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Concise Review: Reactive Astrocytes and Stem Cells in Spinal Cord Injury: Good Guys or Bad Guys?

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ABSTRACT

Spinal cord injury (SCI) usually results in long lasting locomotor and sensory neuron degeneration below the injury. Astrocytes normally play a decisive role in mechanical and metabolic support of neurons, but in the spinal cord they cause injury, exerting well-known detrimental effects that contribute to glial scar formation and inhibition of axon outgrowth. Cell transplantation is considered a promising approach for replacing damaged cells and promoting neuroprotective and neuroregenerative repair, but the effects of the grafted cells on local tissue and the regenerative properties of endogenous neural stem cells in the injured spinal cord are largely unknown. During the last 2 decades cumulative evidence from diverse animal models has indicated that reactive astrocytes in synergy with transplanted cells could be beneficial for injury in multiple ways, including neuroprotection and axonal growth. In this review, we specifically focus on the dual opposing roles of reactive astrocytes in SCI and how they contribute to the creation of a permissive environment when combined with transplanted cells as the influential components for a local regenerative niche. Modulation of reactive astrocyte function might represent an extremely attractive new therapy to enhance the functional outcomes in patients. STEM CELLS *2015;33:1036–1041*

INTRODUCTION

Astrocytes are the most abundant glial cell type in the central nervous system (CNS), playing a decisive role in the mechanical and metabolic support of neurons, and are involved primarily in regulating neural network activity, synaptic plasticity, blood flow, ionic and extracellular space volume homeostasis, and key neurotransmitter and ion concentrations [1, 2].

The definition of reactive astrocytes is related to radical change in the morphological and functional state of astrocytes as a response to a specific acute pathological condition such as spinal cord injury (SCI), brain injury, mechanical lesions of brain parenchyma, ischemia, and infection or neurodegenerative diseases [3]. In this review, we analyze reactive astrocytes and their specific function in the spinal cord upon transplantation of pluripotent stem cells, trying to answer whether these cells exert a beneficial or detrimental function in regenerative processes after injury. We specifically focus on the dual opposing role of reactive astrocytes in SCI and how they contribute to the creation of a permissive environment in synergy with transplanted cells as the key influential components in creating a local regenerative niche.

REACTIVE ASTROCYTES IN SPINAL CORD INJURY: TRADITIONALLY CONSIDERED AS BAD GUYS

Spinal cord injury caused by mechanical trauma (contusion or compression) encompasses various processes that have been divided into two phases. The first phase is characterized by the induction of massive cell loss, with axonal, oligodendrocyte, and neural cell death [4]. Massive loss of oligodendrocytes results in the inability of spared neurons to regenerate their axons [5], leading to neurological dysfunction. As a consequence of blood-brain barrier (BBB) damage and increased permeability, infiltrated cells from the blood invade the medullar tissue, triggering an inflammatory response. In the second phase, weeks after injury, different environmental cues associated with the inflammatory response and cell damage trigger existing astrocytes to undergo different morphological and molecular perturbations. These are mostly related to hypertrophic processes, increased

Table 1. Outline of markers of proteoplasmatic astrocytes and reactive ones

Proteoplasmatic astrocytes	Reactive astrocytes
GFAP	GFAP ↑
S100β	S100β ↑
GLAST	GLAST ↑
GLT1	GLT1 ↑
BLBP	BLBP ↑
AQP4	AQP4 ↑
	CSPGs↑
	Ephrins↑
	Chemokines↑
	Vimentin↑
	Nestin↑

Abbreviations: AQP4, aquaporin 4; BLBP, lipid-binding protein; CSPGs, chondroitin sulfate proteoglycans; GFAP, glial fibrillary acidic protein; GLAST, glutamate-aspartate transporter; GLT1, glial glutamate transporter 1; ↑, overexpression.

cell proliferation, and overexpression of glial fibrillary acidic protein (GFAP), vimentin, and nestin [6], including the complex molecular, temporal, and functional interconnections amongst the various signaling pathways (reviewed in [3, 7]).

These reactive astrocytes then interact with other cell types such as fibromeningeal cells and additional glial cells, contributing to the formation of a glial scar as a mechanical and chemical barrier to axonal regeneration. A more detailed description of glial scar formation can be found in the review by Silver and Miller [8]. The protein pattern of reactive astrocytes is also changed; reactive astrocytes overexpress GFAP and vimentin [6], the proteins that contribute to the formation of the glial scar [6], as well as S100β, nestin, glutamate-aspartate transporter, and glial glutamate transporter 1 (Table 1). Furthermore, reactive astrocytes secrete inhibitory extracellular matrix molecules such as chondroitin sulfate proteoglycans (CSPGs), which inhibit axonal growth and regeneration [8], as well as tenascin, ephrin B2, and Slit proteins. These proteins secreted by reactive astrocytes in synergy with other cell types are traditionally considered to have detrimental roles by virtue of contributing to the formation of the physical and chemical barrier that impedes axonal regeneration. This negative connotation of reactive astrocytes is an old concept and has been used to develop different strategies in order to regenerate the damaged tissue. One strategy is the manipulation of the glial scar using chondroitinase ABC to dissolve and reduce CSPG inhibition as a possible therapy for SCI [9], with moderate success. Application of chondroitinase ABC together with other intervention strategies such as peripheral nerve grafting [10], or Schwann cells and olfactory-ensheathing glia grafts [11] significantly increased axonal growth and improved locomotor outcomes, suggesting possible introduction of this approach in clinical practice [9].

Beneficial Effects of Reactive Astrocytes: Their Neighbors Make Them Good Guys

The detrimental functions of reactive astrocytes mentioned previously are in fact responses to injury with the aim of protecting the nervous system and less affected tissue by healing the damaged area and halting further extension of injury and additional cell damage. At the same time, reactive astrocytes exert detrimental functions in the context of rendering the environment nonpermissive for axonal repair and regeneration. In vitro and in vivo studies show that under normal conditions reactive astrocytes exert their protective role in the CNS in different ways such as: (a) active clearance of extracellular excitotoxic glutamate; (b) protection from ammonium toxicity; (c) protection from nitric oxide toxicity by producing glutathione; (d) degradation of amyloid β peptide; (e) stabilization of extracellular fluid and ion balance; (f) BBB repair; and (g) playing a role in energy (ATP) production via glucose transport. Upon SCI, besides expressing detrimental molecules, astrocytes contribute an important part in endogenous neuroprotection and also secrete growth-promoting neurotrophic factors. These are brain-derived neurotrophic factor, ciliary neurotrophic factor, nerve growth factor (NGF), and basic fibroblast growth factor (FGF-2) as well as the extracellular matrix proteins laminin and fibronectin. In the last 20 years, accumulating evidence from different deletion studies has revealed that reactive astrocytes exert a beneficial function upon injury, putting into question the concept of reactive astrocytes as a purely detrimental and negative phenomenon in SCI.

Genetically manipulated animal models provide further evidence that the loss or attenuation of reactive astrocytes worsens the outcome after CNS insults. While GFAP and/or vimentin knockout (KO) mice show normal developmental and breeding patterns [12], they also exhibit low astroglial activity and increased plastic sprouting of supraspinal axons, including the reconstruction of circuits associated with functional outcomes in a hemisection model of SCI [13]. An elegant transgenic mouse model was developed by Sofroniew and coworkers [14] that allowed the conditional ablation of GFAP-expressing cells using a herpes simplex virus type 1-thymidine kinase (suicide gene) under the control of the mouse GFAP promoter [14]. In this model, it is possible to temporarily ablate dividing scar-forming reactive astrocytes using the antiviral drug ganciclovir in the context of different types of injury [14]. Such early ablation of reactive astrocytes limited scar formation in SCI models while the BBB was impaired, allowing increased immune cell infiltration, in particular macrophages, as well as neuronal death, demyelination, and impaired locomotor function [14]. The same effect of impaired recovery after SCI was observed in animals lacking matrix metalloproteinases (MMPs). MMPs proteolytic enzymes are involved in both, injury and repair mechanisms in the CNS and expressed in reactive astrocytes at the lesion border [15]. These results provide evidence that scar-forming astrocytes have a crucial role in neural protection and damage repair and are essential in tissue protection and preservation of function after mild or moderate SCI.

Okada et al. provided further evidence of a beneficial role of reactive astrocytes [16]. They applied a Cre-loxP system designed for conditional KO of signal transducer and activator of transcription 3 (STAT3) or cytokine signaling 3 suppressor (SOCS3), an intracellular signal transducer for various cytokines [16] involved in the upregulation of reactive astrocytes. The conditional ablation of STAT3 significantly decreased hypertrophy, overexpression of GFAP, and the migration of reactive astrocytes, leading to increased inflammation, demyelization and neural disruption, and poor locomotor outcome [16]. In addition, the deletion of SOCS3, which normally provides negative feedback on STAT3, displayed the opposite outcomes, reducing the lesion and improving motor symptoms

after SCI [16]. Taken together, these findings reveal complex functions of reactive astrocytes that can be beneficial or detrimental for SCI depending on the signaling pathways involved. Proper modification of reactive astrocyte function after SCI could represent a new regeneration state.

The above described studies reveal the role of reactive astrocytes in preserving tissue integrity; however, little is known about how different cell replacement strategies modify the detrimental role of reactive astrocytes at the site of injury and contribute to improved recovery and enhanced axonal regeneration. We review cell transplantation approaches in tissue regeneration after SCI where the role of reactive astrocytes has been demonstrated. Schwann cells, fetal spinal cord, neural stem cells, or neural derivatives of pluripotent stem cells are all considered promising sources for cell replacement therapy after SCI.

Schwann cells naturally populate the growth-permissive peripheral nervous system and their grafts seem to support axonal outgrowth by bridging the lesion gap following SCI, rendering the environment permissive for more extensive axonal outgrowth [17]. These early landmark transplantation studies showed successful regeneration of intraspinal, but not supraspinal axons. The latter have been regenerated when different combinatorial strategies were applied [18]. In most of these studies, analysis of the host spinal cord/Schwann cells bridge interfaces revealed that astrocyte processes elongated in association with Schwann cells and regenerated axons, which correlated with locomotor outcome. For example, in the study of Guest el al. [19], the authors show that transplanted Schwann cells in combination with IN-1 antimyelin antibodies induced migration of astrocytes into the site of a spinal cord transection and supported the axonal growth. Brainstem axons did not regenerate the lesion site and did not enter into the lesion if astrocytes failed to extend their processes. In contrast, Schwann cell grafts that exclude astrocytes are not invaded by CNS axons unless they are supplemented by exogenous neurotrophic factors. Elongation of reactive astrocytes in combination with Schwann cells can be triggered by glial-derived neurotrophic factor or by pretreatment of glial-precursor astrocytes with bone-morphogenetic protein (BMP) [20]. This is a clear example of how appropriate stimulation or modification of the reactive astrocyte response after injury could be exploited for successful restoration of injured spinal cord.

After accumulating evidence that astrocytes are capable of responding to CNS injury by undergoing a process of reactive astrogliosis and providing support for cells and processes in the injured spinal cord, several studies have specifically exploited a cell transplantation strategy using astrocyte progenitors from embryonic or neonatal tissue in SCI models. These studies have addressed the phenotypic diversity or therapeutic application of glial-restricted progenitors (GRP), particularly those derived from human fetal tissue. It has been shown that with proper ex vivo treatment these cells are capable of promoting neuroprotection and repairing damaged spinal cord [20, 21]. GRP can be isolated via the A2B5 surface marker from developing rat spinal cord [22], which are able to give rise to oligodendrocytes and astrocytes and exhibit self-renewal in vitro [22]. Upon treatment with either BMP or CNTF, GRP gave rise to distinct types of astrocytes, but it was shown that only BMP-induced astrocytes exerted regenerative effects on axonal sprouting and locomotor recovery in the rat model of SCI [20, 23]. Human GRP transplanted into various types of SCI survived, migrated, and differentiated toward astrocytes and oligodendrocytes [24]. In addition, these cells limited the formation of the glial scar through reducing CSPGs as well as enhancing axon sprouting [24] and promoted functional and sensory recovery [21]. Although GRPs represent a promising cell source for therapeutic application, the guidelines for their isolation, maintenance, and differentiation remain to be standardized in order to optimize functional recovery after injury, which reveals the lack of well-established standards in preclinical studies using GRPs.

Early studies have shown that multipotent tissue grafts, such as fetal spinal cord [25], that were transplanted into damaged spinal cord, exerted neuroregenerative effects on the host tissue, by modifying scar formation in a manner associated with positive locomotor outcome [26]. These studies highlight the possible contribution of reactive astrocytes to the regeneration of damaged spinal cord tissue upon transplantation of fetal grafts.

Neural stem cells (NSC) are a population of adult stem cells naturally present in adult neural tissue, primarily in the periventricular subependymal layer and the subgranular zone of the dentate gyrus [27]. In the spinal cord, ependymal cells have neural stem cell properties and are located around the central canal [28]. Both brain and cord-derived NSC can be propagated in vitro as neurospheres and differentiate in vitro to form neurons, astrocytes, and oligodendrocytes [29]. In response to injury, their proliferation capacity dramatically increases and constitutes the main pool of new glial cells in the injured spinal cord [30]. The regenerative effects of these cells after proper induction have been shown on different SCI models [29, 31]. In spite of their extensive capacity for neuronal differentiation in vitro, transplanted brain and spinal cord NSC in vivo differentiate almost exclusively into glia, with minimal neuronal differentiation observed [32]. In contrast, several transplantation studies of cells exhibiting astroglial hallmarks have been shown to promote functional recovery after SCI [33, 34].

We recently reported functional motor recovery after transplantation of spinal cord-derived precursor cells, from spinal cord injured donors (ependymal stem-precursor cell induced by the injury, epSPCi), with induced cell proliferative capacity and glial preferential in vivo differentiation [29]. epSPCi in a contusion rat model showed a neuroprotective role by accompanying naked axons within the injured area, suggesting glial progenitor-derived neurotrophic support for axonal regeneration and cell survival [29]. Although NSC are considered to be ideal candidates for cell transplantation studies, due to their inherent neural commitment, low rates of tumorigenesis, and the opportunity for autologous transplantation, their clinical application is questionable as a result of their limited availability and low proliferation rate.

Human pluripotent stem cells, encompassing human embryonic stem cells (hESC) and human-induced pluripotent stem cells (hiPSC), hold great potential as a source for cell replacement therapies in humans. Human embryonic stem cells are pluripotent cells and can be derived from the inner cell mass of an early blastocyst [35]. A solution to the ethical concerns surrounding hESC was offered by the discovery of the Nobel prize winning technology of creating hiPSC from adult somatic cells by ectopic expression of a defined set of

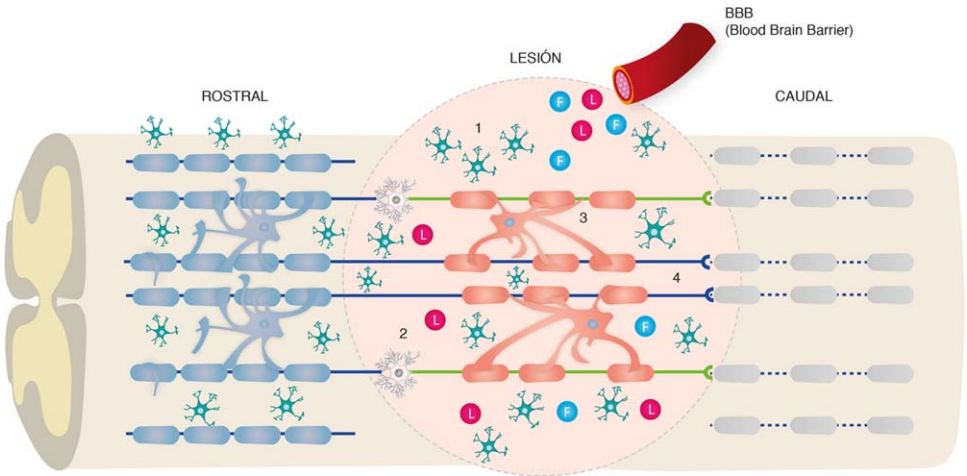

Figure 1. Schematic overview of astrocyte modulation upon cell transplantation. Spinal cord injury triggers the migration of reactive astrocytes (1). Stem cell progenitors transplanted during the acute phase of injury differentiate toward neurons (2), which reconnect the damaged tissue, and oligodendrocytes (3) that remylinate new neurons and sprouted axons (4). Transplanted cells modulate the detrimental role of reactive astrocytes and create a permissive environment for the upregulation of beneficial proteins such as laminins (L) and fibronectins (F).

factors [36]. hiPSC can be easily derived from individual patients allowing the development of customized stem cell therapies, generation of disease-specific stem cell lines, and the possibility to correct the mutant gene in the target cells and return it to the patient [37]. Self-renewal and multilineage differentiation toward virtually any cell type in the human body are two unique properties that make hESC and hiPSC the most promising sources for tissue regeneration. hESC and hiPSC are capable of differentiating toward all cell types, including neurons, glia, NSC, and motoneurons [38]. In the context of cell therapy for SCI, oligodendrocytes, astrocytes, and neurons are of particular interest. As we already mentioned, SCI-induced massive cell death and loss of oligodendrocytes result in demyelination of spared axons leading to locomotor impairment. Delivery of early neural progenitor cells (NPCs) or oligodendrocyte progenitor cells (OPC) as a source for the remyelination processes, including the migration and mature differentiation of these cells, could be a promising strategy for spinal cord repair [39]. The regenerative properties of hiPSC-derived neural progenitors in SCI [40–43] were found to take place through three possible mechanisms: remyelination, axonal regrowth, and trophic support. Transplanted cells survive, migrate, and differentiate toward all major neural cell lineages (astrocytes, neurons, and oligodendrocytes) [44], corroborating the potential of hiPSC and hESC neural derivatives over adult stem cells [45]. Nevertheless, the mechanism of action of the transplanted cells is still unknown.

In the context of the beneficial role of reactive astrocytes in combination with pluripotent stem cell therapy, our recent observation of the regenerative potential of OPC when transplanted into a complete transection rat model of SCI, either alone or in combination with motoneuron progenitors (MP), is of particular interest [45]. We hypothesized that the potential of OPC and/or MP to rescue locomotor activity is due to the presence of heterogeneous cell types present in transplanted progenitors [46]. We identify their possible mechanism through the modification of inhibitory properties of reactive astrocytes [46]. In this study, we analyzed the microenvironment of the lesion site, revealing the molecules and processes that could point to the possible mechanism of action. We showed enhanced astrogliosis 4 months after transplantation of OPC and MP in the acute phase of SCI, possibly as a result of Notch and JAK/STAT signaling activation. At that time point, the GFAP-negative area in the epicentre of the injury site was significantly reduced in transplanted animals, suggesting that JAK/STAT and Notch signaling enhanced astrogliosis in the lesion site resulting in lesion compaction, as has been previously observed [47]. In transplanted rats, we found improved growth of serotonergic and dopaminergic axons in close proximity with transplanted cells and GFAP$^+$ astrocytes. It seems that the prompt contraction of the lesion favors hESC-derived neuronal survival and differentiation and correlates with improved locomotor recovery in these animals [16]. This study corroborates the role of astrogliosis in spinal cord regeneration [14, 23], suggesting that neural protective factors secreted from transplanted cells in synergy with reactive astrocytes could create the neuro-protective environment for neurogenesis observed in transplanted cells [45] (Fig. 1). This particular niche created by transplanted cells seems to alter the detrimental properties of reactive astrocytes. The increase in the expression of beneficial molecules such as NGF, laminins, fibronectin, and neurotrophins, and decreases in detrimental genes such as CSPG, TENASCINS, and genes included in SLIT/ROBO signaling in the lesion site resulted in less inhibitory reactive astrocytes, making them permissive to axonal growth, neuronal progenitor survival, and differentiation [46]. This in turn is associated with the presence of human neurons in the lesion site [45]. This finding corroborates reports of iPS progenitor-derived astrocyte promotion of axonal regrowth via a growth-permissive substrate [43], which is involved in specifically creating a permissive environment where beneficial molecules such as BDNF are expressed. This was recently demonstrated in a study by Romanyuk et al. [41] (Fig. 1). The absence of a growth-promoting cell population in the control group resulted in a lack of axonal regeneration.

Finally, growing lines of evidence suggest that astroglia lineages host neural stem-like cells [48] revealing their in vitro capacity to self-renew and give rise to progenies including all

three neural lineages [7, 48] when grown as neurospheres. Recently, it has been shown that injury reactivates stem cell potential of reactive astrocytes [49] through sonic hedgehog protein [50]. It is of particular interest to understand the mechanisms behind the proliferative response of reactive glia in different injury settings in order to assess the ability of these NSC-like cells to provide endogenous repair for therapeutic purposes.

Therapeutic Interventions to Make Reactive Astrocytes Good Guys

As soon as reactive astrocytes had been recognized as key regulators of spinal cord injury, specific molecular aspects of reactive astrogliosis were targeted for the purpose of therapeutic manipulations [51]. It has been identified that epigenetic regulation of gene expression can be of great therapeutic importance. Acetylation and deacetylation of histones using histone deacetylases (HDACs) and histone acetyltransferases (HATs), respectively, affecting the global gene expression pattern in cells, can be of special interest in the neurological field. For example, pharmacological inhibition of HDAC with valproic acid (VA) increases histone acetylation and improves pathological conditions in various neurological diseases, including SCI [52]. VA was shown to stimulate the release of neurotrophic factors from astrocytes [53], at the same time decreasing autophagy and reducing the expression of inflammatory mediators including tumor necrosis factor-alpha [54]. Curcumin, a major curcumanoid in the spice turmeric and HAT inhibitor is also a prospective drug for neurological diseases. The effects of curcumin have been generally described as anti-inflammatory, antiapoptotic, and antioxidative, improving neurological function in the injured spinal cord of rats [55]. Curcumin exerts its function through inhibiting astrocyte reactivation, which may be beneficial for neuronal survival, as claimed in a study by Lin et al. [55] and other study of various types of injuries [56]. However, future studies are needed to clarify the roles of curcumin in the regulation of signaling pathways and gene expression profiles in astrocytes.

Other potential targets for astrocyte functional modification include Aquaporin 4, a water channel responsible for bidirectional, blood-brain water transport, normally localized on astrocyte endfeet, or different connexins or potassium channels localized to the same site, responsible for maintaining the resting membrane potential and needed for proper K^+ buffering. In the context of astrocyte function, these targets have been well reviewed by Hamby and Sofroniew. [51].

A recent article by Renault-Mihara et al. [57] showed that the beneficial role of reactive astrocytes can be triggered in SCI by inhibiting glycogen synthase kinase 3, a serine/threonine protein kinase, resulting in improved locomotor activity in mice. This study clearly demonstrates the stimulation of astrocyte migration as a feasible therapeutic strategy for traumatic injury in the central nervous system.

Conclusions

The growing evidence summarized here indicates that reactive astrocytes have both active and passive roles in regenerative processes after SCI. Reactive astrocytes per se cannot repair damaged SCI, but can contribute to healing the area by protecting the lesion site from any further extension of damage. It seems that only in the proper environment can these cells modify their own properties and create a permissive niche for endogenous events and exogenous interventions to regenerate the damaged tissue. Also in this review, we reported the novel function of transplanted hESC derivatives on modifying properties of reactive astrogliosis, which was classically considered as detrimental to axonal regeneration, but has also been shown to contribute to a favorable environment for neuronal differentiation of transplanted cells. Overall, a future repair strategy based on combined cell and drug therapy with pluripotent stem cell-derived neural cells that minimize the inhibitory properties of astrocytes while maximizing their growth-promoting properties would be extremely attractive for the treatment of SCI. It will be challenging to "fine tune" the modification of reactive astrocyte function via a selective blockade of molecules that inhibit axonal outgrowth while allowing the formation of a physical barrier to protect the intact tissue.

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Author Contributions

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Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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