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MINI-REVIEW

Pro-regenerative properties of cytokine-activated astrocytes

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Abstract

The prevailing view of the astrocytic response to injury is that reactive astrocytes impede the regenerative process by forming scar tissue. As the levels of many cytokines dramatically increase following CNS insult and as this increase in cytokine expression precedes the production of the glial scar, a long-standing view has been that cytokines diminish neuronal survival and regeneration by stimulating the formation of astrogliotic scar tissue. However, there is a wealth of data indicating that cytokines 'activate' astrocytes, and that cytokine-stimulated astrocytes can promote the recovery of CNS function. Supporting evidence demonstrates that cytokine-activated astrocytes produce energy substrates and trophic factors for neurons and oligodendrocytes, act as free radical and excess glutamate scavengers, actively restore the blood–brain barrier, promote neovascularization, restore CNS ionic homeostasis, promote remyelination and also stimulate neurogenesis from neural stem cells. Accordingly, a re-assessment of cytokine-activated astrocytes is necessary. Here, we review studies that promote the thesis that cytokines elicit potent neuroprotective and regenerative responses from astrocytes.

Keywords: ciliary neurotrophic factor, gliosis, interleukin-1, microglia.

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Astrocytes are the major support cells of the CNS

Astrocytes are dynamic cells that maintain homeostasis in the undamaged CNS. Astrocytes express numerous receptors that enable them to respond to virtually all known neuroactive compounds, including neurotransmitters, neuropeptides, growth factors, cytokines, small molecules and toxins. These receptors enable astrocytes to not only participate in signal processing, but to function as sentinels. Astrocytes also establish and maintain CNS boundaries, including the blood–brain barrier (BBB) and the glial limitans, through interactions with endothelial and leptomeningeal cells. Given their important roles in establishing and maintaining CNS homeostasis, an adaptive response by astrocytes to tissue damage should be expected. Indeed, when astrocytes sense that the homeostasis of the brain or spinal cord has been disrupted, their metabolic activity increases, as well as their production of growth and trophic factors; thus endowing them with a greater capacity to protect other brain cells from energy depletion, toxic-free radicals, ammonia, metals and calcium overload. Additionally, after injury, cytokine-activated astrocytes promote the reformation of essential barriers and reestablishment of CNS ionic homeostasis.

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Abbreviations used: ADNF, activity-dependent neurotrophic factor; BBB, blood–brain barrier; BDNF, brain-derived neurotrophic factor; CNTF, ciliary neurotrophic factor; GS, glutamine synthetase; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor-1; IL, interleukin; LT, lymphotoxin; MIP, macrophage inhibitory protein; MMP, matrix metalloproteinases; MT, metallothionein; NSC, neural stem cell; PAF, platelet-activating factor; TNF-α, tumor necrosis factor; VEGF, vascular endothelial growth factor; VIP, vasoactive intestinal peptide.

Anisomorphic astrogliosis (reactive gliosis) versus isomorphic astrogliosis (astrocyte activation)

The characteristics that are most frequently associated with the astrocytic response to insult involve cellular hypertrophy, astrocyte proliferation, process extension and interdigitation, and increased production of the intermediate filaments glial fibrillary acidic protein (GFAP), vimentin and nestin. This constellation of responses, which pathologists classify as anisomorphic gliosis, is the consequence of gross tissue damaging injuries and results in the formation of a tightly compacted limiting glial margin, termed the astrogliotic scar (Norton *et al*. 1992; Norenberg 1994; Bignami and Dahl 1995; Berry *et al*. 1999). These astrocytic responses to CNS injury have been extensively reviewed with much attention focused on how the components of the scar inhibit neurite outgrowth (Reier and Houle 1988; McKeon *et al*. 1991; Eng *et al.* 1992; Laywell *et al*. 1992; Norton *et al*. 1992; Norenberg 1994; Ridet *et al*. 1997; Raivich *et al*. 1999). These 'reactive' astrocytes may also exacerbate tissue damage as they can release pro-inflammatory cytokines such as tumor necrosis factor (TNF-α) which can inhibit neurite outgrowth and kill oligodendrocytes (Neumann *et al*. 2002; D'Souza *et al*. 1996), and they can produce and release arachidonic acid metabolites, nitric oxide (NO) and reactive oxygen species that can adversely affect cell survival after injury (Dayton and Major 1996). However, microglia produce much higher levels of these cell damaging intermediates than astrocytes; therefore, the astrocytes may not be culpable for the bystander damage that occurs subsequent to reactive gliosis (see below).

There is another type of astrocyte response to insult that is less dramatic and frequently transient which is classified as isomorphic gliosis. This astroglial response is associated with improved recovery from tissue damaging insults. At sites distant from traumatic injury or in regions where the tissue has been merely disturbed (e.g. following axotomy, nerve crush, or after a mild stroke), the astrocytes become larger in size, and undergo nuclear hypertrophy, they transform to a more pronounced stellate shape and they increase their production of many cytosolic enzymes, antioxidants, structural proteins and organelles. Additionally, these 'activated astrocytes' produce soluble trophic and growth factors that enhance the survival of adjacent neurons and glia, as well as coordinate tissue remodeling. Thus, these isomorphic changes should be considered adaptive and beneficial to restoring homeostasis.

Furthermore, an important distinction between isomorphic and anisomorphic gliotic changes is that astrocytes that undergo isomorphic changes revert over time to a premorbid cytoarchitecture, unlike the reactive astrocytes observed in anisomorphic gliosis that produce a permanent glial scar (Bignami and Dahl 1995; Raivich *et al*. 1999). There is abundant evidence linking cytokines to isomorphic gliosis, which we equate with activation. For instance, the cytokines ciliary neurotrophic factor (CNTF) and interleukin-1β (IL-1β) have been shown to induce astrocyte nuclear hypertrophy, which is characteristic of activated astrocytes (Hudgins and Levison 1998; Albrecht *et al*. 2002). Since our laboratories have been studying the effects of these cytokines on astrocyte function for several years, many of the examples cited in this mini-review will be taken from studies that have assessed how IL-1 and CNTF affect astrocytic properties and functions.

Microglia are the first cells to respond to CNS insults and they produce cytokines

Microglia are exquisitely sensitive to their environment and can become activated without obvious signs of neuropathology, suggesting that microglia become activated as a consequence of metabolic stress (Raivich *et al*. 1999). Once activated, microglia release soluble effectors that can directly or indirectly cause damage to neural cells. One of the first effectors to be produced is IL-1β. We showed that IL-1β is produced within 15 min following a cortical injury, and that this IL-1β is produced by activated microglia (Herx *et al*. 2000; Herx and Yong 2001). As the promotor for IL-1 has multiple *cis*-acting regulatory sites including those for hypoxia inducible factor (HIF), nuclear factor-kappa B, cJun, cFos, ATF2, cEBPβ and Elk1 that enables IL-1 mRNA to be transcribed in response to diverse stimuli, IL-1 is classified as an early and dominant injury signal (Auron 1998). As astrocytes become activated subsequent to IL-1β production, one can view the microglial reaction as necessary for the response of astrocytes to an insult. Indeed, supporting this view, astroglial activation is delayed in mice lacking IL-1β as well as in mice lacking the IL-1 type 1 receptor (Herx *et al*. 2000; Basu *et al*. submitted to Basu and Levison).

In addition to producing IL-1β, microglia release IL-3, IL-6, TNF-α, vascular endothelial growth factor (VEGF), lymphotoxin (LT), macrophage inhibitory protein (MIP)-1α, matrix metalloproteinases (MMPs), NO and other reactive oxygen species (Stoll *et al*. 2002). The cytokines IL-1, IL-6, TNF-α and LT alter vascular adhesion molecule expression, which recruits lymphocytes and macrophages to sites of injury. In addition, LT, TNF-α, NO and reactive oxygen species can directly kill cells. Whereas astrocytes also produce these potentially toxic factors, microglia produce much higher levels of these cell-damaging intermediates than astrocytes. For instance, the eicosanoid thromboxane A2 will cause vasoconstriction, which can exacerbate tissue damage after injury. Rat microglia produce more than 300 fold more thromboxane A2 than astrocytes upon elevating intracellular calcium levels with a calcium ionophore (Giulian *et al*. 1996). Similarly, endotoxin stimulated rat microglia express immunohistochemically detectable iNOS

whereas similarly treated astrocytes do not, and endotoxin-stimulated microglia produce approximately 15 fold more NO than the astrocytes (Vincent *et al*. 1996). Analogously, endotoxin stimulated murine microglia produce 95 times more TNF-α than astrocytes (Malipiero *et al*. 1990). Because thromoxane A2, NO and TNF-α are tissue damaging when present at high levels, whereas they may be neuroprotective at lower concentrations, much of the secondary injury observed after an insult is likely not due to the activation of astrocytes, but to the greater harm subsequent to reactive microgliosis. Therefore, the initial response to CNS injury is mediated by microglia, who then recruit other immune cells to clear pathogens and infected or damaged cells. Feedback mechanisms are then initiated to prevent excessive tissue destruction, and there is good evidence that the astrocyte reaction is indeed an important compensatory response to bring about the resolution of the injury response and to restore homeostasis.

Metabolic changes in astrocytes after injury that are adaptive

Astrocytes are essential support cells in the CNS that create and maintain an environment that optimizes neuronal function. As many of these activities sustain metabolic homeostasis, it is logical to expect that they would respond to perturbations of homeostasis with an increase in their housekeeping activities. For instance, astrocytes are one of the few cell types in the brain that can store energy in the form of glycogen. As a consequence of activation, astrocytes become metabolically hyperactive and they metabolize stored glycogen, which sustains their own energy requirements and enables them to support neighboring neurons through the export of glucose or lactate. Importantly, a recent study demonstrated that a mild hypoxic insult increases the basal levels of glycogen within the brain. As such, mild insults 'precondition' the brain and decrease the extent of damage subsequent to more severe traumatic events (Brucklacher *et al*. 2002). Levels of glycogen storage in astrocytes have been shown to be directly regulated by insulin-like growth factor-1 (IGF-1) (Dringen and Hamprecht 1992) which is downstream of IL-1β signaling (Mason *et al*. 2001), which as discussed above is induced after injury, and more specifically after a stroke (O'Donnell *et al*. 2002). Taken together, these data support the hypothesis that astrocytes, by responding to cytokines, comprise an important substrate of preconditioning insults.

Neuronal dysfunction subsequent to brain damage causes the release of glutamate which causes secondary excitotoxic neuronal death, and oligodendroglial progenitor cell death. Astrocytes regulate glutamate levels by actively removing it from the extracellular space and converting it to glutamine. Therefore, the capacity of astrocytes to reduce extracellular levels of glutamate can dramatically impact the extent of neuronal and oligodendroglial damage after an insult. The levels of glutamate transporters and glutamine synthetase increase when astrocytes become activated. Astrocytes possess two glutamate transporters that sequester excess glutamate, GLT-1 and GLAST. Cultured astrocyte GLT-1 levels increase as a consequence of EGF stimulation (Zelenaia *et al*. 2000). Analogously, the levels of cultured astrocytic GLAST have been shown to increase following treatment with fibroblast growth factor (FGF), EGF, and IGF-1 (Suzuki *et al*. 2001). Injury also affects GLT-1 and GLAST expression. GLT-1 levels increase 2.5-fold above control levels three days after the trauma produced by transplanting E18 neocortical tissue into rat cortex (Krum *et al*. 2002). Similarly, GLT-1 and GLAST mRNA expression are induced after cultured astrocytes are physically traumatized (Faden *et al*. 1989; Eng *et al*. 1997). The levels of the glutamate transporters also may be indirectly regulated by cytokines (Aronica *et al*. 2003). *In vitro* studies on cultured astrocytes have shown that metabotropic glutamate receptor expression can be altered in response to FGF-2, TGFα and EGF (Miller *et al*. 1995; Balazs *et al*. 1997; Minoshima and Nakanishi 1999), and as a consequence of mGluR expression the levels of glutamate transporters on the astrocytes increase (Ciccarelli *et al*. 1997; Bruno *et al*. 1998; Ciccarelli *et al*. 1999). Therefore, increased expression of mGluRs, GLAST and GLT-1 post injury better enable astrocytes to sequester excess glutamate. In addition, activated astrocytes synthesize increased amounts of glutamine synthetase (GS), which enables the conversion of glutamate to the harmless amino acid, glutamine. The levels of GS also have been shown to be regulated by cytokines, including NGF and FGF-2, which are downstream of IL-1 stimulation (Loret *et al*. 1989; Kazazoglou *et al*. 1996).

The levels of cytosolic proteins with antioxidant function also increase in activated astrocytes. For instance, the levels of the astrocyte specific antioxidant, glutathione-*S* transferase-μ increases as a consequence of CNTF stimulation (Levison *et al*. 1996). Increased expression of GST-μ enables astrocytes to detoxify xenobiotics and free radicals. Also, the expression of the multifunctional protein ceruloplasmin is increased in astrocytes after injury and as a consequence of IL-1β stimulation (Chang *et al*. 2001; Kuhlow *et al*. 2003). Ceruloplasmin buffers free copper, oxidizes ferrous iron and catalyzes the dismutation of free radicals. Therefore, cytokine-increased levels of ceruloplasmin will protect neurons and glia from sustaining free-radical damage after injury. Metallothioneins I and II (MT-I + II) buffer levels of reactive metals such as zinc and mercury (Hamer 1986), and elevated levels of MT-I + II protect the brain from injury. In many diseases and following injury, MT-I + II expression levels are increased as shown for demyelinating diseases (Vela *et al*. 1997), Alzheimer's disease (Adlard *et al*. 1998), Amyotrophic Lateral Sclerosis (Sillevis Smitt *et al*. 1992) and following

ischemia (Neal *et al*. 1996). IL-6 treatment has been shown to induce astrocytic MT-I + II in a murine cortical cryo-injury model (Penkowa *et al*. 1999) and IL-6-deficient mice have a reduced number of activated astrocytes and a significant reduction in MT-I + II protein levels. Further studies by Penkowa and colleagues showed that targeted IL-6 over-expression in mice reduces brain damage after cryo-injury which correlates with an up-regulation of MT-I + II (Penkowa *et al*. 2003).

Cytokine-activated astrocytes promote angiogenesis

After an injury, angiogenesis and neovascularization must occur to provide nutrients and oxygen. Angiogenesis can be induced by numerous factors, including VEGF, which is a potent mitogen for endothelial cells and is rapidly produced in the brain in response to both hypoxia and cytokines. Several lines of evidence implicate activated astrocytes in VEGF-mediated angiogenesis following CNS injury. It has been shown that VEGF mRNA expression, VEGF protein and one of the VEGF receptors, flt-1, are increased in activated astrocytes after glioma implantation, stab wounds and neural grafting (Krum and Rosenstein 1998). In both stab wound and neural grafting models, VEGF mRNA expression is most highly elevated at 2 days post operation and persists throughout the following 14 days. Importantly, this robust expression is localized to strongly GFAP + astrocytes whose end-feet are in close proximity to blood vessels. The temporal pattern of VEGF mRNA expression is different following glioma implantation with peak expression occurring at 22 days post implantation (Krum and Rosenstein 1998). Generally, VEGF and flt-1 protein levels are coincident with each other.

Platelet-activating factor (PAF) also is synthesized following ischemia and PAF stimulates VEGF expression in cultured human astrocytes (Braquet *et al*. 1989; Yoshida *et al*. 2002). Furthermore, Yoshida and colleagues added desferrioxamine to similar astrocyte cultures to mimic hypoxia *in vitro*, and with the subsequent introduction of PAF, enhanced VEGF mRNA and protein production. Therefore, PAF, which is produced in response to hypoxia/ischemia, stimulates astrocytic production of VEGF to enhance the proliferation of endothelial cells, thus promoting revascularization. A separate set of experiments demonstrated that VEGF mRNA expression increases 2.5 fold after 8 h of hypoxic conditions in cultured C6 rat glioma cells. Interestingly, the half-life of VEGF mRNA increases to 2.4 h in C6 cells grown under hypoxic conditions while the half-life is 40 min under normoxic conditions (Stein *et al*. 1995). This result implies that VEGF expression and half-life in glial cells is increased in response to oxygen demand, thus promoting angiogenesis and preventing hypoxic damage.

Cytokine-activated astrocytes restore the blood–brain barrier (BBB)

The blood vessel endfoot specialization is one of the distinguishing characteristics of a mature astrocyte. The basal lamina secreted by the endothelial cells, as well as the basal lamina formed by the fibroblasts located at the pial surfaces of the brain, are covered by astrocytic end-feet. These contacts are functionally important, as there are bi-directional signals that occur at these sites. In particular, the interactions of astrocytes with endothelial cells induce and maintain the BBB properties of the endothelial cells (Janzer and Raff 1987; Hayashi *et al*. 1997; Abbott 2000). Astrocytes not only maintain the BBB, but they participate in reforming the BBB following CNS injury (Norenberg 1994; Yong *et al*. 1998). Bush *et al*. (1999) confirmed the essential role for astrocytes in BBB repair. They inflicted a stab wound to the forebrain of adult mice that expressed the herpes simplex virus thymidine kinase gene (HSV-Tk) driven by the GFAP promoter and then treated the animals with ganciclovir to selectively ablate reactive astrocytes adjacent to the injury site. In the absence of reactive astrogliosis, Bush and colleagues found that there was a failure of BBB repair, increased leukocyte infiltration and increased neuronal degeneration.

Evidence implicating cytokine-stimulated astrocytes in BBB restoration comes from experiments on IL-1β null mice. BBB repair following CNS lesion in rodents begins to be noticeable only from 5 to 7 days following insult (Shapira *et al*. 1993; Soares *et al*. 1995; Baldwin *et al*. 1996), whereas astrocyte GFAP increases and morphologic changes occur more rapidly. We found that animals lacking IL-1β showed less astrocyte reactivity 2–3 days following cortical lesion, and increased permeability of the BBB at 7 days post lesion, compared with wild-type controls (Herx and Yong 2001). These results link the early activation of astrocytes and their subsequent role in BBB repair following traumatic injury to the inflammatory response, and more specifically to the production of IL-1β.

Cytokine-activated astrocytes promote remyelination

Multiple sclerosis (MS) is a neurodegenerative disease characterized by bouts of demyelination and subsequent remyelination. However, after numerous episodes of de/remyelination, the lesions fail to remyelinate and thus remain demyelinated (Thompson and McDonald 1992). Emerging evidence implicates cytokine-activated astrocytes in the regenerative phases of this disease; in particular, the cytokines IL-1β and CNTF play important roles. These two cytokines are not only functionally implicated, but also their expression after injury correlates temporally. We recently studied the temporal expression of IL-1β and CNTF during the course of demyelination and remyelination in mice

infected with the A-59 strain of the mouse hepatitis virus (MHV-A59), which is a useful animal model for MS (Jordan *et al*. 1989; Messersmith *et al*. 2000). These studies revealed that IL-1β is present early during the course of infection, when demyelination occurs, whereas CNTF is present later, during the remyelination phase (Albrecht *et al*. 2003). These observations are in accordance with our other studies on IL-1β null mice demonstrating that CNTF mRNA and protein are induced with slower kinetics than IL-1β following cortical injury, and that CNTF induction requires IL-1β signaling (Herx *et al*. 2000). Microglia and activated astrocytes can both produce IL-1, whereas the cellular source of CNTF production postinjury is most likely astrocytes, since CNTF has been localized to astrocytes in the normal brain (Stockli *et al*. 1991; Guthrie *et al*. 1997; Dallner *et al*. 2002) and CNTF expression is greatly enhanced in astrocytes following injury (Stockli *et al*. 1991; Guthrie *et al*. 1997; Dallner *et al*. 2002).

Using cuprizone to induce demyelination, Mason and colleagues found that IL-1β null mice failed to remyelinate as rapidly as wild-type mice, apparently due to a delay in oligodendrocyte precursor proliferation that correlated with decreased astrocyte expression of IGF-1 (Mason *et al*. 2001). IGF-1 is a potent growth factor that regulates the proliferation and self-renewal of oligodendrocyte precursors, and is required for differentiation and maturation of oligodendrocyte precursors into mature, myelin producing oligodendrocytes. They determined that the IL-1β null mice expressed lower levels of IGF-1 mRNA compared to wild-type mice, and furthermore, that the IGF-1 protein levels achieved in wild-type astrocytes after injury were absent in the IL-1β null mice after injury. These studies indicate that an abrogated astrocyte reaction due to a lack of IL-1β signaling significantly impairs remyelination.

Astrocytes also become activated after injury by CNTF, and we have recently implicated CNTF in the production of fibroblast growth factor-2, which is a growth factor that can enhance oligodendrocyte precursor proliferation (Albrecht *et al*. 2003). Using *in situ* hybridization and immunohistochemistry on spinal cord tissue from animals recovering from MHV-A59-induced demyelination, we found that CNTF mRNA, but not IL-1β, is induced in regions undergoing remyelination, and is localized to cells exhibiting astrocytic features. In addition, highly enriched cultured mouse spinal cord astrocytes increased their levels of FGF-2 mRNA approximately 2.5-fold above untreated astrocytes in response to CNTF stimulation. IL-1β was without effect (Albrecht *et al*. 2003). These results strongly suggest that CNTF mediates the FGF-2 increase within astrocytes during spinal cord remyelination. Since oligodendrocyte precursors express FGF receptors and oligodendrocyte precursors are present in the penumbra of demyelinated lesions (Redwine *et al*. 1997; Messersmith *et al*. 2000) the FGF-2 that is released from CNTF-activated astrocytes can bind to high affinity FGF receptors on the oligodendrocyte precursors to stimulate their proliferation and thus enhance repopulation of the lesioned white matter.

Cytokine-activated astrocytes promote neuronal survival

Astrocytes produce an enormous array of neurotrophic factors, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), activity dependent neurotrophic factor (ADNF), hepatocyte growth factor (HGF), CNTF, and FGF-2 in response to injury, disease and activation of cytokine receptors (Rudge *et al*. 1995; Schwartz and Nishiyama 1994; Uchida *et al*. 1998; Dreyfus *et al*. 1999; Messersmith *et al*. 2000; Albrecht *et al*. 2002). Experiments demonstrating that IL-1β potently increases astrocytic expression of NGF provided the first evidence that glial activation may promote neuronal survival and perhaps regeneration (Spranger *et al*. 1990; Friedman *et al*. 1990).

Vasoactive intestinal peptide (VIP) is another cytokine that exerts neuroprotective responses from astrocytes and has been shown to promote synaptogenesis. The neuroprotective properties of VIP are due to its actions on astrocytes that express high affinity VIP receptors. Activation by VIP induces the release of the survival-promoting, substance-activity-dependent neurotrophic factor (ADNF) (Brenneman and Gozes 1996). ADNF release from VIP-stimulated astrocytes has been shown to promote the survival of spinal cord neurons and cortical neurons (Gozes *et al*. 1999). Moreover, ADNF protects rat cerebral cortical cultures from β-amyloid peptide neurotoxicity (Brenneman and Gozes 1996; Gozes *et al*. 1996).

Interleukin-6 has been shown to support neuronal regeneration via neurotrophins released by activated astrocytes (Marz *et al*. 1999). Marz and colleagues showed that primary rat astrocytes increase their expression of NGF, NT3 and NT4/5 in response to a fusion protein of IL-6 and soluble IL-6 receptor. Cultured cortical and hippocampal astrocytes respond by increasing their NGF mRNA expression maximally at 21 h after IL-6 and soluble IL-6 receptor stimulation. Interestingly, neocortical astrocytes preferentially produce NT3, whereas hippocampal astrocytes predominantly produce NT4/5.

CNTF also promotes neuronal survival by activating astrocytes. We found that when cultured spinal cord astrocytes are activated with CNTF *in vitro*, they support the survival of a significantly greater number of ventral spinal motor neurons compared to unstimulated astrocytes. Also, CNTF-stimulated astrocytes promote neurite outgrowth better than untreated astrocytes (Albrecht *et al*. 2002). CNTF likely achieves these effects by increasing astroglial neurotrophic factor production, in particular by inducing the expression of FGF-2. Taken together with our data demonstrating that IL-1β is a necessary upstream inducer of astrocytic CNTF production (Herx *et al*. 2000), these results

support the hypothesis that cytokine-stimulated astrocytes increase neuronal survival, aid in the restoration of homeostasis and promote recovery. Consistent with a role for IL-1 and FGF-2 in regeneration, combinatorial treatment with IL-1 and FGF-2 enhances the outgrowth of dorsal root ganglion neuron neurites 2.5-fold versus untreated astrocytes through a three dimensional astrocyte culture, and this treatment also increases the migration of oligodendrocyte precursors through this same matrix (Fok-Seang *et al*. 1998).

Cytokine-activated astrocytes promote synaptogenesis

Astrocytic processes envelope synapses and they release neuromodulators that can regulate synaptic efficacy. Therefore, it is not surprising that astrocyte-derived factors maintain synapses after injury, and that they promote synaptogenesis. When embryonic hippocampal neurons are maintained *in vitro* and stimulated with VIP in the absence of astrocytes, there is no increase in synapse formation (Brenneman *et al*. 1987). However, exposing embryonic hippocampal neuron cultures to ADNF stimulates synapse formation (Blondel *et al*. 2000). As indicated previously, VIP elicits the release of ADNF from astrocytes (Brenneman *et al*. 1990; Gozes *et al*. 1999). Therefore, the VIP-activated astrocyte-derived factor, ADNF, acts directly on neurons to enhance synaptic connectivity and alter neuronal morphology (Blondel *et al*. 2000).

Cytokine-activated astrocytes promote neurogenesis

Adult neural stem cells (NSCs) have the potential to enhance recovery from CNS injury or disease and there is accumulating evidence that cytokine-stimulated astrocytes can promote neurogenesis. Throughout adult life, NSCs are present within the subventricular zone and the dentate gyrus of the hippocampus. By definition, these cells are undifferentiated and multipotent in that they can generate neurons and macroglia. As the progeny of NSCs can migrate beyond their sites of origin and later differentiate into mature neurons and glia these cells hold great promise as substrates for neuroregeneration. Recent research indicates that these cells proliferate in response to CNS insults, and there is emerging evidence that activated astrocytes may regulate their proliferation and eventual fate. Extracellular signals, including cytokines, have been shown to regulate neural stem cell progression from an undifferentiated form to a rapidly proliferating, differentiated form. These extracellular signals are responsible for determining which cell type the progenitors will become. Both *in vivo* and *in vitro* experiments have shown that neural stem cells can be stimulated to proliferate in response to FGF-2 and other factors (Kuhn *et al*. 1997). Since cytokine-stimulated astrocytes release FGF-2 (Albrecht *et al*. 2002), it can be inferred that activated astrocytes promote neurogenesis.

Recent *in vitro* data suggest that astrocytes and astrocyte-conditioned media promote neurogenesis (Song *et al*. 2002; Nakayama *et al*. 2003). Nakayama and colleagues cultured embryonic stem cells with astrocyte conditioned media and found that undifferentiated embryonic stem cells only form spheres that contain NSCs when the medium is conditioned by astrocytes and not by astrogliomas (Nakayama *et al*. 2003). Additionally, Song *et al*. (2002) demonstrated that astrocytes stimulate neurogenesis from adult NSCs. Co-culturing adult NSCs with primary hippocampal astrocytes increases the number of newly formed neurons 10-fold (Song *et al*. 2002). These results suggest that astrocyte-derived factors regulate neurogenesis during development. As it is known that astrocytic production of neurotrophic factors *in vitro* is enhanced following cytokine treatment, an interesting follow-up study would be to determine whether cytokine-activated astrocytes increase the production of neurons from NSCs above that seen from untreated astrocytes.

Conclusion

Astrocytes are essential for maintaining homeostasis throughout the normal CNS. They express numerous receptors, respond to a host of substances and their activity helps to maintain metabolic and ionic homeostasis. We have reviewed studies that support the view that cytokine-activated astrocytes generate an adaptive response post-injury to promote CNS repair. Cytokine-activated astrocytes can break down stored glycogen for glucose or lactate release, increase glutamate transporter levels to prevent excitotoxicity, increase cytosolic antioxidant proteins, promote revascularization, restore the BBB, promote remyelination and subsequent remission from a demyelinating event, enhance neuronal survival through the release

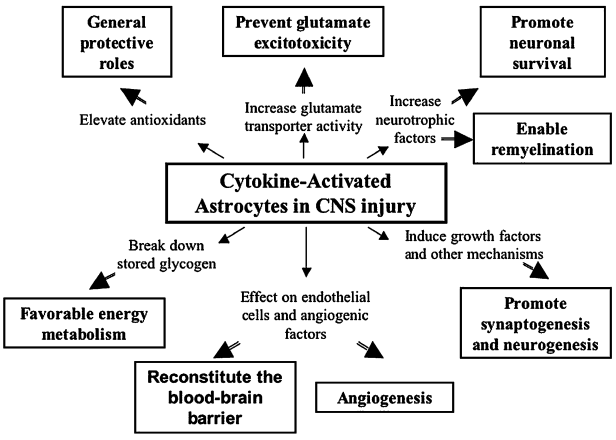

Fig. 1 A schematic depicting how cytokine stimulated astrocytes can prevent CNS injury and promote CNS repair and regeneration.

of neurotrophic factors and promote the formation of new synapses and neurons (Fig. 1). These functions of astrocytes show that the antiquated view of these cells as the source of the glial scar must be updated to recognize the full potential that these cells have to promote recovery from CNS injury and disease.

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