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REVIEW

Spinal Cord Injury Scarring and Inflammation: Therapies Targeting Glial and Inflammatory Responses

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Abstract

Deficits in neuronal function are a hallmark of spinal cord injury (SCI) and therapeutic efforts are often focused on central nervous system (CNS) axon regeneration. However, secondary injury responses by astrocytes, microglia, pericytes, endothelial cells, Schwann cells, fibroblasts, meningeal cells, and other glia not only potentiate SCI damage but also facilitate endogenous repair. Due to their profound impact on the progression of SCI, glial cells and modification of the glial scar are focuses of SCI therapeutic research. Within and around the glial scar, cells deposit extracellular matrix (ECM) proteins that affect axon growth such as chondroitin sulfate proteoglycans (CSPGs), laminin, collagen, and fibronectin. This dense deposition of material, i.e., the fibrotic scar, is another barrier to endogenous repair and is a target of SCI therapies. Infiltrating neutrophils and monocytes are recruited to the injury site through glial chemokine and cytokine release and subsequent upregulation of chemotactic cellular adhesion molecules and selectins on endothelial cells. These peripheral immune cells, along with endogenous microglia, drive a robust inflammatory response to injury with heterogeneous reparative and pathological properties and are targeted for therapeutic modification. Here, we review the role of glial and inflammatory cells after SCI and the therapeutic strategies that aim to replace, dampen, or alter their activity to modulate SCI scarring and inflammation and improve injury outcomes.

Key Words Macrophage · human · chondroitinase ABC (chABC) · azithromycin · glial limitans · traumatic brain injury.

Introduction: Glial Effectors of Spinal Cord Injury Scarring and Inflammation

Neuronal dysfunction underlies the disabilities associated with spinal cord injury (SCI). At the time of injury synaptic connections are lost, demyelination and axon damage disrupts signal propagation, and neurons undergo mechanically induced cell death. The primary injury also activates a secondary cascade of vascular, inflammatory, and biochemical events that further disrupt neuronal function. These primary and secondary injury events activate glia, including astrocytes, fibroblasts, pericytes, Schwann cells, and microglia. The dialog between activated glia and injured neurons underlies endogenous pathological and reparative processes in the injured central nervous system (CNS).

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In the absence of injury, glia support signal transmission and neuronal function. Oligodendrocytes wrap axons with myelin sheaths, insulating the axon to increase action potential conduction velocity and decrease signal decrement. Astrocytes interface with the vasculature and sequester and transport neurotransmitters, ions, and nutrients to neurons to optimize signaling. Pericytes ensheath endothelial cells of the CNS capillaries and can adjust capillary diameter, control vascular coupling, and control neurovascular function [1–3]. Microglia patrol the CNS as resident immune cells and sample the CNS environment phagocytosing potential pathogens while secreting growth and supportive factors.

Following SCI, glia secrete toxins and cytokines in response to the mechanical damage. Tissue initially spared from mechanical trauma is susceptible to secondary damage from these glial by-products [4]. The diverse assemblage of glial cells necessary to maintain healthy CNS function becomes a complicated array of cells now activated with pathological and reparative properties. The mechanical trauma and downstream signaling cascades further drive injury progression by facilitating infiltration of nonresident cells. Immune cells extravasate into the injury site and persist chronically within the

injured spinal cord [5–7]. Fibroblasts either infiltrate from the periphery or differentiate from other resident cells and deposit inhibitory extracellular matrix (ECM) components within the injured spinal cord [2, 8]. Schwann cells migrate through dorsal root entry zones into the lesion epicenter and contribute ECM proteins and growth factors to the lesion milieu [9–12]. Collectively, SCI triggers diverse glial activation and cellular recruitment with complex downstream effects on neuronal function. Here, we will review the cellular effectors contributing to scarring and inflammation following SCI with a specific focus on glial-targeted therapies.

Glial and Fibrotic Scarring After Spinal Cord Injury

SCI activates resident astrocytes and pericytes, as well as recruits infiltrating fibroblasts and Schwann cells from periphery, leading to the development of lasting glial (cellular) and fibrotic (acellular) scars in the injured spinal cord (Table 1). Regarding the various cells of the glial scar, astrocytes surround the lesion site and take up residence in the lesion penumbra [42]. Pericytes and nonpericyte perivascular cells infiltrate into the lesion core where they are closely associated with ECM components such as fibronectin, laminin, and collagen, as well as, traditional fibroblast markers [2, 8, 26, 43]. The exact origin and contribution of these particular cells typically associated with connective tissue (i.e., pericytes, meningeal cells) is an active area of debate [2, 8, 44]; we will collectively refer to these cells as fibroblasts. Schwann cells from nerve peripheral roots infiltrate into the lesion epicenter where they also express fibroblast markers and closely associate with laminin, fibronectin, and collagen deposits [9–12].

The astrocytic and fibroblast/Schwann cell components of the glial scar are strictly separated to the penumbra and lesion core, respectively (Fig. 1). Indeed, many studies use astrocytic boundaries to demarcate regions of frank tissue pathology from more intact penumbral tissues [45]. This interface is sometimes referred to as the "glia limitans." The strict sequestration of cell types is in stark contrast to regenerating species where both ECM components and glial cells cross the lesion site and precede neural regeneration [46–48]. Formation of the glia limitans may be species-specific or driven by cellular interactions, as the phenomenon has been replicated *in vitro* by cocultures of mammalian astrocytes and fibroblasts/Schwann cells that maintain spatial separation and inhibit neurite growth [11, 21, 42].

After injury, proliferating astrocytes thicken cellular processes and surround the lesion with a meshwork of overlapping outgrowths (Fig. 1). Astrocyte activation and subsequent glial scar boundaries are enhanced by the addition of transforming growth factor-beta (TGF-β) [21, 49, 50]. TGF-β increases microglia/macrophage and astrocyte activation and fibronectin and laminin deposition [49]. Signal transducer and activator of transcription 3 (STAT3) is also important for establishing the glial scar border that secludes infiltrating cells to the lesion epicenter [51, 52]. Previous schools of thought simply classified the glial scar as a maladaptation opposing neurite regrowth. More recently, evidence indicates that the glial scar is important for neurotrophin production, debris clearance, blood brain barrier repair, and toxic species sequestration to the injury site [13, 53]. The positive role of the glial scar in SCI responses is reflected by the necessity of a glial bridge for neural regeneration in nonmammalian models [46, 48].

The fibrotic scar, i.e., the acellular components of the scar consisting of deposited ECM materials, influences the cellular

Table 1 Time course of SCI acute inflammatory responses and their effects on SCI progression and repair. Specific references for each row include astrocytes [13–16], Schwann cells [12, 17], meningeal cells [18–20], fibroblasts [2, 8, 21, 22], CSPGs [23–25], fibronectin [26, 27], collagen [28–31], tenascin-C [24, 27], laminin [30, 32–34], microglia [35–37], neutrophils [36, 38, 39], and macrophages [36–38, 40, 41]. For an in-depth review of phases of responses to central nervous system damage, see Burda et al. [14]

	Responder	Onset	Peak	Resolution	Effects on SCI
Glial scar	Astrocytes	< 1 dpi	~14 dpi	Persistent	Segregate spared penumbral tissue and lesion core
	Schwann cells	< 21 dpi	???	???	Support and guide axons
	Meningeal cells	< 3 dpi	14 dpi	Persistent	Oppose neurite and cell infiltration
	Fibroblasts	< 3 dpi	7-14 dpi	Persistent	Deposit ECM (variable effects) and decrease glial mobility
Fibrotic scar	CSPGs	< 7 dpi	~30 dpi	Persistent	Inhibit axon regrowth
	Fibronectin	< 1 dpi	7 dpi	Persistent	Inconclusive
	Collagen	< 1 dpi	7 dpi	Persistent	Variable
	Tenascin-C	1 dpi	8 dpi	30 dpi	Inhibits axon sprouting and leukocyte infiltration
	Laminin	< 1 dpi	7-28 dpi	Persistent	Inhibits axon growth into lesion core
Inflammation	Microglia	< 1 dpi	7 dpi	Persistent	Phenotypic-specific beneficial or detrimental effects
	Neutrophils	< 1 dpi	1 dpi	~3 dpi	Remove debris with potential tissue-toxic bystander damage
	Macrophages	3 dpi	7 dpi	Persistent	Phenotypic-specific beneficial or detrimental effects

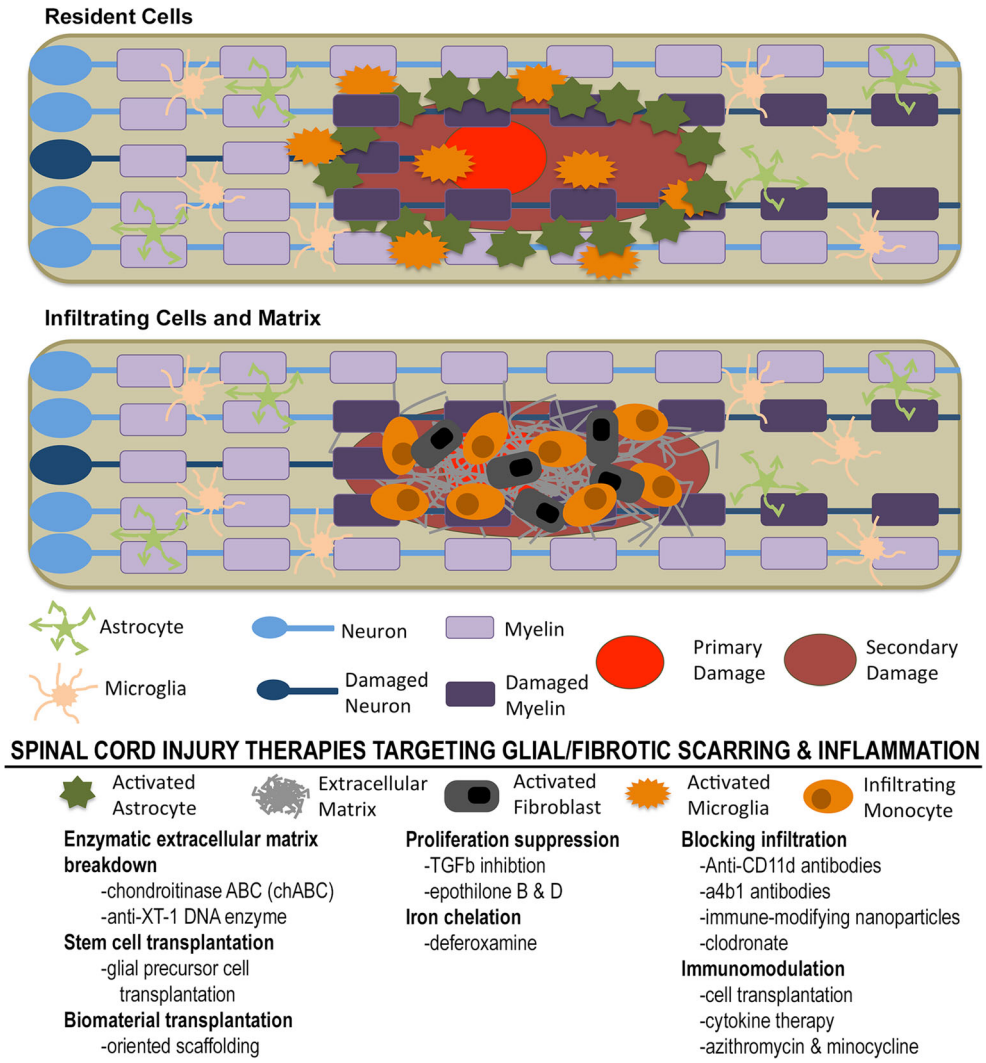

Fig. 1 Schematic of resident and infiltrating glial cells and associated therapies following traumatic spinal cord injury. Resident microglia and astrocytes are activated following injury and form a glial scar surrounding and sequestering the damaged tissue (top). Fibroblasts and inflammatory cells infiltrate into the damaged tissue and deposit extracellular matrix proteins forming the fibrous scar (middle). Activated cells exacerbate damage, leading to an expanded secondary injury. Therapeutic approaches (bold) and example agents (hyphenated) targeting glial activation, scar formation, and inflammation after spinal cord injury (bottom). This therapeutic list is not comprehensive, and references and abbreviations are in the main body of the manuscript

distribution of the glial scar. ECM molecules can increase the rigidity of the environment, create a physical barrier, and provide nonspecific topographical cues, all of which may affect cellular migration (Fig. 1) [54–56]. Additionally, ECM components signal through cell surface receptors to influence cellular activity. For example, tenascin and fibronectin increase matrix metalloproteases (MMPs) in various cell types [57–59] and MMPs influence outcomes of SCI including the infiltration of cells into the injury core [60–66]. Despite the presence of tenascin, fibronectin, and MMPs at the glia limitans, the demarcation remains intact chronically. Overall, investigations into the glia limitans provide interesting pathophysiological descriptions but therapeutic strategies that interfere with the establishment of the scar demarcations or that drive the injury responses toward establishing a glial bridge are limited.

The fibrotic scar is also a critical regulator of axonal regeneration and growth after SCI (Table 1). Cells within the lesion core mediate ECM dynamics through production of ECM components and proteolytic enzymes, especially MMPs [26, 67, 68]. MMPs degrade ECM molecules allowing receptor mediated assembly into dense matrices [26]. Several of the ECM components, such as CSPGs and fibronectin, inhibit neurite regrowth *in vitro*; others, such as laminin, promote greater neurite outgrowth [13, 53, 55, 69–72]. Similarly, removal of inhibitory ECM components, such as CSPGs, improves neurite growth *in vivo* [23, 46–48, 73], whereas removal of other ECM proteins, such as collagens, fails to promote regeneration or recovery [28]. The orientation and stiffness of ECM scaffolds may also act as a physical cue for neurite growth leading to strategies with aligning ECM components to promote directional axon growth [55, 56, 70, 74, 75].

Using transgenic models, researchers have gained insight into therapeutic targets that reduce the inhibitory effects of scarring on SCI repair. Targeted suppression of astrocyte signaling pathways reduces inhibitory scar formation and facilitates axon growth and SCI recovery [76]. Specifically, transgenic approaches have identified astrocyte inhibition of TGF-β/Smad, TLR, JAK/STAT3, and JNK/c-Jun signaling

cascades, among others, as potential SCI therapies [76]. However, depending upon the timing post-injury, astrocyte inhibition also interferes with ECM deposition of growth-supportive substrates and neurotrophins (e.g., laminin, fibronectin, growth factors) thereby reducing endogenous repair processes [77]. Indeed, transgenic models demonstrate that astrocytes play an important role in limiting the spread of secondary injury events early after injury [14]. Although the results of these transgenic models reveal a complex role for scarring after injury, there are ongoing research efforts to target different components of glial and fibrotic scars to improve SCI recovery.

The above cellular components of the glial scar and ECM deposition of the fibrotic scar are primarily derived from observations made in rodent SCI models. By comparison, data is limited regarding SCI scar formation in humans. As in rodents, there is clear cellular demarcation of the glial scar with astrocytes around the lesion border and fibroblasts, Schwann cells, and meningeal cells sequestered within the lesion [9, 10, 78–81]. This inverse relationship between astrocytes and other glial cells within the lesion is similar between species. However, in humans, Schwann cells are the predominant cells type composing the glial scar within the lesion instead of fibroblasts as in rodents [9, 10, 79, 80]. There are also conflicting reports suggesting that the prominence of the astrocytic glial scar varies between species with less astrocytosis in humans [10, 80].

The fibrotic scar, i.e., the acellular components of the scar consisting of deposited ECM materials, is sometimes referred to as the mesenchymal or fibroblastic scar in humans [80]. The composition of the fibrotic scar in humans consists of abundant collagen, laminin, and fibronectin deposits within the lesion core and reduced deposition in penumbral areas of astrocyte activation [10, 80]. Although the distribution and prominence of CSPGs are comparable between species, there is evidence that the cellular specificity and spatiotemporal distribution of specific CSPGs may vary between humans and rodents. For example, in humans, versican and neurocan are found almost exclusively within the lesion and are likely produced by Schwann cells rather than by fibroblasts, glial precursors, meningeal cells, or astrocytes (in the lesion penumbra) as observed in rats [9, 24, 80, 82]. Collectively, there is strong evidence that Schwann cells disproportionately contribute to the glial and fibrotic scar in human *versus* rodent SCI.

Spinal Cord Injury Therapies Targeting the Glial and Fibrotic Scar

Therapies targeting astrocyte activation and the glial scar focus primarily on three approaches (Fig. 1). The first approach is to break down inhibitory extracellular matrix molecules produced by astrocytes with CSPGs being the primary target. The second is to transplant astrocyte stem cells, or glial-restricted precursor cells, into the injured spinal cord to suppress scar formation, facilitate a permissive environment through the release of growth factors, deposit supportive ECM substrates, and increase the effectiveness of stem cell therapies. The third is to manipulate the extracellular matrix through transplantation of biomaterials that provide substrates for axon growth and effectively bypass or alter the inhibitory glial ECM deposition that occurs after SCI.

As mentioned above, the glial and fibrotic scars form a physical and chemical barrier to axon growth. The dense deposition of extracellular matrix presents a physical obstruction for growing axons. In addition, CSGPs and other inhibitory extracellular matrix molecules bind receptors that signal axonal growth inhibition [83]. Transgenic manipulation of SOX9 and N-acetylgalactosaminyl transferase demonstrate the efficacy of reducing CSPGs on SCI neuroprotection and axon regeneration [84, 85]. Therapeutically, enzymatic digestion of CSPGs with anti-XT-1 DNA enzyme or chondroitinase ABC (chABC) facilitates axon regeneration and functional recovery [23, 86, 87]. There is promising converging preclinical evidence of increased axon growth after SCI with the chABC treatment from multiple independent researchers and in combination with other therapeutic strategies (reviewed by [88]). Chondroitinase ABC may also provide anti-inflammatory mediated neuroprotection by reducing pro-inflammatory CSPG stimuli [89–91]. Across a number of different rodent SCI models, chABC treatment improves functional recovery [88]. As mentioned above, the presence and distribution of CSPGs after SCI are similar between rodents and humans, and therefore, optimizations in delivery and safety may lead to successful translation of chABC treatment to humans [88].

Interestingly, glial crosstalk may contribute to the therapeutic effects of chABC treatment. Chondroitinase ABC has immunomodulatory effects when delivered after SCI as shown by the increased predominance of reparative macrophages with treatment [90, 92]. Specifically, the immunomodulatory effects of chABC treatment depend in part on the release of IL-10, an anti-inflammatory cytokine that increases reparative (also called "M2"—see inflammation below) macrophage activation [92]. Blocking IL-10 with neutralizing antibodies reduces chABC-mediated reparative macrophage activation *in vivo* [92]. In addition, IL-10 may play an essential role in the dialog between infiltrating macrophages and astrocyte-mediated ECM depositions after SCI [67, 93]. The immunomodulatory changes associated with chABC treatment highlight the complex interactions among glia cells related to SCI therapies [94, 95].

The cellular sources and extracellular components of the glial and fibrotic scars are still being identified [2, 8, 96].

Other therapeutic approaches targeted to reduce fibrotic and glial scar formation include suppression of TGF-beta, an upstream regulator of fibroblast proliferation; iron chelation to decrease fibrotic ECM formation; and epothilone B and D treatment to limit pericyte and fibroblast proliferation and migration [29, 32, 97, 98]. Of these, the iron chelator, deferoxamine, and epothilone D have viable safety profiles in humans; however, further work is likely required for both agents to understand their mechanisms of action in SCI. The therapeutic effects of epothilone D may be due to microtubule stabilization in axons. Indeed, a recent evaluation of epothilone D mediated SCI recovery was unable to clearly define the anatomical correlates of treatment [99]. Similarly, the therapeutic effects of deferoxamine after contusion SCI are likely multifaceted and include changes not only in scars but also in inflammation and apoptosis [100].

A second therapeutic approach targeting the fibrotic and glial scars is the transplantation of immature astrocytes into the injured spinal cord. Immature astrocytes, either derived from developmentally immature CNS tissue or immature with regard to lineage progression, support axon growth after injury [101]. A comprehensive review of astrocyte-based stem cell therapies was recently written by Angelo Lepore and colleagues [101] and another review in this special edition focuses on cellular transplantation strategies. In our experience, transplanted glial-restricted precursor cells differentiate into both oligodendrocytes and astrocytes and decrease glial limitans formation and proteoglycan expression concurrent with increased axon regeneration and sprouting [102]. Interestingly, we detected no significant changes in overt inflammatory responses with transplantation, but we did not examine the phenotype of infiltrating macrophages [102]. Schwann cell transplantation is also associated with glial and fibrotic scar changes and is discussed in detail in the companion transplantation chapter of this special edition.

A third therapeutic approach involves manipulation of the ECM through the transplantation of biomaterials. A clinical illustration of this approach comes from a small, ongoing phase I trial in China (ClinicalTrials.gov: NCT02352077) [103, 104]. The goal of the clinical trial is to create a permissive extracellular environment through transplantation of a linearly oriented scaffold that serves both as a delivery tool for bone marrow mononuclear cells (BMMCs) [103] or mesenchymal stem cells (MSC) [104] and to orient axon regeneration across the injured spinal cord. The combinatorial approach has the potential to overcome endogenous molecular and physical ECM barriers after SCI. The transplanted cells release growth factors to counter molecular inhibition and the construction of scaffolds with a growth-supportive matrix (i.e. , collagen) facilitates axon growth across physical glial barriers. Preliminary results of the phase I trial in eight patients receiving MSCs with complete chronic SCI report partial recovery of motor function in three patients and changes in autonomic function in six patients with no adverse effects 1 year after surgery [104]. Similar results were reported from 5 patients that received BMMCs, there were no significant adverse effects for 12 months postoperatively and 2 individuals had partial recovery of sexual arousal and somatosensory evoked potentials [103]. Researchers have tested difference biomaterials and cellular sources in preclinical models with promising results, further demonstrating the therapeutic potential of targeting the glial and fibrotic scar after SCI [105].

Acute Inflammation Following Spinal Cord Injury

Spinal cord injury creates cellular debris and releases intracellular proteins that act as potent inflammatory stimuli. These injury-exposed debris signals, also called damage-associated molecular patterns (DAMPs), are normally concealed from immune surveillance within the intact CNS [106]. After injury, DAMPs engage pattern recognition receptors (PRRs) on inflammatory cells used to detect foreign microbes that invade the body [107]. The results are rapid DAMP- and PRR-mediated activation of resident inflammatory cells including astrocytes and microglia [35]. Reactive astrocytes and microglia release a wide variety of oxidative stress regulators, cytokines, chemokines, growth factors and other inflammatory mediators [108]. Microglia also alter cellular morphology and protein expression profiles after SCI. Under normal conditions, microglia have long, thin processes that extend out from the central cell body to sample the extracellular environment. Following injury, microglia retract their processes and assume a more amoeboid morphology better equipped for phagocytosis and debris clearance. These activated cells closely resemble circulating macrophages in their morphology, protein expression profile, and function [109].

Along with the morphological changes comes the release of chemokines and cytokines which serve to recruit peripheral neutrophils and macrophages into the injured spinal cord [110]. Chemokines drive increased expression of selectins and cell adhesion proteins on nearby endothelial cells. Integrin-mediated adhesion of circulating immune cells facilitates extravasation of monocytes and neutrophils into the spinal cord [111]. The first wave of infiltrating immune cells are neutrophils, which, in rodents and humans, peak within the spinal cord around 1 day post-injury (dpi) [2, 5, 6, 38, 39, 80, 112, 113]. Neutrophils perform bactericidal functions as the first line of defense against invaders; however, following SCI, by-products of neutrophil-mediated phagocytosis of opsonized particles and degranulation of proteases including reactive oxygen species are primarily considered cytotoxic [11, 26, 43, 114]. Due to the hallmark presence of myeloperoxidase and their ability to mount a potentially destructive oxidative burst, neutrophils are purported contributors to SCI pathology

in experimental models. However, conflicting studies report varying degrees of neutrophil-mediated oxidative damage following rodent SCI [46–48, 114–116]. Neutrophils persist chronically at low levels in the injured mouse spinal cord but decrease within a week of injury in both rodents and humans [5, 6, 38, 80, 113, 117] coincident with increased monocyte-derived macrophages infiltration into the spinal cord [35].

Infiltrating macrophages contribute proteolytic enzymes, reactive oxygen species, and inflammatory cytokines to the injury microenvironment but also perform necessary functions of debris clearance, cellular remodeling, and production of pro-regenerative factors [109, 110, 118, 119]. The dual beneficial and reparative functions of macrophages make understanding their role in the injury response difficult. Endogenous microglia-derived and recruited monocyte-derived macrophages are also difficult to distinguish in the injured spinal cord. As discussed above, macrophages are very similar to microglia in morphology, protein expression, and function. Indeed, disentangling the two cell types required flow cytometry or genetic methods until very recent identification of protein markers distinct to the microglia [36, 120]. Nonetheless, favorable and unfavorable outcomes are associated with the inhibition of inflammatory cell recruitment following SCI [39, 40].

Researchers now discuss the beneficial *versus* pathological roles of macrophages in SCI through subcategorization of macrophages into a variety of activation states [110]. Categorization of these activation states in SCI has been revisited several times in recent years beginning with the identification of endogenously activated pathological M1, or "classically activated," and reparative M2, or "alternatively activated," macrophages in the injured spinal cord [37]. Alternative activation states are sometimes subdivided into M2a, M2b, and M2c with more recent trends favoring a indistinct view of macrophage phenotype in SCI in which the same cell can exhibit a diversity of both M1 and M2 markers [109, 110, 121].

Regardless of terminology, researchers recognize that macrophages not only can increase axon regeneration and neuronal function but can also exacerbate tissue destruction [119, 122]. Unfortunately, pro-inflammatory M1 macrophages predominate after injury in rodents [110] and there is evidence of a sustained M1-like monocyte activation after human SCI [123]. Due to the diverse role of macrophages in both injury and repair, therapeutically, clinicians and scientists are developing immunomodulatory approaches for potentiating reparative, M2, microglia and macrophage activation within the injured spinal cord. Past experimental and clinical attempts involved transplantation of prestimulated exogenous microglia or macrophages [124–126]. With the identification of endogenously activated reparative microglia and macrophages after SCI [37], more recent immunomodulatory therapeutic approaches are focused on polarizing endogenous cells toward a reparative phenotype.

Neuroprotective Spinal Cord Injury Therapies Targeting Inflammation

To date, only one pharmacological therapy, methylprednisolone, has completed phase III clinical trials with demonstrated efficacy [127]. Interestingly, the therapeutic effect of this corticosteroid is due in part to its anti-inflammatory properties including decreased macrophage activation. Although methylprednisolone remains the only clinically approved treatment for SCI, its use has declined in recent decades. The decline is due to perceived risks associated with corticosteroid treatment (i.e., gastrointestinal bleeding and wound infection) and a potentially limited therapeutic value and treatment window (8 h) [127]. Despite the current debates regarding its use [128], methylprednisolone provides clinical evidence that limiting inflammation, specifically microglia/macrophage activation, is neuroprotective in SCI.

More recently, therapeutics targeting macrophages and microglia primarily focused on two approaches. The first involves targeting infiltrating immune cells through pharmacological macrophage depletion or antibody-based approaches to interrupt endothelial–monocyte interactions with the ultimate goal of reducing macrophage activation in the injury site. The second focuses on immunomodulation and promotion of reparative, M2, macrophages using pharmacological and transplantation therapies.

Antibodies that disrupt monocyte-endothelial cell interactions result in decreased tissue loss and increased functional recovery in rodent models of SCI. Specifically, extensive work by Dekaban, Weaver, and colleagues provides comprehensive evidence that the mechanism of action for antibodies targeted to CD11d/CD18 or $\alpha 4\beta 1$ integrins involve reduced microglia and macrophage accumulation within the injured spinal cord [111, 129–140]. Although neutrophils may also be affected by treatment [141], these data implicate monocyte-derived macrophages, and potentially CD11d expressing microglia, as mediators of secondary injury after SCI. Further, these data also demonstrate that upregulation of selectins and cell adhesion molecules on endothelial cells after injury may potentiate destructive neuroinflammation.

CD11d-mediated depletion is effective in both rats and mice after SCI [142] regardless of injury type (i.e., compression vs contusion) and in various models of traumatic brain injury [131, 132, 143]. In contrast, SCI treatment with clodronate liposomes, a drug that induces selective deletion of phagocytic monocyte-derived macrophages [144], reduces indices of secondary injury but with inconsistent functional recovery [15, 40, 145, 146]. Similarly, depletion of circulating monocytes using silica dust or chloroquine and colchicine leads to improved function after SCI but these effects have not been replicated in 25 years and new evidence suggests mechanisms of action independent of macrophage inhibition [147–149].

The inconsistency in functional recovery between depletion-type approaches and anti-integrin antibodies are likely due to the heterogeneity of monocyte subsets activated by SCI. It is possible that CD11d selectively targets entry of pathological macrophages, whereas more general depletion approaches limit both reparative and pathological populations. Indeed, there is emerging evidence that specific monocyte subpopulations reduce inflammation and scar formation after SCI [93]. Consistent with this concept of heterogeneity, selective depletion of monocytes expressing the macrophage receptor with collagenous structure (MARCO), a receptor associated with pro-inflammatory macrophage activation [150], leads to improved functional recovery and increased axon sprouting after SCI [151]. Further, specific monocyte subsets expressing the fractalkine receptor, CX3CR1, mediate axon retraction and potentiate anatomical and functional impairments after SCI [152, 153]. Natalizumab, an antibody against $\alpha 4\beta 1$, is effective in multiple sclerosis and similar therapies have been evaluated after myocardial infarction and stroke in humans [154–156]. To the best of our knowledge, the effectiveness of anti-integrin antibody therapies for human SCI remains untested. Regarding approaches involving infiltrating myeloid cells in SCI, targeted depletion that accounts for potential functional monocyte heterogeneity may be of the most significant clinical impact [157].

The most direct approach for increasing reparative macrophages and microglia after SCI involves transplanting prestimulated cells into the injured spinal cord. Specifically, transplantation of cultured microglia or macrophages prestimulated by anti-inflammatory cytokines, peripheral nerve segments, or cocultured with skin, to induce reparative phenotypes, increases axon growth and functional recovery after rat SCI [125, 126, 158, 159]. The observations that macrophages may facilitate repair in the injured spinal cord formed the scientific rationale for the ProCord clinical trials sponsored by ProNeuron Biotechnologies. The design and experimental evidence, as well as issues with patient recruitment and demographics for ProCord, have been discussed in detail previously [95, 124, 160, 161]. Briefly, autologous macrophages were isolated from SCI individuals and cocultured in autologous skin biopsies. After activation, these purportedly reparative macrophages were then transplanted into the injured spinal cord. The results of a phase 1 trial on 8 patients indicated that the cells were well tolerated and three patients experienced functional improvements after transplantation [161]. However, a larger scale, phase II trial with 43 participants failed to detect a significant effect of macrophage transplantation and reported a trend toward increased functional recovery in the control group [162]. Although ultimately unsuccessful, the ProNeuron trial demonstrated the therapeutic feasibility of transplantation trials in SCI [163].

The effects of current cellular therapies for SCI (reviewed in the accompanying special issue article) may be due in part to transplantation-mediated macrophage polarization toward reparative phenotypes. For example, MSCs, neuronal stems cells, olfactory ensheathing cells, and Schwann cells may release anti-inflammatory cytokines as transplantation of these cells into the injured spinal cord is associated with activation of endogenous M2-like macrophages and microglia [95, 164, 165]. Transplant-associated changes in macrophage and microglia activation states provide indirect evidence that modulating inflammatory cell phenotypes may be therapeutic.

More direct evidence comes from efficacy associated with the application of cytokines, specifically IL-4, that drive M2 macrophage activation *in vitro* [166]. Either systemic or intraspinal administration of IL-4 after SCI increases production of the anti-inflammatory cytokine, IL-10, coincident with increases in markers associated with M2 macrophage activation [167, 168]. IL-4 administration also reduces iNOS, a purported mediator of M1 neurotoxicity, regardless of administration route [167, 168]. In addition, IL-4 treatment facilitates neuroprotection as indicated by increased tissue sparing and functional recovery [167, 168]. Other anti-inflammatory cytokines and growth factors including intraspinal delivery of IL-37, systemic delivery of granulocyte colony-stimulating factor, and cell-mediated delivery of IL-13 (a hallmark cytokine that induces M2 activation) facilitate similar effects [169–171]. Although not all anti-inflammatory cytokine therapies are effective in SCI [172], data from these preclinical rodent studies indicate that driving increased M2 macrophage activation is a promising therapeutic approach for treating SCI.

The counter approach, blocking pro-inflammatory cytokines that induce M1 activation, is also beneficial in SCI. Specifically, application of MR16-1, a monoclonal antibody against the prototypical pro-inflammatory cytokine IL-6, decreases iNOS- and CD16/32-positive M1 macrophages and increases arginase-1- and CD206-positive M2 macrophages in the injured spinal cord [173]. These immunomodulatory shifts are coincident with increased tissue sparing and functional recovery [173]. The therapeutic effects of IL-6 inhibition may not be due entirely to immunomodulatory changes in macrophage/microglia, as IL-6 inhibition also alters astrocyte activation [174, 175]. Nonetheless, blocking other pro-inflammatory mediators such as TNFα and macrophage migration inhibitory factor (MIF) after SCI facilitates wound resolution and improves recovery [176, 177]. Collectively, the results of the converging approaches of increasing M2 activation through the delivery of anti-inflammatory cytokines and blocking M1 activation through the delivery of blocking antibodies or inhibitors support applying immunomodulatory therapies to treat SCI.

As an alternative to direct manipulation of pro- or anti-inflammatory cytokines, researchers are also investigating immunomodulation using clinically tolerated pharmacological approaches. The list of drugs and natural compounds with

immunomodulatory properties in SCI has grown in recent years and is too extensive to discuss here thoroughly. Some pharmaceutical agents with desirable clinical safety profiles and demonstrated immunomodulatory properties in SCI include the antibiotics minocycline and azithromycin [45, 150, 178] and natural compounds such as docosahexaenoic/omega-3 fatty acids and flavonoids [179, 180]. These immunomodulatory agents facilitate functional recovery and reduce indices of secondary injury. For a more comprehensive review of immunomodulatory therapies in spinal cord injury, see the following reviews: [121, 164, 181].

Conclusion

Scarring and inflammatory responses to SCI include a complex diversity of cells and cellular activities that vary based on injury type, timing, and spatial distribution [182, 183]. Glial and inflammatory cells affect the injury progression with profound impacts on overall neuronal function and SCI outcomes. The importance of SCI glial and fibrotic scarring, as well as inflammation, has naturally led researchers and scientists to target these responses for therapeutic intervention. Although researchers have found variable amounts of success, unfortunately, few therapies make it to clinical trials and there are no mainstream therapies for SCI.

In our opinion, the general aspects of SCI scarring and inflammation in humans are recapitulated in rodent models of injury. Due to the importance of these complex and intertwined SCI responses, animal models are pivotal for building a holistic understanding of SCI progression and repair. However, subtle and potentially therapeutically relevant differences in cellular composition and timing exist among species. Therapies that can account for these differences, as well as the intertwined role that glia and hematogenous myeloid cells play in scarring responses to SCI, may have the greatest potential for translational success. Further, researchers may need to look to transgenic and novel regeneration models to develop a regenerative roadmap that successfully traverses the complex SCI scarring and inflammatory landscape. As we continue to broaden our understanding of the diverse components of the SCI microenvironment, it is likely that we will find endogenous keys to unlock SCI regeneration and repair.

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