Spinal Cord Injury (SCI) is damage to the spinal cord that results in a loss of function such as mobility or feeling. Frequent causes of damage are trauma (car accident, gunshot, falls, etc.) or disease (polio, spina bifida, Friedrich's ataxia, etc.). The spinal cord does not have to be severed in order for a loss of functioning to occur. In fact, in most people with SCI, the spinal cord is intact, but the damage to it results in loss of functioning. The horseback riding injury of 'Superman' actor Christopher Reeve in 1995 generated considerable publicity and focused attention on the tragedy of SCI. Research on spinal injuries at this time was gathering momentum and scientists all around the world were making progress on what had been considered an untreatable injury. This review article summarizes the background on SCI and discusses the cellular and molecular changes that occur in the spinal cord following injury. The article further provides a glimpse into the ongoing cutting edge research that is aimed at developing pharmacological treatments for SCI. Products and compounds undergoing clinical trials are also discussed.

The spinal cord and the brain together make up the central nervous system (CNS). The brain and spinal cord are confined within bony cavities that protect them, but also render them vulnerable to compression damage caused by swelling or forceful injury. The spinal cord coordinates the body's movement and sensation. The spinal cord includes nerve cells, or neurons, and long nerve fibers called axons. Axons in the spinal cord carry signals downward from the brain (along descending pathways) and upward toward the brain (along ascending pathways). Neurons in both the central and peripheral nervous system (PNS) are surrounded by helper cells called glial cells. Glial cells help maintain the cellular environment, and two types, oligodendrocytes in the CNS and Schwann cells in the PNS make myelin sheaths. Myelin sheaths are thick layers of cell membranes that insulate the axons that carry electrochemical signals. Myelin increases the speed of transmission of signals from one nerve cell to the next, and without myelin the signal may deteriorate so much that it does not reach its target at all (fig. 1).

The spinal cord is organized into segments along its length. Nerves from each segment connect to specific regions of the body. The segments in the neck, or cervical region, referred to as C1 through C8, control signals to the neck, arms, and hands. Those in the thoracic or upper back region (T1 through T12) relay signals to the torso and some parts of the arms. Those in the upper lumbar or mid-back region just below the ribs (L1 through L5) control signals to the hips and legs. Finally, the sacral segments (S1 through S5) lie just below the lumbar segments in the mid-back and control signals to the groin, toes, and some parts of the legs. The effects of SCI at different segments reflect this organization (Table 1).

Statistics, incidence and prevalence:

In the US, it is estimated that the annual incidence of SCIs is approximately 10,000 new cases each year. More than 200,000 Americans are estimated to be living today with the disabling effects of such trauma (Source: Spinal Cord Injury Information Network).
Who and how:

SCI primarily affects young adults. Fifty-five percent of SCIs are thought to occur among persons in the 16-30 age group. SCI also occurs in a small percentage of the elderly population due to falls and degenerative diseases of the spine. Overall, about 80% of all persons who suffer from SCI are male. The contributors to SCI include motor vehicle accidents, violence (primarily gunshot wounds), sporting activities (such as diving, skiing, and surfing) and falls. Medical and surgical complications, injury as a result of falling objects and pedestrian accidents also result in SCI (fig. 2). Whatever the cause, the outcome of severe damage to the spinal cord is the same: full or partial paralysis and loss of sensation below the level of the injury. (Source: Christopher Reeve Paralysis Foundation).

Economic impact:

Because spinal cord injuries usually occur in early adulthood, those affected often require costly supportive care for many decades. The individual costs may exceed US $250,000 per year, placing an often-overwhelming financial burden on these individuals and their families. In the US these costs add up to an estimated $10 billion per year for medical and supportive care alone. A typical quadriplegic's expenses can exceed $400,000 in the first year, and annual maintenance costs can average $100,000 after that. Fewer than 30 percent work. Of course, no dollar figure can describe the human costs to spinal cord injured people and their families (Source: Christopher Reeve Paralysis Foundation).

Histopathological changes:

The effects of SCI depend on the type of injury and the level of the injury. SCI can be divided into two types of injury - complete and incomplete. A complete injury means that there is no function below the level of the injury; no sensation and no voluntary movement. Both sides of the body are equally affected. An incomplete injury means that there is some functioning below the primary level of the injury. A person with an incomplete injury may be able to move one limb more than another, may be able to feel parts of the body that cannot be
TABLE 1: RELATIONSHIP BETWEEN THE SPINAL CORD DIVISIONS, THEIR ASSOCIATED NERVES AND THE FUNCTIONS AFFECTED AS A RESULT OF INJURY TO THE PARTICULAR LEVELS

<table>
<thead>
<tr>
<th>Spinal cord divisions</th>
<th>Nerves</th>
<th>Functions affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>CERVICAL</td>
<td>C1-C4</td>
<td>Breathing</td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td>Head-neck movements</td>
</tr>
<tr>
<td></td>
<td>C4-C6</td>
<td>Heart Rate</td>
</tr>
<tr>
<td></td>
<td>C5</td>
<td>Shoulder movement</td>
</tr>
<tr>
<td></td>
<td>C6-C7</td>
<td>Wrist and elbow movement</td>
</tr>
<tr>
<td></td>
<td>C7-T1</td>
<td>Hand and finger movement</td>
</tr>
<tr>
<td>THORACIC</td>
<td>T1-T12</td>
<td>Sympathetic tone, temperature regulation</td>
</tr>
<tr>
<td></td>
<td>T2-T12</td>
<td>Trunk stability</td>
</tr>
<tr>
<td></td>
<td>T11-L2</td>
<td>Ejaculation</td>
</tr>
<tr>
<td>LUMBAR</td>
<td>L2</td>
<td>Hip motion</td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>Knee extension</td>
</tr>
<tr>
<td></td>
<td>L4-S1</td>
<td>Foot motion</td>
</tr>
<tr>
<td></td>
<td>L5</td>
<td>Knee flexion</td>
</tr>
<tr>
<td>SACRAL</td>
<td>S2-S4</td>
<td>Penile erection</td>
</tr>
<tr>
<td></td>
<td>S2-S3</td>
<td>Bowel and bladder activity</td>
</tr>
</tbody>
</table>

moved, or may have more functioning on one side of the body than the other. With the advances in acute treatment of SCI, incomplete injuries are becoming more common.

The types of disability associated with SCI vary greatly depending on the severity of the injury, the segment of the spinal cord at which the injury occurs, and which nerve fibers are damaged. The level of injury is very helpful in predicting what parts of the body might be affected by paralysis and loss of function. The destruction of nerve fibers that carry motor signals from the brain to the torso and limbs leads to muscle paralysis. Destruction of sensory nerve fibers can lead to loss of sensations such as touch, pressure, and temperature; it sometimes also causes pain. Other serious consequences can include exaggerated reflexes; loss of bladder and bowel control; sexual dysfunction; lost or decreased breathing capacity; impaired cough reflexes; and spasticity.

Primary damage:

Nerve cells of the brain and spinal cord respond to insults differently from most other cells of the body, including those in the PNS. The most common types of spinal cord injuries include contusions, compression, and laceration injuries. In contusion injuries, which occur as a result of bruising of the spinal cord, there is focal hemorrhage in the center of the cord that evolves to become a fluid filled cyst. Massive cord compression usually occurs after a non-penetrating injury resulting in maceration and severe distortion of the cord parenchyma. In such injuries, the tissue topography is lost and connective tissue scar and fragments of nerve roots replace the epicenter of the lesion. Laceration injuries result when the spinal cord is directly torn by injury as a result of sharp penetrating objects. The tissue surrounding the injury area is eventually associated with prominent connective tissue (fibrous) scarring. Complete severing of the spinal cord is rare in humans. Whatever the insult, the mechanical damage results in direct tissue disruption, motion stresses to the damaged cord, and persistent compression of the neural tissue. Axons that survive the initial damage usually lose their myelin covering. This
demyelination greatly slows the speed of nerve transmission. Most people with SCI regain some functions between a week and six months after injury, but the likelihood of spontaneous recovery diminishes after six months. Rehabilitation strategies are currently being employed to minimize the long-term disability.

Secondary damage:

Damage to the spinal cord does not stop with the initial injury, but continues for months following trauma. There is no single point at which to begin describing when the intricately intertwined cellular and molecular events occur following SCI. These secondary effects include edema, vascular injury, ischemia, hemorrhage, hyperthermia, inflammation, production of oxidative free radicals, calcium-mediated damage, excitotoxicity, release of proteases and cytotoxic dysfunction. These effects interact in complex ways to exacerbate the primary damage. However, what is encouraging is that scientists are now beginning to understand that each of these harmful processes offers targets for developing therapies.

Within the first few hours after injury, major changes occur in the gray matter where hemorrhage results due to the breakdown of the blood-brain barrier. The initial edema leads to distortion of the small vessels with alteration in perfusion, and after maximum swelling, progressive axonal loss and total tissue destruction occurs. In the next few days, vascular injury, necrosis, inflammation, axonal tears and swelling and vesciculation of myelin sheaths are evident. Although the nervous system is normally an immunologically privileged site, breach of the BBB results in a rapid and robust invasion of inflammatory cells into the damaged nervous system. This inflammatory response is characterized by fluid accumulation, and the influx of plasma proteins, neutrophils, T lymphocytes and macrophages. Microglial cells, which are normally found in the CNS become activated in response to CNS damage.

It is not clear which exact signals control the entry of immune cells into the CNS. Release of a diverse group of diffusible messenger molecules and cell adhesion molecules on the surfaces of cells appear to control the traffic of immune cells into the spinal cord. It is not always clear to what extent immune reactions help or harm prospects for recovery, although immune reactions do appear to cause some secondary damage. What immune cells do once they enter the damaged spinal cord is only now beginning to be understood. Numerous studies have suggested that the initial infiltration of the hematogenous cells maybe due to the secretion of cytokines and chemokines in the injured spinal cord. Others and we have shown that in addition to macrophages, monocytes, and microglial cells, astrocytes, which were once thought to be only structurally supportive, also produce a wide variety of powerful regulatory substances.

The production of chemokines, particularly monocyte chemoattractant protein-1 (MCP-1), has been closely associated with macrophage accumulation in the injured CNS. Whether preventing immune cell entry into the damaged spinal cord would be beneficial for recovery has been a focus of investigation in several laboratories. We have used broad-spectrum chemokine antagonists and antisense oligonucleotides in animal models of

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**Fig. 3: Flow diagram illustration of the cellular and molecular processes that occur as a result of SCI**
contusion and brain injuries to study the role of hematogenous cells in CNS injury. Our studies have shown that a sustained reduction in posttraumatic cellular infiltration is beneficial for tissue survival. Even a modest decrease in the infiltrating cells within the CNS parenchyma was sufficient to suppress gliotic reaction, reduce axonal degeneration in the gray matter and increase neuronal survival in the ventral horns. On the other hand, potentially beneficial substances (such as growth factors) released by the immune cells, and their ability to phagocytose cellular debris may make them essential for promoting regeneration in the damaged CNS.

The extent of tissue degeneration depends on the severity of injury and time interval following trauma. In the few days following injury, glutamate induced excitotoxicity also contributes to the secondary damage. N-methyl D-aspartate (NMDA) and non-NMDA mediated excitotoxicity leads to neuronal and glial cell death. Increased activities of degradative enzymes (hydrolyses and proteases) also contribute to tissue degeneration. The increased understanding of the role of oxidative stress in SCI has led to pharmacological strategies to combat this problem.

Damage to the spinal cord results in cell death. Recent work has shown that the effects of cell death play a role in neuron and oligodendrocyte loss after SCI: necrosis and apoptosis. Necrosis, a relatively uncontrolled process, is believed to occur as a result of primary damage. Apoptosis, (programmed cell death) on the other hand, occurs in days and weeks following injury. The relationship between apoptosis and necrosis, the role that each plays in SCI, the signals that regulate cell death, and the potential to halt death programs are now being explored to find ways of minimizing secondary damage following SCI.

Studies on mapping the positions of apoptotic cells within injured spinal cords have revealed interesting patterns. Apoptosis of nerve cells is largely restricted to sites near the impact zone itself and generally occurs within about 8 h of the trauma. Apoptosis in glial cells, on the other hand, is much more prolonged, with a second wave of apoptosis occurring probably among oligodendrocytes at about 7 d after injury. This wave of secondary death ripples out much further than the original site of injury. These results have provided important clues in defining the time windows during which therapeutic intervention might be beneficial. Since neurons and oligodendrocytes show different temporal patterns of cell death, optimal strategies for saving each of them may be different.

Although cell death is prominent following SCI, it is important to emphasize that damage to axons causes most of the problems associated with the injury, including loss of motor control and sensation. Animal experiments have clearly indicated that recovery of function correlates closely to the number of remaining axons. Following axon injury, axons disconnected from their nerve cell bodies disintegrate by a process called "Wallerian" or "orthograde" degeneration. The inability of the damaged axons to regrow in the injured milieu of the CNS, results in functional loss. Although some axons survive the initial damage, lytic enzymes and other substances released by the infiltrating neutrophils and macrophages result in demyelination. Demyelination starts within the first 24 h of injury and increases over the next several days. Loss of myelin sheath around the spared axons results in slowing the speed of nerve transmission.

Extensive glial proliferation (reactive astrogliosis) is seen within days after injury. The cells sometimes grow so densely that they seem to provide a mechanical obstacle to axonal growth. Studies by Davies et al. show that axons stop at gliotic-injured areas of the spinal cord, supporting the contention that gliosis is an impediment to regeneration. In addition to the physical barrier, glial cells secrete extracellular matrix factors (particularly chondroitin sulfate) that also seem to inhibit axonal growth.

Following weeks after injury, cord damage results in cavitation, numerous axonal spheroids, macrophages and calcific deposits. Following a healing period in which removal of necrotic debris, resolution of edema, revascularization, and reestablishment of the BBB occurs, the later events (months) are associated with the formation of cysts/cavities, glial scar, fibrosis, syringomyelia, spinal root regeneration, schwannosis and varying degrees of axonal degeneration and demyelination.

Treatment for SCI:

Designing effective therapies for SCI has been a challenging problem because spinal cord injuries are heterogeneous in causality, severity, and location of injury. Despite the complications, medical care of SCI has advanced greatly in the last 50 y. Because of general progress in neuroscience, as well as specific advances...
in SCI research, several new ideas are being tested about how changes in molecules, cells, and their complex interactions affect SCI. Findings from other fields, such as development, immunology, and stroke research, are being applied to the study of SCI. Although researchers are wary of giving people false hope for curing SCI, accelerating progress in basic and applied research, has renewed vitality and growing optimism among investigators that, with continued effort, the problems of SCI will be overcome. However, it seems highly likely that different treatments will be required for different types of damage and different stages of recovery. In general, the treatment approaches practiced and envisioned for SCI have been three pronged: containment of damage, promoting regeneration for functional recovery and improving the quality of life.

Current interventions and drug therapy:

Significant advances in recent years, including an effective drug therapy for acute SCI and better imaging techniques for diagnosing spinal damage, have improved the recovery of patients with spinal cord injuries. Current care of acute SCI is focused on minimizing the initial damage and improving the quality of life. Physicians diagnose and relieve cord compression, gross misalignments of the spine, and other structural problems. Second, they minimize cellular-level damage, and finally, they stabilize the vertebrae to prevent further injury. Techniques such as cord irrigation and cooling are being used to reduce secondary damage.

Effective drug therapy for SCI first became a reality in 1990 with the finding that the steroid drug methylprednisolone (MP) can significantly improve recovery. Clinical trials demonstrated that there is an 8 hour window of opportunity for treatment after injury and MP has now moved to standard use. Animal studies on the postulated effects of MP showed that its positive effect might be due to it acting as a free radical formation inhibitor as well as an antiinflammatory/immunosuppressive agent. Other agents, such as trilazid mesylate and naloxone, with improved antioxidant potency were developed. However, after comparing them in clinical trials to MP, MP was found to be the only therapeutic agent effective in reducing neurological deficit after SCI. Other mechanisms by which MP may enhance neurological recovery include proteinase inhibition, reduction of cytokines such as tumor necrosis factor alpha and neurogenerative sprouting.

While people with spinal cord injuries wait for cures to come, neural prostheses are being developed that can help patients regain some use of their limbs and improve quality of life. These electronic and mechanical devices, such as hand-grasp prostheses, connect with the nervous system to supplement or replace lost motor or sensory function. NeuroControl markets a device for quadriplegics that restores the ability to grasp, hold, and release objects with their hands. Freehand System works by using an electrical stimulation to replace the brain's original nerve impulses. Another product on the US market is a tongue-touch keypad, a miniature circuit board attached to a retainer with nine buttons the user presses with his tongue to operate a wheelchair and any electronic system in the house, like lights and the TV. Devices such as prostheses to control bladder function and to help people stand are now in development or planning stages.

In addition to neural prostheses, rehabilitation strategies have been found to greatly improve patients' health and quality of life. One intriguing idea that is gaining momentum is called 'spinal cord training'. Researchers believe that stimulating the injured spinal cord by constant sensory inputs will 'train' the neural system to form alternate pathway connections, which may restore partial function. Some natural rewiring of spinal circuitry after injury has been demonstrated, as has the spinal cord's ability to "learn," i.e., strengthen existing neural patterns, such as reflexes or spinal locomotion rhythm generators, following training. It is clear from the literature that the environmental surroundings of an injured animal, including the amount of sensory input, appear to play a major role in patterns of recovery. For example, supported ambulation exercises on treadmills have been shown to restore locomotion in some people who have been unable to walk for years.

In addition, agents to alleviate or minimize underlying pain, spasticity, muscle weakness, sexual dysfunction and incoordination due to SCI are being used. In some cases, drugs available for other diseases and disorders are being tested for treating these problems. At present, once a patient's condition is stabilized, care and treatment focus on supportive care and rehabilitation strategies. The ultimate hope, of course, is not just to minimize damage, but also to foster recovery.
### TABLE 2: TARGETS FOR THERAPY FOLLOWING SPINAL CORD INJURY

<table>
<thead>
<tr>
<th>PREVENT EXPANSION OF INITIAL DAMAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Deliver agents that block excitotoxic injury</td>
</tr>
<tr>
<td>• Administer anti-apoptotic compounds</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>REPLACE DEAD CELLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Implant cells able to produce lost cell types</td>
</tr>
<tr>
<td>• Deliver substances that can promote differentiation of immature cells into desired cell types</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>PROMOTE AXONAL REGENERATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Administer agents that induce axonal regeneration</td>
</tr>
<tr>
<td>• Implant tissue that can serve as a bridge for axons and encourage them to grow</td>
</tr>
<tr>
<td>• Supply guidance molecules for growing axons to form proper connections</td>
</tr>
<tr>
<td>• Remove barriers for easy regrowth of regenerating axons</td>
</tr>
<tr>
<td>• Deliver agents that neutralize inhibitors of axonal growth</td>
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<table>
<thead>
<tr>
<th>COMPENSATE FOR DEMYELINATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Supply chemicals that prevent nerve impulses from dissipating at demyelinated areas</td>
</tr>
<tr>
<td>• Provide agents that would promote oligodendroglial survival to encourage remyelination</td>
</tr>
<tr>
<td>• Transplant cells that will promote remyelination</td>
</tr>
</tbody>
</table>

**Experimental approaches for regeneration:**

While current treatment focuses on initial damage containment and improving quality of life, a concerted effort from several research laboratories has focused on developing strategies to promote axonal regeneration. Important advances have been made in our understanding of conditions that influence the intrinsic capacity of mature CNS neurons to initiate and maintain a regrowth response. The combination of exogenous support with strategies to alter the terrain at the injury site itself suggests that there are important interactions between them that lead to increased axonal regeneration. These findings indicate that the windows of opportunity for enhancing growth after SCI may be more numerous than previously thought.

According to scientists, one reason regeneration does not occur in the CNS is that the environment of the spinal cord does not encourage repair and growth of axons. The environment may not be helpful for a number of reasons, some of which are related to the chemicals that contact the injured cell while others are related to the physical environment at the site of injury (or the surfaces to which an axon can attach). Some reasons postulated for poor growth include: death of the neuronal/glial cells, insufficient chemical signals to encourage axons to regrow, the presence of chemical signals that stop axon growth and, physical surfaces around the cell that do not support axon growth.

Research activities geared towards finding a treatment for SCI have focused on targeting the different cellular and molecular events that occur following injury. The approaches thus taken to combat these events include preventing expansion of initial damage, cell transplantation to replace the dead cell population, infusion of factors that promote regeneration and directing axons to proper targets, compensating for demyelination and, inhibition of molecules that prevent axonal growth (Table 2).

**Cell transplantation:**

For successful regeneration to occur following SCI, several things must happen. First, damaged nerve cells and supporting cells must survive or be replaced, despite the acute effects of trauma and the conspiracy of processes that cause secondary damage. Replacement of lost cells in the CNS is unlikely without intervention...
because adult nerve cells in the brain and spinal cord cannot divide. Cell transplantation has been attempted to not only replace the lost neurons, but also provide trophic factors or other substrates that may be required for successful regeneration of the axons.

Because neurons die and those surviving cannot divide, one approach is to replace the dead neuronal cell population. Recently, it was found that there is a small pool of progenitor cells within the CNS that can be made to differentiate into mature neurons. Another approach is the use of fetal neuronal cells, which can divide making them an alternate method of replacing the dead neuronal population.

The clinical prospect of using neural precursor cells for reconstructive approaches in the nervous system has received strong impetus from a recent series of important experimental findings. Transplantation studies in the developing brain have demonstrated that migration and differentiation of neural precursor cells are regulated predominantly by environmental signals. Several observations suggest that the mature CNS retains at least some of these guidance cues. These findings, together with recent evidence for the persistence of neural stem cells in the adult mammalian brain, have made precursor cell recruitment a new focus in CNS reconstruction\textsuperscript{40,41}.

Stem cells have received great attention recently as potential treatments for SCI. Stem cells are primitive cells that can be stimulated to mature into many types of cells and thus may be the best chance at reconstructing the damaged SC\textsuperscript{42}. Researchers are working to learn how to control the development of these cells, a key to applying them to restore function\textsuperscript{43}. Acorda Therapeutics has patented stem cells that produce only neurons.

Although transplantation of adult nerve cells shows little promise, fetal nerve cell transplantation has been quite successful in laboratory animals\textsuperscript{44,45}. Grafting of fetal motor nerve cells (neurons that signal muscles to contract) to restore movement in injured rodents has shown significant promise. Some evidence suggest that fetal tissue transplants into spinal cord produce little glial scarring at the interfaces between the transplant and the host cells; yet, in most of these experiments, host axons penetrate into the fetal transplants but do not leave the fetal transplants in large numbers. Human safety trials are underway with fetal cell transplants. These trials have assessed the safety of surgical manipulations and assisted in the calculation of volumes of cell suspensions that might be needed to fill a cyst or area of spinal cord contusion. It is possible that information gained from these clinical studies may help to develop a reasonable plan for the surgical interventions. Unfortunately, these studies have also indicated that only a few of the transplanted cells survive and turn into neurons.

Transplanting cells into the damaged region has also been aimed at improving the environment of the spinal cord so that it is more encouraging to axonal growth. Transplants can promote recovery by providing growth promoting substances as well as a surface over which damaged axons can regrow. Since axons have not been found to grow well on all surfaces, some cells and tissues have been found that encourage nerve-fiber growth.

Majority of the tissue transplant studies have emerged from many decades of animal research with PNS tissue, where regeneration of axons occurs successfully. Glial cells of the PNS, Schwann cells, have been extensively shown to support regeneration in the damaged CNS. Researchers at the Miami Project to Cure Paralysis have developed a bridge made up of Schwann cells, which insulates nerve axons as they carry messages throughout the body. Inserted at the injured ends of a spinal cord, Schwann cells secrete growth factors that encourage regeneration, and allow the normal functioning of regenerated axons by remyelinating them. Methods have already been developed to acquire the cell populations needed for human autotransplantation, and research has shown that these cells effectively elicit neurite growth from adult human CNS cells. Although transplantation studies have repeatedly demonstrated that transplanted Schwann cells promote regeneration in animal models of spinal cord injury, their ability to enhance successful axonal regeneration beyond the extent of the grafts is limited\textsuperscript{46}. Genetically modified Schwann cells secreting growth factors have also been shown to promote CNS regeneration\textsuperscript{47,48}.

Transplantation of other types of genetically modified cells has also been successful in promoting some functional recovery. Among them are fibroblasts, cells that can be easily grown from skin or other tissues. Researchers have shown that genetically engineered fibroblasts are excellent candidates for gene therapy because these cells secrete the growth factors in both \textit{in vitro} and \textit{in vivo} experiments. Using animal models of SCI, these researchers showed that grafting fibroblasts
could promote regeneration, which was associated with improved limb function. Animal studies have also shown that engineered fibroblasts can act as a bridge\(^9\). Fetal tissues from developing animals have also been found to promote growth; these are believed to support growth because they come from the nervous system at a time when axons are growing normally\(^42\). Olfactory ensheathing glia (OEG) has shown great promise as chaperones in guiding growing nerve fibers through the inhibitory areas. OEG transplants like Schwann cells may also myelinate CNS axons. The exact functions of these cells are being studied, as are techniques for obtaining human OEG\(^30\).

Activated macrophages\(^14\) and activated T-lymphocytes\(^81\) have been reported to regenerate and protect spinal cord after injury in animal studies. Engineered nerve and adrenal cell lines are being developed that secrete selected neurotransmitters and/or growth factors for use in cellular therapies to alleviate chronic pain following nerve and spinal injury in rodents\(^32\).

**Growth factors:**

Axonal growth occurs during normal development of the CNS as a result of factors present in the CNS environment. These factors not only promote growth of the axons, but also guide them to the proper target so that effective synaptic connections are established. Some of the growth factors also maintain the health of neurons during adulthood. From a regeneration point of view, several different growth promoting and guiding molecules are being tested to coax the injured axons of the CNS into regrowing and forming the appropriate connections.

The first glimmer of hope for CNS regeneration came in 1991, when researchers at McGill University elegantly showed that lesioned CNS neurons could regenerate if they were provided with a bridge made of tissue from the PNS\(^53\). These studies suggested that the lack of regeneration in the injured CNS was not due to inherent inability of the CNS neurons to grow, but rather the lack of appropriate growth promoting substances in the injured CNS. These studies opened an entire new avenue for tackling SCI. Based on these findings; considerable effort has now been dedicated to finding the right growth promoting molecules that would promote regeneration in the injured CNS. Infusion of individual growth factors or combination of growth factors has been attempted in animal studies to study regeneration.

One important family of growth factors that has shown a great deal of promise in encouraging axonal growth is the neurotrophins. In animal models of SCI, their application has shown improvement in the health of damaged neurons and increased nerve-fiber growth. The main members of this family consist of four proteins: nerve growth factor (NGF); brain derived growth factor (BDNF), neurotrophin 3 (NT-3) and neurotrophin 4 (NT-4). These factors have both paracrine and autocrine activities. In nerve cells, expression of neurotrophic factors is regulated by physiological afferent activity, which implies that these factors play a role in activity-dependent plasticity and survival of the nerve cells. Neurotrophic factor levels are also altered following injury, which suggests that they play a part in the neurodegenerative response and synaptic reorganization as well\(^54\).

NT-3 and BDNF have received considerable attention with respect to SCI. NT-3 and BDNF have both been shown to promote cell survival by blocking apoptosis and stimulate axonal growth. Both factors, alone or in combination have been reported to enhance axonal regeneration in several types of neurons\(^55,56\). On the other hand, some neurons (e.g. optic nerve), do not respond to NT-3. Other studies have shown that although the growth factors promote axonal growth, these axons do not form synaptic connections. In addition, animal studies showed that NT-3 and BDNF infusion into the brain caused several problems such as marked weight loss, reduce water drinking and marked appetite suppression\(^57\).

In addition to the neurotrophins, other growth factors reported to stimulate neuronal growth include fibroblast growth factor (FGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), and ciliary neuronotrophic factor (CNTF)\(^58,60\).

**Guidance molecules:**

Substrate or guidance molecules to promote new growth have also been proposed as possible therapeutic approaches\(^61\). Substrate and guidance molecules may improve targeting once axons have been encouraged to regenerate past the lesion site. These proteins act as highway road maps to steer axons to the correct targets. Diffusible guidance cues are now understood to play an important role in axon guidance. Just as the axons during development grow and make the proper connections, the regrowing axons after injury, once elongated, must construct the highly specialized structures that release neurotransmitters from nerve terminals. Cells that receive signals across synapses also must participate in forming
new synapses at a time when they would not normally do so. Finally, the regenerating spinal cord must insure that synapses of the correct type form only on proper cells and on the appropriate parts of these cells so that the neural circuits will work.

Within the past several years, researchers have discovered new proteins, which influence the growth of axons during nervous-system development. Among these are the netrins, a family of laminin-related secreted proteins that are critical in controlling axon elongation and pathfinding. The identification of the netrins as potent stimulators and orienters of axon growth in the embryo has given rise to the hope that the netrins might be useful for stimulating regeneration of axons in the adult nervous system following injury or trauma. Netrin 1 is a long-range diffusible factor that exerts chemoattractive or chemorepulsive effects on developing axons growing to or away from the neural midline.

Several laboratories have also reported that cellular adhesion molecules (CAMs) play a major role in axonal growth. CAMs are molecules that are present on membrane surfaces and have long been believed to guide growing axons. L1 found in the developing, but not mature CNS, has been shown to strongly stimulate axonal growth following injury in animal studies.

Whether regenerating axons respond to guidance signals in the same manner as developing axons and whether these cues are still present in the adult spinal cord are particularly important questions for spinal cord regeneration. So far, nerve fibers regrowing in experimental animal models of spinal cord regeneration have developed few new synapses, and this may be the limiting factor in recovery of movement. Understanding how synapses develop may reveal whether spinal regeneration stops because regenerating axons lack the ability to form synapses or whether nerve cells below the lesion are un receptive to synapse formation. This may lead to ways of encouraging the formation of new synapses by regenerating fibers.

**Antinhibitory antibodies:**

Just as lack of positive factors has been suggested to impede CNS regeneration, the discovery of molecules within the adult spinal cord, which prevents axons from growing has led to another approach to combat SCI. Significant research efforts are being devoted towards overcoming the inhibition so as to stimulate axonal regrowth and regeneration. Identification of inhibitory molecules in the injured CNS led to the generation of anti-inhibitory antibodies as a potential therapeutic approach to promoting regeneration in the injured CNS.

Fractionation studies of myelin extracts by Schwab and co-workers demonstrated the presence of two inhibitors, NI35 and NI250, of molecular mass 35 and 250 kDa, respectively. NI250 was purified and characterized and is now called Nogo-A, and is a member of the reticulin protein family. The IN-1 antibody, which blocks the inhibitory action of Nogo-A in *in vitro* assays, has been shown to promote axonal penetration across injury sites and long distance growth in the injured spinal cord. It is reasonable to attribute this important effect of IN-1 to inhibition of Nogo-A, although some of the effect could be contributed by blockade of NI35 or other cross-reactive proteins. Unfortunately, IN-1 is a mouse antibody that cannot be given to humans. While attempts to produce a form of IN-1 suitable for human administration have been made several times, none have yielded an antibody that has been effective *in vivo*.

In addition, myelin-associated glycoprotein (MAG), a well-characterized myelin protein, has also been identified as an inhibitor of axonal regeneration. Analysis of knockout mice deficient in MAG, showed, however, that in the absence of MAG, other inhibitors still blocked extensive regeneration. These results suggest one might expect that blockade of any single inhibitory system might not permit more than a modest amount of axon regrowth. Further support for this idea was provided by a recent study which showed that injection of crude myelin extracts in animal studies stimulated much more axonal regeneration than when any one protein alone is blocked. Although myelin injections into humans are impractical because of fears of multiple sclerosis, these experiments suggest that effective therapy will follow once the important inhibitors are molecularly defined. Thus blockade of multiple systems simultaneously—for example, by removal of both MAG and Nogo-A is currently being studied in animal models. Note that IN-1 is a germ-cell line antibody that is coded in our genome and it is possible that vaccination will stimulate the production of our own endogenous IN-1 and other antibodies to enhance regeneration and remyelination.

Other proteins, including members of the semaphorin, netrin, ephrin, and slit axon guidance protein families,
have been identified that inhibit axon growth during development and are candidates for contributing to inhibition in the adult CNS.\textsuperscript{76,77}

Removal of glial scar:

Regeneration not only requires overcoming the CNS neurons’ resistance to grow, but also requires overcoming other barriers. One obvious physical barrier is the scar tissue formed due to the prolific proliferation of the supporting astroglial cells. Glial scar has long been the main theory held by scientists trying to explain why regeneration does not occur in the central nervous system.\textsuperscript{78,79} Several therapies achieved functional regeneration in rodent spinal cords without eliminating glial scars. Elimination of the glial scars does not seem to be sufficient to allow regrowth of axons across injury sites. For example, several optic nerve regeneration studies suggest that growing axons can secrete enzymes that will digest their way through gliotic areas.

Surgical removal of the glial scar has been proposed to promote regeneration. However, some researchers think that surgical removal of glial scars is not a good idea because there is no such thing as atraumatic surgery. Other than removing the portions of the spinal cord that contain scar and applying some therapy that prevents scar formation, surgery will produce more scar. If scars are to be removed, the best approach is probably through a specific drug or factor that targets glial cells. This may be through antibodies or a drug that prevents proliferation of glial cells. At the present, however, there is no convincing therapy that dissolves existing glial scars in spinal cord.

Administration of CM101, a bacterial polysaccharide, was reported by Wamad et al.\textsuperscript{80} to mediate walking recovery in adult rats paralyzed by SCI. CM101 was thought to mediate its positive action by reducing the glial scarring. However, the study was not particularly convincing and no other laboratory has yet reported a replication of this study.

Remyelination:

Although some types of SCIs may require replacement of nerve tissue, other injuries leave some nerve fibers intact. However, these fibers have often lost their myelin and may not conduct nerve impulses at the correct speed. Therapies that restore nerve-impulse transmission in these fibers might help many individuals with SCI recover some sensory and motor function.

One approach to this problem is in clinical testing but awaits FDA approval: the long-term use of the drug 4-aminopyridine (4-AP). For nerve impulses to pass rapidly along axons, it is important that some types of membrane channels be “covered up” by the myelin coating and other channels be left exposed to help electrical current pass through the membrane. Although 4-AP does not replace the myelin, it can interact with membrane channels the same way myelin does to increase the speed of nerve signals. Treatment with 4-AP appears to improve sensory and motor function in some—but not all—patients who have used it in clinical testing.\textsuperscript{81}

Another approach to helping surviving axons in the spinal cord is to transplant cells that make myelin into the damaged region. Under specific conditions, cells like oligodendrocytes and Schwann cells may be able to replace the myelin coating around axons that is lost as a result of SCI. Restoring this coating may improve nerve-signal transmission. Although at present this technology is used only in animal models of SCI, this therapy may someday be available to people as well.\textsuperscript{82,83}

Combination therapy:

While most researchers agree that combination therapies would be necessary to optimize regeneration, only recently have studies actually looked at combination of therapies to determine which are synergistic, complimentary, redundant, or even antagonistic.\textsuperscript{84} An exciting direction of current research deals with genetically engineered Schwann cells or fibroblasts to synthesize and secrete specific neurotrophins in an attempt to provide more robust regeneration. Studies are ongoing; therefore, to determine what combination of cells and growth factors may best be used to promote successful regeneration and restoration of function. This is an extremely active field of research, and scientists agree that a combination of strategies, including transplantation and growth factor administration will most likely be needed for the cure. A combination of NT-3 and the IN1 antibody has shown significant promise in animal studies.\textsuperscript{85} Peripheral nerve grafts in combination with IN 1 antibody and acidic FGF fibrin glue have been shown to improve corticospinal tract regeneration.\textsuperscript{86} Recent data have suggested that the combination of olfactory ensheathing glial cells together with Schwann cells enhance regeneration far beyond the graft site into host target areas.\textsuperscript{87} The use of anti-inflammatory agents or other neuroprotective strategies prior to or in combination with surgical interventions may...
TABLE 3: RANDOMIZED PROSPECTIVE CONTROLLED TRIALS IN ACUTE SCI IN HUMANS

<table>
<thead>
<tr>
<th>Trial</th>
<th>Year</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylprednisolone</td>
<td>1994</td>
<td>No difference</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>1990</td>
<td>MP improved neurological recovery</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>1997</td>
<td>MP improved neurological recovery</td>
</tr>
<tr>
<td>MP trial in Japan</td>
<td>1994</td>
<td>MP improved neurological recovery</td>
</tr>
<tr>
<td>Ganglioside GM-1</td>
<td>1991</td>
<td>GM-1 improved neurological recovery</td>
</tr>
<tr>
<td>Ganglioside GM-1</td>
<td>1998</td>
<td>Final result pending</td>
</tr>
<tr>
<td>Thyrotropin releasing hormone</td>
<td>1995</td>
<td>No difference</td>
</tr>
<tr>
<td>Gacyclidine</td>
<td>In progress</td>
<td></td>
</tr>
<tr>
<td>Nimodipine</td>
<td>1998</td>
<td>No difference</td>
</tr>
<tr>
<td>Decompression</td>
<td>1997</td>
<td>No difference</td>
</tr>
</tbody>
</table>

well be an important new strategy in the field of spinal cord regeneration.

Other treatments:

In addition to targeting specific events that occur during the progression of SCI, several other treatments have also been proposed for SCI. These have been based on either cell culture or animal experiments. Insights gained from studies on other neurodegenerative diseases have also contributed to these treatments. These include: mild lowering of body temperature (hypothermia)\(^\text{98}\) and radiation therapy\(^\text{99,100}\). Electromagnetic fields have also been reported to stimulate regeneration\(^\text{101,102}\).

Among other compounds that have shown promise include inosine, AF-1, agmatine and interleukin-10. Inosine, a purine nucleoside has been shown to induce axon outgrowth from primary neurons in culture through a direct intracellular mechanism\(^\text{83}\). Axogenesis factor 1 (AF-1), derived from the sheath cells of goldfish optic nerves, is a small (700-900 daltons), heat stable, protease K labile peptide. AF-1 stimulated regeneration of goldfish retinal ganglion cells in culture\(^\text{84}\). Agmatine is an endogenous ligand at imidazoline receptors and has been shown to reduce pain associated with SCI\(^\text{85}\). Interleukin-10, a bioactive molecule that naturally limits inflammatory responses has been shown to be neuroprotective when administered soon after injury\(^\text{86}\).

Whether these compounds, either alone or in combination with additional therapies, will constitute SCI treatments still awaits considerable investigation. None of them have been evaluated for SCI therapy in controlled clinical trials.

Clinical Trials:

To date, there have been 10 randomized prospective trials in acute SCI. In four of these trials the role of MP has been examined, in two GM-1 ganglioside (GM-1) has been studied, and in one each thyrotropin releasing hormone (TRH), gacyclidine (GK-11, an n-methyl-D-aspartate receptor antagonist), or nimodipine (a calcium channel blocker) has been examined. In the remaining trial early and late decompressive surgery were compared and assessed\(^\text{97}\). The results of these trials are summarized in Table 3.

Clinical trials are currently ongoing at several centers that are testing novel strategies to improve motor performance in patients with complete and incomplete spinal cord injuries. It is apparent from a vast amount of experimental and clinical literature that rehabilitation strategies after CNS injury improve motor and sensory function. Thus, even with successful regeneration of the injured spinal cord, rehabilitation strategies will be necessary to guide or modify newly growing circuits to perform necessary functions.

Acorda Therapeutics is testing a slow release formulation of 4-aminopyridine (potassium channel blocker) in phase II/III trials. Elan Corporation supplies the formulation. Proneuron Biotechnologies hopes to combat the scar tissue problem with autotransplanted macrophages and is enrolling patients for their autologous activated macrophage therapy. The company believes
that timely augmentation of quantity and potency of macrophages adjacent to the site of injury will assist in the regrowth of nerve fibers, providing neuroregenerative therapy. Pain following SCI remains problematic for a subset of the spinal cord injury population. The mechanisms underlying neuropathic pain are not fully elucidated. Neurontin (gabapentin), a synthetic structural analog of gamma amino butyric acid has been shown to have beneficial effects in the treatment of neuropathic pain. The efficacy of neurontin in the treatment of SCI is being evaluated at a Clinical Rehabilitation Center in New Jersey, USA. In addition, a randomized crossover double-blind trial of neurontin and methadone (a long term analgesic) is also underway. Acupuncture has also been evaluated in the treatment of pain following SCI. Preliminary results have indicated that it is effective in most forms of pain reported by SCI subjects (Source: RehabTrials.org).

An implantable device (called extraspinal oscillating field stimulator) that harnesses electrical fields to stimulate nerve growth in damaged spinal cords is also being tested in humans. A battery-powered device is used to generate the electrical field (about 600 microvolts per millimeter). The human clinical trial will test whether weak electrical fields applied to spinal cord injuries can promote better functional recovery through regeneration of injured spinal cord nerve fibers (Source: news.uns.purdue.edu). Other devices being evaluated in clinical trials include the functional neuromuscular stimulation system, wheelchair mounted robotic arm, functional magnetic micrurition device, and diaphragm placing devices (Source: ClinicalTrials.gov). In addition, clinical trials combating bowel and bladder dysfunction associated with SCI are underway at several centers throughout the US (Source: ClinicalTrials.gov).

There are other candidates that may enter clinical trials at a future date. Among these is Acorda Therapeutics' positive factor (L1 molecule), and M1 IgM antibodies, which probably act by turning on oligodendrocytes, and recruiting scavenger immune cells to dispose of the damaged cells. These may be related to the IN-1 IgM antibodies (Wells, 2000). Neotrophin™ (AIT-082), a purine derivative is a potential candidate by Neotherapeutics for the treatment of acute SCI. It is currently in clinical trials for Alzheimer's disease. Studies have shown that AIT-082 is a potential neurotrophic and regenerative agent. It induces cells in the nervous system to produce certain proteins called "neurotrophic factors". The company states that these results indicate that Neotrophin™ may provide therapeutic benefit in several different neurodegenerative diseases such as Alzheimer's disease, spinal cord injury and stroke.

It is apparent that numerous approaches are being evaluated for the treatment of SCI. Which of these will ultimately be successful will only be determined with time. Whether these therapies would also be useful for treatment of other neurodegenerative disorders, such as those associated with aging or disease will have to be tested.

CURE FOR SCI?

In summary, SCI has made the transition from a condition in which recovery and regeneration were considered impossible to one in which we have a plethora of promising therapies that regenerate the spinal cord of animals. Although several treatments are still in the preliminary stages, the identification of problems, and the explosion in technological advances along with the multitudes of approaches undertaken to tackle this disorder, have given hope that significant functional recovery after SCI is achievable. Researchers believe that a combination of treatments involving protection of CNS soon after trauma, complex transplantation strategies, and rehabilitative retraining will probably yield the most successful results.

REFERENCES
