996), Tsirka et al. (1997), Bhat et al. (1998), Zhang et al. (1998), Koponen et al. (2000), Tian et al. (2000)
Receptors and transporters	Glutamate receptors (GluR4, NR1, mGluR3, mGluT5), glutamate transporter (EAAT2/GLT-1), Immunoglobulin G receptor, thrombin receptors, purinergic receptors	Kondo et al. (1995), Ferrari et al. (1997), Gottlieb and Matute (1997), Biber et al. (1999), Moller et al. (2000), Noda et al. (2000)
Free radicals	Nitric oxide, peroxynitrite, superoxide anions	Chao et al. (1992), Chao et al. (1995a), Colton et al. (1996)
Neurotoxins	AA, APP, glutamate, quinolinic acid, L- and D-serine, unidentified excitotoxin	Piani et al. (1991), Banati et al. (1993), Giulian et al. (1993), Minghetti and Levi (1995), Heyes et al. (1996), Wu et al. (2004)
Growth and trophic factors	NGF, BFGF, TGF-α and -β, GDNF, NT-3, IGF-I	Mallat et al. (1989), Shimojo et al. (1991), Walker et al. (1995), Elkabes et al. (1996), Honda et al. (1999), O'Donnell et al. (2002)
Ion channels	Inward and outward rectifying K-channels	Lyons et al. (2000)

AA, arachidonic acid; APP, amyloid precursor protein; BFGF, basic fibroblast growth factor; COX-2, cyclooxygenase; cPLA2, cytoplasmic phospholipase A; iNOS, inducible nitric oxide synthase; GDNF, glial cell-derived neurotrophic factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; ICAM-1, intracellular adhesion molecule-1; ICE, interleukin-1 converting enzyme; IGF-I, insulin growth factor I; IL, interleukin; MAPK, mitogen-activated protein kinase; M-CSF, macrophage colony-stimulating factor; MHC, major histocompatibility complex; MIP-1, macrophage inflammatory protein-1; MMP, matrix metalloproteases; NGF, nerve growth factor; NT-3, neurotrophin-3; PA, plasminogen activator; PG, prostaglandin; PKC, protein kinase C; PYK2, proline rich protein tyrosine kinase 2; RANTES, regulation on activation normal T-cell expressed and secreted; TGF, transforming growth factor; TNF, tumor necrosis factor; t-PA, tissue-specific plasminogen activator; u-PA, urokinase-plasminogen activator

Swanson, 2004). Phagocytosis of neurons by activated microglia is commonly observed after brain ischemia, but this is likely a result rather than a cause of neuron death because microglia have not been observed to engulf healthy neurons (Kreutzberg, 1996; Streit, 2002). For example, spreading depression in the absence of ischemia does not lead into neuronal damage even though it does induce microglial activation (Gehrmann et al., 1993). Recent studies suggest that neurogenesis after ischemia is also impaired by activated microglia. Inhibition of microglial activation improves neurogenesis and facilitates survival of engrafted progenitor cells in rodent models (Ekdahl et al., 2003; Lacza et al., 2003; Sasaki et al., 2003).

7.2 Trophic and Neuroprotective Functions of Microglia

In addition to their cytotoxic actions, microglia can release a number of trophic factors that promote tissue repair and regeneration (Kreutzberg, 1996) (*Table 9-1*). These factors may be important determinants of outcome after brain ischemia. In cell culture studies, the presence of resting microglia enhances neuron survival and neurite outgrowth (Zhang and Fedoroff, 1996). Resting microglia also improve astrocyte survival in a cell culture model of ischemia through release of GDNF (Lee et al., 2004). In addition, microglia are capable of high-affinity, high-velocity glutamate uptake via their glutamate transporters (Kondo et al., 1995; Lopez-Redondo et al., 2000; Nakajima et al., 2001; van Landeghem et al., 2001), but the biological significance of this during ischemic conditions remains to be established.

In vivo, secretion of the neurotrophic factors GDNF and BDNF by activated microglia promotes dopaminergic sprouting in a model of striatal injury (Batchelor et al., 1999). Similarly, transplantation studies show that engrafted microglial cells in injured spinal cord promote neurite growth, although the mechanism remains to be established (Rabchevsky and Streit, 1997). Microglia increase expression of neurotrophic factors such as BDNF, IL-6, and TGF-β after ischemia (Lehrmann et al., 1998; Suzuki et al., 1999; Lee et al., 2002); however, there is as yet no direct evidence that microglia promote neuronal survival in this setting.

7.3 Interactions Between Microglia and Astrocytes

Astrocytes and microglia are both capable of influencing one another (Kim, 1996). Microglia in the vicinity of an ischemic infarct secrete cytokines and chemokines that recruit additional microglia to the infarct site (Kreutzberg, 1996). Microglial activation also induces astrocytic proinflammatory response (Giulian and Baker, 1985; Kreutzberg, 1996; Tanaka et al., 1999a; Hailer et al., 2001), and astrocytic glutamate transport is affected by nitric oxide and TNF-α released by activated microglia (Trotti et al., 1996; Gegelashvili and Schousboe, 1997; Bezzi et al., 2001). Conversely, astrocytes influence microglial activation and migration. Microglia in coculture with astrocytes or astrocyte-conditioned medium maintain the resting, ramified morphology, but assume the activated, amoeboid morphology in the absence of astrocytes (Giulian et al., 1995; Kloss et al., 1997; Tanaka et al., 1999a). This affect is mediated by TGF-β, M-CSF, and GM-CSF, as evidenced by the effects of neutralizing antibodies (Schilling et al., 2001). Astrocytes also stimulate microglial proliferation and migration after injury (O'Donnell et al., 2002; Zhang et al., 2003).

8 Summary

Astrocytes and microglia have dynamic interactions with neurons and with each other that influence outcome from stroke and other insults. These interactions involve energy metabolism, redox metabolism, neurotransmitter uptake and release, trophic factor support, and formation of toxic intermediaries. The complexity of these interactions precludes a simple dichotomous classification of glia as positive or negative modulators of brain injury. By the same token, however, these complexities suggest that specific interactions between these cell types may be targeted for therapeutic intervention.

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