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Reciprocal Modulation Between Microglia and Astrocyte in Reactive Gliosis Following the CNS Injury

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Abstract Reactive gliosis, also known as glial scar formation, is an inflammatory response characterized by the proliferation of microglia and astrocytes as well as astrocytic hypertrophy following injury in the central nervous system (CNS). The glial scar forms a physical and molecular barrier to isolate the injured area from adjacent normal nervous tissue for re-establishing the integrity of the CNS. It prevents the further spread of cellular damage but represents an obstacle to regrowing axons. In this review, we integrated the current findings to elucidate the tightly reciprocal modulation between activated microglia and astrocytes in reactive gliosis and proposed that modification of cellular response to the injury or cellular reprogramming in the glial scar could lead advances in axon regeneration and functional recovery after the CNS injury.

Keywords Reactive gliosis · Glial scar · Microglia · Astrocyte · Central nervous system

Abbreviations

ATP	Adenosine triphosphate
CD	Cluster of differentiation
ChABC	Chondroitinase ABC
CSPGs	Chondroitin sulfate proteoglycans
CXCL10 C-X-C	motif chemokine 10
FABP7/BLBP	Fatty acid binding protein 7 brain (aka: brain lipid binding protein)
FGFR3	Fibroblast growth factor receptor 3
GFAP	Glial fibrillary acidic protein
Glast	Glutamate aspartate transporter
Id3	Inhibitor of DNA-binding/ differentiation protein 3
IFN-γ	Interferon gamma
IL	Interleukin
KSPGs	Keratan sulfate proteoglycans
MHCII	Major histocompatibility complex class II
NFI A/B	Nuclear factor I protein A and B
PGE2	Prostaglandin E2
Sox9	Sex determining region Y-box 9
TGF-α and TGF-β	Transforming growth factor alpha and beta
TNF-α	Tumor necrosis factor alpha

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Introduction

Reactive gliosis, also known as glial scar (GS) formation, is a reactive cellular process that occurs after injury in the central nervous system (CNS), involving reactive astrocytes, activated microglia, fibroblast, endothelial cells, infiltrating immune cells, and extracellular matrix surrounding the damaged region. The inflammation seems to be a critical step in secondary degeneration after the CNS injury and causal to the GS formation. Growing evidence suggests that cytokines released from microglia, macrophages, and infiltrating immune cells during the acute phase of CNS damage may function as either initial molecular inducers [e.g., interleukin (IL)-6, tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ)] or repressors (e.g., IL-10) of astrocyte proliferation and GS formation [1–4]. On the other hand, molecules released from reactive astrocytes in turn maintain a persistent inflammatory response and modulate the microglial activation during the chronic phase of the CNS injury. For the regenerative studies of spinal cord injury (SCI), reactive astrogliosis has become an important therapeutic target for axonal regrowth and functional recovery [5–7]. In the primary lesion stage of SCI, astrocytes first provide support to the injured area, maintain blood–cord barrier, secrete cytokines, and prevent excitotoxicity. In the secondary lesion stage, astrocytes enter the hypertrophic state (reactive astrocytes) with increased synthesis of intermediate filaments such as glial fibrillary acidic protein (GFAP) and vimentin, which form a physical wall and produce inhibitory proteoglycans [e.g., chondroitin sulfate proteoglycans (CSPGs) and KSPGs) to drive back axonal regeneration [6]. Although the GS represents a physical and molecular barrier to axonal regrowth, it also isolates the injury site from healthy tissue, which prevents further damage due to uncontrolled expansion of inflammation [8, 9]. However, the reciprocal impact of microglia and astrocytes and how it determines the progression of CNS injury are still poorly understood. In this review, we will attempt to address this complex issue by integrating current findings in microglial and astrocytic activation after the CNS injury, which may aid in understanding the fine balance between inflammation and the GS formation.

Origin, Development, and Physiological Functions of Microglia and Astrocytes in the CNS

Microglia

Microglia are widely regarded as the resident mononuclear phagocytes distributed ubiquitously throughout the nervous system, which are typically characterized by ramified morphology in a "resting" state and express certain cell surface antigens, such as CD11b/c, CD14, major histocompatibility complex molecules, chemokine receptors, and several other markers [10]. In mice, microglial progenitors with amoeboid/phagocytic morphology start to colonize in neural tube around E10.5 (i.e., embryonic day 10.5) [11]. Three days later, they are significantly detected within the superficial mantle layer of the spinal cord as well as the subventricular zone in the brain [12]. The precise origin and cell lineage of microglia remain debated. At least two separate "populations" of microglial progenitors exist during the prenatal CNS development. One mainly comes from extravascular progenitors that are of myeloid/mesenchymal progressively developing until the adulthood. The other derives from circulating progenitors—monocytes and/or fetal macrophages that are seeded within the CNS after the fetal circulation has been established at E14. They may also be derived from neuroectoderm similar to oligodendrocytes and astrocytes. However, in the early postnatal and adult CNS, blood-borne precursors only give rise to a small number of perivascular ameboid-like macrophages/microglia, not most of ramified microglia that are widely and stably distributed in the CNS [12, 13]. Microglial progenitors are differentiated and localized along vascular/ventricular margins and white matter during prenatal stages. Around 5 days after birth (~PND5), these microglia are observed in both white matter and gray matter regions, which dramatically proliferate between PND5 and PND15. By PND20, the adult microglia are well matured and distributed throughout the CNS (Fig. 1). Traditionally, microglia are thought to be in a "resting" state to maintain homoeostatic activity in the normal CNS. Recently, accumulating evidence revealed that microglia are highly dynamic to communicate with neurons, astrocytes, oligodendrocytes, and immune cells, which are proposed to be renamed as "surveying" microglia [13, 14].

Astrocytes

Astrocytes, known as astroglia, are the most abundant cells in the CNS. Astrocytes are classically identified as cells expressing the intermediate filament GFAP, a marker of terminally differentiated astrocytes. Although originally defined as gap fillers for the neuronal network, astrocytes have strategic locations, being in closely contact with CNS-resident cells (neurons, microglia, oligodendrocytes, and other astrocytes) and with blood vessels. The initiation of glial specification occurs after the neurogenesis at E11.5 in the rodent CNS. Radial glial cells derived from the neuroepithelium are the primary precursor cells at embryonic stages to generate neurons first, followed by glia. The timing of this neuron–glia switch is temporally–spatially controlled by extrinsic and intrinsic factors [15, 16]. The bone morphogenetic proteins, Delta-Notch, and Jak-Stat pathways are well-known signaling to activate a set of transcription factors that determine the cell fate of astrocytes in the populations of the ventricular zone (VZ). However, the precise timing of astroglial specification remains unclear. Patterning domains in the ventral spinal cord that generate astrocyte have been established in the p1, p2, and p3 domains at the VZ along

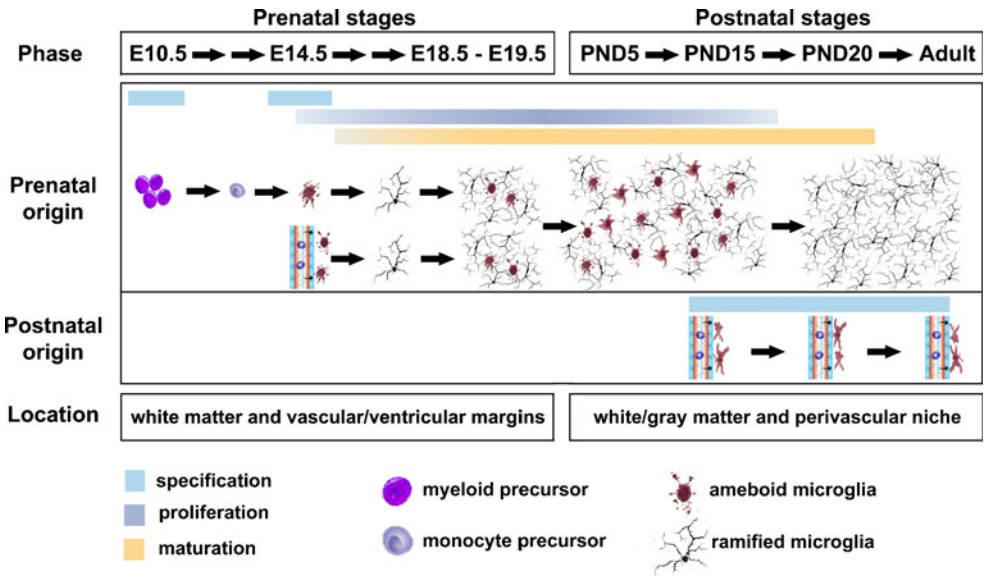

Fig. 1 Origin and development of microglia in the rodent CNS. The myeloid/mesenchymal-derived microglial progenitors start to colonize in neural tube around E10.5. Four days later, the second population of microglial progenitors originates from the circulating blood monocytes and/or fetal macrophages. The proliferating progenitors are differentiated and localized along vascular/ventricular margins and white matter during prenatal stages. Around PND5, these microglia are observed in both white matter and gray matter regions, which dramatically proliferated between PND5 and PND15. By PND20, the microglia are well matured with ramified morphology and stably distributed throughout the CNS. In the early postnatal and adult CNS, blood-borne precursors also generate a small number of perivascular ameboid-like macrophages/microglia

the dorsal–ventral axis which specify three subtypes of ventral white matter astrocytes—VA1, VA2, and VA3, respectively [16, 17]. All newly born astrocytes could be essentially identical, but differentiate into different "shapes" due to their final residential area. The morphology of astrocyte appears to be mature by the third to fourth postnatal week. Two types of astrocytes are identified based on their location in the white versus gray matter [18–21]. Fibrous astrocytes typically showing more classic "star-like" processes with dense GFAP staining populate the white matter. Protoplasmic astrocytes having more thinner and spongiform processes reside in gray matter (Fig. 2). The lack of reliable markers is a major limitation for astrocyte study. GFAP, as a terminally differentiated astrocyte marker, is mainly expressed in the late development of fibrous astrocytes and activated astrocytes under pathological conditions. It is also synthesized in type B multipotent cells at the subventricular zone (SVZ) in the adult rodents. Since neural cells are generated from the neuroepithelium, astrocytes share some markers the same as either neurons [e.g., nuclear factor I protein A and B (NFI A/B), FABP7/BLBP, fibroblast growth factor receptor 3 (FGFR3), and sex determining region Y-box 9 (Sox9)] or oligodendrocytes (e.g., Glast, NFI A/B, FGFR3, Sox9, Id3, and S-100β) at specific embryonic stages [22]. Astrocytes now have been found not only to participate in neurotransmitter regulation, ion homeostasis, blood–brain barrier maintenance, immune responses modulation, and the production of extracellular matrix (ECM) molecules [23, 24], but also to play a number of active roles in cell migration, differentiation, and maturation in the developing CNS, not just as a supportive cell [25]. More recent studies further showed that astrocytes were involved in regulating synaptic plasticity [26] and myelin maturation [14]. In the adult rodent brain, GFAP-positive astrocyte-like cells at the VZ–SVZ (type B cells) serve as stem/progenitor cells that give rise to adult-born neurons in the olfactory bulb [27].

Reactive Gliosis Following the CNS Injury—from Inflammation to Glial Scar

Microglia Activation and Inflammatory Response

Microglia are considered "the tissue macrophages" in the nervous system, owing to their phenotype and reactivity following any disturbance or loss of homeostasis that indicates real or potential danger to the nervous system. It has been reported that a subpopulation of monocytes enters the neural tissue and transforms into microglia after blood–brain barrier damage [28]. Under the pathological conditions of the CNS injury such as infection, ischemia, neurodegenerative disease, trauma, etc., microglia are readily activated and undergo a dramatic transformation from their "surveying" ramified state into an amoeboid morphology [29]. The "surveying" microglia are able to extend or retract cytoplasmic processes within seconds or minutes and reorient their processes within a few minutes. The transformed microglia (activated state) migrate towards the site of lesion in the CNS and form a dense border that seems to seal the lesion and block the spread of the

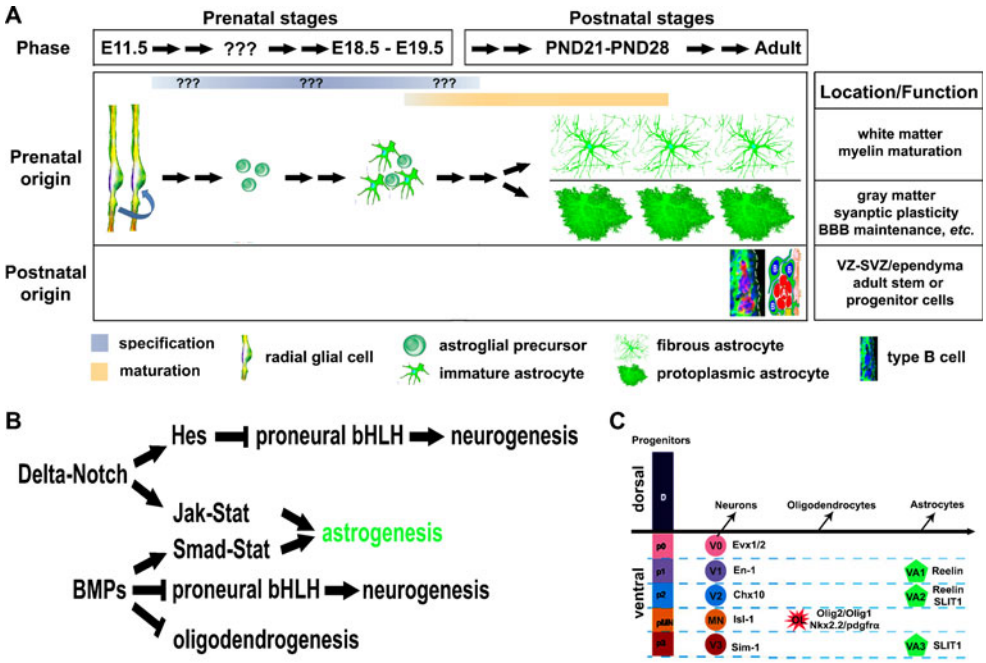

Fig. 2 Origin and development of astrocyte in the rodent CNS. **a** Generation, morphological changes, and physiological functions of astrocyte across developmental time. The initiation of glial specification occurs after the neurogenesis at E11.5 in the rodent CNS. Radial glial cell-derived astroglial progenitors are generated after the neuron–glia switch at E11.5. The precise timing of astroglial specification remains unclear. All newly born astrocytes could be essentially identical, but differentiate into fibrous or protoplasmic astrocyte with respective functions due to their final location in the white matter or gray matter. **b** Signaling pathways that determine the astroglial specification. **c** A schematic illustration of neuronal, oligodendroglial, and astroglial domains in the ventral spinal cord development. The homeodomain code controls the generation of neuron, oligodendrocyte, and white matter astrocyte

damage [30]. In their activated state, they can up-regulate or express de novo distinct profiles of cell surface "phenotypic" markers, which are also found on other mononuclear phagocytes such as macrophages. They serve diverse beneficial functions essential to neuron survival, which include cellular maintenance and innate immunity [31]. Meanwhile, activated microglia are also involved in regulating the CNS development and neurogenesis through the release of trophic and anti-inflammatory factors [13]. However, under the over-activated state, microglia induce detrimental neurotoxic effects by releasing a diverse set of cytotoxic substances, including pro-inflammatory factors such as TNF-α, PGE2, and INF-γ and oxidative stress factors which are toxic to neurons [32–34]. Some experiments have shown that two kinds of functional subsets of monocyte-derived macrophages exist in peripheral blood and may contribute to distinct biological performance in inflammatory diseases [35]. Similarly, different stimulus to microglia may lead to diverse phenotype, referred to as microglial polarization, which results in cells with either pro- or anti-inflammatory properties [36]. In the SCI, the classically activated M1 macrophages/microglia activated by lipopolysaccharide and pro-inflammatory cytokine IFN-γ produce high levels of oxidative metabolites (e.g., nitric oxide, superoxide) and pro-inflammatory cytokines (IL-12, IL-23, IL-1β, and TNF-α) and increase their phagocytic and antigen-presenting capacity [37]. M1 macrophages/microglia not only play essential roles in host defense but also cause the damage to peripheral healthy cells and tissue [38]. Conversely, alternative M2 macrophages/microglia activated by cytokines IL-4 or IL-13 promote angiogenesis, matrix remodeling, and expression of MHCII molecules. They also suppress destructive immunity, nitric oxide (NO), and pro-inflammation cytokines (TNF-α, IL-1β, IL-2, IL-8, IL-12, and CXCL10) release [39–41]. M1 macrophages/microglia express specific antigens such as CD86, CD32, and inducible NO synthase, while M2 can be identified by arginase 1, mannose receptor, and CD206 [38, 39].

Generally speaking, short-term microglial activation is not considered to be detrimental and even plays beneficial effects in CNS injury or diseases. Microglia produce a number of neuroprotective substances in response to injury, including anti-inflammatory cytokines and neurotrophic factors. Transforming growth factor beta (TGF-β) and IL-10 down-regulate the expression of molecules associated with antigen presentation and decrease the production of pro-inflammatory cytokines, chemokines, and nitric and oxygen free radicals [42, 43]. Microglia can also release brain-derived growth factor (BDNF) and insulin-like growth factor1 (IGF1), which led to improved neuronal cell viability [44]. Recent evidence indicates that different cytokines released from activated

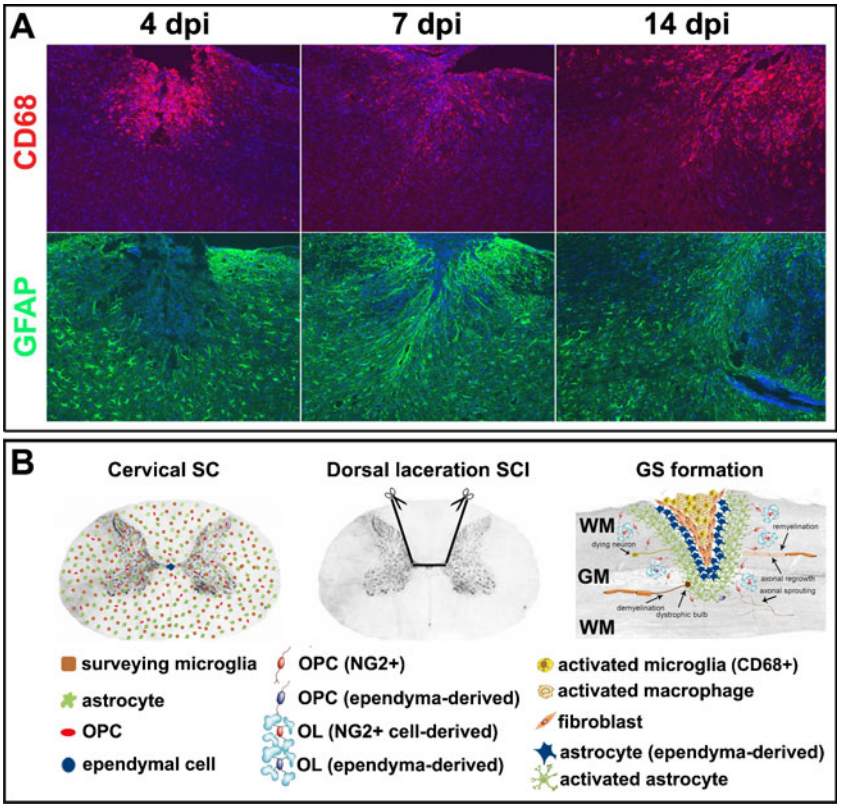

Fig. 3 Reactive gliosis in the mouse spinal cord injury of dorsally cervical laceration. **a** Activation of microglia (CD68+ cells) and astrocytes (GFAP + cells) in the acute stage of SCI. *dpi* days post injury. **b** Major cellular populations in the adult spinal cord and glial scar formation after the spinal cord injury. In the adult spinal cord, "surveying or resting" microglia (*brown*) and astrocytes (*green*) are uniformly distributed as ependymal cells (*blue*) are confined to the epithelium lining the central canal. More adult oligodendrocyte precursor cells (NG2+, *red*) are located in gray matter than those in white matter. After the injury, the resident microglia, infiltrating macrophages, and fibroblasts evenly form the epicenter of the glial scar surrounded by ependymal cell-derived GFAP-negative astrocytes (*blue*) and activated preexisting GFAP-positive astrocytes (*green*). This intense inflammatory response leads to a cascade of secondary damage including dystrophic axons and their demyelination. On the other hand, up-regulation of inhibitory extracellular matrix molecules secreted by microglia and astrocytes, such as proteoglycans, is distributed in an increasing concentration gradient from the lesion penumbra to the lesion center. The inhibitory extracellular matrix molecules impede axonal regrowth and remyelination by the surrounding adult oligodendrocytes and precursor cells that are originated mostly from resident adult oligodendrocyte precursor cells (*red*) and sporadically from ependymal cells (*blue*). Meanwhile, a few axonal sprouting may appear in the area adjacent to the glial scar. *SC* spinal cord, *SCI* spinal cord injury, *GS* glial scar, *WM* white matter, *GM* gray matter, *OPC* oligodendrocyte precursor cell, *OL* mature oligodendrocyte

microglia can stimulate T immune cells to acquire diverse phenotypes with detrimental [45] or beneficial [36, 46] effects in the CNS. In the acute stage of SCI, macrophages/microglia are activated (Fig. 3a) and become the primary source of the pro-inflammatory cytokines IL-1, IL-6, and TNF-α [47]. Most macrophages/microglia are M1 cells, with only a transient and small number showing M2 polarization. cDNA microarray and quantitative real-time PCR analyses showed that M1 and M2 markers were rapidly unregulated after spinal cord injury. The M2 marker—arginase 1—had only a transient increase and returned to normal levels by 7 days post injury. In contrast, in M1 markers, CD16/32 and CD86 expression was maintained for up to 1 month post-injury [38]. The in vivo and in vitro studies indicate that M1 macrophages can directly induce neuronal death and correlate with tissue damage in spinal cord injury as those anti-inflammatory M2 macrophages/microglia probably contributes to the prolonged pro-inflammatory response that has detrimental effects on tissue preservation and cell viability. Furthermore, M1 macrophages may have a negative impact on axon regeneration possibly due to the 17-fold higher expression of CSPGs in M1 than that in M2 cells [48]. In culture, M1-conditioned medium induces stunted, short neurites with multiple branches, whereas M2-conditioned medium promotes extensive, long neurites from dorsal root ganglion cells [38], suggesting that M2 macrophages/microglia may provide a more permissive axon regeneration microenvironment than M1 macrophages in spinal cord injury. Thus, it is very important to understand the diverse phenotype acquired and their regulatory signals of microglial cells responding to the diverse stimulations. It may provide a new therapeutical strategy for the treatment of CNS injury via adjusting the shift of microglial subtypes.

Reactive Astrocytes in the GS Formation

Reactive astrocytes (also known as astrogliosis or astrocytic scar) are the main cellular component of the GS, which is characterized by cellular hypertrophy and an abnormal apparent increase in the number of astrocytes. After the injury, astrocytes are likely to react promptly to the damage, which undergo morphological changes, extend their processes, and increase synthesis of intermediate filament proteins. Up-regulation of intermediate filament proteins, in particular GFAP, vimentin, and nestin in astrocytes, is regarded as the hallmark of astrogliosis. As a major intermediate filament protein in mature astrocytes, significantly increased expression of GFAP has been found in the process of astrogliosis in numerous experimental models (Fig. 3a). The levels of vimentin in astrocytes range from very low to intermediate, depending on the subpopulation of astrocytes. It has been suggested that re-expression of vimentin in reactive astrocytes following the injury is indicative of these cells recapitulating developmental migratory processes [49]. Nestin is regarded as a marker of "neural stem/progenitor cells," which are expressed in both neuronal and glial precursors [50–52]. Nestin-immunopositive cells can be seen in reactive astrocytes in response to the CNS injury [53]. Recent in vivo studies identified two cellular origins of astrocytes in the GS after the SCI—preexisting GFAP-positive astrocytes and ependymal cell-derived GFAP-negative astrocytes [54, 55]. The ependymal cell-derived astrocytes express Sox9 and vimentin but not GFAP. They form the core of the GS surrounded by resident GFAP + astrocytes activated after SCI (Fig. 3b).

Astrocytes perform a serial of protective effects in the CNS injury condition. Activated astrocytes limit the infiltration of peripheral leukocytes/macrophage and activation of local resident microglia by initiating the repair of the damaged blood–spinal cord or blood–brain barrier [56, 57]. They can modulate blood flow by the release of vasoconstrictors [58] and also protect neurons and oligodendrocytes from glutamate excitotoxicity by uptaking excess glutamate in the environment [8, 59]. Deactivation of astrocytes via genetic ablation of GFAP resulted in widespread tissue disruption, pronounced cellular degeneration, and severe persisting motor deficits [9]. These findings show that reactive astrocytes provide an essential ability that protect tissue loss and preserve function after the CNS injury. On the other hand, reactive astrocytes contribute significantly to the release of the inhibitory ECM components after the CNS injury [60], which form a dense GS around the injured lesion to pose physical and chemical barriers [61, 62]. It suggested that ependymal cell-derived astrocytes do not synthesize those inhibitory ECM components [55]. ECM components such as CSPGs, tenascins, and collagen are dramatically up-regulated in the GS after the CNS injury and inhibit axonal elongation and sprouting [63–65]. It has been found that ChABC, a bacterial enzyme that is able to degrade CSPG gradient, can enhance axonal regeneration through the GS after the SCI [66]. CSPGs also influence the properties of oligodendrocyte precursor cells (OPCs). They inhibit the outgrowth of OPC processes, OPC migration, and differentiation [67, 68], which eventually lead to failure of remyelination for regenerated axons. In addition, astrocytes and matrix components create a scaffold for the vascularization network at the injury site where endothelial cells and fibroblasts are recruited to form new capillaries. Thus, modulation of reactive astrocytes and ECM in the GS may be crucial for axonal regeneration following the CNS injury.

Reactive Gliosis and Functional Recovery in the CNS Injury

The CNS lesion may cause locomotor deficits, sensory impairment, and/or chronic neuropathic pain to various extents, depending on the location, range, and severity of the injury. Animal studies have shown that anti-inflammatory treatments significantly ameliorated motor and sensory functional recovery [69–73]. Reducing the infiltration of neutrophils, macrophages, or T cells with neutralizing antibodies [69, 70], depletion of macrophages [71], or anti-inflammatory cytokine therapy [72] in the acute phase decreased the secondary tissue damage with functional improvement. Repression of microglial/macrophage activation by administration of minocycline or CD25 antibodies during either acute or chronic phases increased the neuroperformance after the traumatic SCI [73–75]. Inhibiting the astroglial activation or remodeling the ECM of astrocytic scar enhanced axonal plasticity and regeneration and promoted the functional improvement in the rodent SCI models [76–80]. Recently, accumulating evidence suggests that inflammation and reactive gliosis (both microglia and astrocytes) have emerged as key contributors to pathological and chronic neuropathic pain mechanisms in the CNS injury [72, 81–85]. Thus, anti-inflammatory therapy may also relieve the chronic neuropathic pain [86, 87].

Microglial Modulation on Astrogliosis in the CNS Injury

Although astrogliosis is associated with diverse neurological disorders, the cellular and molecular mechanisms leading to astrogliosis are not yet completely understood. As the first line of defense in the CNS, macrophages/microglia respond immediately to the presence of danger signals, react quickly to increase inflammatory signals, and destroy the infectious agents before they cause damage in neural tissue [88]. They can respond within minutes after injury with production of pro-inflammatory cytokines. Growing evidence suggests that activated macrophages/microglia may contribute to the subsequent activation of astrocytes in the CNS injury. A number of cytokines, chemokines, growth factors, and transcription factors have been identified as triggers and modulators for astrogliosis [89], including TNF-α, IL-1β, IL-6,

IL-10, TGF-α, TGF-β, ciliary neurotrophic factor (CNTF), fibroblast growth factor-2, platelet-derived growth factor, insulin-like growth factor (IGF), leukemia inhibitory factor, monocyte chemoattractant protein-1, endothelin-1, erythropoietin, fibrinogen, matrix metalloproteinase-9, and Sox9. As the most important pro-inflammatory cytokines secreted by macrophages/microglia, IL-1, IL-2, IL-6, and TNF-α play important roles as initial triggers to activate the astrocytes via their receptors in the acute phase of CNS injury [90–93]. IL-1, IL-2, IL-6, and TNF-α have been found to increase GFAP immunoreactivity when they were microinjected into the brain in the neonatal stab-wound mouse model [94]. IL-1 injected into the cerebral cortex of adult rats not only elicits new blood vessel growth but also stimulates GFAP expression as well as hypertrophy of astrocytes [95], indicating that IL-1-secreting inflammatory cells may mediate astrocyte activation in the CNS injury. IL-6 has been reported to link several neurological disorders such as multiple sclerosis and Alzheimer's disease. IL-6 induces the synthesis of neurotrophic factors [nerve growth factors (NGFs)] [96] and inhibits the production of the potentially neurotoxic molecule TNF-α [97] in astrocytes. However, excessive expression of IL-6 mice showed marked gliosis and neurological signs even after mild injury of the spinal cord [98]. In IL-6 knockout mice, reactive GFAP-positive stellar astrocytes and gliosis are drastically inhibited [99]. Blocking the IL-6 signal with IL-6 receptor antibody after the contusive mouse injury model can repress the GS formation at the center of the injured spinal cord by suppressing the transformation of ependymal cells to astrocytes [91]. The in vitro studies showed that TNF-α can promote changes in astrocytes via activation of epidermal growth factor receptor (EGFR) [100] and increase astrocyte proliferation and survival [101]. In transgenic model, overexpression of TNF-α directly enhances the immunoactivity of GFAP and vimentin in hypertrophied astrocytes possessing numerous thick processes via activating the EGFR [102].

Effect of Reactive Astrocytes on Microglial Activation After the CNS Injury

Inflammatory response, mediated largely by macrophages/microglia, has been implicated in several different neurological disorders from acute injuries such as spinal cord injury to chronic neurodegenerative conditions such as Alzheimer's disease. Compared to the rapid microglial response, the astrocytic response usually occurs as a secondary event. A recent study reported a secondary peak of microglia and macrophage presenting in the injured spinal cord at 60 days, with continued elevation through 180 days after SCI, apart from the primary peak in 3 to 7 days [103], suggesting that the secondary signals stimulate microglia and eventually cause such long-time chronic inflammation following the SCI. It demonstrated that astrogliosis or GS components may be involved in the modulation of inflammation following the SCI. Some studies indicated that disruption of the scar or some of its components reduced the numbers of reactive microglia in the lesion area and attenuated monocytic activity [104]. Other studies verified that the glial scar was partially required to maintain inflammatory response under balanced condition. Ablation of active astrocytes inhibits leukocyte infiltration in the spinal lesion area [9, 105]. However, the underlying mechanisms are still ambiguous.

Reactive astrocytes contribute to the release of pro- and anti-inflammatory cytokines such as interleukins (IL-1 and IL-6), TNF-α, TGF-β, and IFN-γ, which may in return activate microglia and cause the secondary injury [106]. The studies in vitro provide certain hints: astrocyte-conditioned medium increases ramification of blood monocytes in culture [107], which was prevented by neutralizing antibodies against astrocyte-derived cytokines [108]. Since active microglia also secrete the same inflammatory cytokines that exert biochemical effects on themselves by way of autocrine or paracrine, it is difficult to determine in vivo if the stimulus signals come from reactive astrocytes. Activated astrocytes produce several growth factors and neurotrophic factors, such as IGF, NGF, BDNF, CNTF, and neurotrophin 3 to support the surrounding cells [109, 110]. They also synthesize and release adenosine triphosphate (ATP), glutamate, reactive oxygen species (ROS) and NO, and ECM proteins such as CSPGs [62, 64]. Like microglia, reactive astrocytes can regulate their own activities in an autocrine or paracrine fashion [6, 57]. Meanwhile, they may play important regulatory roles in the activation, survival, and regeneration of adjacent neurons, oligodendrocytes, and microglia by way of paracrine. ATP, as the second messenger, is actually one of the main responsible messengers in the activation of microglia through purinergic receptors that are expressed prominently on microglia [111, 112]. In response to local brain injury, the ATP released from astrocytes activates morphological changes of local microglia migrating towards the injury site quickly [30, 113]. Another report showed that ATP mediated the calcium signaling between astrocytes and microglia involved in controlling the number and function of microglial cells under pathophysiologic CNS conditions [114]. Ca2 + −dependent glutamate released from astrocytes may exacerbate the neuroinflammation in neurodegenerative disorders [115]. Some data implied that glutamate can act on metabotropic glutamate receptor to suppress some facet of the glutamate export mechanism in the process of activation of microglia [116]. The effect of glutamate on microglia can be reversed by glutamate receptor antagonists in the process of neuroinflammation in some neurodegeneration models [117–119]. All these results indicate that ATP and glutamate released from activated astrocytes directly affect the microglial activity in neuropathological condition. In addition, as

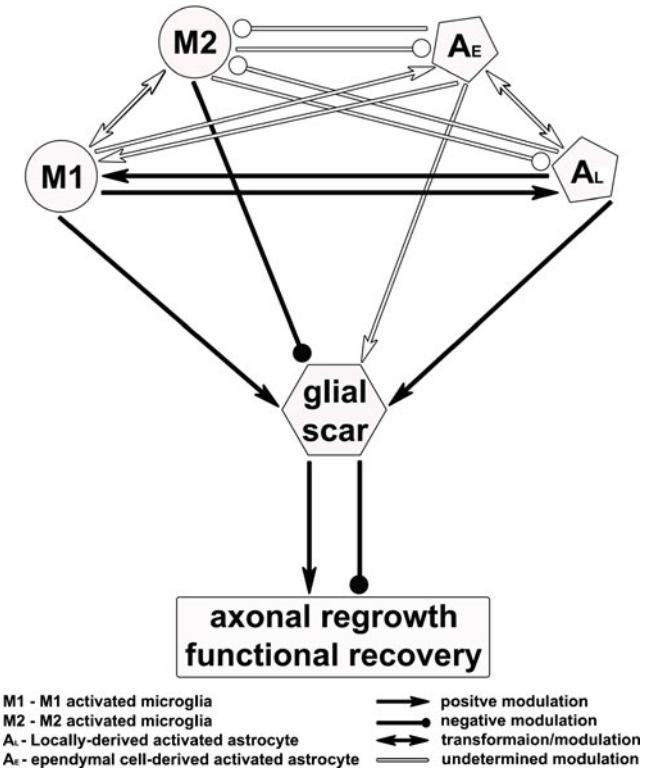

Fig. 4 Hypothetical intermodulation between microglia and astrocytes in reactive gliosis following the CNS injury

products of oxidative stress, ROS and NO are other important mediators of inflammatory processes during microglia activation [120]. After the CNS injury, over-reactive astrocytes at the lesion site form the GS and alter the composition of ECM dramatically. ECM components including CSPGs and tenascins are markedly up-regulated in astrocytes [121, 122]. CSPGs were found to adhere to chemoattractive molecules and growth factors which are needed for recruitment and activation of macrophages [123], immune cells [124], and dendritic cells [125]. These findings suggest that CSPGs may capture these factors and increase their focal concentration to attract more microglia migrating towards the lesion area, thereby enlarging immune response to the CNS damage. CD44 functions as a receptor colocalized in astrocytes and microglia. CD44-neutralizing antibodies can suppress CSPG-induced activation of microglia and modulate the release of neurotrophic factors [126, 127]. Furthermore, inhibition of CSPG production leads to a dramatic effect on the spatial organization of the infiltrating macrophages and resident microglia around the lesion site, decrease of IGF-1 expression, and increase of TNF-α level in the acute stage of the SCI, which enhances the motor functional recovery [127]. On the other hand, it has been reported that activated astrocytes can exert inhibitory effects on microglial activation. TGF-β mainly produced by astrocytes [128] can reduce microglial activation by down-regulating the expressions of molecules associated with antigen presentation, pro-inflammatory cytokines, NO, and ROS [129]. Additionally, astrocytes can restrain the infiltration of the circulating macrophage and other immune cells by repairing the damaged blood–brain and blood–spinal cord barriers [8, 9]. Taken together, accumulative evidence indicates that reactive astrocytes and their products are mostly associated with modulation of inflammatory response by regulating the number, location, and activation of infiltrating monocytes–macrophages and resident microglia.

Conclusions and Prospects

In the CNS, reactive gliosis is a complicated process with both beneficial and detrimental effects on injury recovery. As two major cellular populations of reactive gliosis, microglia and astrocytes can activate each other and have a tightly reciprocal modulation during the GS formation. Either microglia or astrocytes can release a battery of signal molecules to feedback themselves or serve a cross-talk with adjacent brain cells, i.e., neurons, oligodendrocytes, astrocytes, microglia, and infiltrating immune cells. Under the pathological conditions in the CNS, microglia are activated earlier than astrocytes. In acute phase, most subpopulations of macrophages/microglia are pro-inflammatory M1 cells, while only a transient and small number are anti-inflammatory M2 cells. The inflammatory molecules produced by activated M1 microglia activate both preexisting GFAP-positive astrocytes and GFAP-negative astrocytes derived from ependymal cells, which form the GS confining the lesion area in the CNS. The products released from reactive astrocytes may contribute to induce a secondary peak of macrophages/microglia presenting in the lesion site and maintain a persistent inflammatory response during the chronic phase of the CNS injury (Fig. 4). The following points remain poorly understood: (1) the mechanisms to determine the shift between M1 and M2 microglia, (2) the functional difference in preexisting GFAP-positive astrocytes and ependymal cell-derived GFAP-negative astrocytes, and (3) how activated microglia and astrocytes synergistically modulate ECM components in the GS formation. A precise understanding of the underlying mechanisms will have a significant bearing for potential therapeutic use. Modulation of injury response and cellular function in activated microglia and astrocytes with a new balance of protective and inhibitory effects in the injured CNS will likely become the master key to create a nourishing niche for axonal regeneration. In addition, recent studies in vitro show that lineage-specific transcription factors or microRNA can induce differentiated cells (e.g., fibroblasts and astrocytes) to trans-differentiate into functional neurons without going back to the fully undifferentiated state [130–137], which may alternatively provide an in vivo source of neurons and modify the microenvironment for use in cell-based therapies. Thus, cellular components of the GS, including

fibroblasts, astrocytes, microglia, etc., could be reprogrammed onsite and driven toward the neuronal lineage for functional repair.

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Conflict of Interest The authors report no conflicts of interest.

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