Chapter

# Reactive Astrocyte Gliosis: Production of Inhibitory Molecules

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## Abstract

The astrocytic cell responses to injury have been extensively studied in a variety of experimental models, and the term "astrogliosis" is often used to describe the astrocyte reactions to injury. Cells responding in these ways to injury are often referred to as "reactive astrocytes." Glial scarring appears to be a critical feature of wound healing in the central nervous system (CNS), since elimination of the mitotically active contingent of reactive astrocytes leads to increase in the size of the wound. Reactive astrogliosis is a term coined for the morphological and functional events seen in astrocytes responding to CNS injury. The concept of reactive astrogliosis and its molecular and cellular definition in spinal cord injury (SCI) is still incomplete. Producing several inhibitory molecules discourages regeneration of axons in the injured spinal cord. This inhibition is compounded by the poor regenerative ability of most CNS axons. This is probably a more achievable therapeutic target than axon regeneration, and an effective treatment would be of assistance to the majority of patients with partial cord injuries. Of course, understanding about astrogliosis and producing mediators and inhibitory molecules such as signaling pathways help us to develop new treatment strategies for SCI.

**Keywords:** astrogliosis, reactive astrocyte, inhibitory molecule

## 1. Introduction

Astrocytes are the most numerous glial cells in the CNS, which are pivotal for various structural and physiological functions [1]. SCI triggers astrocytes to become reactive and initiate astrogliosis. Reactive astrogliosis is characterized by the proliferation and hypertrophy of astrocytes, which eventually leads to scar formation via the activation of signaling pathways such as Gp-130/activator of transcription 3 (STAT3) and transforming growth factors-beta (TGF-β/Smad) [2]. With the onset of injury, changes occur in the phenotype and morphology of astrocytes. These changes include increasing in their expression of intermediate filaments such as nestin, glial fibrillary acidic proteins (GFAP), and vimentin. Reactive astrocytes also related to the release of pro-inflammatory and anti-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), TGF-β, interferon-gamma (IFN-γ),

and interleukins (IL-1 and IL-6). It is well established that these cytokines can modulate inflammation and also secondary injury [3].

When astrocytes are activated, they change the composition of extracellular matrix (ECM) dramatically. Several ECM components including chondroitin sulfate proteoglycans (CSPGs) and tenascins are markedly upregulated in astrocytes. In addition to these phenotypic changes, astrocytes increase in number and migrate to the site of injury [4].

Therefore, astrocyte reactivity is considered as a part of endogenous mechanisms to restrict the initial tissue injury to the spinal cord and prevent extension of damage into adjacent segments. The pivotal role of reactive astrocytes particularly at first stages of SCI is indicated by recent findings. Ablation of reactive astrocytes or altering with their activation at the time of SCI injury can intensify the damage by elevating tissue degeneration and disrupt to reconstruct blood-spinal barrier (BSB) [5]. However, over time after injury, inhibitory features of reactive astrocytes overcome their constructive properties. This is mostly contributed to the upregulation of inhibitory molecules such as CSPGs that extremely prevent neuroregeneration and neural repair [6].

Astrogliosis may be heterogeneous. Not all astrocytes with the morphological characteristics of reactive astrocytes (i.e., increased GFAP) are present in areas with increased levels of ECM. Perhaps not all astrocytes that react to injury play a role in the failure of CNS regeneration, and that only those astrocytes associated with inhibitory molecules are detrimental to axon growth while those further away from the lesion may be more conducive to neurite sprouting, functional plasticity, and long-distance regeneration [7].

## 2. Functions of astrocytes in a healthy brain

Based on previous studies, astrocytes were for decades considered to be assisting and nurturing neurons. Regarding several studies, the protoplasmic astrocytes divide the whole gray matter of the brain and spinal cord into distinct domains, with blood vessels, neurons, and synapses contained within these domains [8], and the fibrous astrocytes are in the white matter and are in physical contact with oligodendrocytes and have an important role in myelinization; however, astrocyte functions go far beyond assistance and support [9, 10].

During development, they are considered in key developmental and postnatal traces in the CNS. Astrocytes release neurotrophic factors that regulate neuronal development, cell migration, and differentiation [11]. Developing astrocytes guide postmitotic neurons from the ventricular zone to their target destination in developing CNS. Radial glial cells, a subtype of astrocytes, guide new neurons for accurate migration [12]. Astrocytes secrete vascular endothelial growth factor that is necessary for the generation of new blood vessels in rostral migratory stream (RMS) [13]. Besides, astrocytes have connection with blood vessels through their end-feet. They can produce important mediators which contributed to vasoconstriction or vasodilation such as arachidonic acid, nitric oxide (NO), or prostaglandins [14]. Astrocytes play a critical role in the coupling of neuronal organization to signaling circuits. They are involved in hemodynamic responses with neurons through blood flow.

Astrocytes significantly contribute to the establishment and maintenance of blood-brain barrier (BBB) and BSB in the CNS [15]. Astrocytes also clear neurotransmitters such as gamma-aminobutyric acid (GABA), glycine, and glutamate from the synaptic clefts and facilitate normal synaptic transmission [16]. Astrocytes have an important function in regulation of pH in CNS. They set up

proton shuttling through different proteins such as $Na^+/H^+$ exchanger, bicarbonate transporters acting in a sodium-dependent/independent mode, monocarboxylic acid transporters, carbonic anhydrase in both intra-and extracellular spaces, and the vacuolar-type proton ATPase [17].

Astrocytes are actively involved in the synthesis and maintenance of the ECM in the CNS. They produce a number of ECM components with both growth-promoting and inhibitory properties [18]. Astrocytes also express tenascin-C and different CSPGs with growth inhibitory properties [19]. When neuronal maturation begins in the normal CNS, CSPGs are concentrated strongly in the perineuronal nets where they are critical for stabilizing synapses and limiting undesirable plasticity [20].

## 3. Reactive astrogliosis in SCI

After SCI, astrocytes undergo significant cellular, molecular, and functional changes along with profound alterations in their gene expression. The reactions of astrocytes to the injury include hypertrophy of processes and soma and increasing in proliferation and upregulation of intermediate filaments such as GFAP, vimentin, and nestin. These alterations are the important markers of a phenomenon known as reactive astrogliosis [7].

Reactive astrogliosis is also indicated by high production of CSPGs, several cytokines, and chemokines such as IL-1β, IL-6, TGF-β, ciliary neurotrophic factor (CNTF), adhesion molecules, and proteins such as cyclooxygenase2, inducible NO synthase (iNOS), and calcium-binding protein S100β. These factors are considered as the functional markers of astrocyte reactivity whose levels are upregulated following CNS injuries [21].

Astrogliosis can be categorized from moderate changes in astrocytes to high reactivity related to scar formation [22]. In initial stages, there is aberrant hypertrophy of astrocytes and low upregulation of GFAP levels; however, no important proliferative activities usually occur in mild astrogliosis [23]. Mild astrogliosis or "isomorphic gliosis" is seen in the cases of axotomy, chemical lesions, or mild injury where astrocytes are distal to the site of lesion [24]. These alterations can be turned by reducing the triggering effects of upstream signaling molecules. Over time, reactive astrocytes express GFAP highly and show substantial hypertrophy, and some degree of proliferation. These remarkable expansions lead to disruption of particular regions of astrocytes and cause tissue distortion [3]. In intensive injuries, astrocytic processes overlap and become densely packed. At this stage, a glial scar encircles the epicenter of spinal cord lesion. Glial scar that is formed after local disruption of spine parenchyma is invariable and is nominated as "anisomorphic gliosis" [25].

Although astrogliosis is an early important marker of SCI in rodents, in human SCI, astrocyte reactivity is not a prominent property at acute or subacute phases, and astrogliosis seems to evolve over the time and become more evident at intermediate and chronic phases of SCI [26]. The presence of dense astrogliosis at 11 days after SCI that was still evident after 1 year post-SCI has been reported in some evidences [27]. Further investigations for astrogliosis in human SCI are necessary to examine the impact and timing. This is particularly important when translating therapeutic strategies that target astrogliosis from rodent models to human SCI.

Meningeal fibroblasts also contribute to scar formation. In fact, the glial scar formation is adjusted by a cell-cell contact mechanism between reactive astrocytes and meningeal fibroblasts at the spinal cord lesion. Signaling between ephrin-B2 on reactive astrocytes and EphB2 receptors on meningeal fibroblasts appears to carry on this process [28].

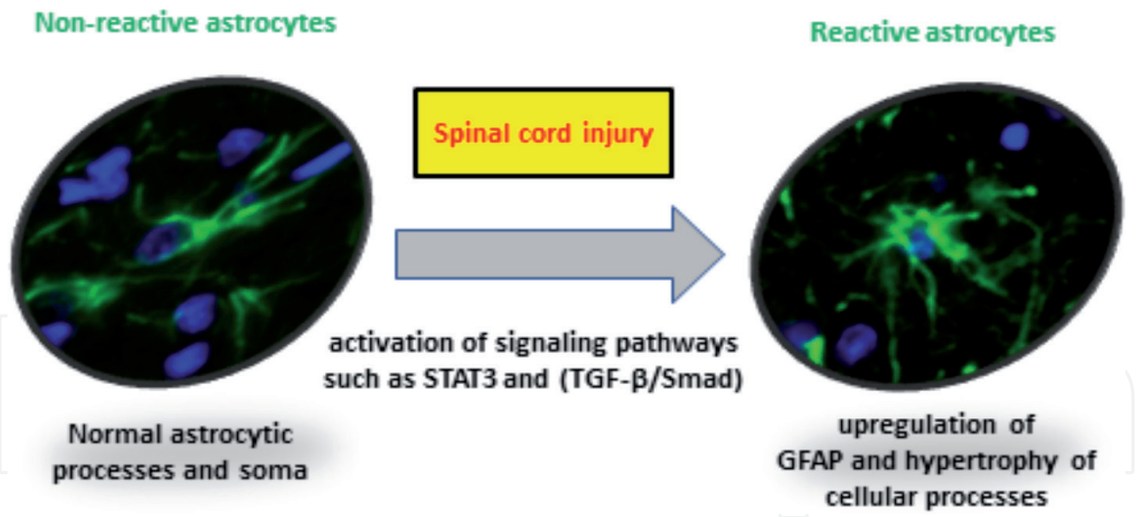


**Figure 1.**
*Reactive astrogliosis is a response of activated astrocytes seen in spinal cord injury and can be triggered through various signaling pathways such as signal transducers and activators of transcription (STAT) and TGF-β/Smad. In most situations, it can be viewed as a defensive reaction counteracting acute stress, restoring the CNS homeostasis, and limiting the tissue damage; however, persisting reactive astrogliosis can be lead to inhibition of neural plasticity and other regenerative responses.*

Reactive astrogliosis can be triggered through several signaling pathways such as signal transducers and activators of transcription (STAT) and TGF-β/Smad (**Figure 1**) [29]. Both beneficial and detrimental effects of SCI can be dependent to which signaling pathways and timing after SCI are involved. Understanding the beneficial and detrimental role of reactive astrocytes will allow us to plan therapeutic approaches.

## 4. Beneficial effects of reactive astrogliosis in SCI

Previously, astrocytes were known to be solely harmful in SCI, and their inhibition or ablation was considered as a therapeutic strategy. Recent studies have provided strong evidence that reactive astrocytes play pivotal roles in SCI repair with protective features [30, 31]. Repair responding by reconstructing the damaged BSB and limiting the infiltration of peripheral leukocytes and activation of resident microglia [32], modulating blood flow by the release of vasoconstrictors and regulating blood vessels diameter [33], uptaking excess glutamate, protecting neurons and oligodendrocytes from glutamate excitotoxicity, and producing antioxidants such as glutathione and defending against oxidative stress [34] are inconsiderable parts of beneficial roles of astrocytes. Reactive astrocytes upregulate the expression of intermediate filaments, GFAP, vimentin, and nestin. Interestingly, in hemisection model of SCI, double GFAP and vimentin knockout mice showed beneficial outcomes [35].

Besides, astrocytes are known to become reactive through STAT3 and suppressor of cytokine signaling 3 (SOCS3) pathways. Some evidences indicated that knockout of SOCS3 or STAT3 in GFAP-Cre or nestin-Cre transgenic models caused limited migration of astrocytes to the site of lesion and interfered with the formation of glial scar. Failure of scar formation in these animals resulted in widespread lesion [36]. Also, astrocytes can promote tissue repair and regeneration as they upregulate their expression of fibroblast growth factor-2 (FGF-2) and S100β in the injured spinal cord [37]. Furthermore, astrocyte polarity and directional migration play an important role in astrocyte ability to react to injury. Recent findings

demonstrated that astrocytes depleted of the small RhoGTPase Cdc42, which is a key regulator of cell polarization, display impaired recruitment to the stab wound lesion, despite their upregulation of GFAP and hypertrophic response [38].

## 5. Detrimental roles of reactive astrocytes after SCI

Glial scar is a major detriment to regeneration of severed axons by upregulating a great number of molecules around the lesion and preventing regrowth of injured axons at the lesion area, including CSPGs, tenascin, semaphorin 3A, keratan sulfate proteoglycans (KSPGs), myelin-associated inhibitors, and ephrins/Eph receptors [6]. Reactive astrocytes and the ECM components generate a dense glial scar around the SCI lesion and create physical and chemical barriers on axonal regeneration. In fact, as axons come in close contact with the glial scar, they form dystrophic end-bulbs and retract without any regeneration [39]. ECM components such as CSPGs [40], tenascins [41], and collagen [42] can be act as main inhibitory factors in axonal regeneration. They could upregulate in the glial scar after SCI and obstruct axonal elongation and sprouting [43].

## 6. Molecular mediators of reactive astrogliosis

### 6.1 STAT3

STAT3 is a member of the Janus kinase STAT family and a transducer of signals for many cytokines and growth factors, such as IL-6, leukemia inhibitory factor (LIF), and CNTF [44]. The effect on astrocyte activation may be mediated via the STAT3 signaling pathway, phosphorylation, and nuclear translocation of STAT3 in astrocytes as well as indirectly through the effects of these molecules on other cell types such as microglia, neurons, or endothelial cells [45]. One of the key mediators of astrocytic scar formation after SCI is STAT3 signaling. STAT3 conditional knockout mice failed to create a glial scar that led to a widespread lesion and poor recovery of function after SCI. Lack of STAT3 activation especially led to the inability of astrocytes to move and migrate to the lesion site. This resulted in exacerbated infiltration of inflammatory cells at the site of SCI. This finding emphasized the importance of STAT3 activation in astrocytes and the impact of reactive astrogliosis in restraining leukocyte infiltration and reducing the initial insult after SCI [36].

### 6.2 Ephrins/Eph receptors

Erythropoietin-producing human hepatocellular (Eph) receptors and ephrin ligands have attracted considerable attention since their discovery, due to their extensive distribution and unique bidirectional signaling between astrocytes and neurons [46]. Eph/ephrin signaling is involved in the glial scar formation in CNS disorders. It has been demonstrated in a model of spinal cord injury that the development of glial scars and the exclusion of meningeal fibroblasts from the site of damage are a result of cell contact-mediated bidirectional signaling cascades, which is stimulated by the interaction of ephrin-B2 and EphB2 with reactive astrocytes and meningeal fibroblasts, respectively [28]. Another previous study demonstrated that ephrin B2 (−/−) mice exhibited a reduction in astrogliosis and an accelerated regeneration of injured corticospinal axons, which resulted in the recovery of murine motor function following spinal cord injury (SCI) [47].

### 6.3 TGF-β

TGF-β signaling is one of the mediators of reactive astrogliosis in SCI. TGF-β has been identified as a key trigger of CSPGs formation in the glial scar [48]. In experimental models of SCI, blockade of TGF-β signaling is shown to attenuate scar formation [49]. Interestingly, blood fibrinogen is a factor that activates TGF-β signaling after CNS injury. After vascular disruption and hemorrhage, blood fibrinogen is released into the CNS tissue, and reactive astrogliosis and CSPGs formation through the activation of TGF-β Smad2 pathway can be activated [50].

### 6.4 Nuclear factor-κB (NF-κB)

Activation of NF-κB transcription factor has been implicated in astrogliosis, although with some sophisticated evidence. In SCI, one study indicated that increased level of NF-κB was found in microglia/macrophages and endothelial cells but not in astrocytes [51]. However, in another study, reactive astrocytes were displayed to express NF-κB. Notably, studies in transgenic mice expressing IkBα, an inhibitor of NF-κB, under hGFAP promoter demonstrated that inactivation of astroglial NF-κB reduced the expression of TGF-β2 and CSPGs as well as other chemokines involved in glial scar formation such as C-X-C motif chemokine 10 (CXCL10) and C-C motif chemokine ligand 2 (CCL2). Moreover, blockade of NF-κB activation in astrocytes has resulted in white matter sparing and improved functional recovery after SCI [52].

### 6.5 Endothelins (ET)

ETs are peptides with vasoactive property. They can modulate reactive astrogliosis in various CNS diseases. ET-1 and its receptors are particularly increased in astrocytes after damage and seem to be one fundamental cause of astrogliosis [53]. In a stab wound injury, ET-1 receptor antagonist BQ788 decreased the activation and proliferation of astrocytes. ET-1 stimulates astrocyte proliferation via the activation of JNK/c-Jun signaling pathway in vitro [54].

### 6.6 Mitogen-activated protein kinase (MAPK)

MAPK and its downstream cascades mediate astrogliosis. It is indicated that c-mos proto-oncogene, which triggers the activation of MAPK signaling, stimulates astrogliosis. Several studies implicated the phosphorylation of extracellular signal-regulated kinase/MAPK in reactive astrocytes in mice and humans [55].

### 6.7 Semaphorin 3A

Semaphorin 3A (Sema3A) is an important secreted repulsive guidance factor for many developing neurons [56]. Sema3A may be secreted from non-neuronal cells such as astrocytes. Sema3A continues to be expressed in adulthood, and expression of its receptor, neuropilin-1 (Nrp-1), can be altered by nerve injury [57]. Sema3As are regarded as one of the major classes of axon repulsive molecules that lead to the failure of axons to regenerate through the neural scar. Thus, interfering with Sema3A signaling can be beneficial for axonal regrowth [58].

### 6.8 Aquaporins

Aquaporins may play a role in the activities of astrocytes after SCI. In particular, recent studies showed that Aquaporin-4 is critical in glial scar formation [59].

In a cortical brain injury, Aquaporin-4 null mice displayed decreased migration of astroglia as a contribution to the injury site and less glial scarring. However, findings from rat SCI indicated biphasic changes in astrocytic Aquaporin-4 levels with preliminary downregulation after SCI and a following long-lasting upregulation in subacute and chronic stages of damage. Further elucidation is needed to understand the impact of Aquaporin-4 in scar formation after SCI [60].

### 6.9 Components of ECM

The ECM comprises the molecules that form the structure of the matrix. There is a huge range of molecules that have been shed from the cell surface or secreted by neurons and glia [22]. Most of these shed or secreted molecules bind to the matrix to some extent, mainly to the negatively charged glycosaminoglycan (GAG) chains of the CSPGs and heparan sulfate proteoglycans (HSPGs). There are two families of cell surface-attached HSPGs, the transmembrane syndecans and the GPI-linked glypicans. Various matrix components, particularly tenascin-C and CSPGs, are upregulated in regions of CNS damage.

Tenascins are abundant in the ECM of developing vertebrate embryos. There are four members of the tenascin gene family: tenascin-C, tenascin-R, tenascin-X, and tenascin-W. Tenascin-C is the most intensely studied member of the family [61]. Tenascin-C is anti-adhesive to many forms of neuron in vitro and inhibits axon growth from many neurons, although it promotes axon growth from some embryonic neuronal types [62]. These dual properties have been assigned to different splice variants of tenascin-C and molecular epitopes within those splice variants [63].

The levels of CSPGs increase dramatically following various CNS injuries, including lesions in the spinal cord, cortex, fornix, and nigrostriatal area [20]. CSPGs are primarily generated by reactive astrocytes and to a lesser extent by oligodendrocytes and monocytes. CSPGs are a family of molecules characterized by a core protein to which the large and highly sulfated GAG chains are attached. The major CSPGs found in the CNS include lecticans (neurocan, versican, aggrecan, and brevican), phosphacan (6B4 proteoglycan), and NG2 [64].

KSPGs are another class of inhibitory ECM molecule, which are associated with spinal cord lesions [65]. Mice lacking GlcNAc6ST-1, an enzyme critical for keratan sulfate (KS) biosynthesis, have enhanced plasticity and functional recovery after SCI [66]. Recent findings show that using KS-specific degradative enzyme, keratanase II (K-II), degrade KSPGs and allow substantial motor recovery in acute phase of SCI [67].

## 7. Conclusion

Beneficial and detrimental effects of astrogliosis have been reported by various researches. It depends on mediators and inhibitory molecules and also signaling pathways involved in SCI. Of course, more studies about astrogliosis as a complex and multifactorial phenomenon in SCI are essential. New strategies are required to minimize the detrimental effects of reactive astrocytes for increasing their beneficial effects and improve repair and regeneration.

Limiting the amount of secondary damage done by inflammation to reduce cavitation, encouraging the production of molecules supportive of regeneration, and decreasing factors inhibiting axon growth will tip the delicate balance of growth-promoting and growth-inhibiting factors to a net environment that supports functional regrowth after CNS injury.

## Conflict of interest

The authors declare that there are no conflicts of interest.



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