Chapter 5
Glial Reactivity After Damage: Implications for Scar Formation and Neuronal Recovery

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It has been known for decades that acute neuronal damage caused by stroke, traumatic injury, infection, subarachnoid hemorrhage, and other central nervous system (CNS) insults sets in motion a complex cascade of events that ultimately leads to the formation of a long-lived glial scar (Fig. 5.1). The ability of glial scars to inhibit axon growth has provided strong motivation to determine the cellular and molecular mechanisms involved in their formation. Moreover, manipulation of injury-induced gliotic scars could be of great clinical relevance and, thus, this has become an important goal in the neurosciences.

PART I: THE CNS REACTION TO INJURY

In this review, we summarize the processes that lead to scar formation and examine what is known about the functional roles for two types of glial cells involved (astrocytes and microglia). We also examine one potential modifier of scar formation, thrombin, and its receptor, protease-activated receptor-1 (PAR-1). Because of space limitations, we do not consider in detail the processes of neuronal death, blood-brain barrier (BBB) breakdown, proteoglycan inhibition of axon growth, or the expression and roles of cytokines in neurodegeneration. We refer the interested reader to several excellent reviews on these topics (6, 17, 75, 233, 246).

Step 1: Neuronal Death

Common pathways of neuronal cell death in response to diverse insults, such as hypoxia, ischemia, or trauma, include early disruption of ion homeostasis, increased release and impaired uptake of neurotransmitters (such as glutamate), excessive neuronal activation, cellular swelling, intracellular entry of divalent cations, and release of nitric oxide and free radicals. These changes in cell physiology lead to both apoptotic and necrotic cell death, and set in motion the development of a gliotic scar (9, 14, 28).

Step 2: Inflammation

The first cell type to respond to injury is the microglia (Fig. 5.2). On activation, these CNS macrophages phagocytose apoptotic cells and necrotic debris; release proinflammatory cytokines, chemokines, and reactive nitrogen species; and up-regulate surface expression of specific receptors, such as major histocompatibility complexes (MHC) I and II. Microglial release of cytokines, such as tumor necrosis factor ƒÑ (TNF-ƒÑ) and interferon ƒ× (IFN ƒ×); and chemokines, such asƒnmacrophage inflammatory protein 1-ƒÑ (MIP1 ƒÑ) and interleukin-8 (IL-8), recruit peripheral white blood cells to the site of damage.

Breakdown of the BBB, which is composed of endothelial cells and astrocytes, occurs concurrently with microglial activation. This breakdown appears in response to the release of various cytokines, reactive oxygen species (ROS), glutamate, adenosine triphosphate (ATP), bradykinins, histamine, and nitric oxide from neurons, activated microglia, and the endothelial cells themselves. Breakdown of this barrier facilitates the translocation of plasma-derived

molecules into the brain (17). Several studies, including our own work, suggest that this influx of blood-derived molecules is a critical step in the formation of a glial scar. Consistent with this notion, areas of greatest glial scarring are often found near regions of the largest BBB breakdown (202). Moreover, breakdown of the BBB contributes to the posttraumatic inflammatory response by increasing extravasation of blood-borne neurotrophins, macrophages, and T- and B-lymphocytes, which may trigger further brain damage (17). Because CNS inflammation is largely propagated by microglia and infiltrating immune cells, neuroinflammation has a complicated effect on CNS health and recovery (72).

Step 3: Oligodendrocyte Precursor Proliferation

Oligodendrocyte precursor cells (OPC) are recruited by inflammation to the site of injury within 2 days of the injury, and their numbers increase for the following 2 weeks (124). Although these cells are activated by neuronal damage, proliferation requires at least some demyelination of neurons (67). Because of the close proximity of OPCs to synapses and nodes of Ranvier, as well as the involvement of OPCs in excitatory transmission, it is not clear whether OPCs are responding to a growth factor released by myelin sheath breakdown or whether OPCs are sensitive to changes in neuronal conduction (22, 40, 42, 123). Regardless of what causes their proliferation, expansion of OPCs inhibits neural cone growth, caused, in large part, by the release of inhibitory extracellular matrix molecules known as chondroitin sulfate proteoglycans (CS-PGs) (45, 72, 125). The family of large CS PGs found in the CNS consists of aggrecan; versican; neurocan; brevican; and phosphacan, a molecule that can exist in two forms: as the receptor tyrosine phosphatase or as the truncated secreted molecule DSD-1/phosphacan (72, 122). CS-PGs appear within a day after injury and persist for several months thereafter (145, 219). In addition to the observation that CS-PG expression is increased in areas of gliosis, it is also reported that axon regrowth stops where the CS-PGs are deposited (62, 63). Further, in vivo and in vitro assays show that blockade of CS-PG signal transduction is permissive for axonal growth (203).

Step 4: Astrocytic Activation

Astrocytes are involved in a wide range of important functions, such as physically supporting CNS vasculature, providing metabolic substrates to neuronal dendrites and synapses, and maintaining ionic and neurotransmitter balance in the extracellular space (165). In response to injury, astrocytes undergo many cellular changes, leading them to adopt a ¡§reactive¡¨ phenotype (Fig. 5.2). As with microglia, the astrocytic response to injury proceeds through several stages and depends on the extent of trauma. Soon after injury, there is a rapid increase in the synthesis of glial fibrillary acidic protein (GFAP) that can extend far from the actual site of damage (2). This is followed by the appearance of small and slender GFAP-positive processes, which in several days become fully stellarized, fibrillary astrocytes. Long-standing hypotheses suggest that reactive astrocytes create a physical barrier between damaged and healthy cells and re-establish an intact BBB (71, 187). However, given the wide array of signaling systems involved in the astrocyte response to injury, it seems likely that additional roles will emerge.

PART II: MICROGLIAL RESPONSES TO CNS INJURY

As the resident macrophages of the CNS, microglia are the primary source of innate and adaptive immune responses within the brain. They are main players in mediating neuroinflammatory cascades, by expressing and/or releasing a number of different cytokines, chemokines, and receptors (see Figure 8.31). Their ability to become activated throughout the course of neuropathic stimuli, such as invading pathogens, cell death, and hypoxia, allows microglia to

respond to, and, at times, contribute to, neuropathology. Although neuroprotective and restorative roles of reactive microglia in models of acute and chronic neuropathologies have been documented, examples of the detrimental effects of microglial activation are evident in recent studies (1, 20, 141, 157, 166, 210). Not only is rapid microgliosis considered the most immediate and harmful of glial responses in the pathogenesis of acute CNS trauma, prolonged microgliosis is also known to exacerbate continuing damage in various neurodegenerative illnesses (88).

Triggers and Regulators of Microgliosis

As illustrated in (Fig. 5.4), microgliosis is characterized by a complex set of events, including changes in microglial morphology, increased proliferation, migration to a site of damage, phagocytosis, antigen processing and presentation, up-regulation of numerous cell surface and secreted signaling molecules, and apoptosis. Substances that trigger microgliosis fall into three categories: 1) those that signal the invasion of foreign organisms, 2) those that signal local cellular damage and death, and 3) regulatory signals from other cells.

In the category of pathogen invasion signals, pathogen-associated molecular patterns, such as bacterial and viral surface proteins, sugars, and proteoglycans, as well as unmethylated DNA, are some of the most potent and reliable activators of microglia (60, 174). In the second category, cell damage and death trigger the presentation of cell injury signals, such as membrane exposure of phosphatidylserine, or the release of ATP and sialic acid¡Vcontaining glycosphingolipids (e.g., gangliosides), which induce proinflammatory cytokine and ROS production in microglia (153, 185). Additionally, hypoxia may be a direct signal for activation, because it triggers microglial expression of a proinflammatory cytokine, IL-1ƒÒ (111). Senile plaque¡Vassociated proteins, such as ƒÒ-amyloid and chromogranin A, are also strong signals that stimulate microglia (49, 52, 147). In the third category of regulatory signals, modulators from other cells, including certain surface molecules, cytokines, chemokines, and proteases, can activate and also regulate the function of microglia (see Figure 8.3). Interestingly, ATP is also an example of a regulatory signal released from astrocytes that can serve as a chemotactic, a mitogenic, and an apoptotic signal for microglia (100, 185, 229).

Microglial Migration and Proliferation

Migration of microglia to sites of infection or injury can be observed in culture in hippocampal slices and in murine models of brain damage (171, 178, 184). Several chemotactic signals mediate the recruitment of microglia, as well as other immune cells, to areas of brain damage and, thus, are essential for the spatial coordination of a local neuroimmune response. Chemokines, such as monocyte chemoattractant protein-1 (MCP-1/CCL2), RANTES/CCL5, MCP-2/IL-8/CCL8, IFN-ƒ×¡Vinducing protein-10 (IP-10/CXCL10), are a family of chemoattractants known to stimulate microglial and other immune cell migration (56, 181). CXCR3, a chemokine receptor for CXCL10/IP-10, has been shown to promote microglial movement to a lesion site in the entorhinal cortex and increase local neuronal loss (184). Among other molecules, ATP, ƒÒ-amyloid, and complement factor 5a are also found to induce microglial migration (100, 117, 171). Chemotaxis will be important in cases of localized brain injury, such as in lacunar strokes (Figure 8.5) , where damage in a small region may recruit outlying microglia to amplify local microgliosis. However, the role of chemotaxis in microglial response to global CNS insults is still unclear.

Cell death is thought to activate microglial proliferation in experimental injury models of excitotoxicity and ischemia (68, 94, 119, 128). However, even in the absence of neuronal loss after transient global ischemia, microglial proliferation peaks within 4 days in the striatum and neocortex, suggesting that microgliosis does not require overt brain injury (128). In addition to cell death signals, various factors, such as macrophage colony-stimulating factor (M-SCF) granulocyte-macrophage colony-stimulating factor (GM-CSF), corticotropin-releasing hormone, and thrombin, can stimulate microglial cell proliferation (113, 130, 154, 212, 238). The release of these factors together with cell death signals may synergistically contribute to the microglial proliferation observed during CNS damage. Interestingly, microglial numbers return to baseline levels 30 days after excitotoxic injury, indicating the removal of activated microglia after damage resolution (94). Cytokines, such as IL-4 and IL-13, induce apoptosis in microglia only after activation by lipopolysaccharide, gangliosides, or thrombin and may play a role in limiting the duration of microgliosis (245).

Phagocytosis and Antigen Presentation by Microglia

Phagocytosis is the receptor-mediated uptake of large extracellular particles and cells, and sets reactive microglia apart from reactive astrocytes. Antibodies and/or complement proteins can bind to and can target various substrates, such as myelin, for either Fc receptor¡Vmediated or complement receptor¡Vmediated phagocytosis, respectively (66, 160, 222). Although apoptosis-triggered phosphatidylserine exposure on the outer membrane leaflet is the most well-known mechanism of microglial phagocytosis of dying cells, other mechanisms for microglial recognition and phagocytosis of cells undergoing nonapoptotic and necrotic death also exist (98). Soluble signals, such as M-CSF, GM-CSF, TNF-ƒÑ, and IL-4, have also been shown to stimulate phagocytic clearance in vitro (154, 206). Antigen processing and presentation, as evidenced by increased MHC I and II surface expression, is also up-regulated in activated microglia after a variety of insults (27, 158). MHC expression is also increased by IFN-ƒ× and decreased by IFN-ƒÒ, indicating a dynamic regulation by cytokines (109). MHC presentation usually peaks with concomitant cytokine up-regulation as well as CD4+ and CD8+ T-lymphocyte infiltration into brain parenchyma, and, thus, initiates the adaptive neuroinflammatory response (26).

Microglial activation and phagocytosis may facilitate additional neuronal cell death, but recent studies showing release of several anti-inflammatory and neuroprotective factors during phagocytosis suggest that not all signals of cellular damage induce proinflammatory reactivity and toxicity in microglia (65, 141, 215). It has not been resolved whether up-regulation of phagocytic function in microglia is beneficial or harmful. The clearance of pathogens, necrotic debris, and apoptotic cells is likely to promote healthy brain function and recovery from minor insults. However, targeting of intact myelin or healthy cells for destruction during unchecked neuroinflammation can be a direct cause of additional neuronal loss during the later phases of brain injury. Indeed, it has been reported that 24 hours after transient exposure to ƒÒ-amyloid peptides, microglia continue to exhibit enhanced phagocytosis of several other unrelated substrates (114).

Soluble Factors Released by Microglia

During brain pathology or trauma, astrocytes, microglia, and infiltrating immune cells release and respond to both proinflammatory and anti-inflammatory cytokines (Figure 8.3). The role of each cytokine in brain damage and recovery has been difficult to analyze because of the antagonism, synergism, and redundancy inherent to the cytokine system (18, 148). Nevertheless, several cytokine mediators have been shown to be up-regulated after a variety of acute and chronic CNS insults, and many of these cytokines exacerbate glial responses to damage and promote neuronal cell loss in numerous brain injury and disease models (6). Among these, IL-1ƒÒ and TNF-ƒÑ are both up-regulated in microglia and astrocytes in cases of neurodegenerative diseases, stroke, epilepsy, brain trauma, and infection (19, 193, 195, 231, 233). Furthermore, both cytokines exacerbate neuronal degeneration in animal and cell culture models of these disorders (6, 175, 209). Specifically, exposure of cultured hippocampal neurons to IL-1ƒÒ induces tyrosine kinase¡Vmediated phosphorylation of NMDA receptor subunits NR2A/B, leading to consequent facilitation of calcium currents and increased neuronal cell death (232). TNF-ƒÑƒnhas also been recognized for its ability to cause cell death by both a direct p55 receptor¡Vmediated mechanism as well as an indirect suppression of survival signaling mechanism (228). TNF-ƒÑƒnrelease by astrocytes and microglia can suppress neurite growth and potentially inhibit neuronal regeneration after injury, whereas transgenic TNF-ƒÑ overexpression in the brain results in neuroinflammation, degeneration, ataxia, and epilepsy (3, 166).

Activated microglia also release a variety of ROS and reactive nitrogen species resulting in oxidative stress and increased neuronal death (33, 51, 79, 143). Like cytokines, these substances are also observed at high levels in various neurodegenerative conditions (8). Inhibition of the inducible nitrogen oxide synthase (iNOS) after lipopolysaccharide administration into the substantia nigra can rescue dopaminergic neurons from cell death whereas microglia-secreted superoxide also contributes to degeneration of dopaminergic neurons (10, 79).

Compared with cytokines, considerably less information exists regarding chemokine and chemokine receptor expression by glial cells during brain damage (15). Chemokine IL-8 release from microglia can promote immune cell infiltration and activation as well as perturb hippocampal synaptic plasticity (242). In general, chemokine inhibition has been effective in decreasing neutrophil and macrophage infiltration, as well as reducing lesion volumes in animal models of cerebral ischemia (21).

PART II: MICROGLIAL RESPONSES TO CNS INJURY

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Neuroprotective Roles of Microglia

Although less recognized, protective effects of microglial activation during CNS injury have also been reported, and these may be therapeutically augmented. Microglial release of growth factors, such as brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor, as well as production of certain cytokines, such as IL-6, transforming growth factor-ƒÒ (TGF-ƒÒ), and IFN-ƒ×, affords neuroprotective properties to microglial responses during brain injury and repair (29, 35, 76, 121, 162, 176, 177, 213, 240). Furthermore, activated microglia and incoming macrophages express excitatory amino acid transporters for glutamate uptake, suggesting a direct role in reduction of excitotoxicity (91, 192). The expression of the antioxidant, glutathione, and glutathione reductase is much higher in microglia than in neurons and astrocytes, and may protect against the ROS produced during normal oxidative metabolism in the brain and during neurodegeneration that is often accompanied by high oxidative stress (8, 97, 134).

PART III: ASTROCYTIC RESPONSES TO CNS INJURY

As previously discussed, astrocytes are versatile cells with a wide array of physiological functions (165). These cells express receptors for nearly all types of neuroactive molecules, including neurotransmitters, cytokines, and toxins. These receptors allow astrocytes to sense and respond to many perturbations to the normal environment. When damage occurs, astrocytes respond by migrating to the lesion and activating the expression of a number of genes (see Figs. 8.3 and 8.4) (39, 126).

Astrocyte Hyperplasia and Hypertrophy

Astrocytes proliferate in response to most forms of injury, at least in part, because of activation of a variety of G-protein¡Vcoupled receptors, including endothelin, thrombin, serotonin, lysophosphatidic acid, and sphingosine-1-phosphate (S1P). Activation of these receptors leads to an increase in intracellular calcium levels and activation of the mitogen-activated protein (MAP) kinase extracellular receptor kinase 1/2 (ERK1/2), which increases mitogenesis (13, 99, 137, 207). This proliferation occurs in astrocytes found close to the site of the lesion, but becomes less prevalent further away (71, 72).

Although originally thought to be the critical step in the formation of the glial scar, evidence now shows that proliferation of astrocytes in gliosis is less important than cellular hypertrophy and thickening and lengthening of processes (32, 205). Any form of CNS damage will cause an increase in astrocytic expression of GFAP, which can be a considerable distance away from the actual site of injury. These reactive astrocytes become much larger and their once delicate processes become thicker, longer, and more numerous.

Eventually, the astrocytic processes will interweave to become the boundary of the glial scar. Because of the density of these processes, it has classically been thought that the physical structure of the scar inhibits axon regrowth. This hypothesis has been revisited and evidence suggests that axonal regeneration through the scar towards a localized source of trophic factors is possible (108). Multiple in vivo and in vitro assays have shown that extracellular matrix molecules associated with the scar tissue itself are inhibitory to regeneration, suggesting that axonal growth inhibition by glial scars may be biochemical rather than physical in nature (63, 73, 146).

Astrocyte Changes in Protein Expression

Once activated, a variety of changes in protein expression is observed in astrocyte populations. Best known is the

increase in GFAP observed in the reactive astrocyte population (190). This intermediate filament, along with vimentin and S 100, are the most commonly used markers to identify activated astrocytes both in vivo and in vitro (190). Interestingly, genetic knockouts of GFAP and vimentin have been shown in vitro to have improved survival and neurite growth, whereas in vivo double knockouts had improved functional and histological recovery after spinal cord hemisection, indicating the negative consequences of changes in astrocytes that require intermediate filaments (149, 150). Likewise, one can also observe up-regulated expression of oxidoreductive enzymes required for increased energy use and metabolism (190). Further, there is increased expression and release of proteases and protease inhibitors that directly aggravate neuronal damage or are neuroprotective (36, 190). Reactive astrocytes also up-regulate a variety of cell-surface receptors, such as epidermal growth factor (EGF) receptors, tyrosine kinase receptors, zinc receptor, and corticotrophin-releasing factor receptor, which serve to aid in cell-to-cell signaling during formation of the glial scar (190). These changes in protein expression can have opposing effects on scar formation and axonal regeneration. For example, expression of the zinc receptor, ZnT-1, in astrocytes is neuroprotective, whereas expression of the corticotropin releasing hormone receptor-1, promotes neurodegeneration (170, 208).

Another molecule up-regulated in injury is tenascin, which is associated with astrocytes and is highly inhibitory to axon growth. In addition to its direct effects on axon growth, tenascin has binding sites for most of the inhibitory CS-PGs. Reactive astrocytes are known to secrete the CS-PG, neurocan, into the extracellular matrix (11, 93, 145). Because many CS-PGs are not attached to the cell surface, tenascin acts as an adapter molecule and may determine whether or not the CS-PGs are retained in the area of damage (72). Through interactions with tenascin and the neural cell-adhesion molecule (NCAM), neurocan can inhibit axon growth (77, 188). Neurocan production in astrocytes is highly up-regulated by cytokines such as TGF-ƒÒ, TGF-ƒÑ, and EGF (72, 131, 132).

Astrocyte Release of Cytokines and Other Factors

Astrocytes are capable of producing a variety of cytokines, including interleukins (IL-1, IL-6, IL-10), and interferons (IFN-ƒÑ, IFN-ƒÒ), tumor necrosis factors (TNF-ƒÑ, TNF-ƒÒ), and a variety of growth factors (fibroblast growth factor, platelet-derived growth factor, nerve growth factor, and EGF) (70, 75, 190). As previously mentioned, 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 has been shown to protect against ischemic and excitotoxic injury, and hippocampal neurons treated with TNF-ƒÑ are less vulnerable to substrate deprivation and excitotoxicity (5, 46, 140). However, in vivo, IL-6 and TNF-ƒÑ have been shown to promote demyelination, thrombosis, leukocyte infiltration, and BBB disruption (70, 75). Thus, the specific contribution of astrocyte cytokine release to these processes in vivo remains to be established.

Finally, astrocytes are known to release several growth factors, such as brain-derived neurotrophic factor and nerve growth factor. Astrocytes are stimulated to produce and release these neurotrophic factors by a variety of signals, including prostaglandins, ƒÒ-amyloid, ischemia, IL-1ƒÒ, TNF-ƒÑ, ROS, histamine, and dopamine. These neurotrophic factors are known to play a critical role in neuronal survival and differentiation (37, 151, 221, 227).

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In the category of pathogen invasion signals, pathogen-associated molecular patterns, such as bacterial and viral surface proteins, sugars, and proteoglycans, as well as unmethylated DNA, are some of the most potent and reliable activators of microglia (60, 174). In the second category, cell damage and death trigger the presentation of cell injury signals, such as membrane exposure of phosphatidylserine, or the release of ATP and sialic acid¡Vcontaining glycosphingolipids (e.g., gangliosides), which induce proinflammatory cytokine and ROS production in microglia (153, 185). Additionally, hypoxia may be a direct signal for activation, because it triggers microglial expression of a proinflammatory cytokine, IL-1ƒÒ (111). Senile plaque¡Vassociated proteins, such as ƒÒ-amyloid and chromogranin A, are also strong signals that stimulate microglia (49, 52, 147). In the third category of regulatory signals, modulators from other cells, including certain surface molecules, cytokines, chemokines, and proteases, can activate and also regulate the function of microglia (see Figure 8.3). Interestingly, ATP is also an example of a regulatory signal released from astrocytes that can serve as a chemotactic, a mitogenic, and an apoptotic signal for microglia (100, 185, 229).

Microglial Migration and Proliferation

Migration of microglia to sites of infection or injury can be observed in culture in hippocampal slices and in murine models of brain damage (171, 178, 184). Several chemotactic signals mediate the recruitment of microglia, as well as other immune cells, to areas of brain damage and, thus, are essential for the spatial coordination of a local neuroimmune response. Chemokines, such as monocyte chemoattractant protein-1 (MCP-1/CCL2), RANTES/CCL5, MCP-2/IL-8/CCL8, IFN-ƒ×¡Vinducing protein-10 (IP-10/CXCL10), are a family of chemoattractants known to stimulate microglial and other immune cell migration (56, 181). CXCR3, a chemokine receptor for CXCL10/IP-10, has been shown to promote microglial movement to a lesion site in the entorhinal cortex and increase local neuronal loss (184). Among other molecules, ATP, ƒÒ-amyloid, and complement factor 5a are also found to induce microglial migration (100, 117, 171). Chemotaxis will be important in cases of localized brain injury, such as in lacunar strokes (Figure 8.5) , where damage in a small region may recruit outlying microglia to amplify local microgliosis. However, the role of chemotaxis in microglial response to global CNS insults is still unclear.

Cell death is thought to activate microglial proliferation in experimental injury models of excitotoxicity and ischemia (68, 94, 119, 128). However, even in the absence of neuronal loss after transient global ischemia, microglial proliferation peaks within 4 days in the striatum and neocortex, suggesting that microgliosis does not require overt brain injury (128). In addition to cell death signals, various factors, such as macrophage colony-stimulating factor (M-SCF) granulocyte-macrophage colony-stimulating factor (GM-CSF), corticotropin-releasing hormone, and thrombin, can stimulate microglial cell proliferation (113, 130, 154, 212, 238). The release of these factors together with cell death signals may synergistically contribute to the microglial proliferation observed during CNS damage. Interestingly, microglial numbers return to baseline levels 30 days after excitotoxic injury, indicating the removal of activated microglia after damage resolution (94). Cytokines, such as IL-4 and IL-13, induce apoptosis in microglia only after activation by lipopolysaccharide, gangliosides, or thrombin and may play a role in limiting the duration of microgliosis (245).

Phagocytosis and Antigen Presentation by Microglia

Phagocytosis is the receptor-mediated uptake of large extracellular particles and cells, and sets reactive microglia apart from reactive astrocytes. Antibodies and/or complement proteins can bind to and can target various substrates, such as myelin, for either Fc receptor¡Vmediated or complement receptor¡Vmediated phagocytosis, respectively (66, 160, 222). Although apoptosis-triggered phosphatidylserine exposure on the outer membrane leaflet is the most well-known mechanism of microglial phagocytosis of dying cells, other mechanisms for microglial recognition and phagocytosis of cells undergoing nonapoptotic and necrotic death also exist (98). Soluble signals, such as M-CSF, GM-CSF, TNF-ƒÑ, and IL-4, have also been shown to stimulate phagocytic clearance in vitro (154, 206). Antigen processing and presentation, as evidenced by increased MHC I and II surface expression, is also up-regulated in

activated microglia after a variety of insults (27, 158). MHC expression is also increased by IFN-ƒ× and decreased by IFN-ƒÒ, indicating a dynamic regulation by cytokines (109). MHC presentation usually peaks with concomitant cytokine up-regulation as well as CD4+ and CD8+ T-lymphocyte infiltration into brain parenchyma, and, thus, initiates the adaptive neuroinflammatory response (26).

Microglial activation and phagocytosis may facilitate additional neuronal cell death, but recent studies showing release of several anti-inflammatory and neuroprotective factors during phagocytosis suggest that not all signals of cellular damage induce proinflammatory reactivity and toxicity in microglia (65, 141, 215). It has not been resolved whether up-regulation of phagocytic function in microglia is beneficial or harmful. The clearance of pathogens, necrotic debris, and apoptotic cells is likely to promote healthy brain function and recovery from minor insults. However, targeting of intact myelin or healthy cells for destruction during unchecked neuroinflammation can be a direct cause of additional neuronal loss during the later phases of brain injury. Indeed, it has been reported that 24 hours after transient exposure to ƒÒ-amyloid peptides, microglia continue to exhibit enhanced phagocytosis of several other unrelated substrates (114).

Soluble Factors Released by Microglia

During brain pathology or trauma, astrocytes, microglia, and infiltrating immune cells release and respond to both proinflammatory and anti-inflammatory cytokines (Figure 8.3). The role of each cytokine in brain damage and recovery has been difficult to analyze because of the antagonism, synergism, and redundancy inherent to the cytokine system (18, 148). Nevertheless, several cytokine mediators have been shown to be up-regulated after a variety of acute and chronic CNS insults, and many of these cytokines exacerbate glial responses to damage and promote neuronal cell loss in numerous brain injury and disease models (6). Among these, IL-1ƒÒ and TNF-ƒÑ are both up-regulated in microglia and astrocytes in cases of neurodegenerative diseases, stroke, epilepsy, brain trauma, and infection (19, 193, 195, 231, 233). Furthermore, both cytokines exacerbate neuronal degeneration in animal and cell culture models of these disorders (6, 175, 209). Specifically, exposure of cultured hippocampal neurons to IL-1ƒÒ induces tyrosine kinase¡Vmediated phosphorylation of NMDA receptor subunits NR2A/B, leading to consequent facilitation of calcium currents and increased neuronal cell death (232). TNF-ƒÑƒnhas also been recognized for its ability to cause cell death by both a direct p55 receptor¡Vmediated mechanism as well as an indirect suppression of survival signaling mechanism (228). TNF-ƒÑƒnrelease by astrocytes and microglia can suppress neurite growth and potentially inhibit neuronal regeneration after injury, whereas transgenic TNF-ƒÑ overexpression in the brain results in neuroinflammation, degeneration, ataxia, and epilepsy (3, 166).

Activated microglia also release a variety of ROS and reactive nitrogen species resulting in oxidative stress and increased neuronal death (33, 51, 79, 143). Like cytokines, these substances are also observed at high levels in various neurodegenerative conditions (8). Inhibition of the inducible nitrogen oxide synthase (iNOS) after lipopolysaccharide administration into the substantia nigra can rescue dopaminergic neurons from cell death whereas microglia-secreted superoxide also contributes to degeneration of dopaminergic neurons (10, 79).

Compared with cytokines, considerably less information exists regarding chemokine and chemokine receptor expression by glial cells during brain damage (15). Chemokine IL-8 release from microglia can promote immune cell infiltration and activation as well as perturb hippocampal synaptic plasticity (242). In general, chemokine inhibition has been effective in decreasing neutrophil and macrophage infiltration, as well as reducing lesion volumes in animal models of cerebral ischemia (21).

Neuroprotective Roles of Microglia

Although less recognized, protective effects of microglial activation during CNS injury have also been reported, and these may be therapeutically augmented. Microglial release of growth factors, such as brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor, as well as production of certain cytokines, such as IL-6, transforming growth factor-ƒÒ (TGF-ƒÒ), and IFN-ƒ×, affords neuroprotective properties to microglial responses during brain injury and repair (29, 35, 76, 121, 162, 176, 177, 213, 240). Furthermore, activated microglia and incoming macrophages express excitatory amino acid transporters for glutamate uptake, suggesting a direct role in reduction of excitotoxicity (91, 192). The expression of the antioxidant, glutathione, and glutathione reductase is much

higher in microglia than in neurons and astrocytes, and may protect against the ROS produced during normal oxidative metabolism in the brain and during neurodegeneration that is often accompanied by high oxidative stress (8, 97, 134).

PART III: ASTROCYTIC RESPONSES TO CNS INJURY

As previously discussed, astrocytes are versatile cells with a wide array of physiological functions (165). These cells express receptors for nearly all types of neuroactive molecules, including neurotransmitters, cytokines, and toxins. These receptors allow astrocytes to sense and respond to many perturbations to the normal environment. When damage occurs, astrocytes respond by migrating to the lesion and activating the expression of a number of genes (see Figs. 8.3 and 8.4) (39, 126).

Astrocyte Hyperplasia and Hypertrophy

Astrocytes proliferate in response to most forms of injury, at least in part, because of activation of a variety of G-protein¡Vcoupled receptors, including endothelin, thrombin, serotonin, lysophosphatidic acid, and sphingosine-1-phosphate (S1P). Activation of these receptors leads to an increase in intracellular calcium levels and activation of the mitogen-activated protein (MAP) kinase extracellular receptor kinase 1/2 (ERK1/2), which increases mitogenesis (13, 99, 137, 207). This proliferation occurs in astrocytes found close to the site of the lesion, but becomes less prevalent further away (71, 72).

Although originally thought to be the critical step in the formation of the glial scar, evidence now shows that proliferation of astrocytes in gliosis is less important than cellular hypertrophy and thickening and lengthening of processes (32, 205). Any form of CNS damage will cause an increase in astrocytic expression of GFAP, which can be a considerable distance away from the actual site of injury. These reactive astrocytes become much larger and their once delicate processes become thicker, longer, and more numerous.

Eventually, the astrocytic processes will interweave to become the boundary of the glial scar. Because of the density of these processes, it has classically been thought that the physical structure of the scar inhibits axon regrowth. This hypothesis has been revisited and evidence suggests that axonal regeneration through the scar towards a localized source of trophic factors is possible (108). Multiple in vivo and in vitro assays have shown that extracellular matrix molecules associated with the scar tissue itself are inhibitory to regeneration, suggesting that axonal growth inhibition by glial scars may be biochemical rather than physical in nature (63, 73, 146).

Astrocyte Changes in Protein Expression

Once activated, a variety of changes in protein expression is observed in astrocyte populations. Best known is the increase in GFAP observed in the reactive astrocyte population (190). This intermediate filament, along with vimentin and S 100, are the most commonly used markers to identify activated astrocytes both in vivo and in vitro (190). Interestingly, genetic knockouts of GFAP and vimentin have been shown in vitro to have improved survival and neurite growth, whereas in vivo double knockouts had improved functional and histological recovery after spinal cord hemisection, indicating the negative consequences of changes in astrocytes that require intermediate filaments (149, 150). Likewise, one can also observe up-regulated expression of oxidoreductive enzymes required for increased energy use and metabolism (190). Further, there is increased expression and release of proteases and protease inhibitors that directly aggravate neuronal damage or are neuroprotective (36, 190). Reactive astrocytes also up-regulate a variety of cell-surface receptors, such as epidermal growth factor (EGF) receptors, tyrosine kinase receptors, zinc receptor, and corticotrophin-releasing factor receptor, which serve to aid in cell-to-cell signaling during formation of the glial scar (190). These changes in protein expression can have opposing effects on scar formation and axonal regeneration. For example, expression of the zinc receptor, ZnT-1, in astrocytes is neuroprotective, whereas expression of the corticotropin releasing hormone receptor-1, promotes neurodegeneration (170, 208).

Another molecule up-regulated in injury is tenascin, which is associated with astrocytes and is highly inhibitory to axon growth. In addition to its direct effects on axon growth, tenascin has binding sites for most of the inhibitory CS-PGs. Reactive astrocytes are known to secrete the CS-PG, neurocan, into the extracellular matrix (11, 93, 145).

Because many CS-PGs are not attached to the cell surface, tenascin acts as an adapter molecule and may determine whether or not the CS-PGs are retained in the area of damage (72). Through interactions with tenascin and the neural cell-adhesion molecule (NCAM), neurocan can inhibit axon growth (77, 188). Neurocan production in astrocytes is highly up-regulated by cytokines such as TGF-β, TGF-α, and EGF (72, 131, 132).

Astrocyte Release of Cytokines and Other Factors

Astrocytes are capable of producing a variety of cytokines, including interleukins (IL-1, IL-6, IL-10), and interferons (IFN-ƒÑ, IFN-ƒÒ), tumor necrosis factors (TNF-ƒÑ, TNF-ƒÒ), and a variety of growth factors (fibroblast growth factor, platelet-derived growth factor, nerve growth factor, and EGF) (70, 75, 190). As previously mentioned, 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 has been shown to protect against ischemic and excitotoxic injury, and hippocampal neurons treated with TNF-ƒÑ are less vulnerable to substrate deprivation and excitotoxicity (5, 46, 140). However, in vivo, IL-6 and TNF-ƒÑ have been shown to promote demyelination, thrombosis, leukocyte infiltration, and BBB disruption (70, 75). Thus, the specific contribution of astrocyte cytokine release to these processes in vivo remains to be established.

Finally, astrocytes are known to release several growth factors, such as brain-derived neurotrophic factor and nerve growth factor. Astrocytes are stimulated to produce and release these neurotrophic factors by a variety of signals, including prostaglandins, ƒÒ-amyloid, ischemia, IL-1ƒÒ, TNF-ƒÑ, ROS, histamine, and dopamine. These neurotrophic factors are known to play a critical role in neuronal survival and differentiation (37, 151, 221, 227).

Neuroprotective Roles of Astrocytes

In addition to the potentially harmful effects listed above, astrocytes also likely to protect recovering neurons and to help re-establish a homeostatic environment. Early hypotheses proposed that astrocytic activation helps recreate the glial limitans to separate neural (healthy) tissue from non-neural (dead) tissue (187). More recently, in a series of elegant experiments examining the protective role of astrocytes, Faulkner et al. generated a transgenic mouse that expressed the thymidine kinase from the herpes simplex virus under control of the GFAP promoter, which allowed selective ablation of astrocytes by administration of gancyclovir (71). Removal of astrocytes in this model system from the site of damage leads to larger lesions and increases breakdown of the BBB and inflammation. Their work suggests that astrocytes may be a necessary part of some healing process in the injured brain (39, 71). However, it remains unclear what role astrocytic debris plays in the exacerbation of injury observed in this model.

Finally, blockade of the TGF-ƒÒ receptor with the proteoglycan decorin prevents glial scar formation in a rat model of cerebral hemisphere ischemia (132, 155). Davies et al. found that using decorin to block TGF-ƒÒ in a spinal cord injury model also decreased the number of reactive astrocytes and their subsequent release of a variety of CS-PGs (61). Yet, despite what might be considered positive effects of TGF-ƒÒ blockade, other data suggests that TGF-ƒÒƒnreceptor blockade has no net effect on neuronal survival (155). TGF-ƒÒƒndoes regulate the production and release of neurocan, but not any of the other CS-PGs studied, consistent with data showing that TGF-ƒÒ blockade does not improve axonal growth, perhaps because of reduction in the release of extracellular matrix proteins, such as laminins, that are necessary for axonal regrowth (155). Clearly, more information is needed to understand the consequences of therapeutic manipulation of gliotic scarring.

PART IV: THROMBIN AND THE GLIAL RESPONSE TO BRAIN INJURY

Thrombin is a multifunctional serine protease first described for its fibrinolytic role in the blood-clotting cascade. In addition to its well-known role in blood hemostasis, thrombin may be involved in degenerative and protective mechanisms in the CNS. Activation of prothrombin to active thrombin and subsequent extravasation into CNS parenchyma has been implicated in the pathology of a number of CNS disorders, such as traumatic brain injury, stroke, and ischemia (4, 57, 58, 74, 85, 168). It has been shown that prothrombin messenger ribonucleic acid (mRNA) is up-regulated after several pathological situations in which glial scar formation is observed, such as cerebral ischemia and spinal cord injury (50, 191). Thrombin activity in the CNS is thought to be primarily regulated by a highly specific inhibitor, protease nexin-1 (85).

Most of these extravascular effects of thrombin are mediated by receptors belonging to the family of PARs, which are proteolytically activated G protein¡Vcoupled receptors that are activated by proteolytic cleavage of the N-terminal exodomain of the receptor. This cleavage unmasks a new N-terminus that functions as a tethered ligand, docking intramolecularly on a receptor site to affect transmembrane signaling. Three protease-activated receptors, PAR-1, 3, and 4, have been identified as responding to thrombin, whereas PAR-2 is activated preferentially by trypsin-like proteases (mast cell tryptase, for example). PAR-1, the most well studied of the PARs, initiates signaling in astrocytes by heterotrimeric G-protein subunits, Gq/G11, Gi/Go, and G12/G13, which activate a variety of cellular pathways, including phosphoinositide hydrolysis, calcium mobilization, tyrosine/MAP kinase, and Rho kinase (139, 207). Because PAR-1 is expressed by both microglia and astrocytes and because PAR-1 activators enter the brain at sites of BBB breakdown, PAR-1 is particularly interesting in the context of glial scar formation (Fig. 5.4) (104, 167, 212, 234).

Thrombin as a Marker in Many Pathological Conditions

Although thrombin levels in the brain are low under normal conditions, disruption of the BBB will result in leakage of thrombin from blood into brain parenchyma. This influx of thrombin can subsequently result in activation of PARs. PAR-1 activation may be an important feature of brain diseases that are characterized by the loss of BBB integrity, such as stroke, head trauma, status epilepticus, human immunodeficiency virus (HIV) encephalitis, and multiple sclerosis (17, 30, 55, 105). Indeed, when bleeding occurs directly within the brain tissue, active thrombin and other proteases will freely penetrate the interneuronal spaces by diffusion until clotting closes the injured vessels and thrombin becomes depleted from the clots or becomes bound by local inhibitors, such as protease nexin-1 (85). Clinical data show that subdural hematomas can elevate thrombin levels 250-fold in cerebrospinal fluid, from 100 pmol/L to 25 nmol/L for a period of more than a week, suggesting that appreciable amounts of thrombin can be generated and persist at sites of cerebrovascular injury (214). More recently, a comparison of levels of the inactive form of thrombin, prothrombin, was performed in the cerebrospinal fluid of 67 individuals from six groups with different neurological disorders; this comparison suggested that the levels of prothrombin are reduced after a traumatic brain injury, suggesting an increase in the activation of prothrombin to thrombin in cerebrospinal fluid of patients (204).

In addition to blood-derived thrombin, prothrombin mRNA is also expressed by cells within the CNS. Although thrombin was only detected at minimal levels in control brain tissue, the immunoreactivity of thrombin in astrocytes was markedly enhanced and more widespread in brains with HIV encephalitis or multiple sclerosis and after spinal cord injury; pathological conditions always associated with high glial reactivity (30, 191). Other potential activators of PAR-1 that are expressed in CNS tissue include plasmin and Factor Xa. However, we do not know whether these activators are up-regulated after brain injury (223, 243).

Thrombin Modulates the Posttraumatic Inflammatory Response

Thrombin has long been associated with inflammation, as shown by Nishino et al., who found that thrombin infusion into the rat caudate nucleus led to infiltration of inflammatory cells, reactive gliosis, scar formation, proliferation of mesenchymal cells, and induction of angiogenesis (168). More recently, a study showed that injection of thrombin into the substantia nigra is neurotoxic and triggers microglial activation and transient expression of iNOS, cyclooxygenase-2 (COX-2), and several proinflammatory cytokines, including IL 1ƒÒ, IL-6, and TNF-ƒÑ (48). Additionally, it seems that thrombin acts as a chemotactic agent for inflammatory cells, such as monocytes, macrophages, and neutrophils (25, 87, 103, 138, 212).

PAR-1¡VMediated Effects of Thrombin in the Glial Scar Formation

An increasing number of studies show a direct correlation between the size of the glial scar area and the extent of BBB breakdown, suggesting a major role of blood components in glial scar formation (202). Among blood-derived proteases, thrombin activation via activation of PAR-1 seems to be important in glial scar formation. Indeed, picomolar concentrations of thrombin or the PAR-1¡Vspecific agonist peptide can stimulate astrocyte proliferation in culture, indicating that thrombin extravasated from vasculature might be mitotic in vivo. Furthermore, the tyrosine kinase inhibitor, herbimycin A, and the kinase inhibitors, staurosporine and H7, can block thrombin-mediated cell

proliferation in vitro (207, 235). Thrombin has also been proposed to up-regulate glial expression of thrombomodulin in in vitro models of injury (179).

We have studied the involvement of PAR-1 in the initiation of astrogliosis in vivo (167, 207). Selective PAR-1 activation can induce proliferation of GFAP-positive cells in the striatum, suggesting that activation of PAR-1 in isolation is sufficient to stimulate proliferation (167). Furthermore, we found that PAR-1−/− mice have a reduced astrocytic response to cortical stab wound at 7 days after injury. These data suggest that PAR-1 activation plays an important role in glial scar initiation after brain injury. The mechanisms by which PAR-1 stimulates glial proliferation and scar formation seem to involve the ability of PAR-1 to induce sustained ERK activation (167). In contrast to the transitory activation of ERK by other cytokines and growth factors, PAR-1 activation is able to induce a sustained ERK activation for many hours (95, 159, 164). This effect requires an interaction between Rho kinase and ERK, and the resulting sustained ERK activation is required to induce cyclin D1 expression and consequent astrocyte proliferation (167). These experiments link PAR-1 activation directly to control of the cell cycle.

PART V. THERAPEUTIC INTERVENTIONS

Experimental efforts to understand reactive gliosis have elucidated some of the complexities of glial responses to injury. Unfortunately, it remains unclear whether gliotic scars are harmful or protective, and how to manipulate glial responses to promote axonal regeneration. Nevertheless, a number of potential targets for intervention have emerged. For example, immunosuppressants, or agents that inhibit proinflammatory intracellular signaling, as well as antioxidants, have been successful in attenuating glial responses to injury and minimizing neuronal cell loss in a variety of injury models in vivo and in vitro. In particular, animal models of Alzheimer¡¦s Disease, excitotoxicity, and ischemia have shown that blockade of microglial and astrocytic activation using minocycline or COX-2 inhibitors can alleviate oxidative stress and reduce neuronal loss (41, 86, 115, 194, 198). Furthermore, frequent use of nonsteroidal anti-inflammatory drugs has been suggested to decrease the risk for Alzheimer¡¦s Disease, and Parkinson¡¦s disease, and clinical trials with nonsteroidal anti-inflammatory drug therapy have provided some promising results (44, 216). Likewise, antioxidant flavonoids, such as wogonin and silymarin, inhibit microglial TNF-ƒÑ, IL-1ƒÒ, and/or nitric oxide release after endotoxin or cytokine stimulation (118, 236). Wogonin additionally inhibits ischemic brain injury and lessens behavioral dysfunction caused by middle cerebral artery occlusion (47).

Studies using animal models have found that anti¡VTGF-ƒÒ antibodies and anti¡VIL-6 receptor antibodies disrupt cytokine signaling and reduce glial scarring by several different measures. However, there was little change in axonal recovery (132, 155, 173). As previously mentioned, the effects of IL-6 and TGF-ƒÒ have a plethora of effects, including increasing receptor expression and inducing further cytokine release. However, interfering with less global, more specific signaling shows both enhanced axonal growth and decreased scar formation. For example, heparin oligosaccharides, which interfere with signaling through the fibroblast growth factor receptor, one of the receptors up-regulated by TGF-ƒÒ, attenuate scar formation and promote recovery (96). Curcumin, a commonly used food spice (turmeric), was recently identified as an inhibitor of activated microglia that functions by suppressing intracellular signaling, thus, reducing iNOS and COX-2 activation and the consequent release of proinflammatory mediators (110). Similarly, curcumin can reduce the responses of other neuroglia, such as astrocytes and OPCs (7). Increased degradation of CS-PG through delivery of metabolic enzymes, such as chondroitinase ABC and xylosyltransferase-1, at the site of injury leads to less scar formation and allows for axonal growth around the core of neural damage observed in animal models of spinal cord injury (31, 92, 144, 156). There have also been several studies aimed at inhibition of proliferation of glial cells that have had moderately positive results. Rhodes et al. found that injecting cytosine arabinoside, a drug commonly used to inhibit proliferation, at the site of a lesion decreased the numbers of microglia and OPCs with little effect on reactive astrocytes and some increase in the number of axons found beyond the site of the lesion compared with controls. However, this effect was short-lived and diminished by 18 days after lesion (189).

Finally, altering serine protease signaling could reveal much about glial¡Vneuronal interactions and improve our understanding of neuropathology and recovery. Blockade of PAR-1 in the nervous system might be a novel therapeutic approach for a range of pathological CNS insults. Striggow et al. showed that the thrombin antagonist, hirudin, protected hippocampal area CA1 pyramidal cells in vivo when applied before the onset of a severe global ischemia in gerbils, but impaired the ischemic preconditioning when applied before each short-lasting attack (211).

Moreover, whole animal studies reveal that continuous infusion of argatroban, a synthetic thrombin inhibitor, significantly reduced the area of infarction and improved neurological deficits after middle cerebral artery occlusion (106, 172). Additionally, several clinical studies have found that argatroban is a safe antithrombolytic to administer within 48 hours of stroke onset, and one clinical study found that argatroban is effective in reducing neurological impairment caused by ischemic stroke (116, 226). All of these data suggest that thrombin inhibitors reduce the neurological deficit associated with stroke. However, more studies are necessary to determine whether the effect of thrombin inhibition is related to a vascular event, glial reactivity, and/or direct neuronal effects. Although the component pathways are complex and at times have opposing actions, they may provide greater therapeutic opportunities and allow for selective targeting of the effects that hinder CNS recovery.

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Fig. 5.1 Glial scarring in human neurological disease. A, hematoxylin and eosin (H & E)–stained tissue section of a resolving cerebral infarction (stroke) showing a cavitary center (asterisk) filled with histiocytes and proteinaceous debris surrounded by a glial scar with numerous hypertrophic astrocytes (arrowheads). B, Luxol fast blue stain (for myelin) of a multiple sclerosis plaque, demonstrating a loss of myelin staining together with a perivascular and parenchymal macrophage infiltrate (asterisk). Abundant reactive astrocytes are present in regions surrounding the plaque (arrowheads). Normal white matter is present in the lower portion and stains blue. C, H & E–stained section of an abscess showing a necrotic center (asterisk) surrounded by numerous inflammatory cells and reactive astrocytes of the glial scar (arrowhead). All photographs: original magnification, ×200.

Fig. 5.2 Cellular components of a glial scar. A, reactive (hypertrophic) astrocytes (arrow) are observed with H & E staining in a region surrounding a cavitary infarct (asterisk), where they have abundant pink cytoplasm and show increased cell density. B, immunohistochemical staining for GFAP in a region surrounding the infarct (asterisk) highlights the reactive astrocytes and their processes (arrow). C, microglia are a morphologically and functionally distinct component of a glial scar. Microglia are small, elongate, and have cigar-shaped nuclei with scanty cytoplasm (“rod cells”; arrowheads). All photographs: original magnification, ×400.

Fig. 5.3 Some of the important substances released and/or expressed by activated microglia and astrocytes. List is not exclusive. Substances made by microglia are in normal font; substances made by astrocytes are underlined; and substances made by both microglia and astrocytes are in bold font

Fig. 5.4 Cartoon representation of activation of microglia and astrocytes. A, microglia respond to variety of signals including pathogen invasion, cytokines, and cell death signals. Once activated, they become less ramified, proliferate, begin secreting proinflammatory cytokines, and up-regulate a number of receptor molecules. Additionally, microglia respond by undergoing apoptosis. B, similarly, astrocytes respond to injury, ischemia, and infection. In contrast to microglia, activated astrocytes have more processes and greatly hypertrophy. Like microglia, astrocytes release proinflammatory cytokines and up-regulate a variety of receptors. However, astrocytes also release a plethora of growth factors.

Fig. 5.5 Immunohistochemistry of human stroke tissue. Postmortem sections of human brain with a lacunar stroke (hematoxylin; A and B). Activated astrocytes (arrows) can be detected around the lesion by the presence of large eosinophilic cytoplasm and eccentrically located nuclei (hematoxylin; B) and GFAP staining (fluorescein isothiocyanate, FITC; C). Double immunofluorescence performed with antibodies to GFAP (FITC; C) and human PAR-1 (Texas Red; D) shows the astrocytic localization of PAR-1; E