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Thirty years ago, patients with spinal cord injury (SCI) and their families were told "nothing can be done" to improve function. Since the SCI patient population is reaching normal life expectancy through better health care, it has become an obviously worthwhile enterprise to devote considerable research effort to SCI. Targets for intervention in SCI toward improved function have been identified using basic research approaches and can be simplified into a list: *1*) reduction of edema and free-radical production, *2*) rescue of neural tissue at risk of dying in secondary processes such as abnormally high extracellular glutamate concentrations, *3*) control of inflammation, *4*) rescue of neuronal/glial populations at risk of continued apoptosis, *5*) repair of demyelination and conduction deficits, *6*) promotion of neurite growth through improved extracellular environment, *7*) cell replacement therapies, *8*) efforts to bridge the gap with transplantation approaches, *9*) efforts to retrain and relearn motor tasks, *10*) restoration of lost function by electrical stimulation, and *11*) relief of chronic pain syndromes. Currently, over 70 clinical trials are in progress worldwide. Consequently, in this millennium, unlike in the last, no SCI patient will have to hear "nothing can be done."

INCIDENCE AND HISTORY OF SPINAL CORD INJURY

Trauma to the spinal cord causes dysfunction of the cord, with loss of sensory and motor function distal to the point of injury. There are approximately 400,000 patients with spinal cord injuries (SCI) in the United States. The data on incidence rate are estimated to be 1 in every 1,000 people per year in the United States, and males are four times more likely than females to be involved. The leading causes of SCI are motor vehicle accidents (47%), sports-related accidents, especially diving injuries (24%), falls (12%), and violence—gun shot, knife injuries (7%). The majority of injuries are divided into three general groups. The first group is young individuals, typically between the ages of 16 and 25 years, who sustained their injury from a motor vehicle collision or other high-energy traumatic accident. The second consists of older individuals with cervical spinal stenosis caused by congenital narrowing or spondylosis. Patients in this second group often sustained their injury from minor trauma and commonly have no vertebral fracture. The third group consists of people with gunshot wounds, which is now the leading cause of SCI in many urban areas [Spinal Cord Injury Information Network (11, 77)].

Thirty years ago, patients with SCI and their families were told "nothing can be done" to improve function beyond a "wait and see what recovers" attitude. This was due in large part to the difficulties that the health care providers faced with patient management, including urinary tract dysfunction (recurrent kidney stones, infections), secondary loss of function such as respiratory and skin (pressure sores), venous thrombosis from inactivity, and cardiac dysfunction stemming from autonomic dysfunction such as hypotension or hypertension. Thus, during World War II, the life expectancy of SCI patients was about three months. In 1966, the life expectancy was 20 years, with renal problems contributing to the majority of deaths. Currently, SCI patients have a life expectancy of 25 to 30 years beyond the initial injury, for which the contributing cause of death is cardiac and respiratory dysfunction. Thus, within the last decade, it has become an obviously worthwhile enterprise to devote considerable research effort to SCI, since this patient population is reaching normal life expectancy.

PATHOPHYSIOLOGY OF SPINAL CORD INJURY

To understand the rationale of the recent advances, it is first necessary to review the pathophysiology of spinal cord injury. There are four general types of spinal cord injury: 1) cord maceration, in which the morphology of the cord is severely distorted; 2) cord lacerations (gun shot or knife wounds); 3) contusion injury, which leads to a central hematomyelia that may evolve to syringomyelia; and 4) solid cord injury, in which there is no central focus of necrosis as in contusion injury. In the first two injuries, the surface of the cord is lacerated and a prominent connective tissue response is invoked, whereas in the latter two the spinal cord surface is not breached and the connective tissue component is minimal (23, 24). Of these four injury types, the contusion injury represents from 25 to 40% of the cases and is a progressive injury that enlarges over time. The most commonly used animal model in SCI research is patterned after the contusion injury (6, 13). Within these four injury types, degree of completeness must be considered, as incomplete lesions will benefit more dramatically from experimental interventions than complete lesions in terms of degree of recovery that can be obtained. It is important to note that the clinical presentation of SCI is most often characterized as an anatomically incomplete lesion, irrespective of initial neurological presentation (37, 73).

There are three phases of SCI response that occur after injury: the acute, secondary, and chronic injury processes (124–126). In the acute phase, which encompasses the moment of injury and extends for the first few days, a variety of parallel pathophysiological processes begins. Upon initial impact or injury, there is immediate mechanical damage to neural and other soft tissue, including endothelial cells of the vasculature. Thus necrosis, or cell death, results from these mechanical and ischemic insults, is instantaneous, and, in a contusion injury, appears to be more predominant in the grey matter of the spinal cord than in the white matter, resulting in a ring of preserved white matter at the contusion site (Fig. 1). After the insult, over the next few minutes, the injured nerve cells respond with an injury-induced barrage of action potentials. Accompanying this are significant electrolytic shifts, principally involving the monovalent and divalent cations Na^+ (intracellular concentrations increase), K^+ (extracellular concentrations increase), and Ca^{2+} (intracellular concentrations increase to toxic levels), that contribute to a failure in normal neural function and spinal shock, which lasts for about 24 hours and represents a generalized failure of circuitry in the spinal neural network. Hemorrhage occurs, with localized edema, loss of microcirculation by thrombosis, vasospasm and mechanical damage, and loss of vasculature autoregulation, all of which further exacerbate the neural injury. Furthermore, compression of the spinal cord occurs as a result of vertebral displacement followed by edema and later by fibrotic responses, contributing further to the neural injury. Because in the best circumstances the time to admission after spinal cord injury is about three hours, the immediate acute injury processes do not offer a clinically useful target for therapeutic intervention unless the Emergency Medical Service can adapt an easy-to-administer intervention, and/or the population adopts a preventative stance, such as taking aspirin once a day to prevent cardiac death after an episode of cardiac ischemia as recommended by the American Heart Association. In contrast, the secondary and chronic injury processes, because these occur
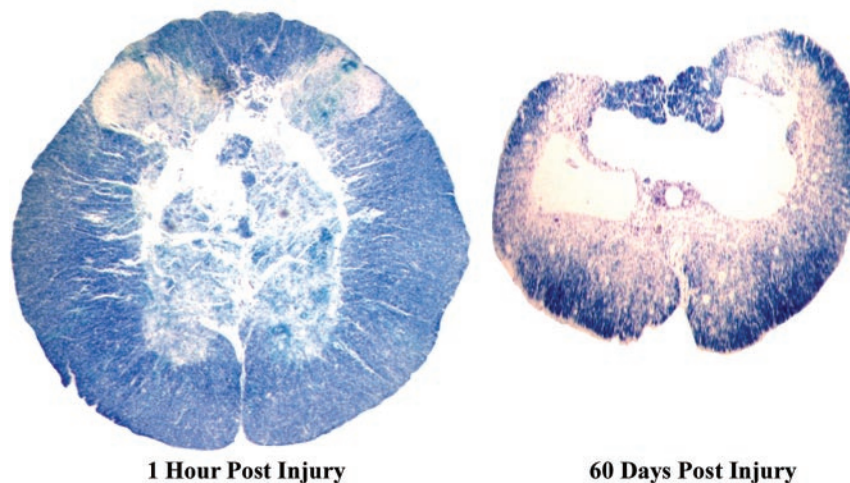
Tissue Loss after Spinal Cord Injury

1 Hour Post Injury   **60 Days Post Injury**

FIG. 1.
Not trivial was the development of an animal model of spinal cord injury (SCI) that mirrored the pathophysiology of human SCI. The most popular model is the rodent contusion model, which produces a necrotic core, principally in the central grey matter, that is surrounded by histologically normal-appearing myelinated fibers and portions of grey matter from both dorsal and ventral horns (*left*). Similar to human SCI pathophysiology, the cell loss continues radially in all directions, so that the lesion expands over time. By 60 days post-SCI, there remains only a thin rim of white matter (*right*). It should be obvious that massive cell death occurs immediately after the initial impact in the central core region. These cells are not rescuable. However, cell death continues to occur over several days and weeks and offers an opportunity for therapeutic intervention to rescue the neural cell populations that are at risk of dying after the first few hours.

within minutes to weeks after injury, are strategically better for therapeutic targets.

In the secondary phase (which occurs over the time course of minutes to weeks), the ischemic cellular death, electrolytic shifts, and edema continue from the acute phase. Within the first 15 minutes after injury, extracellular concentrations of glutamate and other excitatory amino acids reach cytotoxic concentrations that are six- to eightfold higher than normal as a result of cell lysis from mechanical injury and both synaptic and nonsynaptic transport. In addition, lipid peroxidation and free-radical production also occur as a result of glutamate receptor-activated and subsequently mediated pathways. Apoptosis—a secondary, programmable cell death different from necrosis— occurs and involves reactive gliosis that includes increased expression of glial fibrillary acidic protein

(GFAP) and astrocytic proliferation. Neutrophils (which secrete myeloperoxidase) invade the spinal parenchyma from the circulatory system within 24 hours, followed by lymphocytes (which secrete a variety of cytokines and growth factors) that invade and reach peak numbers within 48 hours. The invading inflammatory cells increase the local concentrations of cytokines (cyto = 'cell'; kine = 'small protein') and chemokines (chemotactic cytokine). In addition, inhibitory factors and/or barriers to regeneration are expressed in the perilesion site. The lesion grows in size from the initial core of cell death with cells at risk of dying in the perilesioned region, to a larger region of cell death (Fig. 1). Finally, in the chronic phase, which occurs over a time course of days to years, apoptosis continues in both orthograde and retrograde directions including brain regions; a variety of receptors and ion channels are altered in

expression levels and activation states, scarring and tethering of the cord occurs in the penetrating injuries (about 25% of all SCI); demyelination results in conduction deficits; a cyst forms in a subset of all SCI patients (~20%), and continues to enlarge in a condition called syringomyelia; cut and nearby uncut axons exhibit regenerative and sprouting responses but go no farther than 1 mm; neural circuits are altered due to changes in inhibitory and excitatory input; and in many cell types, permanent hyperexcitability develops, which results in chronic pain syndromes in a majority of SCI patients (32, 33).

INTRINSIC FACTORS OF CNS NEURONS TO REGENERATE

Classically, central nervous system (CNS) neurons were thought to be incapable of regeneration. Early work by Liu and Chambers in 1958 (80) indicated that central projections of primary afferent fibers can sprout in the spinal cord. Thus, for certain neuronal populations, principally those without myelin, regenerative sprouting in the spinal cord can occur (67, 122). Subsequent work by Peter Richardson with Albert Aguayo and others (109) demonstrated that a peripheral nerve bridge provides an environment that promotes axonal elongation, given the stimulus of axotomy or, in some cases, priming the regenerative process by cutting sister processes of the regenerating CNS neuronal population. Essentially, the intrinsic ability of CNS neurons to demonstrate axonal regeneration was supported by the presence of key proteins involved in neurite elongations: growth-associated protein (GAP)-43 (100), expression of the immediate-early genes, and the protooncogene, such as Bcl-2 (122). However, certain populations of regenerating axons do not express GAP-43, suggesting that the expression of growth-related genes is not sufficient for successful regeneration and that extrinsic factors should be targeted. For example, factors should be examined that play an important role in expression of genes in CNS neurons or glial populations that would stimulate regeneration of axons or provide an appropriate environment that is "permissive" for regeneration and prevent neuronal/glial death. It is obvious that this must be a combinatorial approach.

TARGETS FOR INTERVENTION IN SCI

Thus targets for intervention in SCI toward improved function can be simplified into a list: *1)* reduction of edema and free radical production, *2)* rescue of neural tissue at risk of dying in secondary processes such as damage by abnormally high extracellular glutamate concentrations, *3)* control of inflammation, *4)* rescue of neuronal/glial populations at risk of continued apoptosis, *5)* repair of demyelination and conduction deficits; *6)* promotion of neurite growth through improved extracellular environment, *7)* cell replacement therapies, *8)* efforts to bridge the gap with transplantation approaches, *9)* efforts to retrain and relearn motor tasks, *10)* restoration of lost function by electrical stimulation, such as bladder and bowel function or hand function, and *11)* relief of chronic pain syndromes (Fig. 2).

1) Reduction of Edema and Free Radical Production

Several strategies have been applied to acute SCI management. Certainly, the most immediate concern is patient stabilization through hemostasis and decompression and stabilization of the vertebral column to prevent further trauma. This is achieved by a variety of surgical rods, pins, and wires that must be judiciously placed because the surgical procedure involved may produce additional spinal trauma. Within the last decade, one focus of acute treatment has been the reduction of edema and/or the inflammatory response with steroids, the most successful of which is methylprednisolone (MP). The administration of a high dose of MP, if given within eight hours in patients with both complete and incomplete SCI, as proposed by the National Acute Spinal Cord Injury Study (NASCIS-2), has been promising with respect to improved clinical outcome (15). The cellular and molecular mechanisms by which MP improves function are not clear but may involve antioxidant properties, the inhibition of inflammatory response, and/or a role in immunosuppression (16, 58). MP, a glucocorticosteroid, may exacerbate acute neuronal necrosis in the high doses necessary; therefore, a potent inhibitor of lipid peroxidation without glucocorticosteroid activity, tirilazad mesylate (referred to as a lazaroid) might be desirable. Lazaroid has been shown to be neuroprotective and has fewer side effects than MP (57). Other pharmacological compounds with antiox-
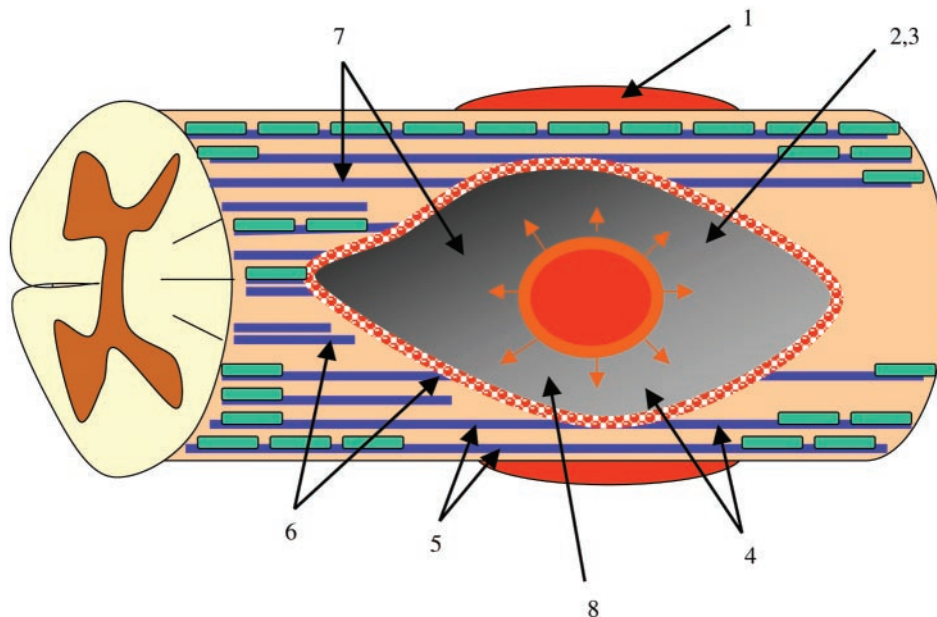
FIG. 2.
Schematic of a piece of spinal cord that has sustained an initial injury (black central oval) that spreads progressively outward and radially (red circular region followed by orange circular region) in zones until it finally reaches the final lesion size (grey shaded area). Blue lines represent axons, and green rectangles are the myelinating cells of the central nervous system, oligodendrocytes. As noted in the text, there are several opportunities for intervention, which correspond to the numbers on the schematic diagram: _1_) reduction of edema and free radical production, _2_) rescue of neural tissue at risk of dying in secondary processes such as abnormally high extracellular glutamate concentrations, _3_) control of inflammation, _4_) rescue of neuronal/glial populations at risk of continued apoptosis; _5_) repair of demyelination and conduction deficits, _6_) promotion of neurite growth through improved extracellular environment, 7) cell replacement therapies, _8_) efforts to bridge the gap with transplantation approaches. Not shown on the schematic diagram but discussed in the text are the _9_) efforts to retrain and relearn motor tasks, _10_) restoration of lost function by electrical stimulation, and _11_) relief of chronic pain syndromes.

idant properties that have been successful in animal models of SCI include cyclosporin A, EPC-K1, which is a phosphate diester linkage of vitamins E and C, melatonin, and high-dose naloxone, which has some demonstrated efficacy in clinical trials (14).

2) Inhibition of Glutamate Toxicity: Stopping the Excitotoxicity Cascade

Different models of SCI have indicated that glutamate receptor antagonists may be useful therapeutic strategies in terms of improved behavioral outcome and neuroprotection. The noncompetitive _N_-methyl-D-aspartate ion channel blocker MK-801 (dizocilpine, Merck) (43, 96, 131) as well as 1,2,3,4-tetrahydro-6-

nitro-2,3-dioxobenzo[_f_]quinoxaline-7-sulfonamide (NBQX), a soluble α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor antagonist (50, 79, 146) have both demonstrated significant improvements in the contusion model of SCI in outcome measures that include measures of improvement in behavior and neuroprotection. In the case of NBQX, the neural protection occurs principally in the oligodendrocyte population (112). In other models of SCI, the NMDA antagonists gacyclidine (GK-11; Beaufour-Ipsen) (45) and agmatine, which also inhibits nitric oxide synthase, have demonstrated neuroprotective effects (151). More recently, the group I metabotropic glutamate antagonists have demonstrated both

behavioral improvements and neural protection after contusion SCI (91).

As more of the secondary pathways activated by the excitotoxicity cascade are elucidated, more intervention opportunities will arise. For example, the production of nitric oxide was recently identified as a major contributor to neural cell death after SCI (81, 114). Although no posttreatment studies exist, pretreatment with N^G-nitro-L-arginine methyl ester (L-NAME) has demonstrated some morphological improvements (153). Another promising intervention is activation of A1 adenosine receptors either by adenosine or a selective receptor agonist in a site-specific manner. Both approaches have demonstrated neuroprotective properties (99).

3) Inflammation: Inhibition by Anti-Inflammatory Agents

Although inflammation is generally held to be an endogenous repair mechanism that is restorative in nature, recent work has demonstrated that the inflammatory cascade produces several pathways that are degradative in nature, such as the prostaglandin pathways. Consequently, anti-inflammatory agents have been tried with some success. One novel idea was the intravenous application of an antiangiogenic compound CM101 one hour after SCI in mice with the intent of preventing inflammatory angiogenesis and inhibiting gliosis. CM101 improved locomotion and appeared to be neuroprotective (133). Administration of the anti-inflammatory cytokine IL-10 has a neuroprotective role, although the improvement in locomotor behavior that was first reported (12) has been retracted by the same group (123). Most likely, improvements in behaviors involving trunk musculature innervation would have revealed differences, since thoracic grey matter was preserved. In three different laboratories, selective cyclooxygenase (COX)-2 inhibitors given systemically have demonstrated significant improvements in both behavioral and neuroprotective outcome measures (56, 107, 150) in the rodent model of contusion SCI. Of the anti-inflammatory agents, the selective inhibition of the inducible COX-2 enzyme appears to be the safest to begin phase I clinical trials. In addition, inhibition of proinflammatory agents, such as inhibition of the IL-1β receptor,

has resulted in improved behavioral outcomes after spinal cord injury (96).

Promising phase I clinical trials for neurotrauma include the application of either whole body hypothermia or local cord cooling (60, 62, 129). Application of hypothermia, either spinally or systemically, is thought to provide protection for neural cells with increased secondary sensitivity to neural death and to reduce secondary inflammation, decreasing immediate mortality. The mechanism may be related to the inhibition of increases in extracellular concentrations of excitatory amino acids. Local spinal cord cooling within eight and a half hours of injury in ten patients produced a better-than-expected rate of recovery of sensory and motor function and reduction in mortality rate compared with more traditional forms of therapy (60), which confirmed earlier studies with eight SCI patients treated by irrigation with 5°C saline for two hours (21).

4) Apoptosis-Rescue from Neural Cell Death

The recognition of delayed cell death after CNS trauma has been described long ago as Cajal's seminal studies early in the twentieth century (35). Because cells die due to a programmed cell death after SCI, an excellent opportunity is present for intervention with factors that could rescue the cells at risk, both neuronal and glial (7, 34, 42, 82). One approach to cell rescue is the inhibition of caspases. Caspases are cysteine-dependent, aspartate-specific proteases that cleave at specific sites within proteins, with a nearly absolute requirement for aspartate at the cleavage site. Unlike other proteases that are degradative in nature, caspases are regulated signaling proteases and are activated by proteolysis and are thought to play an important role in mediating cell apoptosis (18, 41). There is a growing list of companies, including GlaxoSmithKine, Aventis, Idun, and Vertex, that are currently working on novel caspase inhibitors that can be tried in clinical phase I trials (14). These proteins are a part of the bcl-2 oncogene products, which are potent death suppressors, and inhibit programmed cell death in neurons as well as in lymphoma and other types of cancer. However, the Bcl-2 family is composed of both death suppressor and death promoter proteins that are normally in a checked and balanced system (51). Recent work in a model of SCI

has demonstrated rescue by direct injection of a plasmid encoding the Bcl-2 protein into the damaged site (121). Because Bcl-2 is a membrane-bound mitochondrial protein, cells that are rescued must have retrogradely transported the Bcl-2 to the neuronal soma.

Another group of proteins that has received attention for their role in cellular degradation in both necrosis and apoptosis is the calpains. Calpains are calcium-activated proteases that play important roles in cytoskeletal degradation of damaged cells. Thus calpain inhibitors would be expected to reduce or delay apoptosis. Several companies have compounds that demonstrate some improvement in behavior and tissue preservation in models of SCI, such as Taisho Pharmaceutical and Cephalon (14). GM1 ganglioside (Sygen; FIDIA Pharmaceutical), a natural component of cell membranes, is thought to aid in both regenerative responses and have some neuroprotective role, but the mechanism(s) of action is unknown. In a recent multicenter trial of 760 patients, Sygen in combination with MP showed a trend in improved outcomes in terms of improved motor, light touch, and pinprick scores and bowel, bladder, and sacral function (46, 47).

Finally, delivery of exogenous neurotrophins have long been considered therapeutically useful for rescuing cells that lose neurotrophic support due to cell death of projection neurons, the innervation target, or ensheathing cells (oligodendrocytes). Neurotrophins (neuro–'relating to nerve cells'; trophe–'nutrition') that have been administered with some success in SCI include nerve growth factor (NGF), glial-derived neuronotrophic factor (GDNF), cilliary neuronotrophic factor (CNTF), neuronotrophic factor-3 and 4/5 (NT-3, NT-4/5), fibroblastic growth factor (FGF), and brain-derived neuronotrophic factor (BDNF). Delivery systems include intrathecal administration by direct injections through catheters attached to indwelling osmotic pumps, through genetically engineered biological delivery systems such as transduced fibroblasts or immortalized cell lines, and by direct injection of genes into the spinal parenchyma at the injury site (72, 75, 76, 116, 122).

5) Demyelination and Conduction Deficits

The correlation of increased neural death with increased electrical activity offers an opportunity for intervention. It is well known that, concomitant with neural injury, a barrage of action potentials is produced that causes massive release of transmitter substances. This massive release, coupled with the ionic shifts, can result in neural cell death. The strategy of inhibiting the neural injury induced by the increased barrage of action potentials early in the injury phase or by inhibiting the voltage-dependent sodium channels, which provide the ionic basis for the action potential, is novel but may be fruitful. For example, the sodium channel blocker tetrodotoxin demonstrated a rescue of neural tissue and improved behavioral recovery (112). In addition, neural injury and disease may introduce altered ionic channel function on nerve processes that would result in impaired conduction properties (134), which produces persistent hyperexcitability leading to the basis for chronic pain after CNS neural trauma (38, 135). Thus ionic channel blockers may prove effective in attenuating neural damage and dysfunction.

Along these lines, it is important to note that many axons are demyelinated as a result of secondary injury to the spinal cord. Infusion of the fast, voltage-sensitive potassium channel blocker 4-aminopyridine (4-AP) (59, 63, 117) looks extremely promising and is now in phases II and III clinical trials at Acorda (69) and Washington University. 4-AP's utility is based on the exposure of the internodal potassium channels that occurs as a result of demyelination, which considerably alters conduction properties in surviving demyelinated axons. Application of 4-AP blocks potassium channels and partially restores conduction properties. Phase I trials of 4-AP given in doses of 6–30 mg/kg (59, 63, 117) demonstrated increased motor control and sensory ability below the injury and reduction in chronic pain and spasticity and restored voluntary bowel control in a subset of patients. (59, 63, 118). Another strategy for demyelination is the transplantation of cells that may produce new myelin and thus restore conduction deficits. Cells that have been tried with some success in animal models include olfactory ensheathing cells, oligodendrocytes, and Schwann cells (see discussion on Cell Replacement Strategies below).

6) Promoting Axonal Regeneration: Matrix Proteins, Inhibitory Factors, and Neurotropins

Since Cajal's time, it has been understood that nerve cells in the CNS grow a millimeter or so and no

longer. However, nerve cells have the intrinsic machinery necessary for neurite growth; consequently, the external environment must be nonpermissive to neurite growth. There are several strategies that are currently targeted to provide permissive environments to neurite growth. During neural development, a variety of neurite-promoting and guidance molecules exist in the extracellular matrix that are subsequently downregulated in the adult CNS, permitting stable and complex circuitry. Thus, in the adult CNS, the balance of factors that support neurite growth and factors that inhibit neurite growth shifts toward enhanced expression of growth-inhibiting molecules. After SCI, many of the neurite guidance factors are reexpressed and may provide strategies for neurite growth if better understood. The field of extracellular matrix materials has recently exploded, providing families of highly conserved attractant and repulsive guidance molecules such as netrins (152), semaphorins (98), ephrins (74), tenascins (71), integrins, and a variety of matrix proteoglycans, including chondroitin sulfate proteoglycans (CSPG) (149). The extracellular matrix proteins are emerging as key players for the failure of injured central neurons to regenerate. Reports of functional recovery in rats with dorsal column lesions treated with chondroitinase ABC suggests that removal of the glycosaminoglycan CSPG will be useful in regeneration (17). Although it is early in the field of SCI research for this area to have made a contribution, the extracellular matrix proteins will be an important consideration in a combinatorial approach for successful regeneration of axons after injury.

Perhaps the best known of the inhibitory factors is NoGo, which was first detected as a high-molecular-weight inhibitor in myelin (28, 29). The IN-1 antibody, which neutralized the inhibitory protein activity of NoGo, has been shown to encourage long tract regeneration. For example, hybridoma cells secreting IN-1 antibody increased the number of corticospinal axons that grew around a spinal cord lesion (115) and resulted in some improvement in hindlimb function (20). The inhibitory protein NoGo was only recently identified (30). Nonetheless, this promising therapy will soon be in phase I clinical trials sponsored by Novartis. Another myelin-derived growth-inhibitory protein, myelin-associated glycoprotein (MAG), was identified by two laboratories independently (90, 93) and has been characterized both in vitro and in vivo (89).

In the perilesioned region, growth-inhibitory proteins are present at the glial scar and represent an important barrier to axon growth. Reactive astrocytes form many different kinds of CSPG. CSPG proteins that inhibit axonal growth include versican, phosphocan, NG2, and neurocan (89). The CSPGs are thought to be expressed by reactive astrocytes, oligodendrocyte precursor cells, and meningeal cells, among other cell types (89). Consequently, one approach to limiting the inhibitory barrier at the lesion site is to eliminate the astrocytes by a variety of techniques. Direct injection of ethidium bromide, which binds to cellular DNA and RNA and thus kills the cells by disrupting cell function, has been used experimentally, since these techniques, if directed appropriately, will target white matter cells, including glial cells, while leaving axons of passage uninjured. A second approach has been the application of site specific X-irradiation. High doses of X-irradiation have been reported to reduce astrogliosis and improve motor recovery. Finally, chemically specific glial cytotoxins may be useful toward reducing the glial barrier (88); however, these three approaches have the obvious contraindications that a selective removal of astroglial cells is critical and these approaches are likely to remove a considerable number of astrocytes. Because astrocytes have an essential role in general CNS homeostasis, there will certainly be some unintended negative consequences (88). Agents that degradate the CSPG should also prove useful. Reports of improved function in rodents after treatment with chondroitinase ABC appear promising (17).

Another approach to stimulating axonal growth is to use local delivery of neurotropins (neuro = 'relating to nerve cells'; tropin = 'a turning') by techniques described above for neurotrophins. Interestingly, the factors that are neurotrophic also serve as neurotropins for specific cell populations. For example, NGF stimulates the sprouting of fine, primary afferent fiber systems, which, unfortunately, are the pain fibers. NT-3 has been demonstrated to potentiate corticospinal growth (116). It is obvious that subsets of nerve cells will respond differently to each growth factor, so that a combinatorial approach will be needed to encourage robust regeneration of several systems. Recently, treatment with intrathecal infusion of NGF, NT-3, GDNF, and BDNF demonstrated some success in regeneration of cut dorsal roots into the

dorsal root entry zone. Of these, GDNF demonstrated the best anatomical, functional, and behavioral recovery, whereas the mechanisms involved in the other treatments were likely due to collateral sprouting of undamaged primary afferent populations above and below the cut dorsal root (105).

Approaches that act through direct intracellular mechanisms in the nerve cell body to promote neurite growth should prove useful. Recent findings with inosine, a purine nucleoside (10), and cAMP (104) that have demonstrated neurite outgrowth promotion in vitro and in the rodent spinal cord are encouraging. Currently in clinical trials for SCI is the compound AIT-082, a synthetic hypoxanthine derivative containing a para-aminobenzoic acid moiety (Neotrofin; NeoTherapeutics), which had been shown to promote both axonal sprouting and the production of NGF, NT-3, and bFGF in astrocytes in culture systems and in vivo (36). Currently, there are four rehabilitation centers testing Neotrofin in subacute ($\leq$21 days of injury) spinal cord injuries: Ranchos Los Amigos, Gaylord, Craig, and Thomas Jefferson Rehabilitation Centers.

7) Cell Replacement Strategies

After injury, with resultant loss of both nerve cells and cells that provide the myelin for appropriate conduction properties, the obvious solution would be to provide cells that can replace the lost function. A variety of tissues and cells have been added to the adult spinal cord to encourage restoration of function. These include stem cells, olfactory ensheathing cells, (the cells that form the myelin on olfactory nerves), Schwann cells (the cells that form the myelin on peripheral nerves), dorsal root ganglia, adrenal tissue, hybridomas, peripheral nerves, or transplanted conduits of Schwann cells, which would serve as a source for chemical and mechanical guidance. It is postulated that these tissues would rescue, replace, or provide a regenerative pathway for injured adult neurons, which would then integrate or promote the regeneration of the spinal cord circuitry and restore function after injury (154). Because many nerve fibers persist as demyelinated fibers, considerable effort has been directed toward the transplantation of cells that may produce new myelin and thus restore conduction deficits. Cells that have been tried with some

success in animal models include olfactory ensheathing cells (85), oligodendrocytes, Schwann cells (22), and stem cells (86, 141, 154). Altering the secretory products of transplanted cells by modifying the cells with molecular techniques holds great promise in the future (72, 101), particularly when the addition of only one factor will produce improvements of behavior, such as relief of chronic pain (55, 64).

Gene therapy allows the engineering of cells, which combines the therapeutic advantage of the cells in combination with a gene delivery system. For example, if delivery of neurotrophins is desired, a combination of cells that will form myelin and secrete neurotrophins can be engineered to both promote neurite growth and restore nerve function. For example, an attractive cell population for transplant studies is fibroblasts. Advantages of fibroblasts include the following properties: *1*) ability to clinically harvest a patient's own fibroblasts, *2*) ability to culture patient's fibroblasts with rapid proliferation in vitro, and *3*) accessibility to transfection with a desired gene and transplantation. Rodent syngenic fibroblast cells transfected with NGF demonstrated some success with neurite growth of cells that respond to NT3 (52), but these cells eventually become tumorigenic and produce collagen, not normally found in the spinal cord. The fundamental idea of harvesting cells from prospective patients led to targeting other harvestable cell types for gene therapy. Other cells that have been transfected with neurotrophins and demonstrated some success in terms of promoting neurite growth and some behavioral recovery include Schwann cells (72, 94, 136) and embryonic neural precursors (55, 147).

In an effort to enhance beneficial effects of endogenous cell populations, the addition of a patient's own activated macrophages, which have some efficacy in animal models, is in phase II clinical trials in Belgium and the Weizmann Institute (www.proneuron.com). Macrophages from the patient's own blood (autologous macrophages) are activated and implanted at the site of the spinal injury. The rationale is that the patient's own activated macrophages will scavenge degenerating myelin debris, rich in nonpermissive factors, and thus encourage regenerative growth without eliciting an immune response. In another open clinical trial, transplantation of porcine fetal spinal cord cells, pretreated with F(ab$'$)2 immuno-

modulation, will be placed directly into the undamaged spinal cord adjacent to focal spinal lesions. The purpose is to provide cells that will stimulate repair and regeneration in SCI with the hope of improved outcome (www.neuro.wustl.edu/sci/clinicaltrials).

8) Bridging the Gap: Transplant Strategies

As stated above, spinal injury results in a lesion cavity that progresses over time, leaving an anatomically incomplete lesion. Nerve fibers that do demonstrate regenerative growth or collateral sprouting (undamaged nerves cells that sprout) encounter an inhibitory environment as well as a physical gap that requires a permissive bridging substance. Thus bridges such as cells, fetal tissue en bloc, or artificial material have been implanted with some success (22). To date, promising work has been the use of fetal spinal cord transplants into the adult spinal cord in rats, mice, and primates (154). Good host/graft integration is demonstrated in some instances, and appropriate circuitry appears to have been supplied by the fetal transplant by use of morphological and electrophysiological criteria. Moreover, the fetal transplant can chronically alter host metabolic pathways. Numerous studies have demonstrated the survival of transplanted embryonic tissue in the nonimmunosuppressed adult spinal cord. Other research reports the ability of transplanted fetal raphe cells to extend axons through the grey matter of the host spinal cord, form synapses, and demonstrate behavioral improvement (103). Additionally, transplant of fetal tissue into adult spinal cord results in a remarkable decrease or absence of gliosis around the transplant site, even when extensive lesions have been made (19, 65, 66, 127).

There are several clinical trials in Sweden, Florida, Russia, and China in which fetal tissue en bloc has been used in patients with chronic SCI to treat progressive syringomyelia (http://carecure.rutgers.edu/lectures/SCIHope.htm). These patients develop posttraumatic syringomyelia, in which a fluid-filled cavity in the spinal cord progressively increases in size, presumably due to progressive orthograde and retrograde degeneration of neural elements. As a result, the patient's behavioral outcome worsens as the pathophysiology progresses. To interfere with the progressive nature of this condition, en bloc fetal spinal tissue was grafted into the syrinx cavity in the hope that the addition of neurotrophic factors provided by en bloc fetal spinal tissue might result in behavioral improvement. In the case of posttraumatic syringomyelia, outcomes that do not worsen over time, perhaps by inhibiting the progressive neural degeneration, are desirable. These trials demonstrated that the cells survived and some patients demonstrated improved recovery (44, 128, 143).

Implants into the gap composed of Schwann cells, which form the myelin in the peripheral nervous system in which regeneration does occur (22), or multiple grafting of intracostal nerves (31) in rodent models of spinal injury are both approaches in which some regenerating nerve cell axons have been able to grow across the gap, but penetration into the host tissue was not impressive. Some functional recovery was demonstrated in both approaches. Clinical trials in which peripheral nerves are used as bridges are in progress at the University of Sao Paulo, and in Taiwan, where more than 20 patients have received nerve bridges (http://carecure.rutgers.edu/Lectures/SCIHope.htm). Similarly, implanted olfactory ensheathing cells have been shown to align, migrate long distances, and promote functional recovery and regeneration through the lesion site in rodents (106). Human neural progenitor cells and embryonic stem cells have been used in rodent spinal injury models and have demonstrated functional recovery (86, 154). Although considerable work in the field of stem cell biology remains (147), there are some clinical trials in Russia in which fetal stem cells are used as transplant sources for chronic SCI or in combination with olfactory ensheathing glial cells. Other transplant sources include transposition of the omentum (Cuba, China, and Italy) and transplantation of embryonic shark cells in Tijuana, Mexico (http://carecure.rutgers.edu/Lecutres/SCIHope.htm). These latter two approaches lack support from both clinical practitioners and researchers in the United States and are generally thought to lack scientific merit.

Advances in the field of biomatrix material have provided opportunities to bridge the gap with artificial material, such as biodegradable hydrogels or combinations of hydrogels and cells (22), that may promote regeneration. Desired properties of a synthetic bridge are to provide simultaneously a physical substrate for axonal attachment and growth without triggering an-

tigenic host reactions. Although this approach is in its infancy, great strides are possible with polymer chemistry (48).

9) Lost Function: Need to Retrain the Brain by Aggressive Physical Therapy

Supported-ambulation studies at the University of California at Los Angeles are in Phase II clinical trials to demonstrate functional improvements in SCI patients after aggressive treadmill training [National Institutes of Health National Institute of Child Health and Human Development, Dept. of Veterans Affairs (VA); clinicaltrials.gov]. Others investigating supported ambulation include the VA Medical Center in Houston, Washington University, The Miami Project, The Burke Institute, and the University of Bonn (139, 140). In most injuries, caudal gray matter such as that in the lumbosacral cord is essentially uninjured; however, after injury, supraspinal systems do not efficiently communicate information with cervical and lumbar elements to initiate stepping and maintain persistent locomotion. After training with supported ambulation, adaptable circuitry can be trained to interpret complex sensory information associated with load-bearing stepping (5, 39, 40, 61, 137, 138). Because locomotion requires coordination of multisegmental connections and given intrinsic CNS plasticity, a very small biological bridge may produce a dramatic impact on locomotor function (148). Of additional relevance, cellular transplants of either mixed or pure culture offer potential for further adaptability, and transfected transplants can stimulate reorganization of intrinsic and extrinsic spinal neural circuits.

Along these lines, aggressive physical therapy and/or other noninvasive therapies should be used in conjunction with invasive interventions. It is apparent that recovery of locomotion is dependent on sensory input that can "reawaken" spinal circuits and activate central pattern generators in the spinal cord, as demonstrated by spontaneous "stepping" in the lower limbs of one patient (26, 27). Sensory input as would occur in aggressive physical therapy may be important, which is consistent with classical works by Sherrington (120) and Grillner (53) on mammalian locomotor neural circuits. Obstacles to aggressive physical therapy for SCI may be budgetary considerations, biases despite duplicative positive scientific

evidence, and lack of knowledge on the part of the physiatrists and healthcare providers.

10) Recovery of Function through Electrical Stimulation

There are a variety of functional electrical stimulation (FES) approaches that may prove useful given the advances being made in electronics. Because the premise of FES is based on transcutaneous or direct electric stimulation of distal ends of innervating nerves, lower motor neurons and peripheral nerves must be intact. Although some systems aid walking, these systems are physically too tiring due to the high energy requirements of the user. However, FES that contributes to improved standing can greatly improve quality of life for the individual and the caregiver. There is considerable interest in computer-controlled FES for strengthening the lower extremities and for cardiovascular conditioning, which has met with some success in terms of physiological improvements such as increased muscle mass, improved blood flow, and better bladder and bowel function. Concomitant with these improvements, decreases in medical complications such as venous thrombosis, osteoporosis, and bone fractures are reported. Stimulation of the phrenic nerve, which innervates the diaphragm, is used in cases where there is damage to respiratory pathways above the origin of the phrenic nerve (C3 or higher) (87). Open clinical trials are in progress at Washington University in which FES units are implanted in the lower extremities to improve standing for patients with SCI. A similar trial is sponsored by the Federal Drug Administration office and is in development at Case Western Reserve University in which FES units are implanted into the pelvis and legs (clinicaltrials.gov).

In particular, restoration of pelvic visceral function after SCI with the Finetech-Brindley (VOCARE) Bladder System [http://www.finetech-medical.co.uk/bladder_system.html (accessed 2002 May 22)] by electrical stimulation of S2, S3, and S4 roots (the roots of origin of pelvic parasympathetic preganglionic and pudendal nerves) can improve bladder and bowel function. It should be noted that the S2, S3, and S4 dorsal roots must be cut for this treatment to be effective. In another example, the Freehand System [NeuroControl; http://www.neurocontrol.com (ac-

cessed 2002 May 22)], electrical stimulation is used to produce two different types of grasping via the ulnar nerve (lateral grasp) or median nerve (palmar grasp) and can substantially improve the quality of life (102). Therefore, in the FES approach, return of function is incremental, but improvements in the quality of life are immense. Clinical trials using electrical stimulation to activate spinal cord central pattern generator are in progress at the University of Arizona, and electrical stimulation for subacute SCI is in clinical trials at Purdue University and in Dublin, Ireland (http://care-cure.rutgers.edu/Lectures/SCIHope.htm). Finally, robotic devices offer an opportunity for improved function, and two clinical trials are in progress, both US sponsored. The wheelchair-mounted robotic arm is in open clinical trials at the VA Medical Center in Houston. Both trials are sponsored by the Department of Veterans Affairs (clinicaltrials.gov) and robotic-assisted upper-extremity rehabilitation at the VA Medical Center in Baltimore.

11) Treating Chronic Central Pain: "Not All in their Head"

SCIs result in a devastating loss of function below the level of the lesion, in which there are variable motor recoveries, and, in the majority of cases, chronic central pain (CCP) syndromes develop (32, 33), usually within several months to years following injury (33, 108, 111). This pain so greatly affects the quality of life that depression and suicide frequently result (25, 119). Research focused on improving recovery of function, including the reduction of CCP, is essential.

Unfortunately, the study of chronic pain after SCI has been neglected. A problematic issue for the majority of SCI patients who experience CCP is that they are given psychiatric referrals, which do not address the pathophysiological mechanisms that underlie the condition. Although advances have been made in the management of acute SCI, there has been little fruitful progress in efforts to bring an understanding of the pathophysiology of CCP to the development of therapeutic approaches for the SCI patient population. Evidence that neurons in pain pathways are pathophysiologically altered and hyperexcitable and thus exhibit central sensitization (144) after spinal cord injury comes from both clinical and animal literature. Perhaps the first widely cited example of spontane-

ously hyperactive and bursting neurons located in the spinal cord was described by Loeser et al. (83) in a patient who suffered chronic pain. That study suggested that the spontaneously hyperactive neurons might be the generator mechanism for the pain experienced following various forms of deafferentation such as brachial avulsions and spinal cord injury. In subsequent studies, spontaneous neuronal hyperactivity was reported in the thalamic nuclei of patients with chronic central pain syndromes (78, 110), suggesting that spontaneous neuronal hyperactivity may be widely distributed throughout the somatosensory pathways.

Work in animal models indicates that the altered behavioral nociceptive states can be explained by a variety of nonexclusive mechanisms (68, 70), including release of spinal cord nociceptive processing from descending inhibition, a permanent increase in receptor activation triggered by the acute increase in the extracellular concentrations of excitatory amino acids (EAAs) induced by SCI, deafferentation hyperexcitability of spinal neurons and/or thalamic neurons, and disinhibition and/or increased efficacy of previously ineffective synapses. In addition, the development of the chronic pain state correlates with structural alterations such as intraspinal sprouting of primary afferent fibers that provide the substrate for maintained hyperexcitability of dorsal horn neurons. We hypothesize that another mechanism of the pain sequelae involves the EAA receptor-mediated development of hyperexcitability of dorsal horn neurons (9, 145), referred to as central sensitization (33, 142, 144, 145). We hypothesize that important components of these changes involve changes in activation state of excitatory and inhibitory receptors and their transporters (54, 91, 92, 130), areas which we are beginning to explore further.

Within the past decade, recognition of central neuropathic pain has led to the successful utilization of nonopioid analgesics delivered by indwelling pump systems or given orally. Compounds such as the γ-aminobutyric acid B (GABA$_B$) agonist baclofen, once used exclusively in treatment of spasticity (1, 4, 84), and the anticonvulsant gabapentin (Neurontin; Parke-Davis), originally used to treat epilepsy, have had some success with attenuating musculoskeletal (baclofen) and CCP syndromes (gabapentin) (2, 3,

97). The tricyclic antidepressant amitriptyline, shown effective in treatment of dysesthetic pain (113), is also in phase I clinical trials in work funded by NIH. The mechanism of action by which amitriptyline produces analgesia is unclear, but it may be related to inhibition of norepinephrine and serotonin reuptake (8) or other actions (reviewed in Ref. 154). Oral tricyclic antidepressants are used for many CCP syndromes, even those that are refractory to standard therapy including narcotics (49), so results of the amitriptyline trials seem promising.

Most chronic pain studies involve few patients, are poorly designed, and are therefore inconclusive. Few large clinical trails in this area exist. One open clinical trial at the University of Pittsburgh will examine the effects of psychological intervention and physical therapy to improve pain reduction sponsored by NIH-National Institute of Child Health and Human Development (clinicaltrials.gov). Another study, in partnership with Diacrin and Washington University (http://www.neuro.wustl.edu/sci/clinicaltrials.htm), is a phase I clinical trial in which procine fetal neuronal cells are harvested from the lateral ganglionic eminence (LGE) and transplanted into the spinal cord for treatment of chronic pain. The LGE cells produce GABA-secreting cells, which are lost in SCI. Because GABA can inhibit pain circuits (68), the hope is that the LGE cells will attenuate CCP syndromes. Overall, management of chronic pain after SCI is poor and remains a clinical challenge.

FUTURE DIRECTIONS

In the next few years, stem cell therapy will offer opportunities for cell replacement strategies, once the fundamental biology of these cells is known. Additionally, matrix proteins and biomatrix materials will aid in "bridging the gap." Huge advances in the field of electronic circuitry (miniaturization, micromachines) will lead to better FES and robotics devices. Cell transplant strategies will be most useful when one or a few factors are missing; thus chronic pain will benefit first in the current clinical trial applications. Aggressive pharmacological approaches to recovery of function offer another intervention opportunity. For example, the addition of the adenosine antagonist theophylline has been used to enhance residual synaptic drive to spinal respiratory neurons by blocking adenosine A1 receptors (99), an approach that has proved successful in clinical trials. In another example, serotonin administration by genetically engineered precursor cells transplanted in the region below the level of SCI can improve both locomotor and somatosensory function (55). Finally, the application of DNA microarray analysis and proteomics will allow temporal assessment of large numbers of genes and proteins after SCI (95). As a result, more therapeutic opportunities will become available, as many genes, previously thought to be unimportant, will now be able to be identified with these techniques. In addition, the consequences of therapeutic intervention on the expression levels of vast numbers of genes and proteins will be easily assessed and allow easy prediction of efficacious treatments that are likely to have few contraindications. Because the injured cord is a different molecular environment than an uninjured cord, it is necessary to apply techniques that give a large amount of information toward understanding the effect of a single intervention on gene expression. Thus there is considerable work in progress.

In October 1974, the star running back of the Texas Christian University football team, Kent Waldrep, was stopped by a wall of Alabama tacklers and landed head first on the artificial turf (132). His football career ended with the diagnosis of quadriplegia. The prognosis that was told to Kent and his family was that "nothing can be done." But a new career was born, that of an advocate for spinal cord research. As a patient, Kent found unacceptable the notion that SCI was incurable and that nothing could be done. Despite the skepticism and lack of support by the medical community, Kent decided to seek help at the Palenov Institute in the Soviet Union in a program that predated current supported ambulation therapy. Although the intervention did not help him substantially, it did alert Kent to the problem of the long-accepted dogma in the United States that "nothing can be done" for SCI. At age 25, he formed the American Paralysis Foundation, now reorganized as the Kent Waldrep National Paralysis Foundation. The original organization became a model for the much later-formed Christopher Reeves National Paralysis Foundation. Because of his efforts, coupled with the efforts of a few vocal and prolific young researchers in the late 1970s who reexamined old data and designed

new experiments that demonstrated that nerve cells in the spinal cord can sprout and that rescue of a few nerve cells can result in improved function, dogma for SCI treatment changed. Athletes like Dennis Byrd, who received methylprednisolone within eight hours after SCI, are able to walk again. Thus the current dogma is much revised in that nerve cells in the spinal cord can be rescued, can sprout, and can be directed to grow, and appropriate growth and reconnection can be encouraged. In this millennium, unlike the last, no SCI patient will have to hear "nothing can be done."

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