

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

*Cecily E. Hamill, Anna Goldshmidt, Olivier Nicole, Robert J. McKeon, Daniel J. Brat, and Stephen F. Traynelis*

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.<sup>6,17,75,233,246</sup>

### 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.<sup>9,14,28</sup>

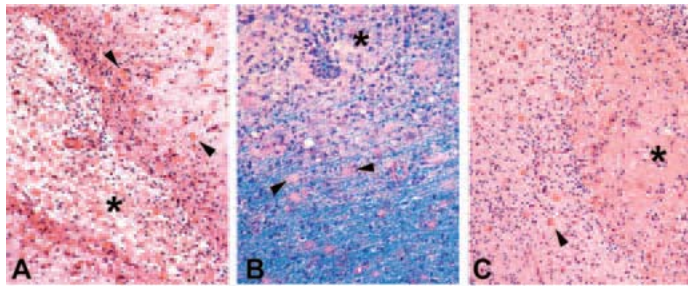
### Step 2: Inflammation

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 and oxygen 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- $\alpha$  (TNF- $\alpha$ ) and interferon- $\gamma$  (IFN- $\gamma$ ); and chemokines, such as macrophage inflammatory protein 1- $\alpha$  (MIP1- $\alpha$ ) and interleukin-8 (IL-8), recruit peripheral white blood cells to the site of damage.

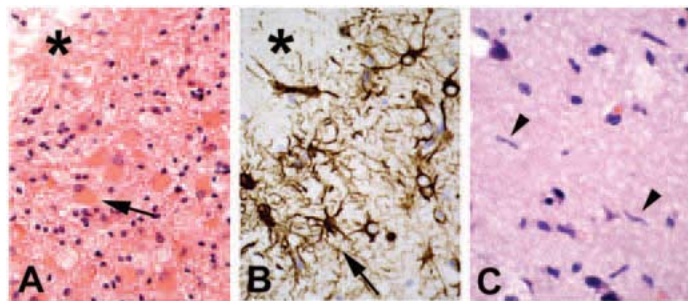
Breakdown of the BBB, which is composed of endothelial cells and astrocytes, occurs concurrently with microglial activation. This breakdown appears in response to the release of various cytokines, reactive oxygen species (ROS), glutamate, adenosine triphosphate (ATP), bradykinins, histamine, and nitric oxide from neurons, activated microglia, and the endothelial cells themselves. Breakdown of this barrier facilitates the translocation of plasma-derived molecules into the brain.<sup>17</sup> 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.<sup>202</sup> 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.<sup>17</sup>

### 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.<sup>124</sup> Although these cells are activated by neuronal damage, proliferation requires at least some demyelination of neurons.<sup>67</sup> 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



**FIGURE 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 cavitory 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,  $\times 200$ .



**FIGURE 5.2** Cellular components of a glial scar. *A*, reactive (hypertrophic) astrocytes (arrow) are observed with H & E staining in a region surrounding a cavitory 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,  $\times 400$ .

responding to a growth factor released by myelin sheath breakdown or whether OPCs are sensitive to changes in neuronal conduction.<sup>22,40,42,123</sup> 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).<sup>45,72,125</sup> 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.<sup>72,122</sup> CS-PGs appear within a day after injury and persist for several months thereafter.<sup>145,219</sup> 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.<sup>62,63</sup> Further, *in vivo* and *in vitro* assays show that blockade of CS-PG signal transduction is permissive for axonal growth.<sup>203</sup>

#### 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.<sup>165</sup> 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.<sup>2</sup> This is followed by the appearance of small and slender GFAP-positive processes, which in several days become fully stellated, fibrillary astrocytes. Long-standing hypotheses suggest that reactive astrocytes create a physical barrier between damaged and healthy cells and re-establish an intact BBB.<sup>71,187</sup> 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 Fig. 5.3). 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.<sup>1,20,141,157,166,210</sup> 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.<sup>88</sup>

### Secreted Factors

Cytokines/Chemokines	Proteases	Growth Factors	ROS/Others
<b>TNF-<math>\alpha</math></b> <sup>70, 82, 106, 120, 126, 190</sup>	<b>MMP-1</b> (Collagenase I) <sup>84</sup>	<b>TGF-<math>\beta</math></b> <sup>53, 70, 190</sup>	<b>Nitric Oxide</b> <sup>51, 82, 107, 109, 126, 190</sup>
<b>IL-1<math>\beta</math>, -6, -10, -12, -15</b>	<b>MMP-2</b> (Gelatinase A) <sup>81, 84, 89, 102, 129, 133</sup>	<b>NGF</b> <sup>126, 190, 248</sup>	<b>Superoxide</b> <sup>225</sup>
<b>IL-16, -18</b> <sup>12, 70, 82, 89, 109, 120, 126, 182, 190, 249</sup>	<b>MMP-3</b> (Stromelysin) <sup>81, 84, 129</sup>	<b>IGF-1</b> <sup>126, 190</sup>	<b>Peroxyntirite</b> <sup>241</sup>
<b>CCL-2</b> (MCP-1), -3(MIP-1 $\alpha$ ), -4(MIP-1 $\beta$ ),	<b>MMP-9</b> (Gelatinase B) <sup>81, 84, 89, 102, 129</sup>	<b>EGF</b> <sup>126, 190</sup>	<b>Glutamate</b> <sup>107, 163, 190</sup>
<b>CCL-5</b> (RANTES), -9(MIP-1 $\gamma$ ) <sup>59, 70, 120</sup>	<b>Cathepsin B, L</b> <sup>78</sup>	<b>VEGF</b> <sup>102, 126</sup>	<b>PGE2</b> <sup>237</sup>
<b>CXCL-8</b> (IL-8), -10 <sup>59, 200</sup>	<b>iPA</b> <sup>201</sup>	<b>BDNF</b> <sup>126, 248</sup>	<b>C1q</b> <sup>138</sup>
<b>LIF</b> <sup>70</sup>	<b>ADAM8</b> (CD156) <sup>199</sup>	<b>ADNF</b> <sup>126</sup>	<b>CS-PG</b> <sup>190</sup>
<b>IP-10</b> <sup>70</sup>	<b>TIMP-1</b> <sup>181</sup>	<b>CNTF</b> <sup>126, 190, 248</sup>	
	<b>PN-1</b> <sup>85, 190</sup>	<b>GM-CSF</b> <sup>70</sup>	
	<b><math>\alpha</math>-Chemotrypsin</b> <sup>190</sup>	<b>GMF</b> <sup>190</sup>	
	<b>Cathepsin D, G</b> <sup>190</sup>	<b>EPO</b> <sup>161</sup>	
		<b>SDF-1</b> <sup>247</sup>	

### Surface Receptors and Cytosolic Proteins

Cytokine/Chemokine	Phagocytosis/Scavenger	Intracellular Proteins/Other
<b>TNFR-I</b> (p55), -II(p75) <sup>24, 107, 190</sup>	<b>Mannose</b> <sup>28, 250</sup>	<b>GFAP</b> <sup>70, 107, 126, 190</sup>
<b>IL-1RI, -1RII</b> <sup>107, 120, 190</sup>	<b>CD36</b> <sup>54</sup>	<b>S-100</b> <sup>190</sup>
<b>IL-2R, -3R, -4R, -5R, -6R, -7R, -8R</b>	<b>SR-A/II, -BI</b> <sup>101, 186</sup>	<b>Vimentin</b> <sup>70, 107, 126, 190</sup>
<b>IL-9R, -10R, -12R, -13R, 15R</b> <sup>107, 120, 190, 197</sup>	<b>MARCO</b> <sup>90</sup>	<b>Aromatase</b> <sup>12</sup>
<b>IFN-R</b> <sup>107, 190, 244</sup>	<b>RAGE</b> <sup>136</sup>	<b>Copper-zinc superoxide dismutase</b> <sup>190</sup>
<b>TGF-betaRI, -betaRII</b> <sup>94, 190</sup>	<b>PtdSer</b> <sup>65</sup>	<b>glutathione peroxidase</b> <sup>126, 127</sup>
<b>CCR-2, -3, -5</b> <sup>15, 69</sup>	<b>TLR-1, -2, -3, -4, -5, -6, -7, -8, -9</b> <sup>174</sup>	<b>iNOS</b> <sup>107, 112, 190</sup>
<b>CXCR-2</b> (IL-8R), -3, -4 <sup>15, 69</sup>	<b>Fc<math>\gamma</math>R</b> <sup>225</sup>	<b>Tenascin</b> <sup>160</sup>
<b>CX3CR1</b> <sup>102, 230</sup>		<b>Glutamine synthetase</b> <sup>81, 190</sup>
		<b>NCAM</b> <sup>190</sup>
		<b>VCAM-1</b> <sup>190</sup>
		<b>ICAM-1</b> <sup>70, 82</sup>
		<b>MHCI, II</b> <sup>26, 70, 82, 120, 156</sup>
		<b>B7.1, B7.2</b> <sup>70, 102, 109</sup>
		<b>CR3/MAC-1/CD11b</b> <sup>82, 120, 152</sup>
		<b>CD40, 45, 68</b> <sup>70, 120, 217, 218</sup>
		<b>PPAR-gamma</b> <sup>23</sup>
		<b>EAAT</b> <sup>91</sup>
		<b>ER</b> <sup>80</sup>
		<b>AR</b> <sup>80</sup>

Metabotropic/Ionotropic	Growth Factor
<b>PAR-1, -2, -3, -4</b> <sup>16, 104, 138, 234</sup>	<b>GM-CSFR</b> <sup>70, 197</sup>
<b>GABA-B1a, -B1b, -B2</b> <sup>43</sup>	<b>M-CSFR</b> <sup>183, 197</sup>
<b>mGluR-2/3, 4, 5, 6, 8</b> <sup>3, 190, 220</sup>	<b>EPOR</b> <sup>161</sup>
<b>Glur2, 3, 4, 5, 7</b> <sup>69</sup>	<b>c-kitR</b> <sup>248</sup>
<b>KA-1, KA-2</b> <sup>69</sup>	<b>FGFR1, 2</b> <sup>190</sup>
<b>P2X, P2Y</b> <sup>164, 215, 224</sup>	<b>CNFR</b> <sup>190</sup>
<b>NT3</b> <sup>142</sup>	<b>trkA, B</b> <sup>190</sup>
<b><math>\beta</math>-adrenergic</b> <sup>190</sup>	<b>EGFR</b> <sup>190</sup>
<b>L-type calcium channels</b> <sup>83</sup>	<b>PGDFR</b> <sup>190</sup>

References: 12, Azcoltia et al., 2003; 15, Bajetto et al., 2002; 16, Balcaitis et al., 2003; 23, Bernardo et al., 2000; 24, Bette et al., 2003; 26, Bohatschek(1) et al., 2004; 27, Bohatschek(2) et al., 2004; 38, Burudi et al., 1999; 43, Charles et al., 2003; 51, Colton et al., 2004; 53, Constam et al., 1992; 54, Coraci et al., 2002; 59, D'Aversa et al., 2004; 64, De Groot et al., 1999; 65, De Simone et al., 2004; 69, Dijkstra et al., 2004; 70, Dong and Benveniste, 2001; 78, Gan et al., 2004; 79, Gao et al., 2003; 80, Garcia-Ovejero et al., 2002; 81, Gardner and Ghorpade, 2003; 82, Gehrman et al., 1995; 83, Geurts et al., 2003; 84, Ghorpade et al., 2001; 85, Gingrich and Traynelis, 2000; 89, Gottschall et al., 1995; 90, Granucci et al., 2003; 91, Gras et al., 2003; 101, Husemann et al., 2002; 102, John et al., 2004; 104, Junge et al., 2004; 107, Kaul et al., 2001; 109, Kawanokuchi et al., 2004; 112, Kim et al., 2004; 120, Lee et al., 2002; 126, Liberto et al., 2004; 127, Lindenau et al., 1998; 129, Liuzzi et al., 2004; 133, Lorenzi et al., 2002; 135, Lue et al., 2001; 136, Lynch et al., 2004; 138, Macfarlane et al., 2001; 142, Martin et al., 2003; 152, Milner and Campbell, 2003; 158, Morioka et al., 1993; 161, Nagai et al., 2001; 163, Nakamura et al., 2003; 164, Neary et al., 2003; 169, Noda et al., 2000; 174, Olson and Miller, 2004; 182, Prinz and Hanisch, 1999; 183, Raivich et al., 1998; 186, Reichert and Rotshenker, 2003; 190, Ridet et al., 1997; 197, Sawada et al., 1993; 199, Schiömann et al., 2000; 200, Si et al., 2004; 201, Siao et al., 2003; 215, Suzuki et al., 2004; 217, Tan(1) et al., 2000; 218, Tan(2) et al., 2000; 220, Taylor et al., 2003; 224, Tsuda et al., 2003; 225, Ueyama et al., 2004; 230, Verge et al., 2004; 234, Wang and Reiser, 2003; 237, Wang et al., 2004; 241, Xie et al., 2002; 244, Yamada and Yamanaka, 1995; 247, Zhang et al., 2003; 248, Zhang and Fedoroff, 1998; 249, Zhao et al., 2004; 250, Zimmer et al., 2003.

Abbreviations: ADAM, a disintegrin and a metalloproteinase; ADNF, activity-dependent neurotrophic factor; AR, androgen receptor; BDNF, brain-derived neurotrophic factor; C, complement; CCL, CC chemokine; CD, cluster of differentiation; c-kitR, c-kit receptor for stem cell factor; CNTF, ciliary neurotrophic factor; CS-PG, chondroitin sulphate proteoglycans; CXCL, CXC chemokine; CX3CR, CX3C chemokine receptor; EAAT, excitatory amino acid transporter; EGF, epidermal growth factor; EPO, erythropoietin; ER, estrogen receptor; Fc $\gamma$ R, IgG receptor; FGFR, fibroblast growth factor receptor; GABA-B, gamma-aminobutyric acid receptor; GFAP, glial fibrillary acidic protein; GluR, ionotropic glutamate receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GMF, glia maturation factor; ICAM, intercellular adhesion molecule; IFN, interferon; IGF, insulin-like growth factor; IL, interleukin; iNOS, inducible nitric oxide synthase; iP, IFN- $\gamma$  inducing protein; KA, kainate receptor; LIF, leukemia inhibitory factor; MACRO, macrophage receptor with collagenous domain; MCP, monocyte chemoattractant protein; mGluR, metabotropic glutamate receptor; MHC, major histocompatibility complex; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase; M-SCF, macrophage colony-stimulating factor; NCAM, neural cell adhesion molecule; NGF, nerve growth factor; NT-3, neurotrophin 3; P2, purinergic receptor; PAR, protease-activated receptor; PGDFR, platelet-derived growth factor receptor; PGE, prostaglandin E; PN, protease nexin; PPAR, peroxisome proliferator-activated receptor; PtdSerR, phosphatidylserine receptor; RAGE, receptor for advanced glycation end products; RANTES, regulated upon activation, normal T cell expressed and secreted; S-100, astrocyte-specific marker; SDF, stromal cell-derived factor; SR, scavenger receptor; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinases; TLR, toll-like receptor; TNF, tumor necrosis factor; iPA, tissue-type plasminogen activator; trk, tyrosine kinase receptor; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor.

**FIGURE 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.

## Triggers and Regulators of Microgliosis

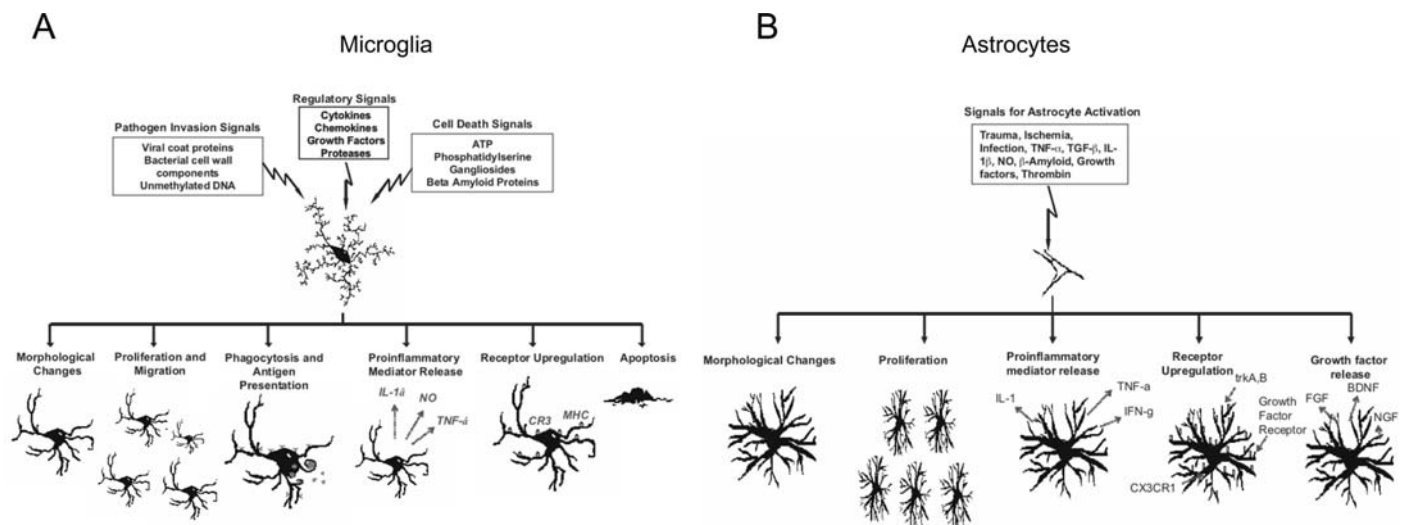
As illustrated in *Figure 5.4* microgliosis is characterized by a complex set of events, including changes in microglial morphology, increased proliferation, migration to a site of damage, phagocytosis, antigen processing and presentation, up-regulation of numerous cell surface and secreted signaling 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.<sup>60,174</sup> 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-containing glycosphingolipids (e.g., gangliosides), which induce proinflammatory cytokine and ROS production in microglia.<sup>153,185</sup> Additionally, hypoxia may be a direct signal for activation, because it triggers microglial expression of a proinflammatory cytokine, IL-1 $\beta$ .<sup>111</sup> Senile plaque-associated proteins, such as  $\beta$ -amyloid and chromogranin A, are also strong signals that stimulate microglia.<sup>49,52,147</sup> In the third category of regulatory signals, modulators from other cells, including certain surface molecules, cytokines, chemokines, and proteases, can acti-

vate and also regulate the function of microglia (see *Fig. 5.3*). 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.<sup>100,185,229</sup>

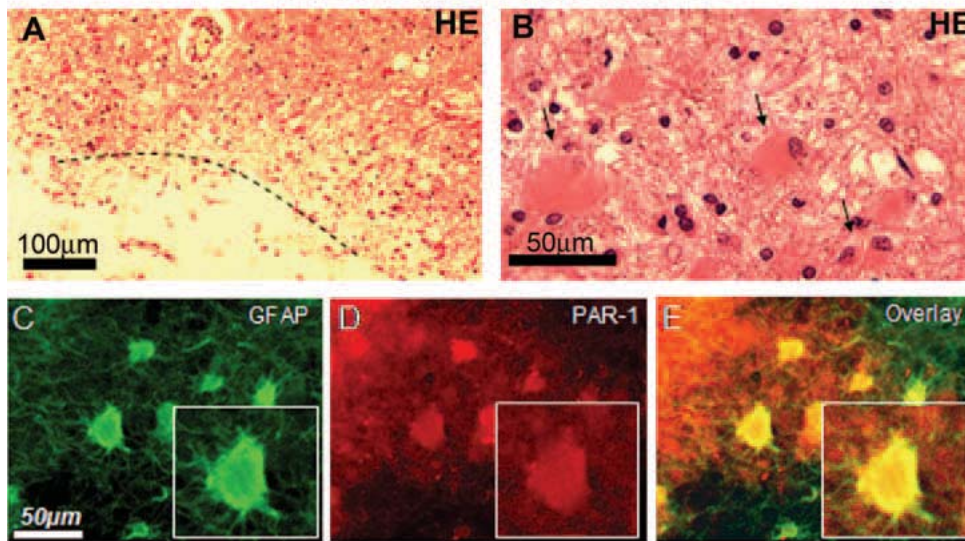
## Microglial Migration and Proliferation

Migration of microglia to sites of infection or injury can be observed in culture in hippocampal slices and in murine models of brain damage.<sup>171,178,184</sup> 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, and IFN- $\gamma$ -inducing protein-10 (IP-10/CXCL10), are a family of chemoattractants known to stimulate microglial and other immune cell migration.<sup>56,181</sup> 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.<sup>184</sup> Among other molecules, ATP,  $\beta$ -amyloid, and complement factor 5a are also found to induce microglial migration.<sup>100,117,171</sup> Chemotaxis will be important in cases of localized brain injury, such as in lacunar strokes (*Fig. 5.5*), 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.



**FIGURE 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.

## HUMAN LACUNAR INFARCT



**FIGURE 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*.

Cell death is thought to activate microglial proliferation in experimental injury models of excitotoxicity and ischemia.<sup>68,94,119,128</sup> 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.<sup>128</sup> In addition to cell death signals, various factors, such as macrophage colony-stimulating factor (M-CSF) granulocyte-macrophage colony-stimulating factor (GM-CSF), corticotropin-releasing hormone, and thrombin, can stimulate microglial cell proliferation.<sup>113,130,154,212,238</sup> 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.<sup>94</sup> 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.<sup>245</sup>

### 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 target various substrates, such as myelin, for either Fc receptor-mediated or complement receptor-mediated phagocytosis, respectively.<sup>66,160,222</sup> 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.<sup>98</sup> Soluble signals, such as M-CSF, GM-CSF, TNF- $\alpha$ , and IL-4, have also been shown to stimulate phagocytic clearance in vitro.<sup>154,206</sup> 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.<sup>27,158</sup> MHC expression is also increased by IFN- $\gamma$  and decreased by IFN- $\beta$ , indicating a dynamic regulation by cytokines.<sup>109</sup> 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.<sup>26</sup>

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.<sup>65,141,215</sup> 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  $\beta$ -amyloid peptides, microglia continue to exhibit enhanced phagocytosis of several other unrelated substrates.<sup>114</sup>

### 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 (*Fig. 5.3*).

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.<sup>18,148</sup> 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.<sup>6</sup> Among these, IL-1 $\beta$  and TNF- $\alpha$  are both up-regulated in microglia and astrocytes in cases of neurodegenerative diseases, stroke, epilepsy, brain trauma, and infection.<sup>19,193,195,231,233</sup> Furthermore, both cytokines exacerbate neuronal degeneration in animal and cell culture models of these disorders.<sup>6,175,209</sup> Specifically, exposure of cultured hippocampal neurons to IL-1 $\beta$  induces tyrosine kinase-mediated phosphorylation of NMDA receptor subunits NR2A/B, leading to consequent facilitation of calcium currents and increased neuronal cell death.<sup>232</sup> TNF- $\alpha$  has also been recognized for its ability to cause cell death by both a direct p55 receptor-mediated mechanism as well as an indirect suppression of survival signaling mechanism.<sup>228</sup> TNF- $\alpha$  release by astrocytes and microglia can suppress neurite growth and potentially inhibit neuronal regeneration after injury, whereas transgenic TNF- $\alpha$  overexpression in the brain results in neuroinflammation, degeneration, ataxia, and epilepsy.<sup>3,166</sup>

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

Compared with cytokines, considerably less information exists regarding chemokine and chemokine receptor expression by glial cells during brain damage.<sup>15</sup> Chemokine IL-8 release from microglia can promote immune cell infiltration and activation as well as perturb hippocampal synaptic plasticity.<sup>242</sup> 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.<sup>21</sup>

### 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- $\beta$  (TGF- $\beta$ ), and IFN- $\gamma$ , affords neuroprotective

properties to microglial responses during brain injury and repair.<sup>29,35,76,121,162,176,177,213,240</sup> Furthermore, activated microglia and incoming macrophages express excitatory amino acid transporters for glutamate uptake, suggesting a direct role in reduction of excitotoxicity.<sup>91,192</sup> 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.<sup>8,97,134</sup>

## PART III: ASTROCYTIC RESPONSES TO CNS INJURY

As previously discussed, astrocytes are versatile cells with a wide array of physiological functions.<sup>165</sup> 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. 5.3* and *5.4*).<sup>39,126</sup>

### 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-coupled 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.<sup>13,99,137,207</sup> This proliferation occurs in astrocytes found close to the site of the lesion, but becomes less prevalent further away.<sup>71,72</sup>

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.<sup>32,205</sup> 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.<sup>108</sup> 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.<sup>63,73,146</sup>

### 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.<sup>190</sup> 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.<sup>190</sup> 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.<sup>149,150</sup> Likewise, one can also observe up-regulated expression of oxidoreductive enzymes required for increased energy use and metabolism.<sup>190</sup> Further, there is increased expression and release of proteases and protease inhibitors that directly aggravate neuronal damage or are neuroprotective.<sup>36,190</sup> 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.<sup>190</sup> 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.<sup>170,208</sup>

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.<sup>11,93,145</sup> 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.<sup>72</sup> Through interactions with tenascin and the neural cell-adhesion molecule (NCAM), neurocan can inhibit axon growth.<sup>77,188</sup> Neurocan production in astrocytes is highly up-regulated by cytokines such as TGF- $\beta$ , TGF- $\alpha$ , and EGF.<sup>72,131,132</sup>

### 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- $\alpha$ , IFN- $\beta$ ), tumor necrosis factors (TNF- $\alpha$ , TNF- $\beta$ ), and a variety of growth factors (fibroblast growth factor, platelet-derived growth factor, nerve growth factor, and EGF).<sup>70,75,190</sup> 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- $\alpha$  are less vulnerable to substrate deprivation and excitotoxicity.<sup>5,46,140</sup> However, in vivo, IL-6 and TNF- $\alpha$  have been shown to promote demyelination, thrombosis, leukocyte infiltration, and BBB disruption.<sup>70,75</sup> 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,  $\beta$ -amyloid, ischemia, IL-1 $\beta$ , TNF- $\alpha$ , ROS, histamine, and dopamine. These neurotrophic factors are known to play a critical role in neuronal survival and differentiation.<sup>37,151,221,227</sup>

### Neuroprotective Roles of Astrocytes

In addition to the potentially harmful effects listed above, astrocytes are also likely to protect recovering neurons and 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.<sup>187</sup> 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.<sup>71</sup> 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.<sup>39,71</sup> However, it remains unclear what role astrocytic debris plays in the exacerbation of injury observed in this model.

Finally, blockade of the TGF- $\beta$  receptor with the proteoglycan decorin prevents glial scar formation in a rat model of cerebral hemisphere ischemia.<sup>132,155</sup> Davies et al. found that using decorin to block TGF- $\beta$  in a spinal cord injury model also decreased the number of reactive astrocytes and their subsequent release of a variety of CS-PGs.<sup>61</sup> Yet, despite what might be considered positive effects of TGF- $\beta$  blockade, other data suggests that TGF- $\beta$  receptor blockade has no net effect on neuronal survival.<sup>155</sup> TGF- $\beta$  does regulate the production and release of neurocan, but not any of the other CS-PGs studied, consistent with data showing that TGF- $\beta$  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.<sup>155</sup> 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.<sup>4,57,58,74,85,168</sup> 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.<sup>50,191</sup> Thrombin activity in the CNS is thought to be primarily regulated by a highly specific inhibitor, protease nexin-1.<sup>85</sup>

Most of these extravascular effects of thrombin are mediated by receptors belonging to the family of PARs, which are proteolytically activated G protein-coupled 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,  $G_q/G_{11}$ ,  $G_i/G_o$ , and  $G_{12}/G_{13}$ , which activate a variety of cellular pathways, including phosphoinositide hydrolysis, calcium mobilization, tyrosine/MAP kinase, and Rho kinase.<sup>139,207</sup> 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).<sup>104,167,212,234</sup>

#### **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.<sup>17,30,55,105</sup> 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.<sup>85</sup> 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.<sup>214</sup> 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.<sup>204</sup>

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.<sup>30,191</sup> 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.<sup>223,243</sup>

#### **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.<sup>168</sup> 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-1 $\beta$ , IL-6, and TNF- $\alpha$ .<sup>48</sup> Additionally, it seems that thrombin acts as a chemotactic agent for inflammatory cells, such as monocytes, macrophages, and neutrophils.<sup>25,87,103,138,212</sup>

#### **PAR-1-Mediated 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.<sup>202</sup> Among blood-derived proteases, thrombin activity via activation of PAR-1 seems to be important in glial scar formation. Indeed, picomolar concentrations of thrombin or the PAR-1-specific 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*.<sup>207,235</sup> Thrombin has also been proposed to up-regulate glial expression of thrombomodulin in *in vitro* models of injury.<sup>179</sup>

We have studied the involvement of PAR-1 in the initiation of astrogliosis *in vivo*.<sup>167,207</sup> 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.<sup>167</sup> Furthermore, we found that PAR-1<sup>-/-</sup> 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.<sup>167</sup> 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.<sup>95,159,164</sup> 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.<sup>167</sup> 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.<sup>41,86,115,194,198</sup> 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.<sup>44,216</sup> Likewise, antioxidant flavonoids, such as wogonin and silymarin, inhibit microglial TNF- $\alpha$ , IL-1 $\beta$ , and/or nitric oxide release after endotoxin or cytokine stimulation.<sup>118,236</sup> Wogonin additionally inhibits ischemic brain injury and lessens behavioral dysfunction caused by middle cerebral artery occlusion.<sup>47</sup>

Studies using animal models have found that anti-TGF- $\beta$  antibodies and anti-IL-6 receptor antibodies disrupt cytokine signaling and reduce glial scarring by several dif-

ferent measures. However, there was little change in axonal recovery.<sup>132,155,173</sup> As previously mentioned, IL-6 and TGF- $\beta$  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- $\beta$ , attenuate scar formation and promote recovery.<sup>96</sup> 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.<sup>110</sup> Similarly, curcumin can reduce the responses of other neuroglia, such as astrocytes and OPCs.<sup>7</sup> 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.<sup>31,92,144,156</sup> 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.<sup>189</sup>

Finally, altering serine protease signaling could reveal much about glial-neuronal 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.<sup>211</sup> 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.<sup>106,172</sup> Additionally, several clinical studies have found that argatroban is a safe antithrombotic 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.<sup>116,226</sup> 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.

## REFERENCES

- Aarum J, Sandberg K, Haerberlein SL, Persson MA: Migration and differentiation of neural precursor cells can be directed by microglia. **Proc Natl Acad Sci U S A** 100:15983–15988, 2003.
- Abnet K, Fawcett JW, Dunnett SB: Interactions between meningeal cells and astrocytes in vivo and in vitro. **Brain Res Dev Brain Res** 59:187–196, 1991.
- Akassoglou K, Probert L, Kontogeorgos G, Kollias G: Astrocyte-specific but not neuron-specific transmembrane TNF triggers inflammation and degeneration in the central nervous system of transgenic mice. **J Immunol** 158:438–445, 1997.
- Akiyama H, Ikeda K, Kondo H, McGeer PL: Thrombin accumulation in brains of patients with Alzheimer's disease. **Neurosci Lett** 146:152–154, 1992.
- Ali C, Nicole O, Docagne F, Lesne S, MacKenzie ET, Nouvelot A, Buisson A, Vivien D: Ischemia-induced interleukin-6 as a potential endogenous neuroprotective cytokine against NMDA receptor-mediated excitotoxicity in the brain. **J Cereb Blood Flow Metab** 20:956–966, 2000.
- Allan SM, Rothwell NJ: Cytokines and acute neurodegeneration. **Nat Rev Neurosci** 2:734–744, 2001.
- Ambegaokar SS, Wu L, Alamshahi K, Lau J, Jazayeri L, Chan S, Khanna P, Hsieh E, Timiras PS: Curcumin inhibits dose-dependently and time-dependently neuroglial cell proliferation and growth. **Neuro Endocrinol Lett** 24:469–473, 2003.
- Andersen JK: Oxidative stress in neurodegeneration: Cause or consequence? **Nat Med** 10:S18–25, 2004.
- Ankarcrona M, Dypbukt JM, Bonfoco E, Zhivotovsky B, Orrenius S, Lipton SA, Nicotera P: Glutamate-induced neuronal death: A succession of necrosis or apoptosis depending on mitochondrial function. **Neuron** 15:961–973, 1995.
- Arimoto T, Bing G: Up-regulation of inducible nitric oxide synthase in the substantia nigra by lipopolysaccharide causes microglial activation and neurodegeneration. **Neurobiol Dis** 12:35–45, 2003.
- Asher RA, Morgenstern DA, Fidler PS, Adcock KH, Oohira A, Braistead JE, Levine JM, Margolis RU, Rogers JH, Fawcett JW: Neurocan is upregulated in injured brain and in cytokine-treated astrocytes. **J Neurosci** 20:2427–2438, 2000.
- Azcoitia I, Sierra A, Veiga S, Garcia-Segura LM: Aromatase expression by reactive astroglia is neuroprotective. **Ann N Y Acad Sci** 1007:298–305, 2003.
- Azmitia EC: Modern views on an ancient chemical: Serotonin effects on cell proliferation, maturation, and apoptosis. **Brain Res Bull** 56:413–424, 2001.
- Back T, Schuler OG: The natural course of lesion development in brain ischemia. **Acta Neurochir Suppl** 89:55–61, 2004.
- Bajetto A, Bonavia R, Barbero S, Schettini G: Characterization of chemokines and their receptors in the central nervous system: Physiological implications. **J Neurochem** 82:1311–1329, 2002.
- Balcaitis S, Xie Y, Weinstein JR, Andersen H, Hanisch UK, Ransom BR, Moller T: Expression of proteinase-activated receptors in mouse microglial cells. **Neuroreport** 14:2373–2377, 2003.
- Ballabh P, Braun A, Nedergaard M: The blood-brain barrier: An overview: Structure, regulation, and clinical implications. **Neurobiol Dis** 16:1–13, 2004.
- Balschun D, Wetzal W, Del Rey A, Pitossi F, Schneider H, Zuschratter W, Besedovsky HO: Interleukin-6: A cytokine to forget. **FASEB J** 18:1788–1790, 2004.
- Basu A, Krady JK, Levison SW: Interleukin-1: A master regulator of neuroinflammation. **J Neurosci Res** 78:151–156, 2004.
- Batchelor PE, Porritt MJ, Martinello P, Parish CL, Liberatore GT, Donnan GA, Howells DW: Macrophages and Microglia Produce Local Trophic Gradients That Stimulate Axonal Sprouting Toward but Not beyond the Wound Edge. **Mol Cell Neurosci** 21:436–453, 2002.
- Beech JS, Reckless J, Mosedale DE, Grainger DJ, Williams SC, Menon DK: Neuroprotection in ischemia-reperfusion injury: An antiinflammatory approach using a novel broad-spectrum chemokine inhibitor. **J Cereb Blood Flow Metab** 21:683–689, 2001.
- Bergles DE, Roberts JD, Somogyi P, Jahr CE: Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. **Nature** 405:187–191, 2000.
- Bernardo A, Levi G, Minghetti L: Role of the peroxisome proliferator-activated receptor-gamma (PPAR-gamma) and its natural ligand 15-deoxy-Delta12, 14-prostaglandin J2 in the regulation of microglial functions. **Eur J Neurosci** 12:2215–2223, 2000.
- Bette M, Kaut O, Schafer MK, Weihe E: Constitutive expression of p55TNFR mRNA and mitogen-specific up-regulation of TNF alpha and p75TNFR mRNA in mouse brain. **J Comp Neurol** 465:417–430, 2003.
- Bizios R, Lai L, Fenton JW 2nd, Malik AB: Thrombin-induced chemotaxis and aggregation of neutrophils. **J Cell Physiol** 128:485–490, 1986.
- Bohatschek M, Kloss CU, Hristova M, Pfeffer K, Raivich G: Microglial major histocompatibility complex glycoprotein-1 in the axotomized facial motor nucleus: Regulation and role of tumor necrosis factor receptors 1 and 2. **J Comp Neurol** 470:382–399, 2004.
- Bohatschek M, Kloss CU, Pfeffer K, Bluethmann H, Raivich G: B7.2 on activated and phagocytic microglia in the facial axotomy model: Regulation by interleukin-1 receptor type 1, tumor necrosis factor receptors 1 and 2 and endotoxin. **J Neuroimmunol** 156:132–145, 2004.
- Bonfoco E, Krainc D, Ankarcrona M, Nicotera P, Lipton SA: Apoptosis and necrosis: Two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. **Proc Natl Acad Sci U S A** 92:7162–7166, 1995.
- Bottner M, Krieglstein K, Unsicker K: The transforming growth factor-beta: Structure, signaling, and roles in nervous system development and functions. **J Neurochem** 75:2227–2240, 2000.
- Boven LA, Vergnolle N, Henry SD, Silva C, Imai Y, Holden J, Warren K, Hollenberg MD, Power C: Up-regulation of proteinase-activated receptor 1 expression in astrocytes during HIV encephalitis. **J Immunol** 170:2638–2646, 2003.
- Bradbury EJ, Moon LD, Popat RJ, King VR, Bennett GS, Patel PN, Fawcett JW, McMahon SB: Chondroitinase ABC promotes functional recovery after spinal cord injury. **Nature** 416:636–640, 2002.
- Brock TO, O'Callaghan JP: Quantitative changes in the synaptic vesicle proteins synapsin I and p38 and the astrocyte-specific protein glial fibrillary acidic protein are associated with chemical-induced injury to the rat central nervous system. **J Neurosci** 7:931–942, 1987.
- Brown GC, Bal-Price A: Inflammatory neurodegeneration mediated by nitric oxide, glutamate, and mitochondria. **Mol Neurobiol** 27:325–355, 2003.
- Buck CR, Juryec MJ, Gupta DK, Law AK, Bilger J, Wallace DC, McKeon RJ: Increased adenine nucleotide translocator 1 in reactive astrocytes facilitates glutamate transport. **Exp Neurol** 181:149–158, 2003.
- Buisson A, Lesne S, Docagne F, Ali C, Nicole O, MacKenzie ET, Vivien D: Transforming growth factor-beta and ischemic brain injury. **Cell Mol Neurobiol** 23:539–550, 2003.
- Buisson A, Nicole O, Docagne F, Sartelet H, Mackenzie ET, Vivien D: Up-regulation of a serine protease inhibitor in astrocytes mediates the neuroprotective activity of transforming growth factor beta1. **FASEB J** 12:1683–1691, 1998.
- Burbach GJ, Hellweg R, Haas CA, Del Turco D, Deicke U, Abramowski D, Jucker M, Staufenbiel M, Deller T: Induction of brain-derived neurotrophic factor in plaque-associated glial cells of aged APP23 transgenic mice. **J Neurosci** 24:2421–2430, 2004.
- Burudi EM, Riese S, Stahl PD, Regnier-Vigouroux A: Identification and functional characterization of the mannose receptor in astrocytes. **Glia** 25:44–55, 1999.
- Bush TG, Puvanachandra N, Horner CH, Polito A, Ostensfeld T, Svendsen CN, Mucke L, Johnson MH, Sofroniew MV: Leukocyte infiltration, neuronal degeneration, and neurite outgrowth after ablation

- of scar-forming, reactive astrocytes in adult transgenic mice. **Neuron** 23:297–308, 1999.
40. Butt AM, Duncan A, Hornby MF, Kirvell SL, Hunter A, Levine JM, Berry M: Cells expressing the NG2 antigen contact nodes of Ranvier in adult CNS white matter. **Glia** 26:84–91, 1999.
  41. Candelario-Jalil E, Gonzalez-Falcon A, Garcia-Cabrera M, Alvarez D, Al-Dalain S, Martinez G, Leon OS, Springer JE (2003) Assessment of the relative contribution of COX-1 and COX-2 isoforms to ischemia-induced oxidative damage and neurodegeneration following transient global cerebral ischemia. **J Neurochem** 86:545–555.
  42. Canoll PD, Musacchio JM, Hardy R, Reynolds R, Marchionni MA, Salzer JL: GGF/neuregulin is a neuronal signal that promotes the proliferation and survival and inhibits the differentiation of oligodendrocyte progenitors. **Neuron** 17:229–243, 1996.
  43. Charles KJ, Deuchars J, Davies CH, Pangalos MN: GABA B receptor subunit expression in glia. **Mol Cell Neurosci** 24:214–223, 2003.
  44. Chen H, Zhang SM, Hernan MA, Schwarzschild MA, Willett WC, Colditz GA, Speizer FE, Ascherio A: Nonsteroidal anti-inflammatory drugs and the risk of Parkinson disease. **Arch Neurol** 60:1059–1064, 2003.
  45. Chen ZJ, Ughrin Y, Levine JM: Inhibition of axon growth by oligodendrocyte precursor cells. **Mol Cell Neurosci** 20:125–139, 2002.
  46. Cheng B, Christakos S, Mattson MP: Tumor necrosis factors protect neurons against metabolic-excitotoxic insults and promote maintenance of calcium homeostasis. **Neuron** 12:139–153, 1994.
  47. Cho J, Lee HK: Wogonin inhibits ischemic brain injury in a rat model of permanent middle cerebral artery occlusion. **Biol Pharm Bull** 27:1561–1564, 2004.
  48. Choi SH, Lee da Y, Ryu JK, Kim J, Joe EH, Jin BK: Thrombin induces nigral dopaminergic neurodegeneration in vivo by altering expression of death-related proteins. **Neurobiol Dis** 14:181–193, 2003.
  49. Ciesielski-Treska J, Ulrich G, Chasserot-Golaz S, Zwiler J, Revel MO, Anis D, Bader MF: Mechanisms underlying neuronal death induced by chromogranin A-activated microglia. **J Biol Chem** 276:13113–13120, 2001.
  50. Citron BA, Smirnova IV, Arnold PM, Festoff BW: Upregulation of neurotoxic serine proteases, prothrombin, and protease-activated receptor 1 early after spinal cord injury. **J Neurotrauma** 17:1191–1203, 2000.
  51. Colton CA, Needham LK, Brown C, Cook D, Rasheed K, Burke JR, Strittmatter WJ, Schmechel DE, Vitek MP: APOE genotype-specific differences in human and mouse macrophage nitric oxide production. **J Neuroimmunol** 147:62–67, 2004.
  52. Combs CK, Karlo JC, Kao SC, Landreth GE: beta-Amyloid stimulation of microglia and monocytes results in TNFalpha-dependent expression of inducible nitric oxide synthase and neuronal apoptosis. **J Neurosci** 21:1179–1188, 2001.
  53. Constam DB, Philipp J, Malipiero UV, ten Dijke P, Schachner M, Fontana A: Differential expression of transforming growth factor-beta 1, -beta 2, and -beta 3 by glioblastoma cells, astrocytes, and microglia. **J Immunol** 148:1404–1410, 1992.
  54. Coraci IS, Husemann J, Berman JW, Hulette C, Dufour JH, Campagnella GK, Luster AD, Silverstein SC, El-Khoury JB: CD36, a class B scavenger receptor, is expressed on microglia in Alzheimer's disease brains and can mediate production of reactive oxygen species in response to beta-amyloid fibrils. **Am J Pathol** 160:101–112, 2002.
  55. Correale J, Rabinowicz AL, Heck CN, Smith TD, Loskota WJ, DeGiorgio CM: Status epilepticus increases CSF levels of neuron-specific enolase and alters the blood-brain barrier. **Neurology** 50:1388–1391, 1998.
  56. Cross AK, Woodrooffe MN: Chemokines induce migration and changes in actin polymerization in adult rat brain microglia and a human fetal microglial cell line in vitro. **J Neurosci Res** 55:17–23, 1999.
  57. Cunningham DD: Regulation of neuronal cells and astrocytes by protease nexin-1 and thrombin. **Ann N Y Acad Sci** 674:228–236, 1992.
  58. Cunningham DD, Pulliam L, Vaughan PJ: Protease nexin-1 and thrombin: Injury-related processes in the brain. **Thromb Haemost** 70:168–171, 1993.
  59. D'Aversa TG, Yu KO, Berman JW: Expression of chemokines by human fetal microglia after treatment with the human immunodeficiency virus type 1 protein Tat. **J Neurovirol** 10:86–97, 2004.
  60. Dalpke AH, Schafer MK, Frey M, Zimmermann S, Tebbe J, Weihe E, Heeg K: Immunostimulatory CpG-DNA activates murine microglia. **J Immunol** 168:4854–4863, 2002.
  61. Davies JE, Tang X, Denning JW, Archibald SJ, Davies SJ: Decorin suppresses neurocan, brevican, phosphacan and NG2 expression and promotes axon growth across adult rat spinal cord injuries. **Eur J Neurosci** 19:1226–1242, 2004.
  62. Davies SJ, Fitch MT, Memberg SP, Hall AK, Raisman G, Silver J: Regeneration of adult axons in white matter tracts of the central nervous system. **Nature** 390:680–683, 1997.
  63. Davies SJ, Goucher DR, Doller C, Silver J: Robust regeneration of adult sensory axons in degenerating white matter of the adult rat spinal cord. **J Neurosci** 19:5810–5822, 1999.
  64. De Groot CJ, Montagne L, Barten AD, Sminia P, Van Der Valk P: Expression of transforming growth factor (TGF)-beta1, -beta2, and -beta3 isoforms and TGF-beta type I and type II receptors in multiple sclerosis lesions and human adult astrocyte cultures. **J Neuropathol Exp Neurol** 58:174–187, 1999.
  65. De Simone R, Ajmone-Cat MA, Minghetti L: Atypical antiinflammatory activation of microglia induced by apoptotic neurons: Possible role of phosphatidylserine-phosphatidylserine receptor interaction. **Mol Neurobiol** 29:197–212, 2004.
  66. DeJong BA, Smith ME: A role for complement in phagocytosis of myelin. **Neurochem Res** 22:491–498, 1997.
  67. Di Bello IC, Dawson MR, Levine JM, Reynolds R: Generation of oligodendroglial progenitors in acute inflammatory demyelinating lesions of the rat brain stem is associated with demyelination rather than inflammation. **J Neurocytol** 28:365–381, 1999.
  68. Dihne M, Block F, Korr H, Topper R: Time course of glial proliferation and glial apoptosis following excitotoxic CNS injury. **Brain Res** 902:178–189, 2001.
  69. Dijkstra IM, Hulshof S, van der Valk P, Boddeke HW, Biber K: Cutting edge: Activity of human adult microglia in response to CC chemokine ligand 21. **J Immunol** 172:2744–2747, 2004.
  70. Dong Y, Benveniste EN: Immune function of astrocytes. **Glia** 36:180–190, 2001.
  71. Faulkner JR, Herrmann JE, Woo MJ, Tansey KE, Doan NB, Sofroniew MV: Reactive astrocytes protect tissue and preserve function after spinal cord injury. **J Neurosci** 24:2143–2155, 2004.
  72. Fawcett JW, Asher RA: The glial scar and central nervous system repair. **Brain Res Bull** 49:377–391, 1999.
  73. Fawcett JW, Housden E, Smith-Thomas L, Meyer RL: The growth of axons in three-dimensional astrocyte cultures. **Dev Biol** 135:449–458, 1989.
  74. Festoff BW, Rao JS, Chen M: Protease nexin I, thrombin- and urokinase-inhibiting serpin, concentrated in normal human cerebrospinal fluid. **Neurology** 42:1361–1366, 1992.
  75. Feuerstein GZ, Wang X, Barone FC: The role of cytokines in the neuropathology of stroke and neurotrauma. **Neuroimmunomodulation** 5:143–159, 1998.
  76. Flanders KC, Ren RF, Lippa CF: Transforming growth factor-betas in neurodegenerative disease. **Prog Neurobiol** 54:71–85, 1998.
  77. Friedlander DR, Milev P, Karthikeyan L, Margolis RK, Margolis RU, Grumet M: The neuronal chondroitin sulfate proteoglycan neurocan binds to the neural cell adhesion molecules Ng-CAM/L1/NILE and N-CAM, and inhibits neuronal adhesion and neurite outgrowth. **J Cell Biol** 125:669–680, 1994.
  78. Gan L, Ye S, Chu A, Anton K, Yi S, Vincent VA, von Schack D, Chin D, Murray J, Lohr S, Pathy L, Gonzalez-Zulueta M, Nikolich K, Urfer R: Identification of cathepsin B as a mediator of neuronal death induced by Abeta-activated microglial cells using a functional genomics approach. **J Biol Chem** 279:5565–5572, 2004.
  79. Gao HM, Liu B, Zhang W, Hong JS: Critical role of microglial NADPH oxidase-derived free radicals in the in vitro MPTP model of Parkinson's disease. **FASEB J** 17:1954–1956, 2003.
  80. Garcia-Ovejero D, Veiga S, Garcia-Segura LM, DonCarlos LL: Glial expression of estrogen and androgen receptors after rat brain injury. **J Comp Neurol** 450:256–271, 2002.

81. Gardner J, Ghorpade A: Tissue inhibitor of metalloproteinase (TIMP)-1: The TIMPed balance of matrix metalloproteinases in the central nervous system. **J Neurosci Res** 74:801–806, 2003.
82. Gehrmann J, Matsumoto Y, Kreutzberg GW: Microglia: Intrinsic immunoeffector cell of the brain. **Brain Res Brain Res Rev** 20:269–287, 1995.
83. Geurts JJ, Wolswijk G, Bo L, van der Valk P, Polman CH, Troost D, Aronica E: Altered expression patterns of group I and II metabotropic glutamate receptors in multiple sclerosis. **Brain** 126:1755–1766, 2003.
84. Ghorpade A, Persidskaia R, Suryadevara R, Che M, Liu XJ, Persidsky Y, Gendelman HE: Mononuclear phagocyte differentiation, activation, and viral infection regulate matrix metalloproteinase expression: Implications for human immunodeficiency virus type 1-associated dementia. **J Virol** 75:6572–6583, 2001.
85. Gingrich MB, Traynelis SF: Serine proteases and brain damage—is there a link? **Trends Neurosci** 23:399–407, 2000.
86. Giovannini MG, Scali C, Prosperi C, Bellucci A, Vannucchi MG, Rosi S, Pepeu G, Casamenti F: Beta-amyloid-induced inflammation and cholinergic hypofunction in the rat brain in vivo: Involvement of the p38MAPK pathway. **Neurobiol Dis** 11:257–274, 2002.
87. Goldsack NR, Chambers RC, Dabbagh K, Laurent GJ: Thrombin. **Int J Biochem Cell Biol** 30:641–646, 1998.
88. Gonzalez-Scarano F, Baltuch G: Microglia as mediators of inflammatory and degenerative diseases. **Annu Rev Neurosci** 22:219–240, 1999.
89. Gottschall PE, Yu X, Bing B: Increased production of gelatinase B (matrix metalloproteinase-9) and interleukin-6 by activated rat microglia in culture. **J Neurosci Res** 42:335–342, 1995.
90. Granucci F, Petralia F, Urbano M, Citterio S, Di Tota F, Santambrogio L, Ricciardi-Castagnoli P: The scavenger receptor MARCO mediates cytoskeleton rearrangements in dendritic cells and microglia. **Blood** 102:2940–2947, 2003.
91. Gras G, Chretien F, Vallat-Decouvelaere AV, Le Pavec G, Porcheray F, Bossuet C, Leone C, Mialocq P, Dereuddre-Bosquet N, Clayette P, Le Grand R, Creminon C, Dormont D, Rimaniol AC, Gray F: Regulated expression of sodium-dependent glutamate transporters and synthetase: A neuroprotective role for activated microglia and macrophages in HIV infection? **Brain Pathol** 13:211–222, 2003.
92. Grimpe B, Silver J: A novel DNA enzyme reduces glycosaminoglycan chains in the glial scar and allows microtransplanted dorsal root ganglia axons to regenerate beyond lesions in the spinal cord. **J Neurosci** 24:1393–1397, 2004.
93. Haas CA, Rauch U, Thon N, Merten T, Deller T: Entorhinal cortex lesion in adult rats induces the expression of the neuronal chondroitin sulfate proteoglycan neurocan in reactive astrocytes. **J Neurosci** 19:9953–9963, 1999.
94. Hailer NP, Grampp A, Nitsch R: Proliferation of microglia and astrocytes in the dentate gyrus following entorhinal cortex lesion: A quantitative bromodeoxyuridine-labelling study. **Eur J Neurosci** 11:3359–3364, 1999.
95. Hashimoto H, Kunugi A, Arakawa N, Shintani N, Fujita T, Kasai A, Kawaguchi C, Morita Y, Hirose M, Sakai Y, Baba A: Possible involvement of a cyclic AMP-dependent mechanism in PACAP-induced proliferation and ERK activation in astrocytes. **Biochem Biophys Res Commun** 311:337–343, 2003.
96. Hayashi N, Miyata S, Kariya Y, Takano R, Hara S, Kamei K: Attenuation of glial scar formation in the injured rat brain by heparin oligosaccharides. **Neurosci Res** 49:19–27, 2004.
97. Hirrlinger J, Gutterer JM, Kussmaul L, Hamprecht B, Dringen R: Microglial cells in culture express a prominent glutathione system for the defense against reactive oxygen species. **Dev Neurosci** 22:384–392, 2000.
98. Hirt UA, Gantner F, Leist M: Phagocytosis of nonapoptotic cells dying by caspase-independent mechanisms. **J Immunol** 164:6520–6529, 2000.
99. Hollenberg MD: PARs in the stars: Proteinase-activated receptors and astrocyte function. Focus on “Thrombin (PAR-1)-induced proliferation in astrocytes via MAPK involves multiple signaling pathways.” **Am J Physiol Cell Physiol** 283:C1347–1350, 2002.
100. Honda S, Sasaki Y, Ohsawa K, Imai Y, Nakamura Y, Inoue K, Kohsaka S: Extracellular ATP or ADP induce chemotaxis of cultured microglia through Gi/o-coupled P2Y receptors. **J Neurosci** 21:1975–1982, 2001.
101. Husemann J, Loike JD, Anankov R, Febbraio M, Silverstein SC: Scavenger receptors in neurobiology and neuropathology: Their role on microglia and other cells of the nervous system. **Glia** 40:195–205, 2002.
102. John GR, Lee SC, Song X, Rivieccio M, Brosnan CF: IL-1-regulated responses in astrocytes: Relevance to injury and recovery. **Glia** 49:161–176, 2004.
103. Jones A, Geczy CL: Thrombin and factor Xa enhance the production of interleukin-1. **Immunology** 71:236–241, 1990.
104. Junge CE, Lee CJ, Hubbard KB, Zhang Z, Olson JJ, Hepler JR, Brat DJ, Traynelis SF: Protease-activated receptor-1 in human brain: Localization and functional expression in astrocytes. **Exp Neurol** 188:94–103, 2004.
105. Junge CE, Sugawara T, Mannaioni G, Alagarsamy S, Conn PJ, Brat DJ, Chan PH, Traynelis SF: The contribution of protease-activated receptor 1 to neuronal damage caused by transient focal cerebral ischemia. **Proc Natl Acad Sci U S A** 100:13019–13024, 2003.
106. Kamiya T, Nito C, Ueda M, Kato K, Amemiya S, Terashi A, Katayama Y: Mild hypothermia enhances the neuroprotective effects of a selective thrombin inhibitor following transient focal ischemia in rats. **Acta Neurochir Suppl** 86:195–198, 2003.
107. Kaul M, Garden GA, Lipton SA: Pathways to neuronal injury and apoptosis in HIV-associated dementia. **Nature** 410:988–994, 2001.
108. Kawaja MD, Gage FH: Reactive astrocytes are substrates for the growth of adult CNS axons in the presence of elevated levels of nerve growth factor. **Neuron** 7:1019–1030, 1991.
109. Kawanokuchi J, Mizuno T, Kato H, Mitsuma N, Suzumura A: Effects of interferon-beta on microglial functions as inflammatory and antigen presenting cells in the central nervous system. **Neuropharmacology** 46:734–742, 2004.
110. Kim HY, Park EJ, Joe EH, Jou I: Curcumin suppresses Janus kinase-STAT inflammatory signaling through activation of Src homology 2 domain-containing tyrosine phosphatase 2 in brain microglia. **J Immunol** 171:6072–6079, 2003.
111. Kim NG, Lee H, Son E, Kwon OY, Park JY, Park JH, Cho GJ, Choi WS, Suk K: Hypoxic induction of caspase-11/caspase-1/interleukin-1beta in brain microglia. **Brain Res Mol Brain Res** 114:107–114, 2003.
112. Kim WK, Hwang SY, Oh ES, Piao HZ, Kim KW, Han IO: TGF-beta1 represses activation and resultant death of microglia via inhibition of phosphatidylinositol 3-kinase activity. **J Immunol** 172:7015–7023, 2004.
113. Koguchi K, Nakatsuji Y, Okuno T, Sawada M, Sakoda S: Microglial cell cycle-associated proteins control microglial proliferation in vivo and in vitro and are regulated by GM-CSF and density-dependent inhibition. **J Neurosci Res** 74:898–905, 2003.
114. Kopec KK, Carroll RT: Alzheimer's beta-amyloid peptide 1–42 induces a phagocytic response in murine microglia. **J Neurochem** 71:2123–2131, 1998.
115. Kunz T, Oliw EH: The selective cyclooxygenase-2 inhibitor rofecoxib reduces kainate-induced cell death in the rat hippocampus. **Eur J Neurosci** 13:569–575, 2001.
116. LaMonte MP, Stallmeyer MJ: Acute ischemic stroke successfully treated using sequenced intravenous and intra-arterial thrombolysis and argatroban anticoagulation: A case study. **J Thromb Thrombolysis** 17:151–156, 2004.
117. Le Y, Gong W, Tiffany HL, Tumanov A, Nedospasov S, Shen W, Dunlop NM, Gao JL, Murphy PM, Oppenheim JJ, Wang JM: Amyloid (beta)42 activates a G-protein-coupled chemoattractant receptor, FPR-like-1. **J Neurosci** 21:RC123, 2001.
118. Lee H, Kim YO, Kim H, Kim SY, Noh HS, Kang SS, Cho GJ, Choi WS, Suk K: Flavonoid wogonin from medicinal herb is neuroprotective by inhibiting inflammatory activation of microglia. **FASEB J** 17:1943–1944, 2003.
119. Lee J, Auyeung WW, Mattson MP: Interactive effects of excitotoxic injury and dietary restriction on microgliosis and neurogenesis in the hippocampus of adult mice. **Neuromolecular Med** 4:179–196, 2003.

120. Lee YB, Nagai A, Kim SU: Cytokines, chemokines, and cytokine receptors in human microglia. **J Neurosci Res** 69:94–103, 2002.
121. Lehrmann E, Kiefer R, Christensen T, Toyka KV, Zimmer J, Diemer NH, Hartung HP, Finsen B: Microglia and macrophages are major sources of locally produced transforming growth factor-beta1 after transient middle cerebral artery occlusion in rats. **Glia** 24:437–448, 1998.
122. Leung KM, Margolis RU, Chan SO: Expression of phosphacan and neurocan during early development of mouse retinofugal pathway. **Brain Res Dev Brain Res** 152:1–10, 2004.
123. Levine JM: Neuronal influences on glial progenitor cell development. **Neuron** 3:103–113, 1989.
124. Levine JM: Increased expression of the NG2 chondroitin-sulfate proteoglycan after brain injury. **J Neurosci** 14:4716–4730, 1994.
125. Levine JM, Reynolds R, Fawcett JW: The oligodendrocyte precursor cell in health and disease. **Trends Neurosci** 24:39–47, 2001.
126. Liberto CM, Albrecht PJ, Herx LM, Yong VW, Levison SW: Pro-regenerative properties of cytokine-activated astrocytes. **J Neurochem** 89:1092–1100, 2004.
127. Lindenau J, Noack H, Asayama K, Wolf G (1998) Enhanced cellular glutathione peroxidase immunoreactivity in activated astrocytes and in microglia during excitotoxin induced neurodegeneration. **Glia** 24:252–256.
128. Liu J, Bartels M, Lu A, Sharp FR: Microglia/macrophages proliferate in striatum and neocortex but not in hippocampus after brief global ischemia that produces ischemic tolerance in gerbil brain. **J Cereb Blood Flow Metab** 21:361–373, 2001.
129. Liuzzi GM, Mastroianni CM, Latronico T, Mengoni F, Fasano A, Lichtner M, Vullo V, Riccio P: Anti-HIV drugs decrease the expression of matrix metalloproteinases in astrocytes and microglia. **Brain** 127:398–407, 2004.
130. Liva SM, Kahn MA, Dopp JM, de Vellis J: Signal transduction pathways induced by GM-CSF in microglia: Significance in the control of proliferation. **Glia** 26:344–352, 1999.
131. Logan A, Berry M, Gonzalez AM, Frautschy SA, Sporn MB, Baird A: Effects of transforming growth factor beta 1 on scar production in the injured central nervous system of the rat. **Eur J Neurosci** 6:355–363, 1994.
132. Logan A, Green J, Hunter A, Jackson R, Berry M: Inhibition of glial scarring in the injured rat brain by a recombinant human monoclonal antibody to transforming growth factor-beta2. **Eur J Neurosci** 11:2367–2374, 1999.
133. Lorenzl S, Albers DS, Narr S, Chirichigno J, Beal MF: Expression of MMP-2, MMP-9, and MMP-1 and their endogenous counterregulators TIMP-1 and TIMP-2 in postmortem brain tissue of Parkinson's disease. **Exp Neurol** 178:13–20, 2002.
134. Love S: Oxidative stress in brain ischemia. **Brain Pathol** 9:119–131, 1999.
135. Lue LF, Walker DG, Brachova L, Beach TG, Rogers J, Schmidt AM, Stern DM, Yan SD: Involvement of microglial receptor for advanced glycation endproducts (RAGE) in Alzheimer's disease: Identification of a cellular activation mechanism. **Exp Neurol** 171:29–45, 2001.
136. Lynch NJ, Willis CL, Nolan CC, Roscher S, Fowler MJ, Weihe E, Ray DE, Schwaebler WJ: Microglial activation and increased synthesis of complement component C1q precedes blood-brain barrier dysfunction in rats. **Mol Immunol** 40:709–716, 2004.
137. MacCumber MW, Ross CA, Snyder SH: Endothelin in brain: Receptors, mitogenesis, and biosynthesis in glial cells. **Proc Natl Acad Sci U S A** 87:2359–2363, 1990.
138. Macfarlane SR, Seatter MJ, Kanke T, Hunter GD, Plevin R: Proteinase-activated receptors. **Pharmacol Rev** 53:245–282, 2001.
139. Mackie EJ, Pagel CN, Smith R, de Niese MR, Song SJ, Pike RN: Protease-activated receptors: A means of converting extracellular proteolysis into intracellular signals. **IUBMB Life** 53:277–281, 2002.
140. Maeda Y, Matsumoto M, Hori O, Kuwabara K, Ogawa S, Yan SD, Ohtsuki T, Kinoshita T, Kamada T, Stern DM: Hypoxia/reoxygenation-mediated induction of astrocyte interleukin 6: A paracrine mechanism potentially enhancing neuron survival. **J Exp Med** 180:2297–2308, 1994.
141. Marin-Teva JL, Dusart I, Colin C, Gervais A, van Rooijen N, Mallat M: Microglia promote the death of developing Purkinje cells. **Neuron** 41:535–547, 2004.
142. Martin S, Vincent JP, Mazella J: Involvement of the neurotensin receptor-3 in the neurotensin-induced migration of human microglia. **J Neurosci** 23:1198–1205, 2003.
143. McDonald DR, Brunden KR, Landreth GE: Amyloid fibrils activate tyrosine kinase-dependent signaling and superoxide production in microglia. **J Neurosci** 17:2284–2294, 1997.
144. McKeon RJ, Hoke A, Silver J: Injury-induced proteoglycans inhibit the potential for laminin-mediated axon growth on astrocytic scars. **Exp Neurol** 136:32–43, 1995.
145. McKeon RJ, Jurynek MJ, Buck CR: The chondroitin sulfate proteoglycans neurocan and phosphacan are expressed by reactive astrocytes in the chronic CNS glial scar. **J Neurosci** 19:10778–10788, 1999.
146. McKeon RJ, Schreiber RC, Rudge JS, Silver J: Reduction of neurite outgrowth in a model of glial scarring following CNS injury is correlated with the expression of inhibitory molecules on reactive astrocytes. **J Neurosci** 11:3398–3411, 1991.
147. Meda L, Cassatella MA, Szendrei GI, Ottos L Jr, Baron P, Villalba M, Ferrari D, Rossi F: Activation of microglial cells by beta-amyloid protein and interferon-gamma. **Nature** 374:647–650, 1995.
148. Mehler MF, Kessler JA: Cytokines and neuronal differentiation. **Crit Rev Neurobiol** 9:419–446, 1995.
149. Menet V, Gimenez y Ribotta M, Chauvet N, Drian MJ, Lannoy J, Colucci-Guyon E, Privat A: Inactivation of the glial fibrillary acidic protein gene, but not that of vimentin, improves neuronal survival and neurite growth by modifying adhesion molecule expression. **J Neurosci** 21:6147–6158, 2001.
150. Menet V, Prieto M, Privat A, Gimenez y Ribotta M: Axonal plasticity and functional recovery after spinal cord injury in mice deficient in both glial fibrillary acidic protein and vimentin genes. **Proc Natl Acad Sci U S A** 100:8999–9004, 2003.
151. Miklic S, Juric DM, Caman-Krzan M: Differences in the regulation of BDNF and NGF synthesis in cultured neonatal rat astrocytes. **Int J Dev Neurosci** 22:119–130, 2004.
152. Milner R, Campbell IL: The extracellular matrix and cytokines regulate microglial integrin expression and activation. **J Immunol** 170:3850–3858, 2003.
153. Min KJ, Pyo HK, Yang MS, Ji KA, Jou I, Joe EH: Gangliosides activate microglia via protein kinase C and NADPH oxidase. **Glia** 48:197, 2004.
154. Mitrasinovic OM, Vincent VA, Simsek D, Murphy GM Jr: Macrophage colony stimulating factor promotes phagocytosis by murine microglia. **Neurosci Lett** 344:185–188, 2003.
155. Moon LD, Fawcett JW: Reduction in CNS scar formation without concomitant increase in axon regeneration following treatment of adult rat brain with a combination of antibodies to TGFbeta1 and beta2. **Eur J Neurosci** 14:1667–1677, 2001.
156. Moon LD, Asher RA, Rhodes KE, Fawcett JW: Regeneration of CNS axons back to their target following treatment of adult rat brain with chondroitinase ABC. **Nat Neurosci** 4:465–466, 2001.
157. Morgan SC, Taylor DL, Pocock JM: Microglia release activators of neuronal proliferation mediated by activation of mitogen-activated protein kinase, phosphatidylinositol-3-kinase/Akt and delta-Notch signalling cascades. **J Neurochem** 90:89–101, 2004.
158. Morioka T, Kolehua AN, Streit WJ: Characterization of microglial reaction after middle cerebral artery occlusion in rat brain. **J Comp Neurol** 327:123–132, 1993.
159. Morita M, Higuchi C, Moto T, Kozuka N, Susuki J, Itofusa R, Yamashita J, Kudo Y: Dual regulation of calcium oscillation in astrocytes by growth factors and pro-inflammatory cytokines via the mitogen-activated protein kinase cascade. **J Neurosci** 23:10944–10952, 2003.
160. Mosley K, Cuzner ML: Receptor-mediated phagocytosis of myelin by macrophages and microglia: Effect of opsonization and receptor blocking agents. **Neurochem Res** 21:481–487, 1996.
161. Nagai A, Nakagawa E, Choi HB, Hatori K, Kobayashi S, Kim SU: Erythropoietin and erythropoietin receptors in human CNS neurons, astrocytes, microglia, and oligodendrocytes grown in culture. **J Neuropathol Exp Neurol** 60:386–392, 2001.

162. Nakajima K, Tohyama Y, Kohsaka S, Kurihara T: Ceramide activates microglia to enhance the production/secretion of brain-derived neurotrophic factor (BDNF) without induction of deleterious factors in vitro. **J Neurochem** 80:697–705, 2002.
163. Nakamura Y, Ohmaki M, Murakami K, Yoneda Y: Involvement of protein kinase C in glutamate release from cultured microglia. **Brain Res** 962:122–128, 2003.
164. Neary JT, Kang Y, Willoughby KA, Ellis EF: Activation of extracellular signal-regulated kinase by stretch-induced injury in astrocytes involves extracellular ATP and P2 purinergic receptors. **J Neurosci** 23:2348–2356, 2003.
165. Nedergaard M, Ransom B, Goldman SA: New roles for astrocytes: Redefining the functional architecture of the brain. **Trends Neurosci** 26:523–530, 2003.
166. Neumann H, Schweigreiter R, Yamashita T, Rosenkranz K, Wekerle H, Barde YA: Tumor necrosis factor inhibits neurite outgrowth and branching of hippocampal neurons by a rho-dependent mechanism. **J Neurosci** 22:854–862, 2002.
167. Nicole O, Goldschmidt A, Sorensen SD, Sastre A, Lyuboslavsky P, Hepler JP, Traynelis SF: Activation of protease activated receptor-1 (PAR-1) triggers glial scar formation after brain injury. **J Neurosci** (in press).
168. Nishino A, Suzuki M, Ohtani H, Motohashi O, Umezawa K, Nagura H, Yoshimoto T: Thrombin may contribute to the pathophysiology of central nervous system injury. **J Neurotrauma** 10:167–179, 1993.
169. Noda M, Nakanishi H, Nabekura J, Akaike N: AMPA-kainate subtypes of glutamate receptor in rat cerebral microglia. **J Neurosci** 20:251–258, 2000.
170. Nolte C, Gore A, Sekler I, Kresse W, Hershinkel M, Hoffmann A, Kettenmann H, Moran A: ZnT-1 expression in astroglial cells protects against zinc toxicity and slows the accumulation of intracellular zinc. **Glia** 48:145, 2004.
171. Nolte C, Moller T, Walter T, Kettenmann H: Complement 5a controls motility of murine microglial cells in vitro via activation of an inhibitory G-protein and the rearrangement of the actin cytoskeleton. **Neuroscience** 73:1091–1107, 1996.
172. Ohyama H, Hosomi N, Takahashi T, Mizushige K, Kohno M: Thrombin inhibition attenuates neurodegeneration and cerebral edema formation following transient forebrain ischemia. **Brain Res** 902:264–271, 2001.
173. Okada S, Nakamura M, Mikami Y, Shimazaki T, Mihara M, Ohsugi Y, Iwamoto Y, Yoshizaki K, Kishimoto T, Toyama Y, Okano H: Blockade of interleukin-6 receptor suppresses reactive astrogliosis and ameliorates functional recovery in experimental spinal cord injury. **J Neurosci Res** 76:265–276, 2004.
174. Olson JK, Miller SD: Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. **J Immunol** 173:3916–3924, 2004.
175. Patel HC, Boutin H, Allan SM: Interleukin-1 in the brain: Mechanisms of action in acute neurodegeneration. **Ann N Y Acad Sci** 992:39–47, 2003.
176. Patterson CE, Lawrence DM, Echols LA, Rall GF: Immune-mediated protection from measles virus-induced central nervous system disease is noncytolytic and gamma interferon dependent. **J Virol** 76:4497–4506, 2002.
177. Pavelko KD, Howe CL, Drescher KM, Gamez JD, Johnson AJ, Wei T, Ransohoff RM, Rodriguez M: Interleukin-6 protects anterior horn neurons from lethal virus-induced injury. **J Neurosci** 23:481–492, 2003.
178. Petersen MA, Dailey ME: Diverse microglial motility behaviors during clearance of dead cells in hippocampal slices. **Glia** 46:195–206, 2004.
179. Pindon A, Berry M, Hantai D: Thrombomodulin as a new marker of lesion-induced astrogliosis: Involvement of thrombin through the G-protein-coupled protease-activated receptor-1. **J Neurosci** 20:2543–2550, 2000.
180. Planas AM, Sole S, Justicia C: Expression and activation of matrix metalloproteinase-2 and -9 in rat brain after transient focal cerebral ischemia. **Neurobiol Dis** 8:834–846, 2001.
181. Platten M, Kretz A, Naumann U, Aulwurm S, Egashira K, Isenmann S, Weller M: Monocyte chemoattractant protein-1 increases microglial infiltration and aggressiveness of gliomas. **Ann Neurol** 54:388–392, 2003.
182. Prinz M, Hanisch UK: Murine microglial cells produce and respond to interleukin-18. **J Neurochem** 72:2215–2218, 1999.
183. Raivich G, Haas S, Werner A, Klein MA, Kloss C, Kreutzberg GW: Regulation of MCSF receptors on microglia in the normal and injured mouse central nervous system: A quantitative immunofluorescence study using confocal laser microscopy. **J Comp Neurol** 395:342–358, 1998.
184. Rappert A, Bechmann I, Pivneva T, Mahlo J, Biber K, Nolte C, Kovac AD, Gerard C, Boddeke HW, Nitsch R, Kettenmann H: CXCR3-dependent microglial recruitment is essential for dendrite loss after brain lesion. **J Neurosci** 24:8500–8509, 2004.
185. Rathbone MP, Middlemiss PJ, Gysbers JW, Andrew C, Herman MA, Reed JK, Ciccarelli R, Di Iorio P, Caciagli F: Trophic effects of purines in neurons and glial cells. **Prog Neurobiol** 59:663–690, 1999.
186. Reichert F, Rotshenker S: Complement-receptor-3 and scavenger-receptor-AI/II mediated myelin phagocytosis in microglia and macrophages. **Neurobiol Dis** 12:65–72, 2003.
187. Reier PJ, Houle JD: The glial scar: Its bearing on axonal elongation and transplantation approaches to CNS repair. **Adv Neurol** 47:87–138, 1988.
188. Retzler C, Gohring W, Rauch U: Analysis of neurocan structures interacting with the neural cell adhesion molecule N-CAM. **J Biol Chem** 271:27304–27310, 1996.
189. Rhodes KE, Moon LD, Fawcett JW: Inhibiting cell proliferation during formation of the glial scar: Effects on axon regeneration in the CNS. **Neuroscience** 120:41–56, 2003.
190. Ridet JL, Malhotra SK, Privat A, Gage FH: Reactive astrocytes: Cellular and molecular cues to biological function. **Trends Neurosci** 20:570–577, 1997.
191. Riek-Burchardt M, Striggow F, Henrich-Noack P, Reiser G, Reymann KG: Increase of prothrombin-mRNA after global cerebral ischemia in rats, with constant expression of protease nexin-1 and protease-activated receptors. **Neurosci Lett** 329:181–184, 2002.
192. Rimaniol AC, Haik S, Martin M, Le Grand R, Boussin FD, Dereuddre-Bosquet N, Gras G, Dormont D: Na<sup>+</sup>-dependent high-affinity glutamate transport in macrophages. **J Immunol** 164:5430–5438, 2000.
193. Rizzi M, Perego C, Aliprandi M, Richichi C, Ravizza T, Colella D, Veliskova J, Moshe SL, De Simoni MG, Vezzani A: Glia activation and cytokine increase in rat hippocampus by kainic acid-induced status epilepticus during postnatal development. **Neurobiol Dis** 14:494–503, 2003.
194. Ryu JK, Franciosi S, Sattayaprasert P, Kim SU, McLarnon JG: Minocycline inhibits neuronal death and glial activation induced by beta-amyloid peptide in rat hippocampus. **Glia** 48:85–90, 2004.
195. Saha RN, Pahan K: Tumor necrosis factor-alpha at the crossroads of neuronal life and death during HIV-associated dementia. **J Neurochem** 86:1057–1071, 2003.
196. Sasaki Y, Hoshi M, Akazawa C, Nakamura Y, Tsuzuki H, Inoue K, Kohsaka S: Selective expression of Gi/o-coupled ATP receptor P2Y<sub>12</sub> in microglia in rat brain. **Glia** 44:242–250, 2003.
197. Sawada M, Itoh Y, Suzumura A, Marunouchi T: Expression of cytokine receptors in cultured neuronal and glial cells. **Neurosci Lett** 160:131–134, 1993.
198. Scali C, Giovannini MG, Prosperi C, Bellucci A, Pepeu G, Casamenti F: The selective cyclooxygenase-2 inhibitor rofecoxib suppresses brain inflammation and protects cholinergic neurons from excitotoxic degeneration in vivo. **Neuroscience** 117:909–919, 2003.
199. Schlomann U, Rathke-Hartlieb S, Yamamoto S, Jockusch H, Bartsch JW: Tumor necrosis factor alpha induces a metalloprotease-disintegrin, ADAM8 (CD 156): Implications for neuron-glia interactions during neurodegeneration. **J Neurosci** 20:7964–7971, 2000.
200. Si Q, Zhao ML, Morgan AC, Brosnan CF, Lee SC: 15-deoxy-Delta<sup>12,14</sup>-prostaglandin J<sub>2</sub> inhibits IFN-inducible protein 10/CXC chemokine ligand 10 expression in human microglia: Mechanisms and implications. **J Immunol** 173:3504–3513, 2004.
201. Siao CJ, Fernandez SR, Tsirka SE: Cell type-specific roles for tissue plasminogen activator released by neurons or microglia after excitotoxic injury. **J Neurosci** 23:3234–3242, 2003.
202. Silver J, Miller JH (2004) Regeneration beyond the glial scar. **Nat Rev Neurosci** 5:146–156.
203. Sivasankaran R, Pei J, Wang KC, Zhang YP, Shields CB, Xu XM, He Z: PKC mediates inhibitory effects of myelin and chondroitin sulfate proteoglycans on axonal regeneration. **Nat Neurosci** 7:261–268, 2004.
204. Smirnova IV, Salazar A, Arnold PM, Glatt S, Handler M, Festoff BW:

- Thrombin and its precursor in human cerebrospinal fluid. **Thromb Haemost** 78:1473–1479, 1997.
205. Smith ME, Eng LF: Glial fibrillary acidic protein in chronic relapsing experimental allergic encephalomyelitis in SJL/J mice. **J Neurosci Res** 18:203–208, 1987.
  206. Smith ME, van der Maesen K, Somera FP: Macrophage and microglial responses to cytokines in vitro: Phagocytic activity, proteolytic enzyme release, and free radical production. **J Neurosci Res** 54:68–78, 1998.
  207. Sorensen SD, Nicole O, Peavy RD, Montoya LM, Lee CJ, Murphy TJ, Traynelis SF, Hepler JR: Common signaling pathways link activation of murine PAR-1, LPA, and SIP receptors to proliferation of astrocytes. **Mol Pharmacol** 64:1199–1209, 2003.
  208. Stevens SL, Shaw TE, Dykhuizen E, Lessov NS, Hill JK, Wurst W, Stenzel-Poore MP: Reduced cerebral injury in CRH-R1 deficient mice after focal ischemia: A potential link to microglia and astrocytes that express CRH-R1. **J Cereb Blood Flow Metab** 23:1151–1159, 2003.
  209. Stoll G, Jander S, Schroeter M: Detrimental and beneficial effects of injury-induced inflammation and cytokine expression in the nervous system. **Adv Exp Med Biol** 513:87–113, 2002.
  210. Streit WJ: Microglia as neuroprotective, immunocompetent cells of the CNS. **Glia** 40:133–139, 2002.
  211. Striggow F, Riek M, Breder J, Henrich-Noack P, Reymann KG, Reiser G: The protease thrombin is an endogenous mediator of hippocampal neuroprotection against ischemia at low concentrations but causes degeneration at high concentrations. **Proc Natl Acad Sci U S A** 97:2264–2269, 2000.
  212. Suo Z, Wu M, Ameenuddin S, Anderson HE, Zoloty JE, Citron BA, Andrade-Gordon P, Festoff BW: Participation of protease-activated receptor-1 in thrombin-induced microglial activation. **J Neurochem** 80:655–666, 2002.
  213. Suzuki H, Imai F, Kanno T, Sawada M: Preservation of neurotrophin expression in microglia that migrate into the gerbil's brain across the blood-brain barrier. **Neurosci Lett** 312:95–98, 2001.
  214. Suzuki M, Ogawa A, Sakurai Y, Nishino A, Venohara K, Mizoi K, Yoshimoto T: Thrombin activity in cerebrospinal fluid after subarachnoid hemorrhage. **Stroke** 23:1181–1182, 1992.
  215. Suzuki T, Hide I, Ido K, Kohsaka S, Inoue K, Nakata Y: Production and release of neuroprotective tumor necrosis factor by P2X7 receptor-activated microglia. **J Neurosci** 24:1–7, 2004.
  216. Szekely CA, Thorne JE, Zandi PP, Ek M, Messias E, Breitner JC, Goodman SN: Nonsteroidal anti-inflammatory drugs for the prevention of Alzheimer's disease: A systematic review. **Neuroepidemiology** 23:159–169, 2004.
  217. Tan J, Town T, Mullan M: CD45 inhibits CD40L-induced microglial activation via negative regulation of the Src/p44/42 MAPK pathway. **J Biol Chem** 275:37224–37231, 2000.
  218. Tan J, Town T, Mori T, Wu Y, Saxe M, Crawford F, Mullan M: CD45 opposes beta-amyloid peptide-induced microglial activation via inhibition of p44/42 mitogen-activated protein kinase. **J Neurosci** 20:7587–7594, 2000.
  219. Tang X, Davies JE, Davies SJ: Changes in distribution, cell associations, and protein expression levels of NG2, neurocan, phosphacan, brevican, versican V2, and tenascin-C during acute to chronic maturation of spinal cord scar tissue. **J Neurosci Res** 71:427–444, 2003.
  220. Taylor DL, Diemel LT, Pocock JM: Activation of microglial group III metabotropic glutamate receptors protects neurons against microglial neurotoxicity. **J Neurosci** 23:2150–2160, 2003.
  221. Toyomoto M, Ohta M, Okumura K, Yano H, Matsumoto K, Inoue S, Hayashi K, Ikeda K: Prostaglandins are powerful inducers of NGF and BDNF production in mouse astrocyte cultures. **FEBS Lett** 562:211–215, 2004.
  222. Trotter J, DeJong LJ, Smith ME: Opsonization with antimyelin antibody increases the uptake and intracellular metabolism of myelin in inflammatory macrophages. **J Neurochem** 47:779–789, 1986.
  223. Tsirka SE, Bugge TH, Degen JL, Strickland S: Neuronal death in the central nervous system demonstrates a non-fibrin substrate for plasmin. **Proc Natl Acad Sci U S A** 94:9779–9781, 1997.
  224. Tsuda M, Shigemoto-Mogami Y, Koizumi S, Mizokoshi A, Kohsaka S, Salter MW, Inoue K: P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. **Nature** 424:778–783, 2003.
  225. Ueyama T, Lennartz MR, Noda Y, Kobayashi T, Shirai Y, Rikitake K, Yamasaki T, Hayashi S, Sakai N, Seguchi H, Sawada M, Sumimoto H, Saito N: Superoxide production at phagosomal cup/phagosome through beta1 protein kinase C during FcgammaR-mediated phagocytosis in microglia. **J Immunol** 173:4582–4589, 2004.
  226. Urabe T, Tanaka R, Noda K, Mizuno Y: Anticoagulant therapy with a selective thrombin inhibitor for acute cerebral infarction: Usefulness of coagulation markers for evaluation of efficacy. **J Thromb Thrombolysis** 13:155–160, 2002.
  227. Vargas MR, Pehar M, Cassina P, Estevez AG, Beckman JS, Barbeito L: Stimulation of nerve growth factor expression in astrocytes by peroxynitrite. **In Vivo** 18:269–274, 2004.
  228. Venters HD, Dantzer R, Kelley KW: A new concept in neurodegeneration: TNFalpha is a silencer of survival signals. **Trends Neurosci** 23:175–180, 2000.
  229. Verderio C, Matteoli M: ATP mediates calcium signaling between astrocytes and microglial cells: Modulation by IFN-gamma. **J Immunol** 166:6383–6391, 2001.
  230. Verge GM, Milligan ED, Maier SF, Watkins LR, Naeve GS, Foster AC: Fractalkine (CX3CL1) and fractalkine receptor (CX3CR1) distribution in spinal cord and dorsal root ganglia under basal and neuropathic pain conditions. **Eur J Neurosci** 20:1150–1160, 2004.
  231. Viviani B, Bartsaghi S, Corsini E, Galli CL, Marinovich M: Cytokines role in neurodegenerative events. **Toxicol Lett** 149:85–89, 2004.
  232. Viviani B, Bartsaghi S, Gardoni F, Vezzani A, Behrens MM, Bartfai T, Binaglia M, Corsini E, Di Luca M, Galli CL, Marinovich M: Interleukin-1beta enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. **J Neurosci** 23:8692–8700, 2003.
  233. Wang CX, Shuaib A: Involvement of inflammatory cytokines in central nervous system injury. **Prog Neurobiol** 67:161–172, 2002.
  234. Wang H, Reiser G: Thrombin signaling in the brain: The role of protease-activated receptors. **Biol Chem** 384:193–202, 2003.
  235. Wang H, Ubl JJ, Stricker R, Reiser G: Thrombin (PAR-1)-induced proliferation in astrocytes via MAPK involves multiple signaling pathways. **Am J Physiol Cell Physiol** 283:C1351–1364, 2002.
  236. Wang MJ, Lin WW, Chen HL, Chang YH, Ou HC, Kuo JS, Hong JS, Jeng KC: Silymarin protects dopaminergic neurons against lipopolysaccharide-induced neurotoxicity by inhibiting microglia activation. **Eur J Neurosci** 16:2103–2112, 2002.
  237. Wang T, Qin L, Liu B, Liu Y, Wilson B, Eling TE, Langenbach R, Taniura S, Hong JS: Role of reactive oxygen species in LPS-induced production of prostaglandin E2 in microglia. **J Neurochem** 88:939–947, 2004.
  238. Wang W, Ji P, Dow KE: Corticotropin-releasing hormone induces proliferation and TNF-alpha release in cultured rat microglia via MAP kinase signalling pathways. **J Neurochem** 84:189–195, 2003.
  239. Westenbroek RE, Bausch SB, Lin RC, Franck JE, Noebels JL, Catterall WA: Upregulation of L-type Ca2+ channels in reactive astrocytes after brain injury, hypomyelination, and ischemia. **J Neurosci** 18:2321–2334, 1998.
  240. Wong G, Goldshmit Y, Turnley AM: Interferon-gamma but not TNF alpha promotes neuronal differentiation and neurite outgrowth of murine adult neural stem cells. **Exp Neurol** 187:171–177, 2004.
  241. Xie Z, Wei M, Morgan TE, Fabrizio P, Han D, Finch CE, Longo VD: Peroxynitrite mediates neurotoxicity of amyloid beta-peptide1–42- and lipopolysaccharide-activated microglia. **J Neurosci** 22:3484–3492, 2002.
  242. Xiong H, Boyle J, Winkelbauer M, Gorantla S, Zheng J, Ghorpade A, Persidsky Y, Carlson KA, Gendelman HE: Inhibition of long-term potentiation by interleukin-8: Implications for human immunodeficiency virus-1-associated dementia. **J Neurosci Res** 71:600–607, 2003.
  243. Yamada T, Nagai Y: Immunohistochemical studies of human tissues with antibody to factor Xa. **Histochem J** 28:73–77, 1996.
  244. Yamada T, Yamanaka I: Microglial localization of alpha-interferon receptor in human brain tissues. **Neurosci Lett** 189:73–76, 1995.
  245. Yang MS, Park EJ, Sohn S, Kwon HJ, Shin WH, Pyo HK, Jin B, Choi

- KS, Jou I, Joe EH: Interleukin-13 and -4 induce death of activated microglia. **Glia** 38:273–280, 2002.
246. Yuan J, Lipinski M, Degtrev A: Diversity in the mechanisms of neuronal cell death. **Neuron** 40:401–413, 2003.
247. Zhang K, McQuibban GA, Silva C, Butler GS, Johnston JB, Holden J, Clark-Lewis I, Overall CM, Power C: HIV-induced metalloproteinase processing of the chemokine stromal cell derived factor-1 causes neurodegeneration. **Nat Neurosci** 6:1064–1071, 2003.
248. Zhang SC, Fedoroff S: Modulation of microglia by stem cell factor. **J Neurosci Res** 53:29–37, 1998.
249. Zhao ML, Si Q, Lee SC: IL-16 expression in lymphocytes and microglia in HIV-1 encephalitis. **Neuropathol Appl Neurobiol** 30:233–242, 2004.
250. Zimmer H, Riese S, Regnier-Vigouroux A: Functional characterization of mannose receptor expressed by immunocompetent mouse microglia. **Glia** 42:89–100, 2003.