NEURON–GLIA INTERACTIONS — OPINION

The bright side of the glial scar in CNS repair

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Abstract | Following CNS injury, in an apparently counterintuitive response, scar tissue formation inhibits axonal growth, imposing a major barrier to regeneration. Accordingly, scar-modulating treatments have become a leading therapeutic goal in the field of spinal cord injury. However, increasing evidence suggests a beneficial role for this scar tissue as part of the endogenous local immune regulation and repair process. How can these opposing effects be reconciled? Perhaps it is all a matter of timing.

Every year more than 10,000 people in the United States alone become victims of spinal cord injury. Owing to the low regenerative capacity of the CNS, most such patients are left permanently paralyzed. For decades there was little hope for treatment. However, in the early 1990s neurologists began treating spinal cord injuries with steroids and other anti-inflammatory drugs. These drugs provided some hope, but they have a narrow therapeutic window and only a modest effect in the best of cases[1]. At approximately the same time, other research groups provided new grounds for optimism when they identified growth-inhibitory components in the injured CNS. Myelin-derived proteins such as nogo A (also known as RTN4), myelin-associated glycoprotein (MAG) and oligodendrocyte myelin glycoprotein (OMG) all inhibit axonal growth[2–4]. Subsequently, additional molecules were identified as growth inhibitors. These molecules have been associated with the glial scar that is actively formed following spinal cord injury.

The glial scar consists predominately of reactive astrocytes, microglia/macrophages and extracellular matrix molecules, especially chondroitin sulfate proteoglycans (CSPGs)[5–7]. CSPGs are a complex family of macromolecules that consist of a core protein and one or more covalently attached glycosaminoglycan chains (GAGs). These polysaccharide chains can vary in their number and composition, and undergo extensive modifications that modulate their biological activity. CSPGs are known mainly for their growth-inhibitory effects, and are secreted by almost all cell types at the injury site (especially astrocytes)[7–9]. The growth-inhibitory effect of the scar tissue is considered to be a major obstacle for regeneration, and to partially explain the lack of effective CNS recovery (FIG. 1).

Various therapeutic approaches have attempted to eliminate and reorganize the chemical components of the glial scar or to regulate its negative effects. These include using degrading enzymes to eliminate scar components (especially CSPGs)[10–13], blocking the activity of the growth inhibitors using specific antibodies[14], blocking the receptors that recognize the growth-inhibitory factors, regulating intracellular signals induced by the growth-inhibitory compounds[15–18], inhibiting astrocyte proliferation to attenuate scar formation[19], applying growth-inducing agents and growth factors to form bridges across the injury site[20,21], and others[22] (FIG. 1). Obviously, all of these approaches have been based on the perception that the glial scar is an obstacle to recovery that should be modified, eliminated, suppressed or circumvented.

However, accumulating evidence indicates that scar tissue and its components might have an important role in the immediate response to CNS injury. In this Opinion article, we propose a framework for reconciling the opposing views regarding glial scar function. We propose that the scar has beneficial effects at one phase of the recovery process and destructive effects at another phase. We focus on the emerging beneficial aspects of the scar, as the destructive features have been extensively discussed in numerous reviews[23–25]. The potential clinical implications of this perception of the scar are also discussed.

General features of the glial scar

The healing process in the CNS, as in the periphery (BOX 1), involves complex cellular and biochemical events, which take place in a tightly synchronized and orchestrated cascade. Although these events overlap in time, each phase of the process is distinct and has its own specific purposes and requirements (FIG. 2). These phases can be referred to as acute (hours to days after injury), sub-acute (weeks) and chronic (weeks and years).

Effects on axonal growth. Scar tissue, and especially CSPGs, is known for its inhibitory effect on axonal growth. Numerous studies attributed the negative aspects of the scar to the growth-inhibitory nature of some of its components. For example, CSPGs have been shown to induce neurite retraction and growth cone collapse *in vitro*[26]. Similarly, studies that compared the effects of different types of astrocytes on neurons revealed that reactive astrocytes, which produce NG2 (also known as CSPG4) in the glial scar, inhibit axonal growth[27,28]. Moreover, the developmental role of CSPGs in the CNS is associated with the formation of boundaries, as they prevent growing neurons from spreading to sites at which they are enriched[29]. Other studies indicated that inducing the degradation of CSPGs using specific enzymes, or inhibiting their formation, results in a dramatic increase in axonal growth and regeneration[10,13,30–32]. Many additional studies have provided evidence of the growth-inhibitory nature of the glial scar (for reviews see REFS 23,24).

Other studies, however, have revealed that proteoglycans vary in their activities, and that the structure of proteoglycans and their availability in the tissue (in either bound or soluble form) are crucial for

their function. Thus, for example, growth-promoting features were demonstrated for over-sulfated CSPGs[33–36] and trophic effects were attributed to several CSPGs in their soluble form[37–39]. Nevertheless, the growth-inhibitory effect of the scar and of the matrix-bound proteoglycans is the most widely studied phenomenon associated with this tissue, and it has led to general support for therapeutic approaches targeted at scar modulation and resolution.

Sealing the site of injury and remodelling the tissue. In injured CNS tissue, neurons that were spared in the primary insult are exposed to a microenvironment that contains toxic factors, such as an imbalance in the levels of excitatory amino acids and ions, reactive oxygen species, free radicals and overwhelming inflammation. This results in further neuronal loss, a process known as secondary degeneration. In this degenerative environment, preventing the spread of toxicity requires the lesion site to be sealed and protective responses to be induced. We suggest that examining some of the features of the glial scar may reveal its potential to carry out part of this 'SOS' activity.

After injury, astrocytes form a dense scar tissue that has been suggested to demarcate the lesion area and separate the injured tissue from its surroundings[40,41]. Moreover, astrocytes have an important scavenging activity, which is crucial for regulating excessive levels of glutamate, K^+ and other ions. Thus, experimental ablation of astrocytes after injury results in a dramatic decrease in the expression of glutamate transporters. Astrocyte ablation was suggested to account for some of the neuronal degeneration processes as the damage observed was similar to the direct excitotoxic effects of excess glutamate[42]. It was also shown that astrocytes can directly protect neurons from nitric oxide toxicity through a glutathione-dependent mechanism[43]. Activated astrocytes produce and secrete CSPGs in the lesion area, creating a diffusion barrier for molecules that are potentially harmful to the spared tissue[44] and thereby attenuating the spread of neurotoxicity[45] and preventing excitatory amino acid-induced neuron death[35].

Trophic and metabolic support are also required to prevent secondary degeneration. Similar to their role in healthy tissue, astrocytes provide trophic support at the injury site[46,47]. This can be crucial for the surviving neurons, which are located mainly at the margins of the lesion and colocalized with the scar. Metabolites, including glucose, nutrients and growth factors such as insulin-like growth factors (IGFs), nerve growth factors[48], brain-derived neurotrophic factor (BDNF) and neurotrophin 3 are produced by astrocytes and support the viability of the surviving cells[46,47,49].

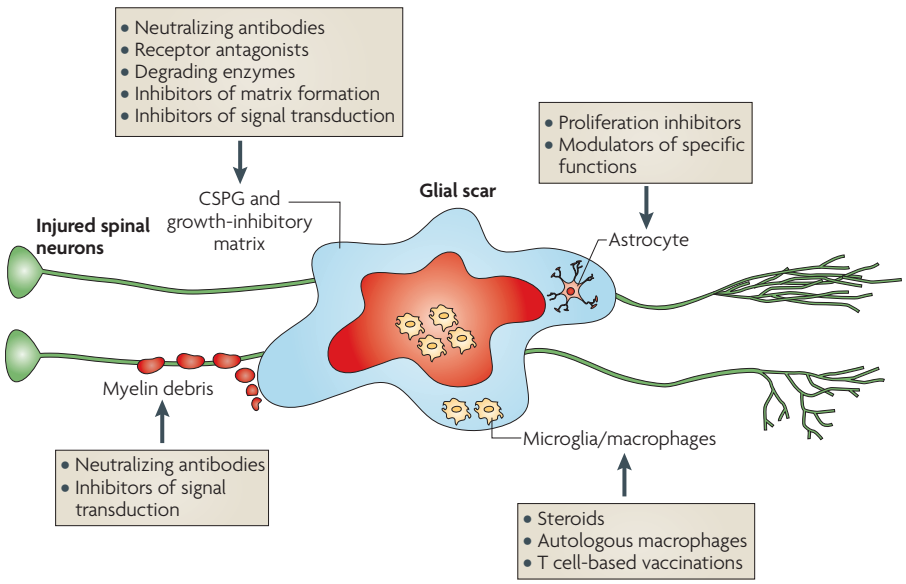

Figure 1 | **Scar components and potential therapeutic interventions.** The major components of the site of injury include myelin debris, the scar-forming astrocytes, activated resident microglia and infiltrating blood-borne immune cells, chondroitin sulfate proteoglycans (CSPGs) and other growth-inhibitory matrix components. All of them are potential targets for therapeutic intervention. Many of the interventions can be optimized by considering the beneficial aspects of the scar tissue and fine-tuning the optimal time window for their application. Each target and the strategies directed at its modulation are shown.

An additional aspect of the glial scar that may be crucial for neuronal survival following injury relates to its activity in filling the gaps in the lesion area, creating a scaffold for the vascularization network. Astrocytes and matrix components stimulate and recruit endothelial cells and fibroblasts in the lesioned area and induce the formation of new capillaries at the site. Ultimately the density of the capillaries that extend into the injured area reaches twice that of uninjured CNS regions[50]. Interestingly, astrocytes also have a direct effect on the intensity of blood flow[51].

In agreement with the potential role of scar tissue in the protective response to injury are the recent experiments performed by several independent groups using transgenic mouse models and specific conditional ablations of reactive astrocytes following injury. These studies revealed that removal of astrocytes from the site of damage leads to larger lesions, local tissue disruption, severe demyelination and neuron and oligodendrocyte death[52,53]. Taken together, these findings indicate that the glial scar might have an important role in the immediate SOS response.

Temporal and spatial control of the local immune response. The effects of the immune system on the CNS are a matter of debate (BOX 2). On the one hand the immune system is an organism's most important protective mechanism; on the other hand, many studies have found immune activity in the context of the CNS to be deleterious. Numerous studies suggest that immune activity can accelerate tissue damage, and that in some cases the immune response itself is the cause of the initial injury. New lines of evidence indicate that the activity of the immune system in the CNS is more complicated than originally thought, and that neural tissue can benefit from the immune response if it is well regulated[54–56]. With the increasing understanding of immune activity in general, and specifically in the CNS, it has become clear that immune cells acquire diverse phenotypes following different types of stimulation. Accordingly, their functional outcome varies. Thus, for example, whereas some macrophages and microglia were reported to interfere with regrowth and to cause neural tissue loss[57,58], other studies reported that these cells can induce growth and support neuronal survival[55,59,60]. Although the primary scope of this Opinion article is not immune activity in the injured CNS, it is important to emphasize that the phenotype acquired by immune cells and

their regulation (regardless of their phenotype) are crucial determinants of the functional outcomes of their activity. Thus, even immune cells that apparently have a beneficial and neuroprotective phenotype must be temporally and spatially restricted. Here, we suggest that the scar tissue can control the functional, temporal and spatial immune activity at sites of axonal injury.

Evidence supporting the notion that the glial scar affects immune activity has emerged from numerous and often conflicting studies. Some results indicated that disruption of the scar or of some of its components reduces inflammation in the lesion area[61] and attenuates monocytic activity. By contrast, other recent studies indicated that the scar is required in order to maintain a balanced inflammatory response. Using a transgenic mouse expressing the thymidine kinase from herpes simplex virus under the control of the glial fibrillary acidic protein promoter, it has been possible to selectively ablate astrocytes by administering ganciclovir[52]. Ablation of the reactive astrocytes from the injury site not only reduced scar formation, it also decreased leukocyte infiltration and the pronounced motor deficits that follow spinal cord injury[52], as well as the neuronal degeneration that follows traumatic brain injury[62]. A recent report demonstrated that selective knockout of signal transducer and activator of transcription 3 (STAT3) in reactive astrocytes limited astrocyte migration and resulted in marked and widespread infiltration of inflammatory cells[63,64], emphasizing the role of astrocytes in immune regulation.

Additionally, astrocytes can contribute to immune regulation through their role in resealing of the blood–brain barrier[52,53], and they have a direct effect on immune cells through the secretion of relevant immune-modulating molecules, such as transforming growth factor-β (TGFβ), tumour necrosis factor-α (TNFα)[65] and proteoglycans.

Proteoglycans, and especially CSPGs, are known for their immune-related activity in the peripheral tissue. Owing to their adhesiveness to chemoattractive agents and growth factors that are needed for recruiting and activating immune cells[66], proteoglycans capture these factors, increasing their focal concentration and thereby targeting the immune response to the damaged area. This is especially important in the context of the injured CNS, which has low tolerance for immune activity: immune cells that are not confined to the damage site can actually cause further damage. By analogy with the elimination of astrocytes, inhibition of CSPG production using xyloside immediately after acute spinal cord injury resulted in an alteration of the immune response, manifested by a significant loss of its compartmentalization and inability of the local immune cells to express IGF1 (REF. 67). These findings are in agreement with other reports indicating that, in the periphery, CSPGs regulate the motility and activation of macrophages[68], dendritic cells[69] and other immune cell types[70].

Together, these studies indicate that different components of the scar affect the immune response: astrocytes are mostly associated with regulating the number of infiltrating cells, whereas CSPGs mainly regulate these cells' spatial localization and activation.

Controlling neurogenesis. Recently it became evident that CNS injuries trigger the proliferation of neural progenitors and stem cells. Increasing evidence indicates that the glial scar and its components are important players in this process. Both astrocytes and CSPGs have been associated with the regulation of neural progenitor cell proliferation and differentiation. Moreover, some data suggest that neural stem cells are actually a specialized type of astrocyte[71,72].

Astrocytes have key roles in controlling multiple steps of adult neurogenesis, from proliferation and fate specification of neural progenitors to migration and integration of the neural progeny into pre-existing neuronal circuits in the adult brain[73]. Neural progenitors are attracted to the CNS by chemoattractive agents such as stromal cell-derived factor 1α (SDF1α) (also known as CXCL12), which is produced by the local astrocytes. Astrocytes are also one of the main sources of molecules such as bone morphogenetic protein (BMP) and WNT, which regulate stem cell proliferation and differentiation[74].

CSPGs also influence progenitor cells under both physiological and pathological conditions. Proteoglycans are enriched in the developing CNS and are involved in key developmental processes: neuronal migration and homing. In the adult CNS, CSPGs are found in constitutively active neurogenic niches[75,76] and contribute to their maintenance[77]. Sulfated proteoglycan structures have been associated with the regulation of stem cell proliferation. Although little is known about the mechanism, it is clear that it is not limited to charge-based interactions[77]. Various proteoglycans, and especially CSPGs, were reported to affect neural stem cell fate[76], survival and maturation[78]. Tenascin C, an extracellular glycoprotein, was reported to affect stem cell migration as well as proliferation[79]. *In vitro* studies revealed that CSPGs promote the proliferation of neural stem cells in a highly specific manner in response to fibroblast growth factor 2 (FGF2) but not epidermal growth factor (EGF)[76], and responsiveness to growth factors was shown to be modulated by tenascin C[78]. Thus, it seems that the nature of the growth factors and their concentration in CSPG-created niches determine the extent of self-renewal and the fate of these proliferating cells. It was recently shown that injection of chondroitinase ABC (ChABC), a CSPG-degrading enzyme, into the telencephalic ventricle caused a reduction in cell

Box 1 | Scar tissue in peripheral wound repair

Wounds in the peripheral tissue (for example, skin tissue) can be healed by a spontaneously occurring cascade of complex biochemical events that take place in a carefully orchestrated manner. These events overlap in time, although they can be categorized into separate phases: the inflammatory, proliferative and remodelling phases. The scar tissue and proteoglycans have been reported to have essential roles at every phase of wound healing. In the inflammatory phase, clotting takes place to maintain homeostasis and various factors are released to attract cells that phagocytose debris, bacteria and damaged tissue and to initiate the proliferative phase of wound healing. Most of the growth factors and cytokines that are involved in the wound healing process are immobilized at the cell surface and in the extracellular matrix through proteoglycan binding. In addition in peripheral tissue, small matrix fragments directly stimulate both angiogenesis and the phagocytic activity of macrophages. Similarly, in the CNS, molecules that degrade chondroitin sulfate proteoglycans act as regulators of microglial activity and stimulate neuroprotection and axonal growth[85]. The proliferative phase, also called the 'reconstruction' phase, is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization and wound contraction. The unique adhesive properties of proteoglycans promote the migration of immune cells within the scar tissue as well as their proliferation and differentiation. Cross links between collagen and proteoglycans strengthen the tissue and provide a scaffold for wound contraction. The last phase, in which maturation and remodelling take place, is also the longest and includes resolution of the scar tissue, removal of the blood vessels and rearrangement of the tissue.

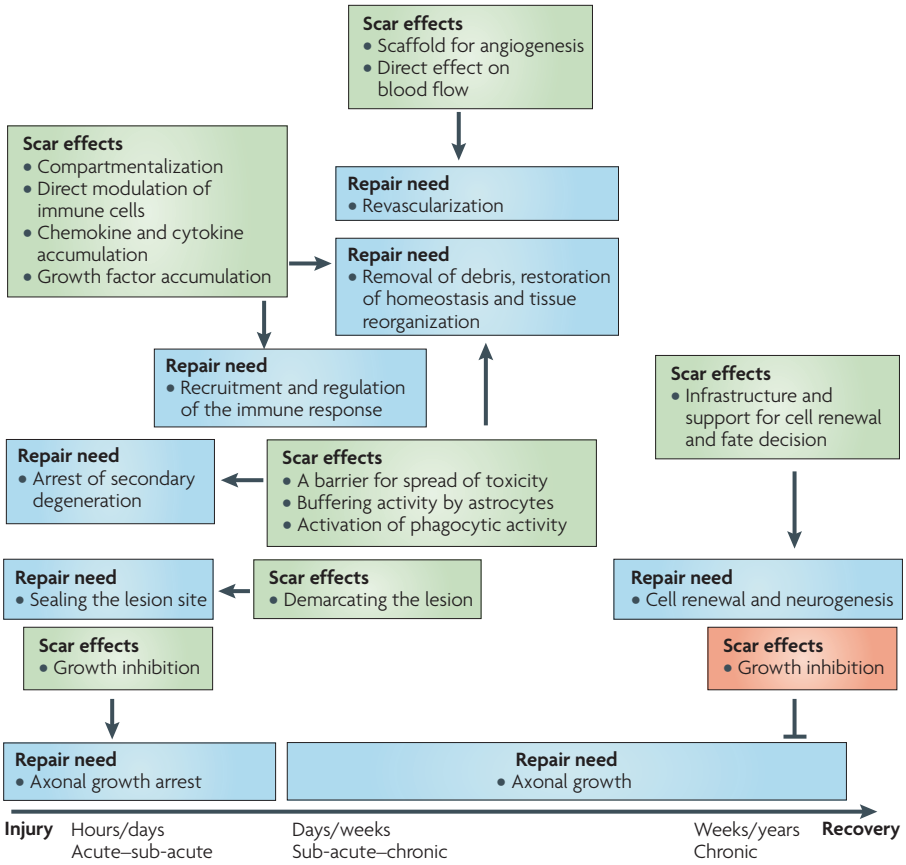

Figure 2 | **The repair process as a function of time and the effects of the scar.** The repair process can be divided into distinct phases (acute, sub-acute and chronic). The main requirements for repair (tissue rescue and neuronal protection) at each phase are plotted on a general timeline in blue boxes. Green boxes and arrows indicate the potential beneficial effects of the scar tissue on each repair requirement. The red box indicates the harmful growth-inhibitory properties of the scar tissue on axon regrowth at later (chronic) phases of the repair process. As discussed in the text, growth inhibition by the scar tissue in the early phases after the injury (acute and sub-acute) might be essential for the preservation of neurons that are capable of regrowth.

proliferation in the ventricular zone and a decrease in self-renewing radial glia[80]. Another study showed that concomitant transplantation of neural stem cells and ChABC in a neonatal/perinatal hypoxia–ischaemia rat model reduced brain damage[81]. Although the mechanisms that mediate the effects of CSPGs on stem cells are not clear, it is evident that CSPGs have a regulatory effect on both endogenous and exogenous stem cells. Some evidence suggests that the effects of CSPGs on neural stem cells might be partly mediated through CD44 or Toll-like receptors (TLRs) expressed on the stem cells. TLRs expressed on neural progenitor cells regulate their proliferation and cell-fate decision in the adult brain and are involved in the active adult neurogenic niche and in the postnatal eye[82,83].

These findings indicate that the glial scar might be an important player in the neurogenic processes that follow injury. Nevertheless, it is important to emphasize that much is unknown in this field, including the functional relevance of the neurogenic process, the extent to which new neurons are formed, the role of the newly formed cells and the mechanisms that regulate post-injury neurogenesis.

Reconciliation: timing and balance

Despite the glial scar having been extensively characterized, there is still not a uniform view of its properties and function in CNS recovery. As discussed above, although the scar participates in sealing the lesion site and modulating immune activity, it is also a major growth inhibitor (FIG. 2).

Many studies have reached opposing conclusions regarding the effects of this tissue and its components on repair. Studies in this field differ in many aspects, including the type of manipulation used (genetic manipulation, inhibitors of synthesis, or neutralization/degrading enzymes[32]), the timing and efficiency of the treatment and the severity of the injury. A study comparing the different scar-removal methods might help to reconcile the confounding findings in this field.

Enzymatic degradation of CSPGs has been a leading therapeutic approach, and the results of this manipulation serve as key evidence supporting the destructive effects of CSPGs in the injured CNS. However, it is possible that processes, other than the elimination of the CSPG from the injury site, that occur as a consequence of scar ablation with ChABC are responsible for some of the beneficial effects of these treatments. It was shown that soluble GAG chains[37,39] and products of the enzymatic degradation of CSPG, especially by ChABC, are potent neuroprotective agents and can induce neuronal growth[37,39,84,85]. This may explain, at least in part, the different and apparently opposing outcomes of CSPG-degrading treatments and pharmacological inhibition of CSPG formation by xyloside: although both enzymatic degradation and inhibition of synthesis lead to lower levels of CSPG, these treatments differ fundamentally in the additional components that remain in the tissue.

In addition, the enzymatic degradation of the scar tissue is rarely complete. It is therefore possible that at least modest levels of CSPGs are spared. Such reduced levels might support survival in the acute recovery phase following injury and not have a negative effect on regrowth in the sub-acute and chronic phases.

In our opinion the timing of scar generation and degradation are crucial in determining its effects. We suggest that the scar tissue is required in the acute phase after injury for sealing and cleaning the injury and restoring homeostasis. All of these processes are made possible by the unique features of the scar tissue. Consistent with this time-dependent view of the scar, application of xyloside on day 0, 2 and 7 after spinal cord injury resulted in destructive, beneficial or no effects, respectively[67]. This is consistent with studies using enzymatic degradation. Degradation can only be induced after CSPG has been formed, and thus by its very nature has a delayed effect. In agreement with this, it was shown that astrocyte ablation at different time points after injury had distinct effects on the outcome. Astrocytes in the acute phase after injury are crucial for recovery, whereas the presence of these cells in the chronic phase is inhibitory[63].

Box 2 | **The inflammatory dilemma in the CNS**

Inflammation (which in Latin means 'to set on fire') has a negative reputation in the context of the CNS, but it evolved as a protective mechanism against harmful stimuli and is essential for subsequent repair in injured tissue. The CNS, however, is an immune-privileged site and as such it was considered for years to be sequestered from the protective potential of the immune system[54,55]. Moreover, most evidence of immune activity in the CNS was related to autoimmune pathologies, further supporting the suggestion that the immune and nervous systems are segregated under normal physiological conditions. Nevertheless, increasing evidence over the past decade has slowly changed this perception. We now recognize that the CNS, like any other tissue, requires both innate and adaptive immune responses for its maintenance, repair and renewal, but these responses must be tightly controlled[89–93]. Microglia and blood-borne monocytes have distinct roles and phenotypes depending on their location (relative to the scar) and phase in the repair process[94,95]. Therefore, the different components of the glial scar might not only prevent the damage that immune cells can potentially cause, but also enable the benefit of immunity to be manifested.

None of these results contradict the fact that scar tissue inhibits growth and regeneration. What we suggest is that the growth inhibition itself might be beneficial, in a time-dependent manner. It is likely that growth inhibition in the early stages after the injury, rather than being an obstacle to recovery, is actually essential for the preservation of neurons that are capable of regrowth. Enabling axonal regrowth in the chaotic and metabolically unbalanced environment that is the injured CNS in its acute phase of recovery might cause more damage than benefit. Conversely, once homeostasis has been restored and balance achieved, recovery necessitates axonal regrowth and reconnection; this regrowth is inhibited by the scar tissue.

Although it was long ago suggested that scar tissue might have a beneficial role, especially in the re-establishment of glial boundaries, the growth-inhibitory nature of the scar has led to the common overall perception that it is a barrier for regeneration. Here, we propose that such a generalized view of the scar tissue ignores its positive and protective effects in the acute phase. In our opinion these features should support any potential therapy.

Implications for CNS injury repair

Recognition of the beneficial aspects of the glial scar has major clinical implications. Most treatments that are currently being developed to eliminate scar formation in an attempt to support CNS recovery are designed for administration immediately after the injury. In our opinion, delaying the application of these treatments might allow the natural reparative properties of the scar to be manifested in the acute phase. We suggest that, at least during the first 24–48 hours following injury, the glial scar is crucial for recovery. Thus, treatments that inhibit scar formation or enhance its breakdown should be introduced at the later stages. Moreover, when considering additional treatment modalities, such as growth induction, they should be carefully timed to meet the changing needs of the tissue. Consistent with this model, delaying treatments that promote cell growth might improve their efficacy: for example, intrathecal application, 7 days after injury, of a competitive nogo 66 receptor antagonist that neutralizes the outgrowth-inhibitory effect of nogo 66 induced the regeneration of corticospinal axons[86]. Another study showed that regeneration from supraspinal pathways and recovery of motor function were dramatically increased when transplants of fetal spinal cord and neurotrophins were delayed until 2–4 weeks after transection, rather than being applied in the acute phase[87]. These studies, although they did not directly address scar resolution, highlight the potential of delayed treatment in CNS injuries, which may be especially important in the case of scar-resolving therapies.

Conclusions and future directions

Repair after CNS injury is an ongoing and dynamic process. Accordingly, the requirements for an effective repair process vary with the recovery stage, and differ greatly between the early acute phase and the later chronic phase of recovery. Thus, the glial scar and its components have a key role in the acute phase after injury in sealing the lesion site, restoring homeostasis, preserving spared tissue and modulating immunity, but this same tissue constitutes an obstacle to recovery in the later periods. Therefore, the detrimental aspects of the glial scar seem to be a result of insufficient resolution or excessive scar formation, rather than a response to its mere presence. Such an unbalanced response may be an outcome of an evolutionary process in which recovery from major CNS traumas was not a strong driving force[88], as surviving individuals would have been unlikely to successfully compete for reproduction. Small traumas and mild injuries induced stronger evolutionary forces; in these cases, the need to restore homeostasis, even at the price of losing a limited number of neurons, overrides the need for regrowth. Thus, it is possible that the available repair mechanisms, including glial scar formation, were not optimized over the course of evolution to address severe injuries. Nevertheless, by appreciating the potential of this scar tissue in repair, it might be possible to derive maximal benefit from its existence and develop therapies aimed at resolving scar tissue or inducing regeneration by fine-tuning the optimal time window for their application.

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DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene

BDNF | BMP | EGF | FGF2 | IGF1 | MAG | neurotrophin 3 | NG2 | nogo A | OMG | SDF1α | STAT3 | Tenascin C | TGFβ | TNFα |

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