Therapeutic Interventions Following Mammalian Spinal Cord Injury

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Every year in the United States spinal cord injuries (SCIs) occur in approximately 12,000 individuals, resulting in chronic, debilitating functional deficits in most of these patients. Owing to the extremely high costs associated with hospitalization, subsequent rehabilitation, and outpatient care, it is becoming evident that effective treatments for SCI could drastically reduce health care costs and, more importantly, improve the quality of life for thousands of individuals. In this review, we will briefly discuss the pathological events that contribute to the poor regenerative capacity of the injured spinal cord and describe experimental methods that are being used to both minimize tissue damage and promote the regrowth of injured spinal cord axons.

THE PATHOLOGY OF SCI

The pathophysiology of acute SCI involves a complex cascade of events resulting in compromised function below the level of the injury. This is due to significant neuronal death and the failure of damaged spinal axons to either propagate signals or regenerate. Damage caused by the initial trauma seems to be immediate and irreversible, which can have catastrophic consequences leading to paraplegia or quadriplegia. This primary injury is followed by a progressive wave of secondary damage that destroys neighboring intact nerve fibers critical for limb function below the injury site. Microcirculatory impairments and rupture of the blood–spinal cord barrier create a harmful microenvironment with increased levels of extracellular ions, excitatory amino acids, and metabolic stress induced by free radical formation (Figure, A). A critical event that contributes to the loss of intact nerve fibers following SCI is the immediate and prolonged death of oligodendrocytes. Ironically, however, these and other specialized glial cells can express inhibitory molecules that produce a “nonpermissive” environment and hinder regeneration of severed spinal cord axons.

GLIOSIS AND ABORTIVE REGENERATION

The morphological activation of astrocytes and microglial cells (also termed reactive gliosis) is systematically associated with injury to the nervous system. Reactive gliosis controls the extracellular microenvironment in the spinal cord by regulating the blood–spinal cord barrier and ionic concentrations, and it plays a critical role in the uptake and metabolism of the potentially excitotoxic amino acid glutamate (Figure). Moreover, it also produces various growth factors and cytokines, some of which are neuroprotective and aid the survival of damaged neurons. Activated microglia-derived brain macrophages also promote neovascularization of the lesion, which is a critical step in the wound-healing process, by ensuring the delivery of trophic factors and nutrients to support cell migration and growth into the damaged area. In addition to being beneficial for the regenerative process, reactive neuroglia also express and secrete molecules that are potentially detrimental for regeneration.

In the first week after SCI there is dramatic proliferation of microglia-derived

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Schematic illustrations of (A) contusive spinal cord injury showing the pathological and cellular reactions resulting from early cytotoxic events, (B) neuron with an injured axon encountering reactive neuroglial cells, which secrete factors known to contribute to growth inhibition, and (C) a neuron successfully extending the growth cone of its axon through a supporting neuroglial matrix expressing proregenerative factors. A, Despite the presence of surviving neural elements above the injury site, a ruptured blood–spinal cord barrier leads to extravasation of blood cells and plasma constituents, including high levels of iron and glutamate, with ensuing hemorrhagic necrosis. Mononuclear phagocytes clear cellular debris and secrete factors involved in immunological responses to tissue repair, but they also express potentially toxic molecules such as reactive oxygen (O²) species. This contributes to further destruction of proximal neurons and demyelination of neighboring intact axons, with ensuing apoptotic events distal to the injury site. Collectively, these events result in the physiological and morphological activation of microglia and astrocytes, which soon completely surround the necrotic region and govern the regenerative success or failure of injured axons. B, An injured axon undergoing Wallerian degeneration encounters numerous growth-inhibitory obstacles. Demyelination itself releases the potent oligodendrocyte growth-inhibitory molecules myelin-associated glycoprotein (MAG) and Nogo-A (Nogo). The neuroglial cicatrix that forms at the severed nerve endings expresses both noxious agents (NO) and potentially harmful cytokines such as tumor necrosis factor-α (TNF-α), as well as the inhibitory extracellular matrix molecules tenascin-R (Tenascin) and chondroitin sulfate proteoglycan (CSPG). C, Axonal growth cones have the potential of extending into the neuroglial matrix if appropriate growth-promoting substrates such as laminin and cell adhesion molecules (CAMs) are present. The local production of growth factors and cytokines by reactive neuroglia helps to create a proregenerative extracellular milieu. In addition, increasing cyclic nucleoside levels (such as cyclic adenosine monophosphate [cAMP]) in the growth cone may convert inhibitory stimuli into growth promotion.
brain macrophages and an enormous recruitment of blood-derived monocytes (Figure, A). The primary functions of these macrophages during tissue repair include removal of dead tissue and debris through phagocytosis as well as lipid recycling. Brain macrophages also aid the progression of wound healing and gliosis with the release of cytokines, growth factors, and extracellular matrix molecules. Additionally, these phagocytes remove putative inhibitory molecules produced by oligodendrocytes by clearing myelin debris from the injury site. Activating resident microglia by injecting pyrogenic bacterial lipopolysaccharide into the injured spinal cord reduces cavitation and density of the astrocytic scar in addition to enhancing both vascularization and neuritic regeneration.3

Injury-induced cytokines produced by reactive microglia initiate a cascade of cellular responses that greatly influence other neuroglia, including astrocytes.1,3 These reactive astrocytes have the responsibility of not only repairing the damage, but also must reestablish the integrity of the microenvironment surrounding the lesion to maintain the function of nondamaged circuits. Thus, they function to both promote regeneration adjacent to the damaged region while preventing growth into the lesion. Initially, astrocytic processes delineate the necrotic area, forming a dense glial lining with basal lamina completely enveloping infiltrating mesodermal elements such as fibroblasts and Schwann cells. This serves to restore the blood–spinal cord barrier and ionic homeostasis in the injured area, but the dense astroglial scars are also thought to inhibit successful axonal growth because of their deposition of neurite growth–inhibiting extracellular matrix molecules, most notably, chondroitin sulfate proteoglycan (Figure, B).2 Mature oligodendrocytes also express potent inhibitory molecules that significantly contribute to the poor regenerative potential of severed spinal cord axons.

Two relatively modest regenerative processes occur after SCI. Terminal sprouts are often formed by cut axons, and collateral sprouts may come from adjacent undamaged axons. Passage of axonal sprouts through damaged spinal cord tissue depends on the clearance of inhibitory molecules and the presence of appropriate guidance cues. Changes in the balance of positive vs inhibitory factors alter the local environment and ultimately influence the amount of regeneration that takes place in the injured spinal cord.

The ongoing challenge for research focused on spinal cord regeneration is to modulate the astrocyte’s response to injury so as to gain from its potential neurotrophic effects while at the same time tempering its scarring effect. Future successes in this challenging field of research will stem from invaluable information obtained from using current methods that encompass pharmacological intervention, cell transplantation, and gene therapy.

WHAT ARE APPROPRIATE MODELS FOR EXPERIMENTAL SCI?

Numerous methods of SCI exist for the rodent. The most commonly used include transection, resection, hemisection, or aspiration lesions. These lesions definitively cut axon tracts, but these types of injuries are not typically seen clinically. Reproducibility is very difficult to attain, and few studies have demonstrated significant regeneration of supraspinal pathways across a transection site unless a cellular or prosthetic graft is used to “bridge” the host-lesion interface. In this regard, using these models of SCI in combination with transplantation experiments have been instrumental in discovering what cell types are proregenerative or growth-inhibiting.

Compression injuries to the spinal cord precipitated by fractured vertebrae following blunt-force trauma are the most common type of SCI seen clinically.3 The weight-drop technique for contusion SCI in dogs was first developed by Alfred Allen Gardner, using these models of SCI in multiple paradigms has greatly advanced our current understanding of the pathophysiology following spinal cord trauma. Equally important is the need to standardize behavioral outcome measures for the different injury models because nonstandardized assessment of functional recovery adds to the complexity of correlating behavioral recovery with histological alterations.

In summary, it must be emphasized that there is no particular model of SCI that can address all aspects of regeneration or functional recovery. In fact, the use of multiple paradigms has greatly advanced our current understanding of the pathophysiology following spinal cord trauma. Equally important is the need to standardize behavioral outcome measures for the different injury models because nonstandardized assessment of functional recovery adds to the complexity of correlating behavioral recovery with histological alterations.

PHARMACOLOGICAL THERAPY FOLLOWING SCI

Numerous studies over the past several years have explored novel pharmacological therapies for the treatment of SCI, some of which have been tried clinically. Ideally, effective treatments should create the type of milieu (at the injury site and in adjacent areas) that promotes regenerative activity on the part of the surviving neural elements. Therefore, any therapeutic intervention designed to foster regeneration of injured spinal axons must first diminish the secondary tissue damage to provide a requisite cellular substrate for regenerating axons.

Current clinical approaches to reduce the spread of cell death and
tissue degeneration following the initial injury include early pharmacological intervention with high-dose steroids.\textsuperscript{5,6} The synthetic glucocorticoid methylprednisolone sodium succinate is the standard treatment following SCI in humans based on its reported neuroprotective effects. Unfortunately, the functional improvements reported in early human trials remain controversial, and benefits of methylprednisolone sodium succinate are limited to a narrow therapeutic window (first 8 hours) and primarily to incomplete injuries. Despite its clinical use, it is not fully understood how methylprednisolone sodium succinate produces its beneficial actions. Reductions in edema, inflammatory cytokine production, and lipid peroxidation are thought to play roles, but the beneficial effects of methylprednisolone sodium succinate range from moderate improvements to none at all, depending on the animal model of SCI used and the severity of the injury.\textsuperscript{6,7} Similarly, ganglioside GM\textsubscript{1} (Sygen; FIDIA Pharmaceutical Co, Troy, Mich) has recently been used clinically without conclusive demonstration of significant functional recovery in rodent models of SCI, and its mechanisms of action are not at all clear.\textsuperscript{3,7}

Although not used clinically, various pharmacological interventions have demonstrated significant anatomical and behavioral effects after experimental SCI in rodents. Some of the more novel therapies have included cycloheximide, α-melanocyte–stimulating hormone, tacrolimus (FK-506), iloprost, tetrodotoxin, clenbuterol, and blockers of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and/or kainate and N-methyl-D-aspartate (NMDA) receptors.\textsuperscript{5,7,8} In light of evidence that free radicals contribute to the pathogenesis of trauma to the central nervous system, others have used the free radical scavengers α-phenyl-N-tetraetyl-nitroamine (PBN) and lazaronid, albeit with limited success in SCI models.

Paradoxically, damage to the spinal cord is accompanied by the production of both proinflammatory mediators as well as factors that promote neuroprotection and self-repair (Figure, C). The temporal progression of neuronal apoptosis and degeneration provides a window of opportunity for augmenting the potentially beneficial effects of protective factors, such as nerve growth factor, neurotrophin-3, brain-derived neurotrophic factor, basic fibroblast growth factor, and interleukin 10.\textsuperscript{1,2,8-10} Of these factors, basic fibroblast growth factor seems to have more success than the others following acute SCI, based on its neuroprotective and proregenerative properties.

Oligodendroglia express at least 2 proteins that may directly inhibit axonal regeneration: myelin-associated glycoprotein and Nogo-A.\textsuperscript{2,9} Nogo-A is an endoplasmic reticular protein with minor expression on the surface of oligodendrocytes (Figure, B). On injury and myelin degradation, Nogo-A may be released with its inhibitory domain exposed to regenerating axons. An antibody (IN-1) designed to neutralize the inhibitory function of Nogo-A was shown to significantly increase the length that some axons regenerated; however, recovery of motor and sensory function was insignificant.\textsuperscript{9} Although individually these interventions demonstrate small effects, they function to alter only one component of a very complex series of events (Figure). This suggests that future therapies that combine various agents to reduce tissue destruction, promote neuronal survival, and increase axonal regeneration may prove the most efficacious in enhancing functional recovery.

TRANSPANTATION

Fetal Spinal Cord Tissue and Stem Cells

Over the years, neural tissue transplantation has served as an experimental tool for investigating the nature of various cellular interactions in the injured spinal cord and determining whether it promotes functional recovery.\textsuperscript{11} While this approach seems to be promising, based on the neuronal viability and plasticity of embryonic tissue the collective results have demonstrated varying degrees of success with respect to recovery or regeneration. Graft-mediated functional recovery may be achieved if combined with other cell types or interventions designed to stimulate axonal growth, such as growth factor treatment.\textsuperscript{7} The isolation of donor cells using specific culturing protocols and isolation techniques in tissue culture allows the greatest control over transplant composition. In this regard, the recent advent of embryonic stem cell isolation has provided a method for grafting progenitor cells (most notably neurons) into the chronically injured spinal cord. Such grafts have been reported to improve functional recovery, but the mechanisms by which the transplanted cells ameliorate behavior are unclear.\textsuperscript{1} Specifically, because stem cells are pluripotent, it is not known whether their differentiation into neurons or glial cells is responsible for these improvements.

Schwann Cells and Olfactory Ensheathing Cells

The success of peripheral nerve grafts in promoting spinal cord regeneration can be directly attributed to the accompanying Schwann cells, which normally insulate axons in the peripheral nervous system. Schwann cells that are grafted into lesions of irradiated spinal cords to eliminate endogenous proliferating glia are very capable of remyelinating spinal axons, thereby restoring normal conduction properties. This is also true of olfactory ensheathing cells (unique glia isolated from the olfactory bulb, which demonstrate similar morphological and physiological properties to Schwann cells). Both glial populations can reduce posttraumatic cavitatory and astrocytic scar formation when injected into the site of an acute compression lesion.\textsuperscript{12,13} Importantly, after being placed into resection cavities of spinal cords, considerable axonal growth occurs through polymeric tubes filled with cultured Schwann cells seeded in a special biomatrix.\textsuperscript{14} This growth response is augmented with co-grafted olfactory ensheathing cells.\textsuperscript{15} More recently, genetically modified Schwann cells engineered to secrete trophic factors have been...
shown to enhance these regenerative responses. \(^6\) Despite significant ingrowth of brainstem and spinal axons through the Schwann cell and/or olfactory ensheathing cell grafts, only 2 studies reported functional improvement following transplantation of olfactory ensheathing cells alone. \(^7,8\)

**Oligodendrocytes**

Transplantation of cultured oligodendrocytes into the injured spinal cord can lead to sufficient remyelination of denuded axons so that normal axonal conduction properties are restored, often resulting in significant behavioral improvements.\(^19,20\) However, oligodendrocytes also have a negative influence on regenerating spinal cord axons through their expression of inhibitory molecules. In this context, while grafting oligodendrocytes following SCI seems to be an approach to remyelinate and rescue denuded axons, it also introduces the possibility of increasing the inhibitory milieu for regenerating axons.

**Astrocytes**

Contrary to the theory that astroglial scars are the major impediment for successful axonal regeneration, under certain circumstances astrocytes can also provide a matrix to support axonal growth during development and regrowth in the injured spinal cord. Transplantation of cultured astrocytes into the injured spinal cord results in their extensive migration, and they are reported to enhance remyelination and reduce scar formation.\(^21\) Additionally, grafting immature astrocytes into the injured adult rat spinal cord can stimulate axonal regeneration,\(^2\) suggesting that reintroducing an immature glial environment at the lesion site improves regenerative responses.

**Microglial Cells**

Unlike other neuroglial cell populations, microglial cell transplantation has not been extensively investigated until recently. While some have proposed that microglial cells exacerbate lesions of the central nervous system through production of putative cytotoxic molecules, they also secrete a variety of beneficial cytokines and growth factors.\(^3\) Accordingly, grafting cultured microglial cells into the injured spinal cord promotes the ingrowth of microvascular elements, neuritic processes, peripheral Schwann cells, and many other heterotypic cellular elements.\(^3\) When introduced with fetal spinal cord transplants, cultured microglial cells also enhance the regeneration of primary sensory axons into the grafts following dorsal root injuries. These results strongly support the concept that posttraumatic microgliosis and brain macrophage formation are critical for postinjury tissue repair, neuronal regeneration, and neuritic outgrowth. This may be reflected in the transplantation of peripheral macrophages that, when stimulated with sciatic nerve-conditioned medium, have recently been shown to promote functional recovery following complete spinal cord transection.\(^23\)

**Genetically Modified Fibroblasts**

The use of genetically modified fibroblasts designed to secrete certain molecules of interest is another novel approach in the field of transplantation.\(^19,20\) While these cells do not provoke adverse immunological reactions in the host spinal cord, they have been shown to promote various cellular responses, including remyelination and regeneration.\(^24,25\) It is important to note that while these cells eventually disappear over time or lose their secretory properties, their survival after a critical period of recovery may not be necessary.

**GENE THERAPY**

Transference of DNA that encodes therapeutic genes directly into cells that surround the lesion could enhance the resident cells to increase their production of beneficial proteins. Genetically modifying endogenous cells both reduces the prospect of further disruption of the cellular continuity outside the damaged regions and modifies the growth-supportive nature of this terrain. Genetically modifying cells to express neurotrophins or growth factors within the damaged brain or spinal cord increases neuronal survival, sprouting, and regeneration.\(^10\) The overexpression of these factors most likely increases the intrinsic ability of axons to grow and potentially allows the growth cone to overcome the elevated levels of inhibitory molecules surrounding the wound site.\(^10\) Long-term expression of these genes can be achieved primarily by viral-derived vector systems, such as lentiviruses, adenoviral-associated viruses, and potentially gutless forms of adenoviruses.

**CONCLUSIONS**

The neuroglial response after SCI results in a balance of conflicting signals that function to (1) isolate the damaged region while preventing long-distance regeneration of severed axons through the lesion itself and (2) support sufficient growth to re-establish a functional environment for local circuitry. Individual interventions attempt to tip the balance of these signals in favor of increasing neuronal survival and growth promotion. However, to significantly increase functional regeneration, multiple strategies are required to compensate for the myriad of detrimental factors produced after injury.

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