The reversal of paralysis following SCI is among the most daunting challenges in all of neuroscience research. Despite significant improvements in the early medical and surgical management of SCI, coupled with a vastly improved understanding of SCI pathophysiology, there remain no effective treatments to improve neurological outcomes following SCI. Although a number of pharmacological agents (such as methylprednisolone sodium succinate and the related compound tirilazad mesylate, GM-1 ganglioside, thyrotropin-releasing hormone, gacyclidine, naloxone, and nimodipine) have been investigated in large, prospective, randomized, controlled clinical trials, all have failed to demonstrate convincing neurological benefit, despite the promise that these agents demonstrated in preclinical animal studies of SCI. The aim of this review is to provide an overview of the current understanding of spinal cord injury pathophysiology and discusses important emerging regenerative approaches that have been translated into clinical trials or have a strong potential to do so. The pathophysiology of spinal cord injury involves a primary mechanical injury that directly disrupts axons, blood vessels, and cell membranes. This primary mechanical injury is followed by a secondary injury phase involving vascular dysfunction, edema, ischemia, excitotoxicity, electrolyte shifts, free radical production, inflammation, and delayed apoptotic cell death. Following injury, the mammalian central nervous system fails to adequately regenerate due to intrinsic inhibitory factors expressed on central myelin and the extracellular matrix of the posttraumatic gliotic scar. Regenerative approaches to block inhibitory signals including Nogo and the Rho-Rho-associated kinase pathways have shown promise and are in early stages of clinical evaluation. Cell-based strategies including using neural stem cells to remyelinate spared axons are an attractive emerging approach.

**Epidemiology of SCI**

Acute traumatic SCI occurs worldwide with an estimated annual incidence of 15–40 cases per million and...
is associated with severe physical, psychological, social, and economic burdens on patients and their families. It has been estimated that the lifetime cost of medical care and other injury-related expenses for a 25-year-old patient with SCI who suffers a high cervical tetraplegia is ~ US $3 million. At the societal level, SCI is particularly troubling because it predominantly occurs in young, otherwise healthy individuals, with injury occurring with the greatest frequency in those between 15 and 25 years of age. The male-to-female ratio for SCI is ~ 4 to 1. During the previous 3 decades there has been a subtle shift in SCI demographics with the average age at injury increasing from 28.7 to 37.6 years and the percentage of injuries occurring in the elderly (those > 60 years of age) increasing from 4.7 to 10% of SCI cases. Common causes of SCI are motor vehicle accidents (50%), falls and work-related injuries (30%), violent crime (11%), and sports-related injuries (9%). Flexible regions of the spinal column are most susceptible to injury and accordingly, injuries most commonly occur in the cervical spine where they are associated with the most devastating neurological impairments. A recent report from the US National Spinal Cord Injury Database found that 56% of all SCI cases occur in the cervical spine.

### Pathophysiology of SCI

A prerequisite to the development of effective therapies for SCI is a detailed understanding of the pathophysiological processes occurring following injury and how various components interact to result in the functional deficits observed. This understanding is complicated by the finding that several elements of the injury response—notably the inflammatory response and reactive astrogliosis—have been found to act pathologically while also serving some neuroprotective or neurorestorative functions.

The pathophysiology of SCI is best described as biphasic, consisting of a primary and secondary phase of injury (Table 1). The primary phase involves the initial mechanical injury during which failure of the spinal column (fracture and/or dislocation) directly imparts force to the spinal cord, disrupting axons, blood vessels, and cell membranes. This is followed by the delayed onset of a secondary injury phase involving vascular dysfunction, edema, ischemia, excitotoxicity, electrolyte shifts, free radical production, inflammation, and delayed apoptotic cell death. Whereas neurological deficits are present immediately following the initial injury, the secondary injury phase results in a protracted period of tissue destruction.
Spinal cord injury pathophysiology and regenerative approaches

Due to the many inherent difficulties associated with studying human postmortem tissue, almost all of our detailed understanding of SCI pathophysiology has been obtained through animal studies. The pathophysiology of SCI has been extensively studied in many experimental animal models including mice, rats, cats, and nonhuman primates. While the overall picture of SCI emerging from each species investigated has been equivocal, some significant differences exist between the human condition and rodent models widely used in preclinical studies, such as the extent of glial scarring.66

Primary Injury Phase

The most common form of acute SCI is a compressive-contusive–type injury in which displaced components of the vertebral column, including intervertebral discs and ligaments, exert force on the cord causing both immediate traumatic injury and often sustained compression.46 The common physical mechanisms of primary injury include shearing, laceration, acute stretching, and sudden acceleration-deceleration injuries.8 Primary injury mechanisms rarely transect or fully disrupt the anatomical continuity of the cord. Axons are commonly found to traverse the lesion site, often occupying a “subpial rim” of spared yet demyelinated or dysmyelinated long-tract axons.23,101,114,130,156 The existence of these spared axons crossing the injury site is highly significant therapeutically and represents the neural substrate on which many emerging therapeutic strategies will act. It is also encouraging, because in animal studies substantial neuronal function has been sustained with the preservation of as few as 5% of the original number of axons.94,79 Therefore, approaches (such as remyelination) to enhance the function of residual neural tissue may maximize the potential neurological recovery in a given patient, even in the absence of significant neural or axonal regeneration. Rehabilitative therapy is also essential to maximizing neurological recovery in patients with SCI.48 Although beyond the scope of this review, rehabilitative approaches, such as weight-assisted treadmill training or functional electrical stimulation,131 are and will continue to be a vital component in future treatment regimes for SCI (for review, see reference 48). One particularly promising area of research in SCI rehabilitation worthy of brief discussion is the development of techniques to stimulate motor neuron pools and networks caudal to the lesion. Pioneering work by Grillner63–65 identified neuronal networks intrinsic to the spinal cord, termed “central pattern generators,” that mediate locomotor output including walking in vertebrates. Building on this work, Chau and colleagues30 have demonstrated that in the cat these “spinal locomotor” central pattern generators can be activated both pharmacologically by clonidine or by sensory input through treadmill training46 to result in functional locomotion with full weight support, even after complete transection of the spinal cord. Therefore, rehabilitation programs should be designed to improve both voluntary and involuntary aspects (spinal locomotor central pattern generators) of motor control to maximize functional recovery following SCI.

Secondary Injury Mechanisms

A number of pathophysiological processes are triggered by the primary mechanical injury leading to the prolonged secondary injury phase. These events of this secondary injury process can be usefully divided temporally into multiple contiguous phases: the immediate, acute, intermediate, and chronic stages of SCI (Table 1).

Immediate Phase (0–2 Hours)

The immediate phase begins at the time of injury, lasts ~ 2 hours, and represents the primary phase of injury in the biphasic SCI model.118 This phase is dominated by the immediate results of the injurious event itself. The traumatic severing of axons, the immediate death of neurons and glia, and the accompanying (poorly understood) phenomenon of spinal shock44 result in instantaneous loss of function at and below the level of injury for complete injuries.

The first detectable pathological change following injury is a generalized swelling of the spinal cord often with hemorrhaging in the central gray matter, in which cells immediately begin undergoing necrotic death due to direct mechanical disruption of cell membranes or ischemia resulting from vascular disruption. Disruption of the microvasculature also leads to hemorrhage (largely petechial) in the surrounding white matter.79,151 Hemorrhage and swelling combine to produce cord ischemia that may extend for many spinal segments both rostral and caudal to the injury. Interestingly, gross morphological or MR imaging abnormalities of the spinal cord can be absent during the immediate phase with the exception of patients suffering from severe compressive or lacerative injuries.6,118

Although profound histopathological changes may not yet be grossly observable in this early injury phase, other injurious pathophysiological processes have been initiated. For example, the activation of microglial cells begins almost instantaneously following injury46 with the upregulation of the proinflammatory cytokines TNFα and IL-β, detectable within minutes of injury.123 Levels of extracellular glutamate can reach excitotoxic levels within minutes of the injury.163

Acute Phase

The acute phase is the period in which the secondary injury processes become dominant. For practical reasons, it is the SCI phase likely to be most amenable to neuroprotective interventions, as it is typically the earliest point at which patients arrive at an appropriate center to receive possible treatment. The acute phase is subdivided into early acute and subacute stages.

Early Acute Phase (2–48 Hours). The early acute phase of SCI can be considered to last from 2 to 48 hours following injury. This phase is characterized by continuing hemorrhage, increasing edema, and inflammation, and marks the onset of additional secondary injury processes including free radical production, ionic dysregulation, glutamate–mediated excitotoxicity, and immune-associated neurotoxicity that contribute to further axonal injury and cell death. Vascular disruption, hemorrhage,
and the resulting ischemia are central constituents of this secondary injury cascade. The vascular mechanisms underlying the prolonged ischemia are not fully understood, although major spinal arteries are typically intact following injury. The disruption of the microvasculature, loss of normal autoregulatory mechanisms, global hypotension, and increased interstitial pressure underlie the prolonged hypoperfusion seen after SCI.187,190,195 The ischemia results in cytotoxic cell swelling that affects both neurons and glia. Axonal swelling is common, leading to action potential blockade.29

The following sections describe elements of the secondary injury cascade prominent during the early acute phase of injury and important potential therapeutic targets.

Ionic Dysregulation and Excitotoxicity. The loss of ionic homeostasis immediately following SCI and excitotoxicity are closely related processes that each significantly contribute to the propagation of cellular injury after SCI. The loss of appropriate ionic homeostasis is a central feature of both necrotic and apoptotic cell death following injury. Specifically, the dysregulation of Ca\(^{++}\) ion concentration is a common element in cell death140 and initiates a number of damaging processes including the activation of calpains, mitochondrial dysfunction, and free radical production culminating in cell death.

Excitotoxicity is a result of the excessive activation of glutamate receptors leading to the influx of Na\(^{+}\) and Ca\(^{++}\) through the NMDA and alpha-amino-3-hydroxy-5-methyl-isoxazolepropionate/kainate receptors. It is believed to play a role in the death of both neurons and glia following many forms of neurotrauma, including SCI (for review, see reference 119). Following injury, extracellular levels of glutamate rise rapidly due to direct injury to cells and failure of energy-dependent transporters, notably the Na\(^{+}\) K\(^{+}\) adenosine triphosphatase membrane transporter that normally functions to regulate extracellular concentrations of ions, glutamate, and other molecules.91 The prominent role of excitotoxicity in the propagation of secondary injury has led to significant interest in pharmacological means to attenuate it with antagonists of NMDA and non-NMDA receptors.

Free Radical Mediated Injury. Free radical mediated injury is an important contributor to secondary damage following SCI with radical-mediated lipid peroxidation contributing to axonal disruption and the death of both neurons and glia. Lipid peroxidation is a self-perpetuating free radical reaction causing membrane damage leading to cell lysis, the dysfunction of organelles, and contributing to calcium dyshomoeostasis through the oxidation of membrane lipids. The detection of ROS peaks at roughly 12 hours following injury and remains elevated for ~1 week, returning to basal, preinjury levels 4–5 weeks after injury.46,164 The production of ROS is associated with both ischemia immediately following injury and subsequent reperfusion.190 Whereas a variety of radical species contribute to damage, with classic examples such as hydroxyl radical and the hydroxyl radical, it has recently been shown that a key mediator of ROS-mediated injury is the peroxynitrite radical generated by the reaction of nitric oxide and the superoxide radical.164 Peroxynitrite has been directly associated with the induction of apoptosis in neurons in rat SCI.4 Highlighting the importance of ROS in SCI pathology is the demonstrated neuroprotective effects of antioxidant compounds such as tempol and selective inhibition of peroxynitrite-mediated reactions by uric acid.144 The modest neuroprotective effects of the now standard methylprednisolone treatment following SCI are also believed to be due, at least in part, to the inhibition of lipid peroxidation.53,187 Therefore antioxidant therapy is an important area of continuing investigation for neuroprotective compounds for SCI.

Membrane Lipid Oxidation. Following injury, the membrane lipids are the initial targets of radical-mediated peroxidation. The membrane lipids are composed of a variety of fatty acids with different chain lengths and unsaturation, and are highly susceptible to oxidative degradation. The products of lipid peroxidation include hydroperoxides, aldehydes, ketones, and hydrolysis products of the now oxidized fatty acid. The formation of these products causes peroxidation to continue and can also contribute to the damage of other tissue components. The products of lipid peroxidation also have a profound impact on cellular function and viability. The resulting ROS can cause membrane damage leading to cell lysis, the dysfunction of organelles, and free radical production culminating in cell death.

Many inflammatory mediators involved in the secondary injury cascade are known to have profound effects on vascular permeability. Two inflammatory cytokines (TNF\(\alpha\) and IL-1\(\beta\) are upregulated following SCI125 and are known to increase vascular permeability.141 Other compounds released by glial cells or invading immune cells believed to play a role in increasing BBB permeability include ROS, nitric oxide, histamine, matrix metalloproteinases, and elastase.46 Because some hydrophilic agents may not be able to cross the BBB and gain access to the injured spinal cord, methods for manipulating the BBB permeability may have some therapeutic utility.46 For many neuroprotective agents administered at early time points when the BBB remains disrupted, the issue of the BBB permeability may not pose a major problem.

Inflammatory Mediators and the Cellular Immune Response. The early acute stage involves infiltration by inflammatory cells and continuing activation of resident microglia. The inflammatory process following SCI is highly complex and involves numerous cellular populations, including astrocytes, microglia, T cells, neutrophils, and invading monocytes. A multitude of noncellular mediators, including TNF\(\alpha\), interferons, and ILs also play important roles.46,156,127 The field of neuroinflammation following SCI has grown immensely in recent years and has revealed a process of tremendous complexity, with some aspects of the inflammatory response contributing deleteriously to further secondary injury, and others contributing beneficially to the removal of cellular debris and enhancement of the environment for regenerative growth.46
The dual nature of the immune response following SCI is well demonstrated by the multiple roles observed for TNFα following CNS injury. Tumor necrosis factor-α is key inflammatory media that is significantly upregulated in neurons, glia, and endothelial cells following SCI. The inhibition of TNFα signaling using neutralizing antibodies has been demonstrated to improve functional neurological recovery following SCI. However, TNFα signaling has also been demonstrated to have a neuroprotective role both in vitro and following SCI in TNFα-deficient mice who display higher numbers of apoptotic cells, increased lesion size, and worsened functional recovery when compared with wild-type mice. These conflicting effects highlight the need for further characterization of the inflammatory response and a clear delineation of the deleterious and beneficial aspects to target therapeutically. The study of the diverse components of the immune response in human SCI is complicated by the difficulty in obtaining pathological samples at early time points after injury, a task that was recently achieved by Fleming and colleagues in their elegant description of the cellular response to human SCI.

Cell Death and Demyelination. Cell death after SCI may occur by necrosis or apoptosis. Death of neurons at all stages of injury occurs mainly through necrosis, with little evidence to date that apoptosis plays an important role in neuronal death after human SCI, although it has been observed in animal SCI. In contrast to this, oligodendrocytes—which, like neurons, are highly sensitive to ischemic injury—readily undergo apoptosis following SCI. This process is at least partly dependent on the activation of oligodendrocyte Fas receptors by activated microglia expressing Fas ligand and signaling through the p75 neurotrophin receptor.

Because apoptotic death is an active process, as it is both energy dependent and requiring de novo protein synthesis, it represents a feasible target for therapeutic intervention by selective inhibition. Recent work in our laboratory has demonstrated the potential efficacy of interfering with Fas-mediated apoptosis. Following SCI, Fas ligand is expressed by microglia and invading lymphocytes while the FasR is predominantly found on oligodendrocytes. The interaction of Fas and FasR initiates apoptosis through the activation of the caspase cascade leading to proteolysis and DNA cleavage by effector caspase-3, -7 and -9. This failure of transplanted cells to survive when transplanted either immediately at the time of injury or after the subacute phase has also been observed by Keirstead and coworkers using human ESC–derived oligodendrocyte progenitors.

The phagocytic response is maximal during the subacute period and is likely beneficial in removing cell debris from the lesion area and may promote axon growth to some degree through the removal of growth-inhibitory components of myelin debris. The phagocytic response observed following PNS injury is significantly greater than that of the CNS and may account for some degree of the difference in regenerative ability.

Following SCI, astrocytes in the lesion core display cytotoxic edema and undergo necrotic cell death in the hours to days following injury. A somewhat delayed astrocytic response begins in the subacute phase, in which astrocytes at the periphery of the lesion become hypertrophic and proliferative, correlating with a dramatic increase in the expression of the astrocytic intermediate filament glial fibrillary acidic protein. These reactive astrocyes grow multiple, large cytoplasmic processes that interweave to form the astrocytic (gliotic) scar. This scar represents both a physical and chemical barrier (see be-
low) to axonal regeneration. Although prominent following rodent SCI, the extent of astroglial scarring is markedly decreased in humans. The reason for this difference is unclear and the consequence on the clinical relevance of various treatment strategies to reduce astrocytic scarring in rodent models is an important question that must be addressed. Despite the production of the scar, astrocytes also serve beneficial functions following SCI such as promoting the reestablishment of ionic homeostasis and reestablishing the integrity of the BBB, which is important for the resolution of the edema and in limiting the infiltration of immune cells.

Intermediate Phase (2 Weeks to 6 Months)

The intermediate phase is characterized by the continued maturation of the astrocytic scar and by regenerative axonal sprouting. In rat models of contusive SCI, corticospinal tract axons display sprouting from 3 weeks to 3 months following injury, whereas sprouting reticulospinal fibers are observed to sprout from 3 to 8 months postinjury. Although these regenerative attempts are obviously insufficient in producing significant functional recovery in severe SCI, it is nonetheless encouraging evidence that regenerative potential does exist in the adult spinal cord. The differential responses of various tracts to injury is a particularly interesting observation and the elucidation of the mechanisms behind this phenomenon is of great interest, as it may reveal that different strategies are needed for different neuronal populations.

Chronic Phase (> 6 Months)

The chronic phase begins ~ 6 months following injury and continues throughout the lifetime of the patient with SCI. The chronic phase is characterized by the maturation/stabilization of the lesion including continued scar formation and the development of cysts and/or syrinxes. The process of Wallerian degeneration of injured or severed axons is ongoing and it may take years for severed axons and their cell bodies to be fully removed. Despite some instances of improved neurological function many years following injury, it can be considered that at ~ 1–2 years postinjury, the neurological deficits have stabilized and the lesion has fully matured. The lesion itself is characterized by cystic cavitation and myelomalacia, representing the final stage of necrotic death after SCI.

The lesion, unfortunately, may not remain static, and syrinx formation in as many as 30% of patients with SCI can cause delayed neurological dysfunction (ascending paralysis, brainstem symptoms) and neuropathic pain. At this chronic stage, therapeutic strategies are aimed to encourage regeneration/sprouting of disrupted axons, promote plasticity with rehabilitation strategies, and improve the function of demyelinated axons with pharmacological measures or cellular transplantation substrates that may potentially remyelinate. Despite much success with stem cell–based approaches when applied subacutely, the successful application of stem cells during this chronic injury phase of injury has not been demonstrated.

As detailed in the preceding sections, SCI pathophysiology involves many mechanistically distinct processes that interact in poorly understood ways to both limit and enhance recovery following injury. This dichotomy is well demonstrated by the astrocytic response, which serves to reestablish the BBB, restore ionic homeostasis, and limit the infiltration by cytotoxic immune cells while

### TABLE 2: Summary of SCI therapies under development and proposed mechanism of actions underlying functional recovery

<table>
<thead>
<tr>
<th>Approach</th>
<th>Effect</th>
<th>Mechanism of Action</th>
</tr>
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<tbody>
<tr>
<td>molecular approaches</td>
<td></td>
<td></td>
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<tr>
<td>soluble Fas receptor</td>
<td>reduced apoptosis</td>
<td>competitive inhibition of FasL-FasR signaling</td>
</tr>
<tr>
<td>IN-1 (anti-Nogo antibodies)</td>
<td>enhanced regenerative growth of axons</td>
<td>inhibition of myelin–associated inhibitors signaling through NgR</td>
</tr>
<tr>
<td>C3-transferase (Cethrin)</td>
<td>enhanced regenerative growth of axons</td>
<td>inhibition of Rho-ROCK signaling</td>
</tr>
<tr>
<td>ChABC</td>
<td>reduced glial scarring</td>
<td>enzymatic degradation of CSPG side chains</td>
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<tr>
<td>cellular approaches</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schwann cells</td>
<td>remyelination</td>
<td>remyelination (peripheral myelin)</td>
</tr>
<tr>
<td></td>
<td>neuroprotection?</td>
<td></td>
</tr>
<tr>
<td>OECs</td>
<td>support of regenerative axon growth, neuroprotection</td>
<td>integration through astroglial scar production of neurotrophic factors?</td>
</tr>
<tr>
<td>NSCs, NPCs, and OPCs</td>
<td>remyelination</td>
<td>oligodendrocyte differentiation/remyelination production of neurotrophic factors?</td>
</tr>
<tr>
<td></td>
<td>support of regenerative axon growth neuroprotection?</td>
<td></td>
</tr>
<tr>
<td>BMSCs and HSCs</td>
<td>remyelination?</td>
<td>transdifferentiation?</td>
</tr>
<tr>
<td></td>
<td>support of regenerative axon growth? neuroprotection?</td>
<td>unknown</td>
</tr>
</tbody>
</table>

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also severely limiting the ability of axons to regenerate and diminishing functional recovery through the formation of the astrocytic scar. An important consideration for understanding the pathophysiology of human SCI is that each injury is unique in cause and the resultant damage. The contrast between the homogeneity of rodent experimental SCI models and the highly heterogenous human SCI population adds to the challenge of successfully translating treatments to the clinic.

Emerging Regeneration and Repair Strategies

The next half of this review will focus on novel therapies under development to induce axonal regeneration or sprouting, and cellular-based strategies to promote remyelination of spared axons (Table 2). The failure of axonal regeneration within the CNS is the result of both the growth inhibitory nature of the mammalian CNS and a relatively low intrinsic regenerative competence of CNS neurons. The ability of CNS neurons to regenerate over long distances after axotomy was first established through the pioneering work of Richardson and colleagues in the 1980s, in which injured CNS axons were found to regenerate into and through growth-permissive peripheral nerve grafts. The obvious therapeutic potential of these findings for SCI was substantiated with Cheng and associates’ seminal (albeit difficult to reproduce) demonstration in 1996 that such peripheral nerve transplantation into a rat spinal cord transection model could promote hindlimb recovery. Incredible optimism followed that such “bridging” strategies would prove helpful for patients with SCIs, although this approach has been largely replaced by cell-transplantation strategies.

In recognition that there are 2 distinct aspects to regenerative failure after CNS injury—the limited intrinsic regenerative potential and the inhibitory extrinsic environment of the injured CNS—great efforts have been undertaken to characterize both. On the intrinsic side, the focus of investigators has been to identify and characterize the molecular signals required to stimulate axonal growth following injury, signals that are clearly more strongly expressed and successfully acted upon in the PNS. Several regeneration-associated genes have been found to be upregulated after axonal injury, notably L1, c-fos, and c-jun, and the 43 kD growth-associated protein. Despite these responses, the degree and extent of their expression is apparently insufficient to promote a strong regenerative response in the CNS in contrast to that observed for axotomized PNS neurons. One factor implicated in limiting the regenerative response in CNS axons is a decrease in the levels of intracellular cAMP in mature versus developing neurons. The elevation of intracellular cAMP lev-
els using cAMP analogs or the phosphodiesterase inhibitor rolipram has been shown to increase axonal sprouting and reduce the effects of myelin-associated inhibitors (for review, see reference 68).

On the extrinsic side, it is well accepted that multiple inhibitory molecules exist that make the injured CNS a nonpermissive environment for axonal growth. These molecules are divided into inhibitors associated with CNS myelin and inhibitors associated with the astrocytic glial scar (Fig. 1).

**Myelin-Associated Inhibitors**

The growth inhibitory nature of CNS myelin was first demonstrated in vitro by Schwab and Thoenen through their observation that cultured sympathetic ganglion neurons would extend neurites on PNS myelin but not CNS myelin. Based on this proof-of-principal experiment, subsequent investigators have identified multiple components of CNS myelin that inhibit axonal growth including Nogo, *M. G. Fehlings et al.*

One outstanding question regarding the use of anti-Nogo treatments (or other myelin inhibitors) is the functionality of the axonal sprouting that might be induced. Myelin-associated inhibitors likely evolved as a means to maintain the structural integrity of synaptic networks formed during development and limit sprouting following developmental pruning and myelination. Whereas anti-Nogo and related strategies promote and enhance regenerative growth, they can also stimulate the aberrant sprouting of uninjured axons or the inappropriate growth of regenerating axons. Whether this overall increase in plasticity is beneficial remains to be seen, although there is a possibility that it could lead to the development (or exacerbation) of neurological symptoms such as autonomic dysreflexia or neuropathic pain.

**Targeting the Rho-ROCK Pathway**

As mentioned above, the discovery of NgR was rapidly followed by the surprising finding that it was the target for not just Nogo but also for MAG and OMgp. Given that the Rho-ROCK signaling pathway is an important downstream mediator of the Nogo receptor, it represents an important point of convergence for multiple inhibitory signals and a potentially efficient therapeutic target that could block the inhibitory effects of multiple distinct myelin-associated inhibitory molecules. Lisa McKerracher and colleagues have developed this concept and used the C3 enzyme from *Clostridium botulinum* (which selectively inactivates Rho via adenosine diphosphate ribosylation, without affecting the associated guanosine triphosphatases Rac or Cdc42) to demonstrate that Rho inactivation could enhance CNS regeneration in an optic nerve injury model. It was later demonstrated that Rho-ROCK inhibition promoted axonal sprouting and improved locomotor function in an incomplete thoracic transection model of SCI in the mouse. Interestingly, the behavioral recovery observed in these animals was very rapid (within days), which was highly suggestive that it could not be mediated by axonal regeneration or sprouting alone. Further study identified a role for activated Rho in apoptotic cell death after SCI and the inhibition of Rho by C3 was found to significantly inhibit this key aspect of secondary injury. These 2 distinct activities of C3-mediated inhibition of Rho were the impetus to commercialize a C3-like enzyme with improved permeability characteristics under the trade name Cethrin (Alseres Pharmaceuticals, Inc.). This approach has been evaluated in a Phase I/IIa open-label human clinical study and has shown preliminary promise (see article by Hawryluk et al. in this issue).

**Gial Scar–Associated Inhibitors**

The glial scar is the result of reactive astrogliosis and is a trademark response to CNS injury of all kinds. The astrocytes that comprise the scar secrete a number of growth inhibitory extracellular matrix components known as CSPGs. The CSPGs include the molecules neurocan, versican, aggrecan, brevican, phosphacan, and NG2. Structurally, CSPGs consist of a core protein to which sulfated GAG side chains are covalently linked.

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Although the GAG side chains are considered the main source of inhibition by CSPGs, it has been postulated that the protein core also contributes to the inhibition of axonal regeneration by acting as a physical barrier to axon elongation, due to high affinity interactions with other extracellular matrix components such as laminin, fibronectin, and neural cell adhesion molecules.

The furthest developed strategy for overcoming this glial barrier is by direct degradation of the CSPGs with a bacterial enzyme known as ChABC that cleaves the GAG side chains from the CSPG core. This approach has been evaluated in numerous in vivo animal models of SCI, although rather infrequently in the most clinically relevant contusion models in which axonal regeneration and sprouting are much more difficult to anatomically assess than in the simpler transection models. Nonetheless, numerous investigators have reported increased sprouting and improved behavioral recovery after ChABC treatment. The possibility of making the CNS environment more permissive to axonal growth has encouraged numerous investigators to use ChABC in combination with other therapies such as fetal tissue transplant, peripheral nerve transplant, neural precursor cells, and Schwann cells to potentially maximize the capacity for remodeling and plasticity. In the chronic injury setting, the addition of ChABC to a cellular transplant may indeed be required to address the well-established glial scar.

Interestingly, in another example of convergence between multiple distinct signaling molecules, the inhibitory interactions between axons and CSPGs may also be in part mediated by the Rho-ROCK pathway, creating the potential that targeting Rho-ROCK and its downstream mediators could also have beneficial effects in helping regenerating axons overcome the glial scar.

**Cell-Based Regenerative Strategies**

A surprisingly diverse range of cell types are currently under investigation as potential treatment strategies for SCI and there is considerable variability in the strength of the scientific rationale for various cell types. Overall the therapeutic premise behind the use of cell-based approaches is based on 2 central concepts: 1) to directly replace cells lost due to injury (oligodendrocytes or neurons); and 2) to influence the environment in such a way as to either enhance or support axonal regeneration, provide neuroprotection, or both. Neurons and oligodendrocytes represent the cell types that are lost due to necrotic and apoptotic death after injury, and various cell substrates (such as stem cells, progenitor cells, and Schwann cells) offer the potential for replacing these lost cell populations within the injured cord. The potential of these cells to remyelinate denuded axons around the injury site has in particular sparked great interest and has shown the most promise to date. Additionally, such cell substrates may express a number of supportive growth cues, such as neurotrophic factors.

Although tremendous optimism abounds regarding the therapeutic potential of cellular substrates for SCI (stem cells in particular), it is important to maintain a realistic perspective on the basic requirements for success and the challenges associated with translation into clinical practice. Stem cells provide a good starting point, as the ability of these cells to differentiate into neurons, oligodendrocytes, or astrocytes in vitro has captured the imagination of basic scientists, clinicians, and patients alike. However, for any cellular substrate to succeed therapeutically, it must meet a number of challenges. A successful cell type must survive following the transplant, differentiate (if not already differentiated) down the correct cell lineage, successfully integrate into the cord tissue, migrate appropriately, physiologically behave in the manner intended, and must be produced in such a way to abide by the Good Manufacturing Process guidelines required for any therapeutic product. One specific area of concern is the potential for contamination of cells with animal antigens such as cell-surface glycoproteins or with viruses. These concerns are now being addressed by the establishment of fully chemically defined culture conditions free of any nonhuman reagents such as fetal bovine serum. Considering that the ideal conditions for cell survival, differentiation, and function that can be generated in vitro will be difficult (if not impossible) to duplicate in vivo within the injured spinal cord, there is good reason for the optimism surrounding cell transplantation to be tempered by the realization of the many obstacles that must be overcome prior to the successful translation of cell-based strategies for SCI. Additionally, many questions remain unresolved regarding the application of this technology, including—on a cell-specific basis—the definition of the optimal time postinjury to perform such a transplant, the patient population most appropriate for the therapy, the techniques required for implanting the cells, and the methods for tracking the fate of the cells in vivo after transplantation.

Given this perspective, the most near-term therapeutic aim for any cell-based therapy is to promote improved axonal function by remyelinating injured demyelinated axons. The following sections will review the cell types that have shown the greatest promise in this regard. It is recognized that in addition to remyelinating denuded axons, these cells may also yield other, still poorly defined growth-promoting effects such as the secretion of neurotrophins or other growth factors.

**Cell-Based Remyelination Strategies**

The transplantation of glial cells (oligodendrocytes, Schwann cells, and OECs) or their progenitors from a number of distinct sources has shown great potential for remyelinating demyelinated axons and improving recovery in animal models of SCI. In the setting of cell transplantation studies, remyelinated axons are distinguished from natively myelinated axons by unique structural characteristics including a reduced myelin thickness, shortened internode length, and loss of the linear relationship in the “myelin ratio” between axonal diameter and myelin sheath thickness. Whereas these differences are useful experimentally to quantify remyelination, it is unclear whether this morphologically abnormal myelin is on its way to being fully remyelinated or, if not, if function will be completely restored. This latter question of
whether these remyelinated axons are truly functional is rather fundamental (and as yet unanswered) in the field, and is unfortunately difficult to address in in vivo animal studies. The following sections describe the cellular substrates that have been used in SCI research for their ability to remyelinate.

Schwann Cells

Schwann cells are the myelinating glia of the PNS. The seminal work of David and Aguayo40,41 using peripheral nerve grafts provided early evidence that Schwann cells could support axonal regeneration of CNS neurons after SCI and ushered in the current era of neuroregeneration research for SCI. The use of intact peripheral nerve segments as bridges to facilitate axonal regeneration within the injured spinal cord has continued33,89 and has even been described in a case report of a patient with chronic paraplegia; after the repair, significant functional improvement was reported in the patient, who was previously confined to using a wheelchair but was able to ambulate independently using a walker after the procedure.34 Despite these impressive claims, it is difficult to draw strong conclusions from such a single case report.

The use of Schwann cells for transplantation has been the research focus of Bunge and coworkers165 at the Miami Project to Cure Paralysis. Using a contusive SCI model in the rat, these investigators have shown that Schwann cell transplantation can result in enhanced axonal regeneration and remyelination by transplanted cells, which was associated with a statistically significant, though very small, improvement in hindlimb function. Schwann cells are an attractive cellular candidate because they can be easily harvested from peripheral nerves (such as the sural nerve), expanded in vitro, then transplanted back into patients in an autologous fashion. Although the ability of Schwann cell transplants to enhance remyelination and support regenerative axon growth has been independently demonstrated by various groups,62,123,126,165 axons typically do not extend beyond the growth-permissive grafts to reinnervate the host spinal cord. Therefore, it is likely that the modest functional improvements observed in such experimental paradigms are attributable to remyelination of existing spared demyelinated axons, and not a result of axons making functionally meaningful synaptic connections with distal targets after regenerating through and out of a Schwann cell graft. This fact casts some significant doubt on the ultimate therapeutic utility of Schwann cell transplantation. However, the proven ability of Schwann cells to integrate into the injured cord parenchyma may make them well suited to serve as a delivery mechanism for other therapeutic molecules such as growth factors.

A novel source of Schwann cells has been identified with the recent discovery and isolation of so-called skin-derived precursor cells, which are multipotent stem cells that have been derived from the dermis of adult mice64 and subsequently humans.155 Skin-derived precursor cells can be differentiated in vitro to produce myelinating Schwann cells, which have been shown to remyelinate axons and produce modest functional recovery following SCI in the rat.166 Although these cells offer the advantage of harvesting transplantable cells from a simple skin biopsy procedure, it is not yet known whether there will be a therapeutic advantage over Schwann cells derived from peripheral nerves.

Olfactory Ensheathing Cells

Olfactory ensheathing cells are a specialized glial cell originally described as olfactory nerve Schwann cells due to their morphological similarities. Olfactory ensheathing cells are unique in their ability to facilitate the passage of new axons from regenerating olfactory receptor neurons (which reside in the PNS within the olfactory mucosa) over long distances up to a mitral/tufted cell target neuron within the olfactory bulb glomeruli (CNS). This ability to seemingly escort axons across the “PNS to CNS barrier” has made OECs an extremely attractive potential transplantation substrate in SCIs, recognizing that despite the propensity of CNS axons to enter Schwann cell grafts (a PNS environment), it was difficult to get them to exit and reenter the hostile CNS environment to make functionally appropriate synapses.

Cotransplantation experiments with Schwann cells housed within guidance channels with OECs at either end revealed that the OECs could indeed facilitate enhanced regeneration of axons back into the spinal cord.133,134 This finding was followed by the reporting of axonal regeneration and functional recovery in a full transection SCI model after OEC transplantation.133 Interest in these cells has since grown immensely (for review see reference 132). Almost a decade of further work has curtailed the enthusiasm surrounding these cells to some extent, because it has become evident that some of the regeneration observed in OEC transplantation experiments may in fact be attributable to invading endogenous Schwann cells or by reportedly pure cultures of OECs becoming contaminated with Schwann cells.17,18 Because the 2 cell types both express the majority of cell markers (such as p75) used to identify OECs. On the issue of myelination, under certain in vitro conditions, OECs (which never myelinate in vivo) have been reported to be capable of myelinating axons.45 However, the ability of the OEC to myelinate in vivo after transplantation into the injured spinal cord remains a subject of controversy, with the weight of evidence indicating that the OECs, in fact, do not form myelin.17 Whether they remyelinate in vivo or not, it appears that OECs are capable of creating an environment that is permissive for axonal growth, secreting factors that may be neuroprotective, modifying the glial scar by their interaction with astrocytes, and promoting angiogenesis.138 This potential has led to their use in numerous clinical trials outside North America (see Hawryluk et al. in this issue). Although the human trials reported to date have failed to demonstrate any significant benefits,45 there is hope that further optimization of culture and transplantation techniques and identification of appropriate time points for treatment postinjury will maximize the potential efficacy of OECs for SCI. Olfactory ensheathing cells are also a promising candidate for combinatorial cell-based strategies (OECs in combination with neural precursor cells) based on their apparent ability to integrate into the glial scar and enhance the growth permissiveness of the injured spinal cord.4

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**Neural Stem Cells and Glial Progenitors**

Neural stem cells are self-renewing, multipotent cells capable of producing all 3 neural lineages (neurons, astrocytes, and oligodendrocytes). Progenitor cells have a limited capacity for self-renewal and typically are unipotent, producing only 1 mature cell type of a given lineage. This distinction is an important one and use of the term “stem cell” has become increasingly confusing as it is often used in reference to any proliferating immature cell type in the CNS or other tissue. Although a detailed discussion of the characterization and definition of specific populations of stem and progenitor cells with therapeutic potential for SCI is well beyond the scope of this review, it is important to recognize that relevant biological differences may exist between seemingly interchangeable cell populations derived from embryonic or adult sources. For example, NSCs with the ability to survive and differentiate into mature, myelinating oligodendrocytes when transplanted into the demyelinated or injured rodent spinal cord have been derived from the embryonic CNS of mice and the postnatal and adult CNS of mice, rats, and humans. More importantly for developing therapies, NSCs and committed OPCs have been produced from both murine and human ESCs. The ideal source of myelinating cells for transplants is a matter that remains to be determined, although ESC-derived OPCs will likely emerge as the most clinically feasible source.

**Embryonic Stem Cell–Derived NPCs and OPCs**

Embryonic stem cells are pluripotent (that is, they can produce cell types from all 3 embryonic germ layers: mesoderm, endoderm, and ectoderm), self-renewing cells derived from the inner cell mass of blastocyst stage embryos. First derived from murine embryos, ESCs have subsequently been produced from human embryos. Due to their pluripotency in the undifferentiated state, ESCs require in vitro differentiation to commit them to a neural or glial fate prior to transplantation. Undifferentiated ESCs, or those that are not fully committed to the neural lineage, form teratomas following transplantation. Over the past decade, significant advances have been made in the ability to preferentially drive ESC differentiation into neural and specifically oligodendroglial lineages. First achieved using murine ESCs, the production of oligodendroglial lineage cells from ESCs followed by myelination has been demonstrated in vitro and following transplantation into the demyelinated or injured spinal cord. After the original demonstration using murine ESCs, efforts began to produce human ESC–derived NPCs and OPCs. The production of a high-purity population of OPCs from human ESCs was first achieved by Hans Keirstead and colleagues. They subsequently demonstrated that these human ESC–derived OPCs resulted in remyelination and significantly improved locomotor function when transplanted 1 week after SCI in the rat. Currently, this approach is being considered to begin preliminary clinical trials (see Havryluk et al. in this issue). The recent demonstration of the induction of an ESC-like pluripotentiality in human adult somatic cells (skin fibroblasts) using the expression of specific transcription factors (Sox2, klf4, c-Myc, and Oct4) has opened up new possibilities for stem cell–based approaches for CNS injury and all other conditions as the potential for production of patient-specific cells of any type now exists. These genetically “reprogrammed” cells are termed iPSCs. Currently, the viral transfection techniques required to induce the pluripotent state in somatic cells would exclude clinical application. However, further development of this technology, such as the identification of small-molecule agents to mimic the actions of transcription factors, may one day make this a clinically viable cell source that would obviate the need for donor cell harvesting or immune suppression. Although this technology remains in its infancy, work to date has indicated that iPSCs behave similarly to bona fide ESCs in their responses to differentiating signals and show no epigenetic abnormalities indicating that they are likely not associated with any risks greater than those of normal ESCs. The development of iPSCs is one of the most exciting developments within stem cell biology to date and continued investigation into their potential use as a source of NSCs or glial progenitors for use in SCI is highly warranted and the first data from their application in SCI models are eagerly anticipated.

**Adult-Derived NSCs**

The discovery of self-renewing NSCs in the adult mammalian CNS by Reynolds and Weiss and Weiss and colleagues revolutionized our view of the once thought static adult CNS. Following their discovery in the early 1990s, these cells were recognized as a potential means to promote the repair of the injured CNS. It has now been established that self-renewing NPC can be isolated from the subventricular zone throughout the neuroaxis, and have been isolated in humans, including being successfully isolated endoscopically from the ventricular wall during routine neurosurgical procedures. The Fehlings laboratory has demonstrated the therapeutic potential of adult murine NPCs derived from the subventricular zone of the forebrain to remyelinate axons and improve locomotor recovery following contusive SCI in the rat. To more directly assess the correlation between NPC–mediated remyelination and improved axonal functional, the effect of NPC transplantation on the conduction properties of the spinal cord of the congenitally dysmyelinated shiverer mice was investigated. Shiverer mice lack central myelin due to a genetic deletion in the gene encoding myelin basic protein, a key structural component of CNS myelin. It was observed that NPC–derived oligodendrocytes remyelinated axons in the shiverer spinal cord (Fig. 2), and these axons have improved axonal conduction measured electrophysiologically, and had normal-appearing nodes of Ranvier based on the establishment of the correct molecular organization of K and Na channels and the node-associated protein Caspr (contactin-associated protein).

**Limitations of Remyelination Therapies for SCI**

Remyelination strategies are among the most promising SCI therapies under development and could prove to be the first embryonic stem cell–based therapies to under-
go clinical trials in North America for any human condition. Although preclinical data in experimental SCI are highly encouraging, it must be understood that the potential functional improvement associated with any remyelinating therapy is limited by and a function of the number of spared, demyelinated axons present. As discussed earlier, the extent to which chronically demyelinated axons persist in human SCI is an unresolved issue. Therefore, within the highly heterogenous SCI population the efficacy of such treatments could potentially be highly variable among patients based on the characteristics of each specific lesion. Furthermore, the timing and methodology of such interventions remains to be optimized.

Bone Marrow Stromal and Hematopoietic Stem Cells

Adult mammalian bone marrow contains both HSCs and BMSCs, which are also known as MSCs. Both of these cell populations are unique, because they are ostensibly dedicated to the generation of hematopoietic cells such as erythrocytes and lymphocytes (HSCs) and mesenchymal cells such as osteocytes and chondrocytes (MSCs), and they both have been reported to have the ability to differentiate into neural lineage cells given the appropriate in vitro and in vivo conditions. The fact that they offer the potential for autologous transplantation, and that techniques for their harvesting from patients or donors are well established, make them appealing cell substrates for SCI repair.

The transplantation of HSCs into the injured spinal cord has been reported to promote functional recovery, but the mechanism behind this improvement (outside of merely surviving and differentiating) has not been well described and remains largely unknown. The transplantation of marrow stromal cells, on the other hand, has been shown to induce remyelination in the demyelinated spinal cord, although the myelin that was observed shared properties of both CNS and PNS myelin. The transplantation of MSCs into models of traumatic SCI has been inconsistently reported to result in some improved behavioral outcomes; however, in vivo the differentiation into neural lineage cells to support the remyelination is not particularly convincing, suggesting that other mechanisms may account for the enhanced myelination and functional improvements observed.

Conclusions

The structural and molecular pathology of SCI are formidable challenges to successful neural repair, challenges that we have yet to surpass, despite the best efforts of the scientific and clinical communities. A more comprehensive understanding of the complex biological processes, particularly in human cases of SCI, will be essential to our development of highly efficacious therapies. Efforts to induce regeneration and repair of the injured cord have led to translatable therapies directed at inhibiting myelin inhibitors, targeting intracellular messenger systems that mediate growth cone dynamics, and degrading the glial scar. Additionally, cell transplantation strategies have enormous momentum due to unparalleled scientific and public interest, although arguably many scientific and clinical aspects of a therapeutic transplantation paradigm still require resolution. The modest functional improvements obtained from the experimental therapies currently being developed clearly illustrates the need for combinatorial approaches that combine neuroprotective, neuroregenerative, and rehabilitative approaches in the hope of optimizing the recovery of individuals with traumatic SCI.

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