

## Review Article

# Spinal cord injury: From inflammation to glial scar

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## Abstract

**Background:** Glial scar (GS) is the most important inhibitor factor to neuroregeneration after spinal cord injury (SCI) and behaves as a tertiary lesion. The present review of the literature searched for representative studies concerning GS and therapeutic strategies to neuroregeneration.

**Methods:** The author used the PubMed database and Google scholar to search articles published in the last 20 years. Key words used were SCI, spinal cord (SC) inflammation, GS, and SCI treatment.

**Results:** Both inflammation and GS are considered important events after SCI. Despite the fact that firstly they seem to cause benefit, in the end they cause more harm than good to neuroregeneration. Each stage has its own aspects under the influence of the immune system causing inflammation, from the primary to secondary lesion and from those to GS (tertiary lesion).

**Conclusion:** Future studies should stress the key points where and when GS presents itself as an inhibitory factor to neuroregeneration. Considering GS as an important event after SCI, the author defends GS as being a tertiary lesion. Current strategies are presented with emphasis on stem cells and drug therapy. A better understanding will permit the development of a therapeutic basis in the treatment of the SCI patients considering each stage of the lesion, with emphasis on GS and neuroregeneration.

**Key Words:** Glial scar, spinal cord inflammation, spinal cord injury, spinal cord injury treatment

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## INTRODUCTION

Spinal cord injury (SCI) is an important cause of neurologic disability after trauma and although prevention programs have been initiated, there is no evidence that the incidence is declining.<sup>[44]</sup> Furthermore, no satisfactory treatment is currently available.<sup>[17,43]</sup>

Spinal cord (SC) inflammation seems to be the most important landmark during the secondary lesion after

SCI. For otherwise glial scar (GS) in chronic stage is the cause of limitation on regeneration.

Currently, there is a multiplicity of interventions to promote recovery from an SCI: treatments immediately following the trauma (treating inflammation and limiting initial degeneration) and long-term procedures (stimulating axonal growth, promoting new growth through substrate or guidance molecules, blocking molecules that inhibit regeneration, supplying new cells

to replace lost ones, and building bridges to span the lesion cavity).<sup>[5,6,18]</sup>

The resolution of inflammation is a highly controlled and coordinated process that involves the suppression of proinflammatory gene expression, and of leukocyte migration and activation, followed by inflammatory-cell clearance by apoptosis and phagocytosis.<sup>[18]</sup>

Following inflammation stage into the SC begins GS formation that will cause limitation in the neural regeneration.<sup>[20]</sup>

In the present review, the author stresses the importance of GS as a tertiary lesion and comment on aspects of its morphology and why it inhibits neuroregeneration. In addition, several topics are showed for a better understanding why it goes on, as well as possibilities of management, as follows: mechanism of injury, role of inflammatory mediators, axonal degeneration and demyelination, lack of recovery and regeneration, morphology of GS, and current management of SCI (with emphasis on stem cells).

A better understanding of this subject and its scientific application may be important in the development of future therapy and in the recovery of the victims of SCI and their associated complications.

## MECHANISM OF INJURY

Currently, the pathophysiology of SCI is established in 2 stages: primary and secondary lesions.

Laceration, contusion, compression, and concussion represent primary lesion, due to the physical and mechanical trauma to the SC mainly causing structural disturbance.<sup>[44]</sup> In the majority of cases of human SCI, the mechanism of the primary lesion is acute compression or laceration of the SC due to displacement of bone or disc into the canal during fracture dislocation or burst fracture of the spine. Residual neural connections usually persist after this stage indicating potential for recovery, despite that functional loss may be complete.<sup>[44]</sup>

In the following stage, secondary lesion mainly causes functional disturbance, in the presence of ischemia and microvascular damage,<sup>[5,6,44]</sup> glutamatergic excitotoxicity, oxidative stress, and inflammation.<sup>[24]</sup> It is not so clear when this stage begins and finishes.

Most of the cell death in consequence of SCI is due to secondary lesion and begins centrally and affects the cell body first.<sup>[44]</sup> Another important reason is swelling and hemorrhage into the cord leads to vascular disturbance, with emphasis on vascular mechanisms, causing depression in oxygen and nutrients support.<sup>[44]</sup>

Cells from the immune system, such as neutrophils and monocytes/macrophages, migrate to the injury site and

produce small molecules called cytokines or interleukins that trigger cells of the immune system to respond to the injury.<sup>[9]</sup> The death of oligodendrocytes, glial cells that produce myelin, causes axons to lose their myelination, which greatly impairs the conduction of action potential, messages or renders the remaining connections useless.<sup>[24]</sup> Astrocytes are the primary support cells of the brain and SC that make and secrete proteins called neurotrophic factors. They also break down and remove proteins or chemicals that might be harmful to neurons (glutamate, a neurotransmitter that in excess causes cells to become overexcited and die due to excitotoxicity). Both glutamate and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) are released immediately after SCI causing cell death.<sup>[24]</sup> Within several weeks of the initial injury, the tissue damage has been cleared away by microglia, and a fluid-filled cavity surrounded by a GS is left behind. Molecules that inhibit regrowth of severed axons are now expressed at this site.<sup>[20]</sup> The cavitation established at this moment acts as a barrier to the reconnection of the two sides of the damaged SC. Although SCI causes complex damage, an amount of the basic circuitry to control movement and process information can remain intact, because the SC is arranged in layers of circuitry.<sup>[20]</sup>

The fact is that the inflammatory process may act as an inhibitory element within the SC after the injury, especially in the beginning of the process. Therefore, it is important to understand how the inflammatory mediators regulate the development of the secondary lesion mentioned above.

## ROLE OF INFLAMMATORY MEDIATORS

The immune system is in dynamic equilibrium with the inflammatory responses (mediated by T helper type 1 cells, interleukin (IL)-1 $\beta$ , interferon- $\gamma$ , and TNF- $\alpha$ ) being balanced by anti-inflammatory responses (mediated by T regulatory type 1 cell, T helper type 3 cells, IL-4, IL-10, and transforming growth factor- $\beta$ ).<sup>[9]</sup>

Cytokines are essential effector molecules of innate immunity that initiate and coordinate the cellular and humoral responses. Cytokine macrophage migration inhibitory factor (MIF) has been discovered to carry out important functions as a mediator of the innate immune system.<sup>[42]</sup> MIF is expressed by a broad spectrum of cells and tissues, including monocytes and macrophages and it is rapidly released after exposure to proinflammatory mediators and induces proinflammatory biological responses that act as a regulator of immune responses. In fact, during the acute phase of SCI, expression of mRNA coding proinflammatory cytokines IL-1 $\alpha/\beta$ , IL-6, and TNF- $\alpha$ , that is released and increase 6–12 h after the injury with peak at 4 days causing apoptosis in neurons and oligodendrocytes.<sup>[42]</sup>

TNF- $\alpha$  might act cooperatively with glutamate by inducing cFOS to cause cell death in the SCI.<sup>[24]</sup> The neuroprotective effects of the anti-inflammatory IL-10 following SCI have been demonstrated that acute administration of IL-10 reduces TNF- $\alpha$  synthesis in the SC and promotes functional recovery.<sup>[7]</sup>

Members of IL-6 superfamily are represented by leukemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF). They are known to cause the differentiation of neural stem/progenitor cells into astroglia by activating the gp130/Janus kinase (JAK)/signal transducers and activator of transcription (STAT) pathway during acute phase of SCI.<sup>[42]</sup> This will cause astrocytes to be reactive and detected by differentiation of nestin-positive stem cells.<sup>[18]</sup> At this moment, there is a change of inflammatory phase to scar formation. Thus, a deposition of chondroitin sulfate will be detected by the expression of chondroitin sulfate proteoglycans (CSPGs), glial fibrillary acidic protein (GFAP), and vimentin by astroglia. This event will result in scar formation (detected by expression of CSPGs by microglia, macrophages, and oligodendrocyte precursors) and inhibition of axonal regeneration (binding of nogo A, myelin-associated glycoprotein (MAG), and oligodendrocyte myelin glycoprotein (OMgp) to nogo receptor).<sup>[18,42]</sup>

On the other hand, IL-1 which is a proinflammatory cytokine mediates a diverse range of effects through activation of T cells, induction of expression of acute-phase proteins and direct effects on the nervous tissue.<sup>[1]</sup> It was demonstrated that IL-1 is a key inflammatory mediator that is increasingly expressed after SCI.<sup>[45]</sup>

In consequence of the inflammatory process causing harm to SC, the axons lose myelin and become degenerated, which is an important fact in the way of the chronic lesion.

## AXONAL DEGENERATION AND DEMYELINATION

In the peripheral nervous system (PNS), spontaneous regeneration is possible in some cases, but axons in the central nervous system (CNS) are lacking in this possibility. This lack of axonal regeneration is explained by the presence of axonal growth inhibitors, myelin-associated proteins and GS in the CNS.<sup>[5,6,18]</sup>

Apoptotic molecules, such as p21, Bcl-2, and Bax, that are in the cascade of the p53 pathway were evaluated in a model of SCI. Double-staining with glial cell markers revealed that p53 immunoreactivity was localized in microglia, oligodendrocytes, and astrocytes, but not in neurons. Thereby, double-staining of p53 and Bcl-2, Bax, or biotin nick end labeling (TUNEL) are expressed all within the first 3 days of injury.<sup>[41]</sup>

Axonal degeneration after SCI is due to glial, in particular oligodendroglial, apoptosis. Activation of the FAS and p75 death receptor pathway may be involved in initiating this process.<sup>[14]</sup>

Remaining axons connect the SC above and below the lesion and might provide some functionality; however, demyelination of these axons compromises their function.<sup>[14,18]</sup>

Neutralizing reactive astrocytes, preventing their migration and scar formation, after SCI, led to cessation of repair along the blood–nervous system barrier. The resulting effect is a massive infiltration of inflammatory cells causing loss of neurons and oligodendrocytes.<sup>[42]</sup>

Unless demyelination and axonal degeneration stop, the following step will begin causing lack of regeneration.

## LACK OF RECOVERY AND REGENERATION

According to the literature, neurogenesis does not occur in normal situation or after trauma, even though the existence of endogenous neural stem cells in the adult SC is possible.

Although following SCI, endogenous tissue repair has been observed as a possibility of spontaneous recovery, in either animal experiments,<sup>[5,6]</sup> especially due to ependyma-derived cells,<sup>[49]</sup> or clinical study,<sup>[11]</sup> several factors take part in inhibiting the regeneration of axons.<sup>[18]</sup> These include GS formation and the evidence of inhibitory proteins, such as CSPG, nogo A isoform, Rho and Rho Kinase, extracellular matrix (ECM), MAG, and OMgp.<sup>[18]</sup>

Transplantation of neural stem/progenitor cells (NSPC) could contribute to the repair of injured SC in adult rats and monkeys, but in some cases, however, most of the transplanted cells had adhered to the cavity wall and had failed to integrate to the host SC. It was because of CSPGs, a known constituent of GS that is strongly expressed after SCI, as an inhibitor of NSPC integration *in vivo*.<sup>[27]</sup> Digestion of CSPGs by chondroitinase ABC (C-ABC) promoted the migration of transplanted cells after SCI. *In vitro*, the migration of NSPC-derived cells was inhibited by CSPGs and this inhibitory effect was attenuated by C-ABC pretreatment.<sup>[27]</sup> *In vivo*, C-ABC treatment combined with NSPC transplantation into injured SC revealed that C-ABC pretreatment promoted the migration of the transplanted cells, whereas CSPGs-immunopositive scar tissue around the lesion cavity prevented their migration into the host SC in the absence of C-ABC pretreatment. Furthermore, this combined treatment significantly induced the outgrowth of a greater number of growth-associated protein-43-positive fibers at the lesion epicenter, compared with NSPC transplantation alone.<sup>[27]</sup>

Nogo is an important inhibitor to axon regrowth.<sup>[18]</sup> Nogo, especially nogo A isoform, is an integral membrane protein related to myelin localized in CNS, but not in PNS.<sup>[18]</sup> *In vitro* characterization of nogo has demonstrated its function as a potent inhibitor of axon elongation.<sup>[21]</sup> *In vivo* neutralization of nogo activity results in enhanced axonal regeneration and functional recovery following CNS injury as well as increased plasticity in uninjured CNS fibers.<sup>[21]</sup> Rho and Rho kinase are myelin inhibitors that also have important influence in the lack of recovery.<sup>[18]</sup>

The scarring process associated with the ECM molecules contributes to the failure of axon regeneration, especially at the border of the GS, in contact with the fibronectin-positive tissue matrix, being the real barrier to prevent axonal regeneration.<sup>[13]</sup>

The levels of CSPGs might reduce when the scar tissue becomes older or quiescent and that axon repulsion might be caused by the mechanical control of the GS tissue.<sup>[13]</sup>

The intervention treatments may promote anatomical recovery, although this recovery may be different in histology from the normal nervous tissue in the SC.

In fact, in the chronic phase GS arises as an inhibitory factor to neuroregeneration. For a better understanding how it goes on, aspects of its morphology must be stressed.

## MORPHOLOGY OF GLIAL SCAR

The importance of an acute astrocytic response to control the inflammation during SCI is relevant and the formation of GS is necessary, despite the reduction in axonal sprouting.<sup>[20]</sup> Inflammation in acute stage is related to a vascular damage causing a secondary lesion.<sup>[44]</sup> GS formation, in turn, inhibits axonal regeneration at 4 weeks after SCI.<sup>[26]</sup> Therefore, GS presents itself as a tertiary lesion.

Considering GS formation and its morphology, little is known about the behavior of a single reactive astrocyte, but it has been demonstrated that after SCI the ependyma cells from central canal may proliferate and differentiate into astrocytes, oligodendrocytes, and other cells involved in the tissue repair.<sup>[3,16,29,33,38]</sup>

Scarring remains as a barrier to overcome because of its increasingly recognized physical heterogeneity and its mix of beneficial and harmful effects on neural regeneration.<sup>[18]</sup>

Two components of the scar must be considered: fibrous scar and GS.

Fibrous scar results from the resolution of the inflammatory process after the trauma and its structure is constituted by connective tissue that may cause a physical barrier over all tissues involved.<sup>[18,20]</sup> Fibrous scar contains

extracellular matrix (ECM) proteins that are potent inhibitors of axonal regrowth and numerous strategies have been developed to overcome this inhibition, such as neutralizing antibodies, agents that prevent their synthesis or those that digest them.<sup>[18]</sup>

For otherwise GS results when reactive, hypertrophied astrocytes form a physical barrier at the periphery of the lesion, walling off lesioned tissue from healthy tissue.<sup>[20]</sup> GS is constituted by astroglial cells and is a more challenging therapeutic target than a fibrous scar because GS promotes and inhibits axonal regrowth at the same time,<sup>[18]</sup> represents a physical and molecular barrier to axonal regeneration, and has become an important target for regeneration research in chronic SCI.<sup>[18,20,48]</sup> Thereby, GS could be considered as causing a tertiary lesion.

Hu *et al.*, 2010, published a very important study where morphologic results showed that the formation of GS was defined at 4 weeks following SCI, when the cavity and GS were formed. According to them and under microscopic observation, the lesioned area became disorganized on the first day, with parenchymal hemorrhage, necrosis, and edema. At 1 week, the region of swelling and degeneration had enlarged and there was no clear border between normal and injured tissue. At 2 weeks there were hemorrhage absorption, liquefaction, and initial cavity formation. At 4 weeks, a large cyst with an amorphous material, trabeculae inside the cavity and liquid formed surrounded by a GS. At 8 and 16 weeks, the lesion was similar to that one at 4 weeks. Using immunohistochemical and axonal tract tracing techniques, they observed that NF-200-labeled neurons and axons presented with interruption in the injured cord, although there were a few number of axons. At first week, the axons at the lesion site became fewer, with fewer nerve fibers in the spared tissues around the injury epicenter. For otherwise, larger numbers of oriented axons in the region rostral to the lesion appeared to be growing. At 2 weeks, more axons seemed to regenerate and to regrow into the vicinity of the lesion. The potential of axon regeneration remained even at 4 and 8 weeks after SCI. By tracing the axonal tract, some regenerative axons could grow according to this method. However, most of the axons could not penetrate the GS that restricted the axon extension. At 4 weeks after injury, the morphology of reactive astrocytes became typical, with a large somatic body, thick processes, and intensive GFAP labeling. Consistent with these changes, the GS and cavity appeared at 4 weeks. Double labeling with NF-200 and GFAP showed that activated astrocytes were cross-linked to form a barrier that obstructed the extension of regenerative axons. Although a few axons could be seen to regrow into the outer layer of the GS, the NF-200-positive axons showed little possibility to penetrate the GS, especially the inner layer.<sup>[26]</sup> Therefore, GS is the most important barrier to neuroregeneration.

Despite the fact that GS is a mechanical barrier, inhibitory



molecules in the forming scar and methods to overcome them have suggested molecular modification strategies to allow neuronal growth and functional regeneration.<sup>[20]</sup>

It was investigated whether glial responses following an SC lesion was restricted to a scar formation close to the wound or they could be also related to widespread paracrine trophic events in the entire cord.<sup>[15]</sup> An SC hemitranssection was performed in adult rats at the thoracic level. Seven days and 3 months later the SCs were removed and submitted to immunohistochemistry of GFAP and OX42, markers for astrocytes and microglia, as well as of basic fibroblast growth factor (bFGF), an astroglial neurotrophic factor. Computer-assisted image analysis was employed in the quantification of the immunoreactivity changes. The results indicated that glial reaction close to an injury site of the SC was related to wounding and repair events. Although gliosis had constituted a barrier to axonal regeneration, glial activation far from the lesion could contribute to neuronal trophism and plasticity in the lesioned SC favoring neuronal maintenance and fiber outgrowth.<sup>[15]</sup>

The most important class of axon growth-repulsive molecules associated with CNS scar tissue formation is the family of CSPGs and the pharmacologic digestion of CSPGs in such lesion model results in enhanced axonal regeneration and a significant functional recovery.<sup>[11]</sup>

In consequence of SCI, undifferentiated nestin-positive cells arise from the central canal. These cells proliferate and migrate to the site of the lesion where they differentiate into astroglia. The problem is that these cells, due to some factors, will result in scar tissue.<sup>[42]</sup> LIF and CNTF stimulate the differentiation of neural progenitor stem cells into astroglia by activating gp130, JAK, signal transducers, and the STAT pathway.<sup>[25]</sup> These reactive astroglia also express CSPGs, which inhibits axonal growth and also regeneration. STAT3 signaling is a critical regulator of certain aspects of reactive astrogliosis and provides additional evidence that scar-forming astrocytes restrict the spread of inflammatory cells after SCI.<sup>[25]</sup> Besides the reactive astrocytes, the oligodendrocyte precursor cells, microglia, and macrophages also contribute to the formation of a GS.<sup>[18]</sup>

Therefore, in accordance with the above-mentioned information, the most important aspect to be considered after inflammation stage is how to control GS. Based on experimental and human clinical trials new strategies have been targeted to neuroregeneration after SCI considering an environment capable of receiving an appropriate therapy.

## CURRENT MANAGEMENT OF SPINAL CORD INJURY FOCUSING ON NEUROREGENERATION

Developing translational strategies, such as molecular

agents, viral-mediated gene transfer, and cellular transplants are being evaluated in several studies.

Currently, experiences in the literature are presented focusing on grafts, engineering, and replacement therapy. Therapeutic strategies include neural stem cells, embryonic stem cell, embryonic raphe nuclei cells, fetal SC from embryonic day 14 (E14/FSC) that consists of neuronal (NRP) and glial (GRP) restricted precursors, bFGF-2, GRP cells, oligodendrocyte-type 2-astrocyte (O-2A) progenitor cells, Schwann cell-seeded channels, Schwann cells derived from bone marrow stromal cells (BMSC-SC), peripheral nerve graft, and engineered tissue for grafting (autologous tissue derived from preligated peripheral nerves).<sup>[18,28,32,34,35,36,38,42,43,48]</sup>

Some therapeutic strategies mentioned above are presented in the following sections in a systematic fashion, considering especially stem cells, autologous tissue, photochemical to scar, and drug therapy.

### Therapeutic strategies using stem cells

#### Human embryonic stem cells

Human embryonic stem cells (HESC) are considered to be an abundant source for pluripotent human stem cells, but there are some problems related to the use of these cells in research and medicine, due to ethical, moral, religious limitations, and risk of mutations. These cells can be propagated in culture in an undifferentiated state but can be induced to differentiate into specialized cell types.<sup>[47]</sup> Recent studies have provided methods related to the transcriptional control of embryonic stem cells, including the regulatory circuitry underlying pluripotency.<sup>[47]</sup>

The transcription factors OCT4, SOX2, and NANOG have important roles in early development and are required for the propagation of undifferentiated embryonic stem cells in culture.<sup>[10]</sup>

#### Neural stem progenitor cells

Neural stem progenitor cells (NSPC) may be an important potential graft material for cell therapeutics after SCI. The use of NSPC-enriched population derived from human fetal SC (embryonic week 8 to 9) and expanded *in vitro* by neurosphere formation seems to be a feasible alternative.<sup>[34]</sup> According to this experience, it was seen that NSPC labeled with BrdU or culture medium (CON) were transplanted into the adult primate marmoset SC, after contusion injury at C5 level. Grafted NSPC survived and migrated up to 7 mm far from the lesion epicenter. Besides, double-staining with TuJ1 for neuron, GFAP for astrocyte, or CNPase for oligodendrocyte and BrdU revealed that grafted NSPC differentiated into neurons and oligodendrocytes 8 weeks after transplantation, and more neurofilaments were observed in BrdU than those of CON. Furthermore, behavioral assessment of forelimb muscle strength using a bar grip test and amount of

spontaneous motor activity using infrared rays monitoring revealed that the grafted NSPC significantly increased both of them compared with those of CON.<sup>[34]</sup>

#### *Umbilical cord blood stem cells*

Human umbilical cord blood stem cells (UCBSC) have been shown to differentiate into neural cells *in vivo* and *in vitro*. It is supported that this source downregulates apoptotic genes, Fas and tissue plasminogen activator (tPA) and blocks activation of caspases 3 and 8. In cases of SCI, UCBSC could control apoptosis, demyelination, and scar formation.<sup>[42]</sup>

Umbilical cord blood is a potential vast source of primitive hematopoietic stem and progenitor cells available for clinical application.<sup>[12]</sup> Culture of pluripotent cord blood cells added to retinoic acid (RA) and brain-derived neurotrophic factor (BDNF) result in differentiation along neural lineage. UCBSC may be differentiated into neural cells on animal serum media, including Dulbecco's modified Eagle's medium (DMEM) augmented with 10% fetal bovine serum (FBS), FBS added to fetal calf serum and endothelial growth factor (EGF), 10% FBS, granulocyte-macrophage colony-stimulating factor (GM-CSF) or L-glutamine. In the same way, media containing DMEM, BDNF, EGF, nerve growth factor (NGF), RA, dBcAMP (dibutyryl cyclic AMP), collagen and fibronectin have been applied in some experiments in course.<sup>[42]</sup>

There are several trials using UCBSC currently in progress.

#### *Placental derived stem cells*

Placental tissue presents great interest as a source of cells for regenerative medicine because of the phenotypic plasticity of many of the cell types isolated from this tissue. Besides, it is readily available without invasive procedures and its use does not elicit ethical problem.<sup>[37]</sup> According to Parolini *et al.*, 2008, and data presented during the First International Workshop on Placenta Derived Stem Cells, the following points became evident: (1) Cells isolated from placental tissue should be verified to be of fetal origin; (2) four regions of fetal placenta could be distinguished: amniotic epithelial, amniotic mesenchymal, chorionic mesenchymal, and chorionic trophoblastic. From these regions, the following cell populations were isolated: human amniotic epithelial cells (hAEC), human amniotic mesenchymal stromal cells (hAMSC), human chorionic mesenchymal stromal cells (hCMSC), and human chorionic trophoblastic cells (hCTC); (3) cells from each layer demonstrated variable plasticity, which characterized them as stem cells; (4) according to criteria proposed for bone marrow-derived mesenchymal stromal cells, mesenchymal cells isolated from fetal membrane should be termed mesenchymal stromal cells. Minimal criteria for defining hAMSC and hCMSC were established as follows: adherence to plastic, formation of fibroblast colony-forming units, a specific

pattern of surface antigen expression, differentiation potential toward one or more lineages, and fetal origin.<sup>[37]</sup>

Experimental trials should be performed to evaluate the immunomodulatory and angiogenetic effects of placental stem cells on functional improvement in SCI.

#### *Bone marrow stromal cells*

Adult bone marrow is a source of stem cells with power to differentiate in osteocytes, chondrocytes, myocytes, hepatocytes, epithelial linings, glia, neurons, and Schwann cells.

In an average bone marrow harvest, only 0.125% of the cells are in fact bone marrow stromal cells (BMSC), and an age-dependent inverse correlation with number of cells isolated in the first passage has also been demonstrated. However, it has also been noted that sufficient BMSC can be successfully cultured for an auto transplant from SCI patients.<sup>[28,38]</sup>

BMSC delivery routes include intravenous, intra-arterial, intrathecal, and lumbar puncture with evidences that these cells migrate mainly to the injury site.  $\beta$ -mercaptoethanol and NGF induce BMSC to express neural markers and differentiate along neural lines, as well as to express an array of growth factors and cytokines to support sprouting axons.<sup>[28]</sup>

It was evaluated whether transplantation of Schwann cells derived from BMSC would promote axonal regeneration and functional recovery in completely transected SC in adult rats. BMSC were induced to differentiate into Schwann cells *in vitro*. A 4 mm segment of rat SC was removed completely at the T7 level. An ultrafiltration membrane tube, filled with a mixture of matrigel (MG) and BMSC or MG alone, was grafted into the gap. In the MG and BMSC group, the number of neurofilament and tyrosine hydroxylase-immunoreactive nerve fibers was significantly higher compared with the MG alone group, although 5-hydroxytryptamine or calcitonin gene-related peptide-immunoreactive fibers were rarely detectable in both groups. In the MG and BMSC group, significant recovery of the hind limb function was recognized, which was abolished by transection of the graft 6 weeks after transplantation. These results demonstrated that transplantation of BMSC promoted axonal regeneration of injured SC, resulting in recovery of hind limb function in rats. Currently, BMSC are the main source in stem cell-based therapy in many neurologic diseases, including SCI, because the immune rejection is small and there is the possibility of using autografts.<sup>[28]</sup>

The effect of SC-derived NSPC after delayed transplantation into the injured adult rat SC with or without earlier transplantation of BMSC was evaluated. Either BMSC or culture medium was transplanted immediately after clip compression injury. NSPC or culture medium was transplanted 9 days after injury.

Transplantation of the BMSC resulted in a trend toward improved survival of the NSPC, but there was no increase in function. Transplantation of adult rat NSPC produced significant early functional improvement after SCI, suggesting an early neuroprotective action associated with oligodendrocytes survival and axonal ensheathment by transplanted NSPC.<sup>[38]</sup>

### Therapeutic strategy using autologous tissue

#### Olfactory ensheathing cells

Olfactory bulb-derived (central) ensheathing cell (OB) transplants have shown significant promise in rat models of SCI, as well as the use of lamina propria-derived (peripheral) olfactory ensheathing cells (LP) in both experimental and clinical trials.<sup>[39]</sup> OB and LP exhibit morphologic and antigenic similarities *in vitro*, and, after transplantation, OB and LP attenuate lesion and cavity formation and promote angiogenesis, endogenous Schwann cell infiltration, and axonal sprouting. However, an increased mitotic rate and migratory ability of LP *in vitro* was observed *in vivo* due to their migration within the SC, reduced cavity formation and lesion size, and stimulated outgrowth of axonal subpopulation when compared with OB.<sup>[39]</sup> For otherwise, there is an important aspect concerning remyelination, that is, olfactory ensheathing cells (OEC) do not myelinate axons in their native environment.<sup>[40]</sup> Therefore, it is not clear how the myelination happens.

According to the literature, it would be preferable to obtain reparative cells from an olfactory mucosal biopsy via intranasal endoscopy rather than requiring the more invasive intracranial approach to remove an olfactory bulb.<sup>[46]</sup> But, when compared with previous findings with bulbar cells, the mucosal cell cultures contained only 5% of OEC and a conversely much larger proportion of fibroblasts. These cell preparations showed minimal migratory ability and failed to form complete bridges across the lesion.<sup>[46]</sup>

There are a great number of primitive stem cells in the OB with power for regeneration as mentioned above. Therefore, OEC can be considered as a nonembryonic source to promote neuroregeneration in cases of SCI. Another advantage is that they can be used as autografts.

Actually several trials with OEC in SCI must be carried out to consider this possibility as effective.

#### Photochemical scar ablation with rose Bengal

Because the GS in SCI is irregular in shape, it is not feasible to ablate it by surgical removal or laser surgery. However, photochemical method with rose Bengal, a molecule commonly used for biological staining, injected into the cavity at the injury site, in rats, has been shown to be capable of ablating an existing GS without significant harm to other cord regions and the locomotion in a chronic contusion model. The scar ablation might

provide a permissive environment for the regenerating axons when enriched by cellular or drug therapy.<sup>[48]</sup>

### Drug alternatives and therapeutic benefits for spinal cord injury and scar

Numerous alternatives have been presented in the literature addressing the fibrous aspect of the scar, for example, neutralizing antibodies for ECM inhibitors, xyloside and iron chelation, and chondroitinase avidin-biotin peroxidase complex.<sup>[18]</sup>

Triptolide, component of the traditional Chinese herb, attenuated inflammation, inhibited astrogliosis and promoted SC repair in a model SCI in rats. Triptolide was shown to protect astrocytes by blocking the JAK2/STAT3 pathway *in vitro* and *in vivo*.<sup>[43]</sup>

A *Clostridium botulinum* protein, C3 transferase, which inhibits Rho, was modified to create a therapeutic known as cethrin and that allow central neurons to overcome inhibitory elements to axon regrowth. Neurotrophins or cethrin may enable axons to overcome the inhibitory signals in the fibrous scar.<sup>[18]</sup>

Therapeutic strategies by using TrkA-IgG reduced initial apoptotic cellular response to the injury and aberrant afferent plasticity that occurred weeks after injury and, subsequently, the development of autonomic disorders.<sup>[32]</sup>

It has been demonstrated that the selective, time-limited action of a monoclonal antibody (mAb) to the CD11d subunit of the CD11d/CD18 integrin, delivered intravenously during the first 48 h after SCI in rats, markedly decreased the infiltration of neutrophils and delayed the entry of hematogenous monocyte-macrophages into the injured cord.<sup>[22]</sup> This treatment restored normal serotonergic projections to the dorsal, intermediate, and ventral horns of injured SC and reduced mechanical allodynia and enhanced locomotor recovery.<sup>[35]</sup>

Intravenous immunoglobulin has been found useful in the treatment of various clinical entities and its effect has been associated with inhibition of complement-mediated tissue damage and the ability to scavenge deleterious products.<sup>[2]</sup>

Glatiramer acetate, as an immune modulator, has been shown to reduce the delayed cell death, according to its protective effects on secondary degeneration in rats, after crush injury to the optic nerve.<sup>[8]</sup>

Data have revealed that statins may reduce vascular inflammatory responses,<sup>[23]</sup> promote angiogenesis,<sup>[30]</sup> modulate cytokine production,<sup>[36]</sup> and decrease oxidative stress.<sup>[4]</sup>

It has been demonstrated that minocycline prevents caspase upregulation, reduces apoptosis in mouse models of Huntington's disease and familial amyotrophic lateral

sclerosis.<sup>[19]</sup> Because apoptosis also occurs after SCI, its prevention may be useful in improving recovery. So, it was analyzed minocycline neuroprotective effects over 28 days following contusion SCI and it was found significant functional recovery compared to tetracycline. This study showed histology, immunocytochemistry, and image findings that indicated statistically significant tissue sparing, reduced apoptosis, and microgliosis, and less activated caspase-3 and substrate cleavage, decreased TNF- $\alpha$ , as well as caspase-3 mRNA expression.<sup>[19]</sup> The use of minocycline also reduced microglia activation, caused antibody blockade of the CD95 (FAS) ligand and the blockade of glycosphingolipid-induced iNOS (inducible nitric oxide synthase), and reduced neuronal and glial apoptosis, with concomitant improvement in neurological function and appear to enhance the efficacy of cell transplantation strategies.<sup>[5,6]</sup>

To find out whether phospholipase A2 (PLA2) plays a role in the pathogenesis of SCI (using biochemical, Western blot, histological, immunohistochemical, electron microscope, electrophysiological and behavior assessments) it was investigated a SCI model and PLA2 activity, expression, and cellular localization after the injury, and the effects of exogenous PLA2 on SC, neuronal death *in vitro* and tissue damage, inflammation, and function *in vivo*.<sup>[31]</sup> After SCI, both PLA2 activity and cytosolic PLA2 expression increased significantly, with cytosolic PLA2 expression being localized mainly in neurons and oligodendrocytes. Both PLA2 and melittin, an activator of endogenous PLA2, induced spinal neuronal death *in vitro*, which was substantially reversed by mepacrine, a PLA2 inhibitor. When PLA2 or melittin was microinjected into the normal SC, the former induced confined demyelination and latter diffuse tissue necrosis. Both injections induced inflammation, oxidation, and tissue damage, resulting in corresponding electrophysiological and behavioral impairments, and the PLA2-induced demyelination was significantly reversed by mepacrine.<sup>[31]</sup>

These studies based on information mentioned above may provide a solid platform to proceed to well-designed human studies on SCI.

## CONCLUSION

Future studies should stress the key points where and when inflammation and GS present themselves as an inhibitory factor to neuroregeneration. Considering GS as an important event after SCI, the author defends GS as being a tertiary lesion. Current strategies are presented with emphasis on stem cells and drug therapy. A better understanding will permit the development of a therapeutic basis in the treatment of the SCI patients considering each stage of the lesion, with emphasis on GS and neuroregeneration.

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