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Restoring Function After Spinal Cord Injury: Promoting Spontaneous Regeneration with Stem Cells and Activity-Based Therapies

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Once thought impossible, nervous system repair is now entering the realm of feasibility. Three important concepts are shortening the path to successful restoration: (1) it is not necessary to cure a nervous system injury; (2) a disproportionate return of function can result from a small degree of regeneration; and (3) substantial loss of spinal cord tissue, particularly gray matter, does not preclude near-normal long-tract function.

This article summarizes the importance of continuous cross-talk between the laboratory and clinic, which is pragmatically possible only with one focused management for reaching readily achievable goals that improve quality of life. The authors also advocate attention to the logistics of potential therapies, because no one benefits from treatments that medical insurers or consumers reject because of fiscal or time constraints. The spectrum of regenerative strategies that scientists are studying, when ranked by feasibility, range from the currently impossible, such as long-tract reconnection, to the more feasible, such as optimizing spontaneous regeneration by modulating patterned neural activity. The authors contrast two similar regenerative approaches that seem to be quite divergent: transplanting stem cells to repair the spinal cord and optimizing spontaneous regeneration to restore function. Both approaches have common cellular mechanisms of regeneration; currently, however, better evidence exists for maximizing regeneration with activity-based restoration therapies than with direct application of stem cells or growth factors. The authors note that strategies once holding great excitement now seem limited and complex, whereas others that once were considered unimportant may be more practical. Rehabilitation traditionally focused on social reintegration is now giving way to inclusion of restoration of function by means of regeneration.

Neurosurgery is at the front line of care and treatment of catastrophic neurologic injuries, including spinal cord trauma. Advances in acute trauma care, interventional surgery, instrumentation for advanced spine stabilization, and rehabilitation have vastly reduced morbidity and mortality after spinal trauma [1–5]. Neurosurgical

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care is also entering the realm of restoration of neural function through optimization of regeneration.

Although neural regeneration is an active research field today, no current treatments can aid regeneration after spinal cord injury (SCI). This article reviews the feasibility of spinal cord repair and provides an overview of the range of strategies scientists are taking toward regeneration. The major focus of this article is the future role of stem cell transplantation and simpler rehabilitative restorative approaches designed to optimize spontaneous regeneration by mobilizing endogenous stem cells and facilitating other cellular mechanisms of regeneration, such as axonal growth and myelination.

The five phases of spinal cord injury

SCI is a debilitating condition. Approximately 1 million individuals in the United States are living with SCI, and approximately 12,000 new cases occur every year. According to the Centers for Diseases Control and Prevention (CDC), traumatic SCI costs the nation an estimated $9.7 billion each year [3].

The description of the mechanisms of SCI has conventionally been divided into acute and chronic phases, based on timing of relevant mechanisms of injury. It is useful to evaluate such mechanisms in terms of five phases, however:

1. Development
2. Acute injury (primary and secondary phases)
3. Subchronic injury
4. Chronic injury
5. Aging

For example, the response and recovery of the central nervous system (CNS) to injury is different depending on age at the time of injury, with the best potential for recovery expected with injuries occurring in the developing nervous system and the worst when injury occurs in the aged CNS. These differences are substantial, ranging from the expectation of near full recovery when the injury occurs at birth to that of little recovery occurring in the aged CNS [6–8]. As part of this concept, it is important to understand that maintenance of the CNS is an important function of the normal CNS. This is important, because the chronic consequences of CNS injury are predicted to impair simple CNS maintenance and not just plasticity and regeneration. For pragmatic reasons of this discussion, the authors include maintenance of the CNS within the chronic phase of injury. An understanding of the events within the five phases of SCI is relevant to understanding the limitations and advantages of potential treatments when administered during the different phases of injury. For example, progenitor cells transplanted during the acute injury phase are vulnerable to the same set of cell death mechanisms predominant during the secondary phase of acute injury.

The pathophysiology of the acute phase of traumatic SCI begins when a mechanical force fractures or dislocates the vertebrae and ligaments of the spinal column that normally protect the spinal cord. The authors briefly outline relevant mechanisms of acute SCI, and interested readers are referred to several in-depth reviews [1,3,9]. Damage to blood vessels results in microhemorrhage in the central gray matter, which spreads radially and axially. The resulting hypoxic and ischemic events deprive gray and white matter of oxygen and nutrients necessary for neural cell survival and function. Swelling rapidly occurs at the injury level, and because the bony spinal canal has a fixed diameter, pressure on the cord climbs higher than venous blood pressure. This leads to a venous stroke, deregulation of blood flow, and imbalance of extracellular electrolytes. The immediate effect of trauma and these events is spinal shock, which typically resolves in the first 72 hours after SCI but, in rare cases, can last for weeks [10]. After spinal shock resolves, reflexes return, but there remains variable loss of motor and sensory function. This loss is caused by interruption of the motor and sensory circuits that cross the lesion in surrounding white matter. Preserved function below the injury level is attributable primarily to residual axons, with preserved functional myelination, crossing the injury level in the remaining but damaged outer white matter tissue.

The events involved in primary injury trigger a series of pathophysiologic changes, collectively known as secondary injury, that progressively destroy spinal cord tissue [11,12]. The release of glutamate, an excitatory neurotransmitter, from damaged cells of the CNS is a primary cause of the excitotoxic component of secondary injury [13]. Overactivation of glutamate receptors on neurons results in increased calcium influx and dyshomeostasis, which, in turn, leads to increased protease activity, loss of mitochondrial function, and increased oxidative stress. A high vulnerability to excitotoxicity has long been restricted to

neurons, but recent discoveries that oligodendrocytes, the myelinating cells in the CNS, share a high vulnerability to glutamate toxicity has heralded a large series of studies extending this observation and demonstrating the importance of this mechanism to white matter injury [14,15]. Unlike neuronal excitotoxicity, which is mediated predominantly by *N*-methyl-D-aspartate (NMDA) receptors, mature oligodendrocytes are sensitive to excitotoxicity mediated by non-NMDA receptors, primarily α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate receptors, particularly under the conditions of acidosis that are achieved with hypoxia-ischemia associated with the venous stroke of SCI [16]. Oxidative stress (eg, free radicals) triggers damage to DNA, lipid membranes, and cytoplasmic proteins, resulting in necrotic and apoptotic cell death in the more normal tissue surrounding the damaged spinal cord tissue [17]. Concurrently, neutrophils [18] and, later, lymphocytes and macrophages [19] invade the lesion site by way of the disrupted blood-brain barrier as part of the inflammatory response. Increased levels of inflammatory cytokines and chemokines accompany this invasion. Microglial cells are also recruited and activated to become part of the immune reaction [20–22]. In addition, activated astrocytes become hypertrophic and express glial fibrillary acidic protein (GFAP) and several extracellular surface molecules and cytokines. Collectively, the events that occur during secondary injury result in continued and selective cell death and demyelination in the previously intact tissue immediately adjacent to the injury epicenter, which leads to increased lesion size and the start of scar formation. Despite these scientific advances, there still only exists one treatment for primary and secondary injury, methylprednisolone, and this has been challenged recently (for a detailed review, see the article by McDonald and Sadowsky [3]). Pharmacologic companies have targeted several promising candidates within the secondary injury phase, including prevention of white matter injury by means of antagonists of non-NMDA receptors as well as amelioration of the cascade of inflammatory factors. Interested readers are referred to more detailed descriptions of the acute and secondary injury mechanisms and the active development of drugs to prevent the cascade of accelerating injury characteristic of secondary injury [3].

The subchronic stage of SCI, which follows primary and secondary injury, is characterized by delayed apoptosis of white matter oligodendrocytes extending in a time-dependent longitudinal manner from the injury epicenter. In rodents, this stage peaks 1 week after injury, but in human beings, it may last for months [17]. Because oligodendrocytes myelinate multiple axon segments (1–60), loss of one oligodendrocyte leads to additional demyelination and loss of function even in axons spared by the initial traumatic injury [23]. Loss of a single segment of myelin renders an axon dysfunctional; therefore, a large subset of axons crossing the lesion eventually become nonfunctional despite the axon remaining physically intact. In addition to the progression of dysfunction, another prominent feature of subchronic SCI is the maturation of a scar around the lesion. This scar tissue forms a cellular and molecular barrier to axonal regeneration [20].

Compared with the acute and subchronic injury phases, there has been relatively little work on the next stage of injury, chronic SCI. By the chronic injury phase, the scar is well formed and consists of several cell types, such as the reactive astrocytes, meningeal fibroblasts, Schwann cells, microglia, and macrophages that have invaded the scar, as well as accumulating cellular debris [20,24]. As well as acting as a physical barrier, scar tissue expresses growth-inhibiting molecules that limit axonal regeneration, including chondroitin sulfate proteoglycans (CSPGs) expressed by reactive astrocytes [25–27], myelin-associated molecules (myelin-associated glycoprotein [MAG], myelin oligodendrocyte glycoprotein [MOG], and Nogo-A) [26,28], tenascins (meningeal cells) [26,29], semaphorins (meningeal cells) [26,30], and ephrins (astrocytes) [26,31]. Damaged myelin and myelin debris at and near the scar also contain growth inhibitors, such as the neurite growth inhibitor Nogo-A [26,32], MAG [26,33], oligodendrocyte myelin glycoprotein (OMgp), semaphorin SEMA4D [26,34], and ephrin B3 [26,31]. During development of the nervous system, these inhibitors are used to guide growing connections to their correct location. After injury, however, these roadmap signs are rearranged in a nonsense manner, leading to misguided and aberrant regeneration. Not all is bad, however, because recent work suggests that scar tissue may also have beneficial effects. For example, it may protect the cord by establishing a barrier between the lesion and the adjacent, healthier tissue. Likewise, cellular components of the scar, such as reactive astrocytes, may also have a neuroprotective role [35].

The end result of SCI is evident by the chronic phase of injury and it is important to understand

the injury characteristics typical of chronic SCI. Typically, the loss of central gray matter is confined to one to one and one-half segmental levels of the spinal cord, leading to central cavitation. The end result is a fluid-filled cyst with imaging characteristics compatible with cerebrospinal fluid (CSF) contents, or the cord collapses around the loss of tissue, resulting in an hourglass-type shape with the minimal diameter located at the spinal segment of maximal injury. Despite the cavitations, almost all injuries are characterized by a preserved donut-like rim of white matter at the injury epicenter. This is an important concept to understand, because the degree of functional recovery depends on the functional axons crossing the lesion in this remaining donut-like rim of white matter. A factor relevant to understanding residual function is an additional element of scar formation, namely, the dural scarring that leads to a permanent connection of the cord to the overlying dura. Because the cord is normally freely mobile within the spinal canal, restricted motion attributable to dural scarring produces unusual forces on the cord, such as when the neck is bent or associated with the normal multimillimetric movement of the cord with breathing and with the cardiac cycle. These types of dynamic forces can produce microscopic injury, which may limit optimal regeneration and recovery.

An underappreciated factor in chronic SCI is the relative lack of neural activity below the injury site. Because the cord does not have spontaneous activity like the brain, neural activity below the injury level is primarily determined by limb movements and sensory stimuli transduced by the moving limbs. Based on developmental studies, this deficit in neural activity is predicted to hinder most of the cellular mechanisms important for regeneration. For example, reduction of growth factor expression hinders regeneration. Furthermore, given the overlap of the cellular and molecular mechanisms governing plasticity and regeneration, substantial reduction of neural activity is predicted to limit the body's ability to maintain the functionality of the spinal cord circuitry as well as to offset the accelerated aging associated with catastrophic CNS injury [1,12].

The presence of progenitor cells in the spinal cord capable of division and differentiation into neurons, astrocytes, and oligodendrocytes has been known for longer than half a century. It has only been recently, however, that the field has begun to understand the importance of progenitor cells and the idea that they participate in the normal maintenance of the CNS. These mechanisms include production and replacement of cells lost to normal aging and cell turnover; production of growth factors and cytokines; and gene regulation important for daily plasticity, learning, and maintenance of voltage-gated and ligand-gated channels as well as myelination. Therefore, to maximize the benefits of any potential curative treatment, the day-to-day cellular mechanisms maintaining the system must be optimized (see the section on activity-based restoration therapies).

In addition to loss of motor and sensory function, other important impairments complicate the chronic phase of SCI. They include pain, bladder and bowl dysfunction, sexual dysfunction, and autonomic dysreflexia (blood pressure, heart rate, temperature regulation, and neuroendocrine dysfunction) [36]. Chronic dysfunction of these systems contributes to the progressive worsening of overall health. Autonomic nervous system dysfunction can cause bradycardia and hypotension and can contribute to neuroendocrine dysfunction and thermoregulatory problems. For example, mismatching of microvascular blood flow in such organs as the liver, spleen, and muscle leads to end-organ dysfunction and failure [37]. Interested readers are referred to in-depth reviews of the complications of SCI [38,39]. To determine the full impact of potential regenerative treatments, future research should include these problems as outcome measures in addition to the typical measures of sensory, motor, and gait function.

Functional impairment after SCI depends on the level and severity of the injury (ie, is the injury in gray matter or extending into white matter, and what descending and ascending white matter axonal pathways are damaged?). In human beings, injury severity and prediction of functional recovery, based on initial neurologic examination between 72 hours and 1 week after SCI, is best described by the American Spinal Injury Association (ASIA) impairment scale (Table 1; Box 1). When an injury damages mostly gray matter at one segment, for example, sensorimotor disturbances are confined to the tissues and organs that send or receive impulses at that level of the cord. White matter damage at the same level disrupts descending and ascending axonal pathways, however, preventing motor commands from the brain from reaching levels below the injury and stopping sensory signals from below the level of injury from traveling upward to the brain. Therefore, identifying noninvasive imaging parameters

Table 1
American Spinal Injury Association impairment scale

Scale	Grade	ASIA impairment scale
A	Complete	No motor or sensory function is preserved in the sacral segments S4–5
B	Sensory incomplete	Sensory but not motor function is preserved below the neurologic level and includes sacral segments S4–5
C	Motor incomplete	Motor function is preserved below the neurologic level, and more than half of the key muscles below the neurologic level have a muscle strength grade of <3
D	Motor incomplete	Motor function is preserved below the neurologic level, and at least half of the key muscles below the neurologic level have a grade of 3 or greater
E	Normal	Motor and sensory functions are normal

Box 1. Summary of recovery in ASIA A through D patients

ASIA A

1. Most (60%–90%) regain one motor level [44].
2. From 0% to 11% improve by one or more ASIA grades [45–50].
3. From 4% to 10% may undergo late conversion (after 30 days) to ASIA B or better [51,52]. This can occur up to 2.5 years after injury.
4. Most motor recovery occurs during the first 6 months after injury, with the greatest rate of change during the initial 3 months. Motor strength can continue to improve during the second year.
5. Muscles graded 1 through 3 in the zone of partial preservation (ZPP) recover useful motor function.

ASIA B through D

6. Of ASIA B patients, sacral preservation of pinprick denotes a better prognosis for some recovery of functional ambulation than the ability to sense light touch: 66% to 89% for pinprick versus 11% to 14% for light touch [52–56].
7. Of ASIA C patients, 52% to 76% recover to ASIA D or E compared with 20% to 28.3% of all ASIA B patients [44,46,47].
8. Central cord syndromes (CCSs) generally have a favorable prognosis [6,57–59], but age is a key determinant, with patients younger than 50 years of age having a better prognosis for ambulation than patients older than 50 years of age (97% versus 41%) [56,58,60].
9. Brown-Sequard syndrome (BSS) lesions recover more than CCSs, which recover more than anterior cord syndromes [44].

that accurately quantify the extent of gray and white matter damage and determine which tracts are still functional is an essential step to predicting outcomes and tailoring rehabilitative and future curative therapies. The poor prognosis in SCI is beginning to give way to hope for substantial recovery of function, even in the chronic phase of injury. Potential for recovery (see Box 1) was commonly held to be limited to the first 6 months to 1 year after injury. Recent discoveries indicate that substantial delayed recovery is possible, however, even long after injury. Actor and activist Christopher Reeve heralded a series of research studies after publication of his widely viewed delayed recovery [40,41]. Despite no recovery in the first 5 years after his C1/C2 ASIA A complete SCI, he recovered to 70% of normal sensory function and 20% of normal motor function (function based on ASIA standards in the first 5 years was less than 5% sensory function and 0% motor function). Most importantly, this recovery dramatically changed his quality of life [41]. Later work more clearly associates his recovery with participation in a home-and activity-based restoration therapy program [1,12,41–43]. The functional benefits were also accompanied by substantial physical benefits, including a 10-fold reduction in infections and use of antibiotics, reversal of severe osteoporosis, reversal of muscle

wasting, and reduction of fat volume [41]. This observation led to development and completion of a formal clinical trial to evaluate the efficacy of functional electrical stimulation (FES) cycling and activity-based restoration therapy (ABRT). with functional and physical outcomes similar in magnitude to those illustrated for Reeve (Table 2) [43].

Aging is an important underrepresented phase in SCI. It is generally known by clinicians that slow progressive loss of function is normal in the setting of chronic SCI. The underlying mechanisms are not understood, however. One concept is that the injured CNS undergoes accelerated aging much like the body, representing a shifting balance of cell production versus cell loss to favor net loss as well as impairments in mechanisms of cellular repair. Understanding the mechanisms contributing to this decline should aid in developing regenerative treatments and offsetting such accelerated aging.

Imaging the injured spinal cord

The lack of noninvasive methods for quantifying and characterizing the severity of injury has hampered SCI research and the development of human therapies. Currently, conventional MRI (T_1 weighted [T_1w] and T_2 weighted [T_2w]) is limited (Fig. 1) because MRI data do not correlate well with patients' potential for regeneration and recovery. This is perhaps not surprising, because conventional imaging does not distinguish between functional and nonfunctional tissue or normal and scar tissue. The realization that most individuals with SCI have substantial connections in the outer donut-like rim of white matter at the injury center was more widely accepted after the observation of delayed recovery in Christopher Reeve [40,43]. The development and optimization of noninvasive MRI-based methods capable of differentiating between functional tissue and scar tissue are important to quantify severity and provide better recovery prognostication. Such MRI tools should improve the design of clinical trials and help to establish regenerative treatment plans.

Table 2
Clinical benefits of Functional Electrical Stimulation in Subjects with spinal cord injury

Neurologic Benefits of FES therapy	
ASIA motor score	↑
ASIA light touch score	↑
ASIA pin, prick score	↑
Reduction of Neurologic Complications after FES Therapy	
Spasticity	↓
Antispasmodic Medications	↓
Physical integrity benefits of FES therapy	
Muscle mass	↑
Fat content	↓
Glucose intolerance	↓
HDL	↑
Lipid metabolism	↑
Functional benefits of FES therapy	
FIM	↑
Strength	↑

Abbreviations: ASIA, American Spinal Injury Association; FES, functional electrical stimulation; FIM, functional independence measure; HDL, high-density lipoprotein; ↑, increase or enhancement; ↓, decrease or degradation.

MRI in acute spinal cord injury

Many studies have attempted to correlate imaging results with neurologic and functional outcomes in acute SCI but with little success. With conventional MRI (T_1w and T_2w), only evidence of cord transection [61] or substantial intramedullary hemorrhage during acute SCI has predictive value for irreversible neurologic injury [62–67]. Although the location of the hemorrhage within the acutely injured spinal cord matches the clinical level of injury [62], the size of the hemorrhage does not predict the extent of functional loss or disability [63,68]. In acute SCI, cord swelling (focal widening) and cord edema are also roughly proportional to injury severity [63,69,70]. A high degree of cord compression and abnormal intensity on T_1w images (cord contusion) correlate positively with a poor neurologic outcome, whereas hyperintensity on T_2w images is useful for clinical follow-up, with early resolution of the T_2w changes associated with a more favorable prognosis [71]. A normal MRI scan predicts the best prognosis [62]. Similarly, such imaging characteristics in acute SCI (hemorrhage and edema) relate to broad classifications of disability, such as those determined by the Functional Independence Measure (FIM) [72]. Only complete spinal cord transection has close to 100% positive predictive value and high specificity on conventional MRI. The sensitivity is relatively low, however, and false-negative rates for all other changes are excessive [61]. Some authors have reported a lack of association between signal intensity changes

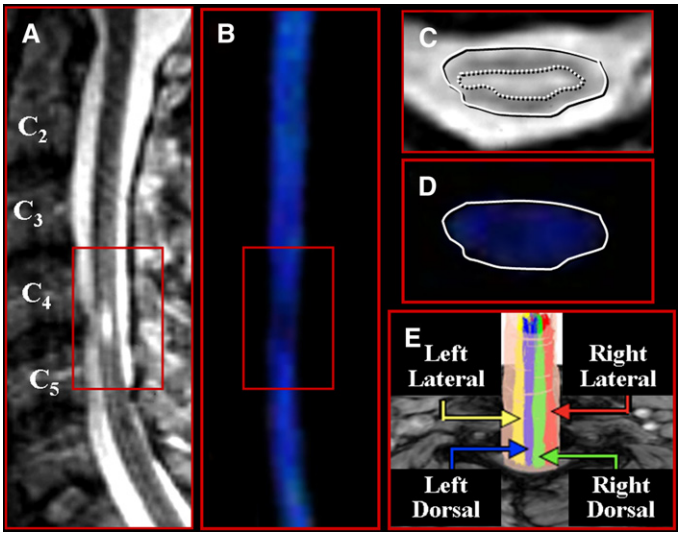

Fig. 1. Conventional and advanced structural diffusion tension imaging (DTI) MRI of injured cord and DTI imaging of normal cord. A C4/C5 spinal cord injury sustained 20 years ago can now be imaged with conventional T_2w MRI (*A, C*) and advanced structural DTI MRI (*B, D*). (*A*) Sagittal view shows a fluid-filled cyst at the level of the injury. (*C*) Cross section of the damaged spinal cord at C4 is shown. Cell death occurs from the inside out, leaving a doughnut-shaped injury on cross-sectional imaging. (*B*) Sagittal view (as in *A*) but demonstrating DTI to reveal vertical axons (*blue*). (*D*) Cross section of same view (as in *B*) demonstrates DTI at the injury level epicenter. (*E*) Three-dimensional view illustrates clinically relevant tracts in the normal cervical spinal cord. Blue and green represent the left and right dorsal columns, respectively. Yellow and red represent the left and right lateral columns, respectively. By magnetization transfer–weighted imaging normalized by cerebrospinal fluid (MTCSF), a high-resolution image is used for region-of-interest (ROI) seeding, allowing us to quantify the integrity of specific white matter tracts in the human spinal cord. (*Adapted from* McDonald JW. Repairing the damaged spinal cord: from stem cells to activity-based restoration therapies. Clin Neurosurg 2004;51:209; with permission.) (DTI images courtesy of Michael V. Johnston, MD, Kennedy Krieger Institute, Baltimore, MD.)

on spinal cord MRI and an individual's recovery [73].

MRI in chronic spinal cord injury

Imaging in chronic SCI is in its infancy [74], and additional studies are needed. One problem is that MRI is insensitive to signals in the chronically injured cord because of lack of inflammation, hemorrhage, and swelling. The recent development of advanced structural imaging techniques, such as diffusion tensor imaging (DTI) and magnetization transfer (MT), is beginning to overcome these problems. In fact, lack of inflammation is advantageous for these types of imaging profiles. Therefore, advanced MRI techniques are preferable to conventional MRI for visualizing chronic SCI. The development and application of functional MRI (fMRI), currently useful in the brain, to the spinal cord should revolutionize the field of regeneration. To date, translation of brain fMRI to the spinal cord has been hampered by the spinal cord's small size, mobility, and artifacts induced by pulsatile CSF flow. Understanding recent advances in MRI technology and imaging is important to the discussion of regeneration.

DTI provides a unique image contrast called diffusion anisotropy [75–80]. This reveals tissue organization at the microscopic level based on the average motion of water molecules (see Fig. 1B, D, E). Previous studies have assessed the feasibility of using DTI to quantify white matter lesions in human patients with SCI [81–86]. A confounding factor is the poor resolution resulting from the field strength of 1.5 T. In human studies, mean diffusivity (MD), a DTI-metric, has been shown to be more sensitive than T_2w MRI in detecting cervical lesions of the spinal cord [87]. MD has also been reported to improve diagnostic sensitivity in chronic SCI [87,88]. Factional anisotropy (FA), another DTI-metric, can

be used to track nerve fibers in the spinal cord and brain stem and is also more sensitive than T_2w MRI in detecting cervical spinal lesions. FA was shown to be a potential prognostic imaging metric for patients' clinical outcomes after treatment, but the authors of that study noted that larger studies are needed [83].

MT-based imaging of the brain and spinal cord has shown great potential for clinical assessment in chronic demyelinating diseases, such as chronic SCI, multiple sclerosis, and genetic leukodystrophies. A recent development in MT imaging—use of CSF as a tract-specific motion-insensitive internal standard—has enabled this modality to distinguish gray and white matter regions in the spinal cord better and to be a more sensitive indicator of myelin integrity [89,90]. In contrast to cord atrophy, as measured by T_1w and T_2w images, MT-weighted imaging normalized by CSF (MTCSF), an MT-metric, correlates well with neurologic function as measured by the Expanded Disability Status Scale (EDSS) and great toe vibration threshold [89,90] in patients with noninflammatory demyelination conditions. Furthermore, MTCSF, unlike current clinical MRI, is sensitive enough to identify white matter abnormalities in the dorsal column of patients with abnormal vibratory sense [89,90].

The use of MTCSF high-resolution images for region-of-interest (ROI) seeding is allowing us to quantify the integrity of specific white matter tracts in the human spinal cord more precisely. Ongoing studies are determining whether DTI- and MT-metrics (FA, MD, eigenvalues, and MTCSF, respectively) can classify the severity of chronic SCI.

"N of 1" examples provide useful information for regeneration. For example, application of advanced structural imaging, such as DTI and MT imaging, to the spinal cord of individuals who have recovered from severe SCI to ASIA D (near-normal function) and only have a white matter rim remaining at the injury epicenter is useful in understanding that a cure does not require complete repair of the CNS and demonstrates just what elements are required for curative recovery. In animal models of SCI, estimates suggest that 10% to 15% of axons surviving injury in the donut-like rim of white matter crossing the lesion are sufficient to allow walking. Preliminary data from such MRI studies in human patients with SCI and cure-like recoveries indicate that the conclusions from animal studies provide an accurate indication in human beings; specifically, less than 15% of functional connections (ie, loss of three quarters of the cord at the injury epicenter) are sufficient for walking (see Fig. 1). Larger scale studies across the ASIA impairment scale are important for developing structural criteria for grading injury.

Repairing the injured spinal cord

The spontaneous regenerative events that follow SCI suggest that the CNS has the potential to repair itself. Perhaps the difference in regenerative abilities between reptiles and mammals is not that mammalian evolution produced a limited capacity for regeneration but, rather, that the advanced mammalian CNS has a regeneration equation that is more heavily weighted by nonoptimized determinants of regeneration. Assuming the latter, identification of the key variables requiring optimization is of paramount importance, because optimizing such factors would predictably aid spontaneous regeneration and recovery (Fig. 2). Such strategies are important to maximize future curative treatments.

Growing evidence indicates substantial but limited cellular and molecular mechanisms for self-repair. In animal models of SCI, genesis of new neurons has not been observed but proliferation in the ependymal and periependymal canal gives rise to precursor cells that differentiate toward glial lineages [91–93]. New neurons are born and added in at least two CNS areas: the olfactory bulb and the hippocampal dentate gyrus. In addition, the response of existing neurons to injury is compatible with limited self-repair mechanisms. Neurons upregulate their expression of regeneration-associated proteins, such as growth-associated protein-43 (GAP-43 [94,95]; β1-tubulin and βII-tubulin [96,97]; and cell adhesion molecules, including L1 [98]. Several growth-promoting molecules, such as fibroblast growth factor-2 (FGF-2) [99], ciliary neurotrophic factor (CNTF) [99], glial growth factor-2 (GGF-2) [99], glial-derived neurotrophic factor (GDNF), and vascular endothelium growth factor (VEGF), are released into the injury environment. These events could positively influence axon regeneration at the site of injury. In fact, axons sprout spontaneously, and these sprouts can persist for long periods [100–102]. Moreover, remyelination by surviving oligodendrocytes, maturing oligodendrocyte precursors [23], or invading Schwann cells [103] also occurs after injury. At present, the degree to

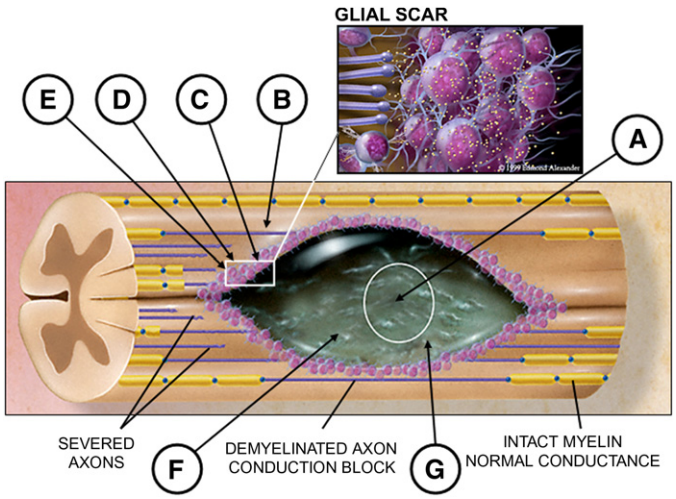

Fig. 2. Barriers to regeneration and common strategies of spinal cord repair. (*A*) Prevention of progression of secondary injury: necrotic and apoptotic cell death would be prevented by antiexcitotoxic drugs and antiapoptotic treatments. (*B*) Compensation for demyelination: chemicals that prevent conduction block in demyelinated areas and agents that encourage surviving oligodendrocytes to remyelinate axons would be provided. Lost oligodendrocytes would be replenished. (*C*) Removal of inhibition: agents that block the actions of natural inhibitors of regeneration or drugs that downregulate expression of inhibitory proteins would be provided. (*D*) Promotion of axonal regeneration: growth factors that promote regeneration (sprouting) of new axons would be provided. (*E*) Direction of axons to proper targets: guidance molecules would be provided, or their expression would be increased in host cells. (*F*) Creation of bridges: bridges would be implanted into the cyst, which would provide directional scaffolding that encourages axon growth. (*G*) Replacement of lost cells: cells capable of generation of all cell types (progenitor cells or ES cells) would be implanted. Substances that induce undifferentiated cells to replace dead cells would be provided. Transplanted cells to deliver regenerative molecules would also be used. (Reproduced with permission of Edmond Alexander. Alexander and Turner Studio, Grayton Beach, FL; 2002.)

which these cellular and molecular events contribute to endogenous restoration of function after SCI is unclear. It is apparent, however, that they are not sufficient for substantial recovery of function. Exciting research is beginning to demonstrate that the mechanisms of spontaneous regeneration are malleable to optimization. Recent work suggests that many of the mechanisms of spontaneous regeneration may share common approaches to optimization, such as neural activity.

This raises the question of how much of the cord has to be restored to achieve significant recovery of function. In the feline model of SCI, rough estimates of retention of 10% of white matter axons seem to be sufficient for recovery of spontaneous walking without external support [104,105]. In the Macaque monkey (*Macaca fascicularis*), 25% of spinal tissue spared allows the animal to regain functional use of its hind limbs [106]. Additional human observations further support the concept that only a fraction of the nervous system is required to retain function. Good examples of this include (1) postmortem analysis of spinal cord partial transections completed for pain relief; (2) hemispherectomies for tumor resection or intractable epilepsy; and (3) recent analysis of damaged but fully recovered individuals despite substantial SCI, as evidenced by a central cyst at the injury epicenter. The clinical practice of neurosurgeons illustrates multiple N of 1 examples supporting these concepts, and it is useful to discuss several key examples. Postmortem histologic examination of cancer patients whose spinal cords had been transected for pain relief showed that greater than 50% transection affected locomotion only weakly or temporarily [107], indicating that more than half of the cord is not required for normal function. This is understandable when one considers the gait central pattern generator (CPG), a minicomputer governing leg patterning control. Additionally, complete loss of central gray matter, including partial damage to the outer rim of white matter at the injury level, can be associated in human patients with an ASIA D classification, which includes the ability to walk (see

Fig. 1) [3,43]. Furthermore, other studies of brain function indicate that near-normal function can occur even after a hemispherectomy. A hemispherectomy is completed under conditions in which function is progressively lost because of pathologic findings that can be eliminated by removal of the affected cortex. Recovery from a hemispherectomy is extremely good in the young nervous system in keeping with SCI data in newborns. More recent work demonstrates that hemispherectomies can be performed in a much older nervous system (including the mid-teens in human patients) with recoveries similar to those of the newborn CNS [108]. Therefore, an important concept in regenerative medicine is that a disproportionate return of function can occur with limited regenerative repair. The reasons accounting for this include the presence of pattern generators governing complex function, redundancy in major projection pathways to the cord, and the ability of the CNS to undergo marked plasticity by transferring functional control of systems that have lost their control centers. Perhaps the best understood is the concept of CPGs, particularly in the spinal cord. For example, a CPG governing aspects of functional control of walking exists in the distal lumbar cord [109–111]. The CPGs are evolutionarily conserved across the spectrum of animals to human beings. Obviously, walking on two small feet is more complex than quadripedal locomotion; yet, the human CPG for gait exists and has been shown to be automatable by sensory stimuli or epidural or intraparenchymal single-point electrophysiologic stimulation. The CPG is localized diffusely in the lumbar cord. A similar pattern generator exists for arm and hand motion with walking as well as for breathing, both of which are located in the cervical spinal cord. Because most SCIs occur in the cervical and thoracic areas of the cord, they leave the lumbar gait CPG intact. Multiple groups have pioneered harnessing the potential of the gait CPG for retraining walking in those recovering from SCI or other disorders causing gait difficulties [109–114]. Considerable progress has been made, enabling most individuals classified as ASIA C to recover to open-ground walking. Although a recent multicenter trial evaluating partial body weight–supported walking (PBWSW) failed to reach its primary end points [115,116], considerable interest exists in the field. The Christopher Reeve Foundation has created a NeuroRecovery Network designed to utilize and test the usefulness of PBWSW [117]. The authors' group has recently discovered a robust example of communication from the lumbar gait CPG to the arm and hand CPG. Individuals with C1 to C5 SCI exhibit upper arm movements that are in correct gait-like phase with the legs when the legs are actively cycled using an FES bicycle. This suggests that the arm and hand CPG is located in the distal cervical spine or upper thoracic cord. A key characteristic of this observation is that this link of CPGs only occurs with active FES-induced cycling but not with passive cycling. Understand the underpinnings of this cross-talk is important in aiding understanding of repair strategies. CPGs can respond and adapt to different sensory inputs, akin to the observation that a decapitated chicken is able to run upright and adapt to stabilization sensory input appropriately. Therefore, many of the complexities of function are governed by CPGs, and regenerative strategies need to take advantage of these minicomputers for design of optimal repair strategies.

Repair strategies aimed at functional repair after spinal cord injury

The pathophysiology of SCI indicates that repair of lost function requires a multifaceted approach (see Fig. 2; Table 3). Thus, investigators have transplanted various cellular substrates in efforts to repair the injured spinal cord. Substantial experience has been obtained with fetal-derived CNS cells, embryonic-derived CNS cells, and immature CNS-derived cells as well as a host of progenitor cells from various organs. Transformed cell lines, to enable limited replication, were a major source of tools used for transplantation, but superior tools, primarily adult-derived progenitors and particularly embryonic stem (ES) cell–derived progenitors, have now replaced transformed cell lines. This large body of work is well reviewed and is outside the focus of this review; however, interested readers are encouraged to read two excellent reviews in this area [118,119]. The authors concentrate on transplantation of ES cells, although they include several proof-of-principle experiments involving other sources of stem cells. The reasons are multifold. First, ES cells offer one of the greatest tools for understanding regeneration. ES cells were the basis of the transgenic mouse era that revolutionized neuroscience, having been discovered in 1981. A similar revolution is occurring today at the tissue culture level because of these incredible tools. ES

Table 3
Barriers to regeneration and strategies to overcome

Barriers	Mechanisms	Strategies
Tissue lost (injury epicenter, typically cystic)	Cell replacement	Transplantation
	Bridging the gap	Peripheral nerve transposition
	Endogenous cell replacement	ABRT to stimulate endogenous cell birth and survival
	Appropriate cell differentiation	Deliver growth factors or ABRT to induce neuronal differentiation
Demyelination	Cell replacement	Transplantation
	Stimulation of endogenous oligodendrocyte precursors to myelinate	ABRT or growth factor delivery
	Overcome conduction block	Delivery drugs that enhance conduction (ie, 4-AP, HP-184)
Scar and inhibitory molecules	Antibody therapy	IN-1 antibody
	Infusion of enzymes	Chondroitinase ABC, sialidase, hyaluronidase
		ABRT to inhibit expression of inhibitory effects
Lack of growth molecules	Restoration of key molecules	Overexpression of growth factors by genetically altered transplanted cells
		ABRT to stimulate cellular release
Guidance molecules	Cell transplantation	Overexpression of growth factors by transplanted cells
	Induction of endogenous expression	Deliver of agents to induce signaling
		ABRT to induce molecular expression

Abbreviations: ABRT, activity-based restoration therapy; 4-AP, 4-aminopyridine.

cells are the only cells capable of double-allele deletion and insertion, offering unprecedented tools for scientific discovery. ES cells can also make every other stem cell and progenitor cell.

The authors contrast the regenerative use of ES cells with a noncellular approach to optimizing spontaneous regeneration, ABRTs. Although, on the surface, these approaches seem to be divergent, they share many similar cellular and molecular mechanisms, and curative treatments, such as ES cell transplantation, are expected to have synergistic benefits with other strategies that optimize spontaneous regeneration and recovery, such as ABRT rehabilitation.

Transplanting embryonic stem cells as therapy for spinal cord injury

Several unique features make ES cells primary candidates for transplantation-based therapies to repair or treat SCI. First, ES cells can replicate indefinitely while maintaining genetic stability (Fig. 3), although it is essential to examine the karyotypes of cultured cells before transplanting them, because genetic instability may occur, as with all dividing cells that undergo multiple passage times in the artificial conditions of culture. Second, ES cells, being pluripotent, can differentiate into every cell type in the mammalian body (see Fig. 3; Figs. 4 and 5). Third, ES cells can be genetically manipulated in unprecedented ways not possible in any other later staged stem or progenitor cell or somatic cell. They can be engineered to express functional markers or other genes of therapeutic value in a controlled or conditional manner. Fourth, they have low immunogenicity (propensity to induce an immune response), which may render them less susceptible to rejection by the host's immune system [120]. These features position ES cells as an indispensable tool in biomedical research and suggest ES cell transplantation as a potential treatment for SCI and other diseases. Once the general safety issue of avoiding the transmission of animal pathogens through transplantation has been overcome, the successful use of ES cells for treating SCI in a clinical setting depends on the cells' ability to avoid graft rejection. ES cells offer additional

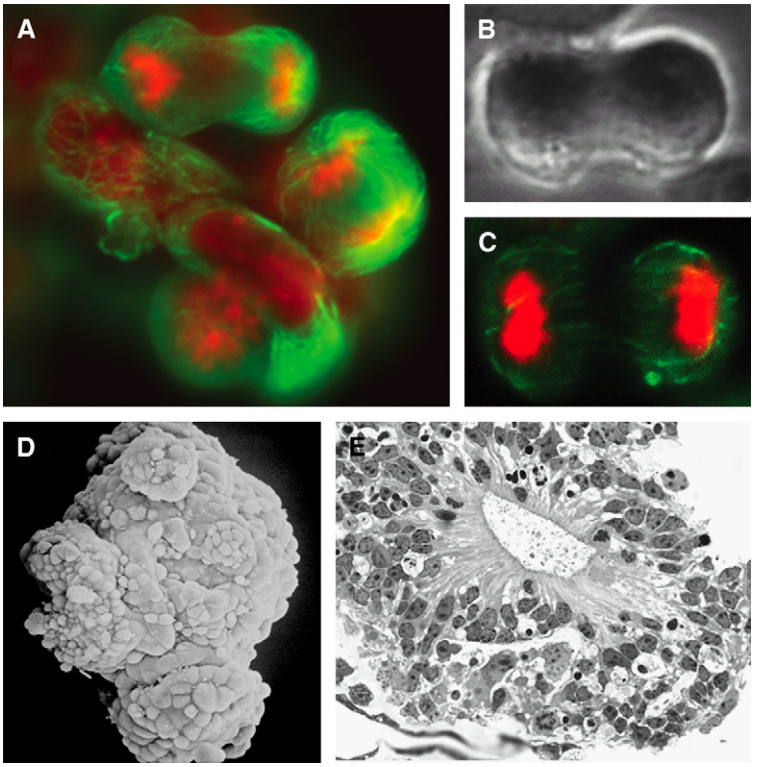

Fig. 3. Murine ES cells in culture. (*A–C*) Undifferentiated mouse ES cells dividing in a culture dish. (*A, C*) Immunofluorescence images demonstrate antiβtubulin (*green*) and anti-DNA (Hoechst; *red*). The phase image (*B*) of an identical field corresponding to the immunofluorescence image (*C*) is shown. (*D*) Scanning electron microscope (SEM) image shows a 4−/4+ embryoid body derived from ES cells, characterized as floating clusters of undifferentiated cells. (*E*) Early notochord-like structure from thin plastic section electron microscopy (EM) from a cross section through an embryoid body derived from mouse ES cells. (*Adapted from* McDonald JW, Becker D, Holekamp TF, et al. Repair of the injured spinal cord and the potential of embryonic stem cell transplantation. J Neurotrauma 2004;21(4):387; with permission.)

genetic advantages for the problem of immunogenicity and rejection.

In previous experiments involving murine neuronal progenitors (derived from ES cells) that were transplanted into a contusion rat model of SCI 9 days after the injury, the authors demonstrated that ES cells (1) survive for weeks after transplantation, (2) do not form teratomas in the spinal cord, (3) differentiate into the three cell types of the neuronal lineage (neurons, oligodendrocytes, and astrocytes), and (4) induce functional improvements [15]. Such improvements could occur by means of several mechanisms other than simply replacing lost neural elements. First, ES cells can secrete neurotrophic factors at the injury site, and neurotrophic factors, such as neurotrophin-3 (NT-3) and brain-derived neurotrophic factor (BDNF), promote cell survival and induce proliferation of oligodendrocytes and myelination of regenerating axons in animal models of SCI [118]. Second, differentiated derivatives of ES cells can remyelinate surviving axons as well as regenerating axons (Fig. 6) [23]. More specifically, oligodendrocyte precursors derived from human embryonic stem (hES) cells survive transplantation in the contused rat spinal cord [121]. Transplantation of oligodendrocytes derived from hES cells 7 days (but not 10 months) after injury leads to axonal remyelination and partially restores locomotion in the injured animals. In a proof-of-principle experiment to demonstrate that remyelination by transplanted cells correlates with functional improvements in a mouse model of contusion SCI in an immune-compromised mouse, human fetal-derived neuronal progenitors were shown to differentiate into oligodendrocytes

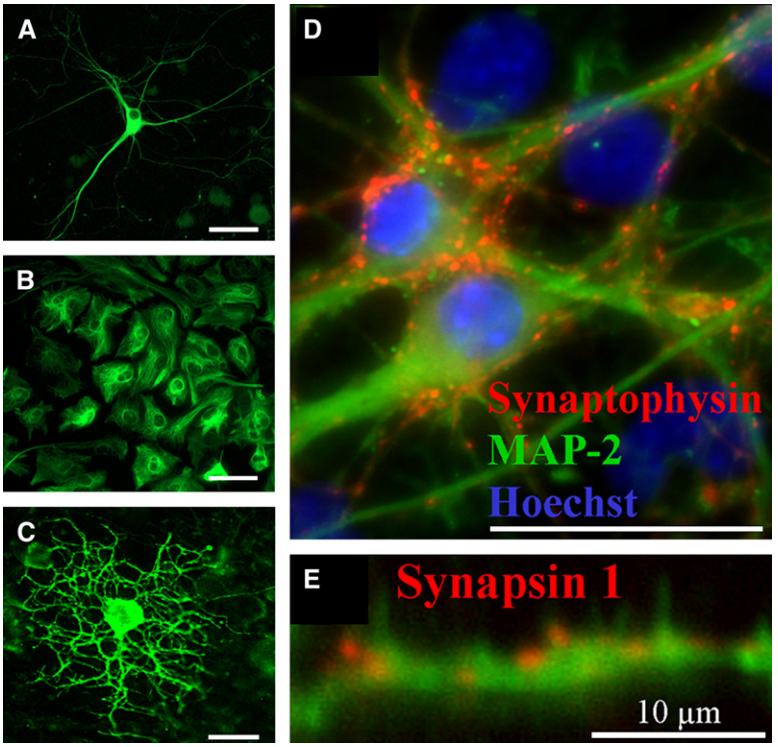

Fig. 4. ES cells differentiate into the principal types of neural cells, and neurons spontaneously create complex neural circuits. Neurons (anti–β-tubulin) (*A*), astrocytes (anti-GFAP) (*B*), and oligodendrocytes (anti-O1) (*C*) are shown. ES cells that have been induced to become neural precursors spontaneously form thousands of synaptic contacts with each other, creating functional neural networks. ES cells induced with retinoic acid were cultured in defined medium for 9 days and then immunolabeled for receptive dendrites (mitogen-activated protein-2; *green*) and presynaptic markers (synaptophysin; *red*). (*D*) ES cell-derived neurons (*green*) covered with multiple synapses (*red*) are depicted. Synapses formed not only at cell bodies but at dendrites. (*E*) High-power image of a dendrite with presynaptic markers is shown. Scale bars = 10 μm. (*Adapted from* Liu S, Qu Y, Stewart TJ, et al. Embryonic stem cells differentiate into oligodendrocytes and myelinate in culture and after spinal cord transplantation. Proc Natl Acad Sci USA 2000;97(11):6128; with permission.)

and remyelinate. In this study, transplantation was associated with functional recovery [122]. The functional recovery was reversed when the transplanted cells were ablated, indicating that the transplanted cells were responsible for recovery. Which of the transplanted cell–derived cells was responsible for recovery remains unknown. Murine ES cell–derived neuroprogenitor cells also survive after transplantation in a rat spinal cord contusion injury model and differentiate into terminally mature oligodendrocytes that remyelinate axons (see Fig. 6). These cells can also remyelinate CNS axons in the shiverer mouse [123], suggesting that remyelination after injury can occur without additional signals beyond those supplied by the transplanted cells and those associated with the injured cord (see Fig. 6). Third, transplanted ES cells and their differentiated derivatives can improve function after SCI by integrating themselves into a host's motor and sensory circuits. Although not performed in the spinal cord, a proof-of-principle experiment showed that hES cells implanted in the brain ventricles of embryonic mice differentiated into functional neural lineages and generated mature active human neurons that seem to integrate into the host adult mouse forebrain [124]. Thus, signals for neuronal differentiation may be highly conserved. Moreover, cholinergic motor neurons differentiate from mouse ES cells and hES cells [125] when transplanted into embryonic chick CNS, injured rodent spinal cord, and an animal model of chronic motor neuron deficiency (newborn sciatic nerve axotomy). These studies further indicate

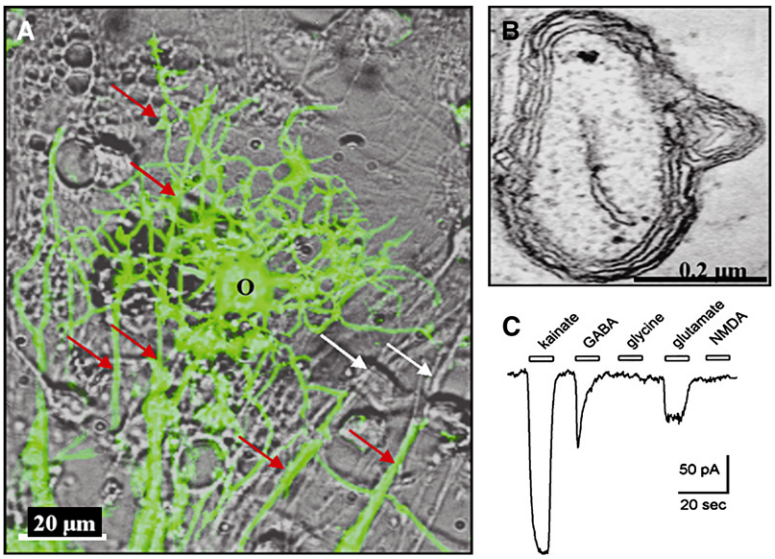

Fig. 5. ES cells produced mature oligodendrocytes with normal anatomic features of myelination and physiologic response to neurotransmitters. (*A*) Cultured ES cell–derived progenitors differentiate into the three principal types of neural cells, and ES cell–derived oligodendrocytes myelinate individual segments of multiple passing axons, just as oligodendrocytes normally do in vivo. White arrows point to axons. Red arrows point to O1 immunoreactive axon segments. (*B*) After 7 days in vitro, normal early patterns of myelination are already present by electron microscopy (EM) analysis. (*C*) ES cell–derived oligodendrocytes demonstrate the presence and absence of agonist gated currents, as assessed by patch-clamp electrophysiology, similar to oligodendrocytes cultured from the neonatal or adult CNS. (*Adapted from* Liu S, Qu Y, Stewart TJ, et al. Embryonic stem cells differentiate into oligodendrocytes and myelinate in culture and after spinal cord transplantation. Proc Natl Acad Sci USA 2000;97(11):6128; with permission.)

that the ES cell–derived motor neurons were capable of sending out axons through ventral roots and sciatic nerves to form neuromuscular junctions with their peripheral muscle targets. The new cholinergic innervations correlated only partially with improvement of motor function, however [126]. In another experiment, murine ES cell–derived motor neurons were treated with dibutyryl cyclic adenosine monophosphate and transplanted into the sciatic nerve of rats, and the rats were treated with subcutaneously injections of rolipram, a phosphodiesterase type 4 inhibitor. This combination of treatments aimed to improve cell survival and axonal extension and to neutralize the inhibitory effects of myelin. The transplanted cells secreted elevated levels of GDNF at the sciatic nerve to attract axons of the transplanted motor neurons toward distal target organs. As a result, the transplanted motor neurons extended axons that reached host muscle, wherein they formed physiologically and functionally active neuromuscular junctions mediating functional improvements [127]. These experiments point to the need for more research on combinatorial approaches to facilitate the regenerative effects of transplanted cells derived from ES cells. Pragmatically, however, combinatorial drug and cell strategies have major barriers at the US Food and Drug Administration (FDA) approval level and are unlikely to move to human treatment quickly.

Additional work has shown promise in transplanting ES cell–derived progenitors into the injured brain, including stroke, traumatic brain injury, and neurotoxin-induced injury [128] (see the articles elsewhere in this issue by Dempsey and Kalluri and Richardson and colleagues). This field is also at an important phase in trying to delineate the cellular mechanisms responsible for recovery. Today, the best that has been done is to correlate anatomic regenerative findings with behavioral recovery, but critical ablation and functional blocking studies are required to identify regenerative mechanisms definitively. Suffice it to say that ES cell transplantation has multiple functions beyond simply replacing cells lost. These include production of growth factors to foster differentiation and prevent cell death, release of metalloproteases to overcome inhibitory effects of scar tissue, and contribution to chimeric blood

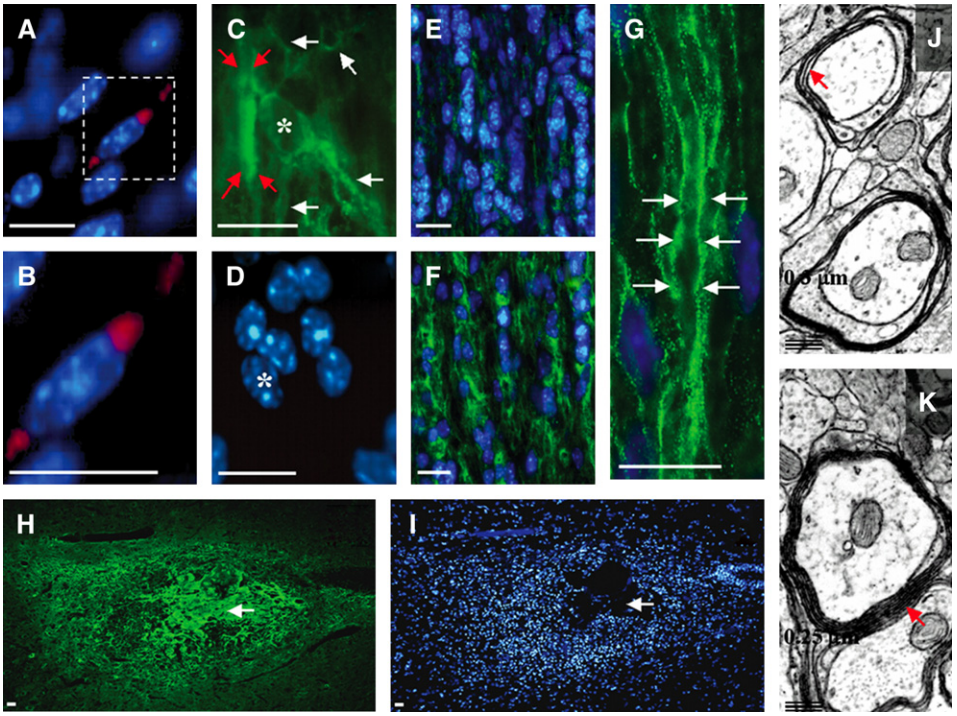

Fig. 6. Cells derived from ES oligospheres can migrate and myelinate axons when transplanted into dysmyelinated spinal cords of adult shiverer mice. Such mice lack the gene for myelin basic protein (MBP). Transplanted cells were identified by cell tracker orange (CTO), epifluorescence, or immunoreactivity for MBP. (*A, B*) CTO-labeled cells aligned with native intrafascicular oligodendrocytes in white matter. (*C, D*) ES cell–derived (MBP+) oligodendrocyte (*asterisk*) with longitudinally oriented processes (*white arrows*) is shown. (*C*) Arrows mark probable myelination around an adjacent axon. (*E*) Little MBP immunoreactivity is seen in white matter in a longitudinal section of spinal cord from a mouse that received sham transplantation. (*F*) Gradient of MBP immunoreactivity centers on the site of ES cell transplantation. (*G*) High magnification of intrafascicular oligodendrocyte nuclei and MBP immunoreactivity, which are two indications of axonal myelination (*white arrows*), in white matter from a mouse transplanted with ES cells. The spatial distribution of MBP immunoreactivity 1 month after ES cell transplantation is shown at low magnification (*H*), with corresponding Hoechst 33,342 counterstaining (*I*). White arrows indicate the center of the transplant site. (*J*) Transmission electron microscopy (EM) shows four loose wraps of myelin, the maximal number of layers typically seen around axons in control animals (*arrow*). (*K*) Transmission EM shows nine or more compact wraps around axons from the transplanted area (*arrow*). Shiverer mutant mice lack a functional *MBP* gene required to form mature compact myelin; therefore, the presence of mature compact myelin is a standard for transplant oligodendrocyte-associated myelin. Scale bars = 10 µm (*A–I*) and 0.3 µm (*J, K*). (*From* McDonald JW, Liu XZ, Qu Y, et al. Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. Proc Natl Acad Sci USA 2000;97(11):6130; with permission.)

vessel formation. The latter is an important observation, because organogenesis and tissue formation have been linked to vasculogenesis [129]. Blood vessels are the conduit that progenitor cells migrate along, and they give rise to key developmental signals for proliferation, migration, and differentiation. Therefore, the scientific path for the next decade is clear, and going beyond correlation is important to understand cellular mechanisms of regeneration definitively. The tools of the ES cell should allow the field to unravel these queries. Much progress is anticipated.

Immunogenicity of embryonic stem cells

Transplantation of cells, tissue, and solid organs between genetically nonidentical individuals causes the immune system to reject the graft and can also create a problem of graft-versus-host disease. The proteins responsible for tissue incompatibility, the histocompatibility antigens or alloantigens, fall into at least three distinct classes: the major histocompatibility complex (MHC) class I and class II antigens, the minor histocompatibility complex (mHC) antigens, and the ABO

blood group antigens [130]. The most prominent causes of graft rejection are mismatches between graft and host MHC antigens, which are recognized by host T lymphocytes [131]. T lymphocytes do not engage in graft rejection unless costimulatory events help them to mature, however. After MHC-mismatched transplantation, antigen-presenting cells (APCs) migrate to the regional lymph nodes, wherein they encounter and stimulate naive or memory-allospecific T lymphocytes to proliferate and then differentiate into effector T lymphocytes through clonal expansion. Essential to this process are the costimulatory molecules CD80 and CD86 [132].

Weak expression of MHC class I alloantigens in human blastocysts (the source of hES cells) indicates that the ES cells constituting the inner cell mass have low immunogenicity [133]. Recent work has more directly tested and demonstrated a lower than expected immunogenicity for hES cells [120,134]. Fluorescence-activated cell sorting (FACS) has shown that cultured hES cells express low levels of MHC class I alloantigens, no MHC class II alloantigens, and no CD80 and CD86 costimulatory molecules [135,136]. Expression of MHC class I alloantigen increases up to fourfold when the cells differentiate into embryoid bodies, which represent primarily neural progenitor cells. Even when hES cells differentiate in teratomas, however, their levels of MHC class I alloantigens are at least 10-fold lower than those on somatic cells [136]. Undifferentiated ES cells do not express MHC class I alloantigens, and they express only low levels after prolonged differentiation [137].

Interestingly, exposure of teratomas derived from hES cells to interferon (IFN)-α, -β, and -γ (a cytokine family that is released during an immune response) induces expression of MHC class I alloantigens to levels seen in somatic cells [136]. Only IFN-γ induces MHC class I alloantigen expression in undifferentiated hES cells, however [136]. Clinically, this is an important distinction because it suggests that therapeutic ES cell transplantation should be deferred until inflammation has subsided at the injury site and the blood-brain barrier has recovered from the breach of injury, which typically takes longer than 1 week after injury.

Current experiments involving ES cell transplantation into animal models of SCI (xenotransplantations or MHC-mismatched transplantations) use immunosuppression to avoid rejection, such as cyclosporine or FK-506 [138]. Under these conditions, it is impractical to study the reactions of the host immune system to the graft. This reaction has been studied in various strains of immunocompetent mice that received transplants of hES cells, however. Intramuscular injection of hES cells did not elicit a host immune response in the first 48 hours after transplantation [120]. The mice rejected the hES cell–derived cells 1 month after transplantation, however. Transplantations into immune-deficient mice have shown that T lymphocytes play a key role in the xenorejection of undifferentiated hES cells and their derivatives. In the humanized (chimera) mouse model, which enables evaluation of direct immune rejection pathways, undifferentiated hES cells and their differentiated derivatives escaped rejection [134]. Thus, hES cells and their differentiated derivatives do not elicit direct allorejection, and they seem to have low immunogenicity compared with somatic cells [134].

The CNS is considered to be immunologically privileged, which would help to prevent cell rejection [139]. Many injury conditions, particularly SCI, are associated with breach of the blood-brain barrier. Thus, fetal-derived dopaminergic neurons transplanted into brains of patients with Parkinson's disease escape immune rejection and provide functional recovery for several years [140]. Nevertheless, evidence exists for graft rejection in these studies, with limited long-term transplant viability. To date, the factors that account for this variability in transplant acceptance or rejection are not well understood. Consequently, MHC-mismatched hES cells transplanted into the injured spinal cord after the blood-brain barrier has been reconstituted may be protected from rejection [130]. The data taken together clearly indicate that issues of rejection of hES cell–derived cells are a primary barrier to optimizing regeneration and recovery. Before hES cells can be used for treating SCI in a clinical setting, more careful evaluation of these rejection issues is required in animal models of SCI transplanted with fully differentiated derivatives of ES cells, such as oligodendrocytes, neurons, and astrocytes. Careful evaluation is necessary, because "absence of evidence is not evidence of absence." Decades of solid organ, bone marrow, and umbilical cord blood transplantation as well as trials of xenotransplantation in human patients clearly indicate that immunologic responses are present and that they are predicted to be a major limitation to successful transplantation for optimizing regeneration and recovery. An additional advantage of ES cells is that, theoretically, it is

possible to minimize the immunogenic response by making the alleles for the three major classes of immune determinants homozygous instead of heterozygous. The reduced genetic variability would limit the immune response. Using this strategy and an analogy to blood type groups, it is possible to develop a set of universal donor ES cells (eg, 10 lines), with each representing a spectrum or class of the immunogenic continuum and each able to be transplanted into individuals whose genetic group closely resembles that of the individual ES cell (Fig. 7). The major advantage of this concept is that such hES cells could be used for all types of transplantation (not just CNS) as well as for other organ repair. An international group (International Neural Transplantation Team [iNTT]) has been assembled to tackle the universal donor concept as a proof of principle in nonhuman primates (see Fig. 7; Fig. 8).

Activity-based restoration therapy rehabilitative strategies in subjects with spinal cord injury

The neural cellular mechanisms of mammalian CNS development are highly activity dependent. The spinal cord below the level of injury experiences the consequences of reduced neural activity after SCI (Fig. 9). This is attributable to altered and reduced input from descending brain centers as well as to reduced sensory input from the periphery because of the consequences of reduced mobility. The dramatic reduction in neuronal activity in the circuitry below the level of SCI injury causes physiologic deficiencies leading to long-term problems stemming from deteriorated cardiovascular conditioning, altered lipid ratios with primary reduction in high-density lipoproteins (HDLs), accelerated osteoporosis and critical loss of bone density, and muscle wasting (see Table 2). This leads to complications, including

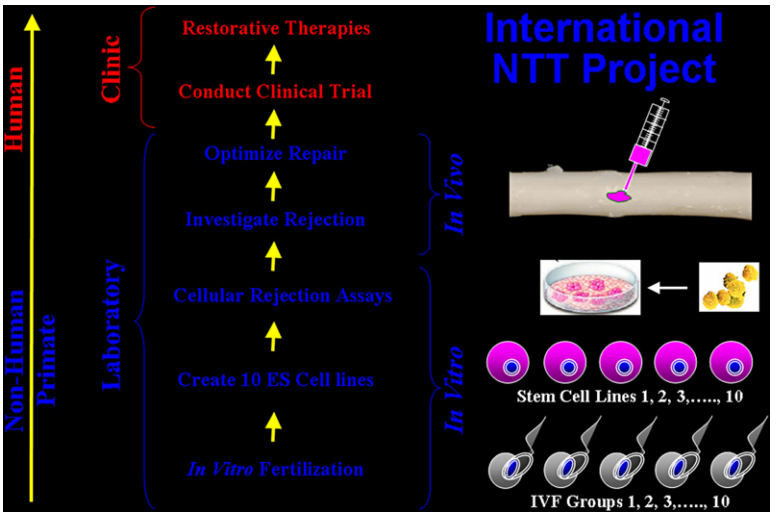

Fig. 7. Universal donor primate ES cells for transplantation repair: from bench to clinic. The Universal Donor ES Cell Project of the iNTT is to be completed in new world nonhuman primates and their ES cells. The project is divided into in vitro and in vivo components. The ultimate goal of this project is to move from nonhuman primate ES cells to hES cells and to develop curative therapies for human patients with the disorder of paralysis. Therefore, in the big picture, this project represents the laboratory phase of the study, whereas the end goal is the clinical phase. The primate project is divisible into four phases: phase I through phase III in vitro and phase IV in vivo. In phase I, 10 ES cell lines are to be created by in vitro fertilization (IVF), with each representative of one of the 10 immunologic classes based on human leukocyte antigen (HLA) genomics. In phase II, additional ES cell lines are to be created by subdividing a single genomic class (ie, group 2 primate genomic class). In phase III, primate ES cells are to be differentiated into neural progenitors and reacted with immune blood cells obtained from primates representing each of the 10 HLA genomic classes, with the prediction that within-class reactions are not likely to cause rejection, whereas across-class reactions are likely to produce immune rejection when assayed in vitro. Based on results from phase III in vitro immunologic studies, proof-of-principle parallel examination of rejection potential is to be completed in vivo by transplanting primate ES cell–derived progenitors into the SCI site. Successful project completion would produce a sufficient world reaction to create a ground swell similar to that of the Human Genome Project, which represents the clinical phase of the big picture.

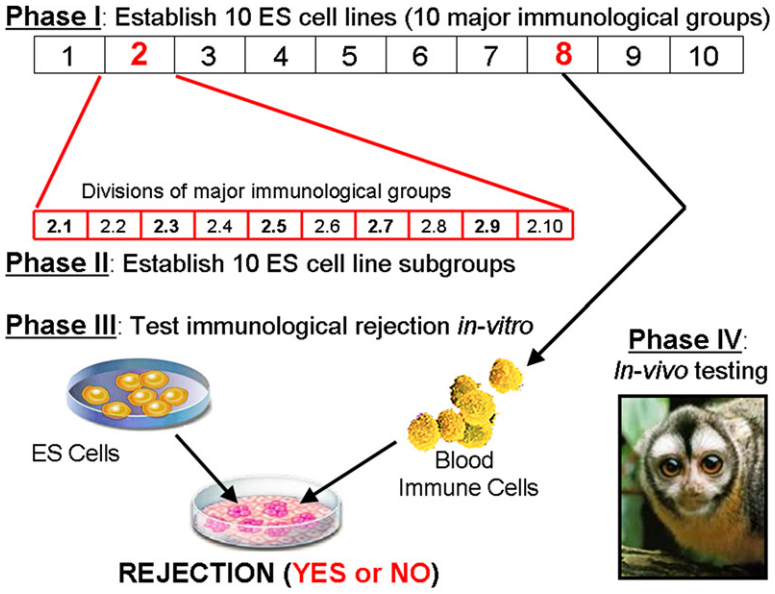

Fig. 8. Testing the concept of universal donor ES cells. Genomic depiction of the MHC groups divisible into 10 groups defined by human leukocyte antigen (HLA) and immunologic genomics (project phase I); each major group (1–10) is divisible into 10 subgroups (ie, 2.1–2.10; project phase II). ES cells from primates are to be created for each of these genetic groups, and primates are to be identified for each of these subgroups based on immunologic factors identified in phase I. Similarly, ES cells from group 2 (G2) are to be exposed to blood-derived immunologic cells from group 8 (G8), with the expectation of there being rejection when ES cells are exposed to blood from primates outside G2, whereas similar exposure of a G2.1 ES cell to blood from the G2.3 primate is expected to elicit minimal response (project phase III). In keeping with these culture data, similar experiments in vivo involving transplantation predict long-term cell survival of transplanted ES cells occurring only in within group transplantation but not outside group transplantation (project phase IV).

enhanced cardiovascular stroke risks, development of glucose intolerance and diabetes, pathologic bone fractures, and skin breakdown. Physical rehabilitation and the known benefits of exercise can theoretically ameliorate these problems [115,141,142]. So why do state-of-the-art rehabilitation facilities not offer exercise and its known and substantial benefits to those who would benefit most—those completely paralyzed? Clinicians have been unaware of how to effectively exercise individuals with complete paralysis. It is true that the benefits of passive movements or weight bearing are limited in completely paralyzed individuals, because passive movements offer no substantial resistance training and force generation is a major determinant of physical reconditioning. Although studies show some physical benefit of passive movements, they are limited and time-inefficient (ie, locomotor training in SCI) [110,114,143]. Additional studies have begun to use resistance training with FES-induced movements against resistance in single limbs, with promising physical muscle rebuilding [144]. It is possible to develop more efficient and effective approaches to allow physical reconditioning in the home, however (Fig. 10). For those with disabilities, time is the greatest obstacle to participation. The International Center for Spinal Cord Injury (ICSCI) has pioneered a novel neurorestoration program that is home based and accomplishable in less than 6 hours per week. The principal goals of the program are to optimize spontaneous regeneration based on ABRT methods while simultaneously maximizing the physical integrity benefits of exercise (see Table 2). Clinical studies have demonstrated predictable and substantial reversal of physical deterioration and reduced medical complications by participation in the ABRT home-based program [1,41,43,142]. Individuals with chronic SCI spend 1 to 6 weeks at the ICSCI center for the following reasons: (1) medical and rehabilitative review and development of a treatment plan for optimization, (2) training in ABRT methods, (3) development of an

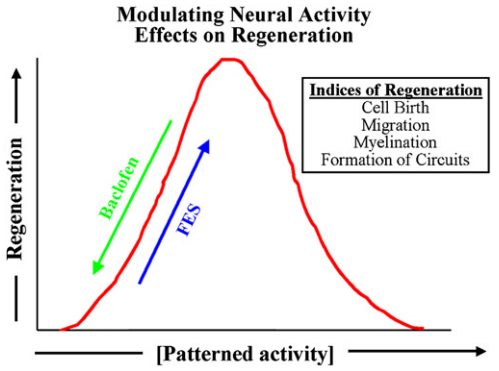

Fig. 9. ABRT hypothesis for optimizing spontaneous regeneration. The hypothesis is that optimal neural activity is required to maximize spontaneous recovery of function after nervous system injury. Data from normal development of the nervous system demonstrate that too little or too much neural activity disrupts important processes of development, including those expected to occur with regeneration, such as cell birth, cell survival, fate and phenotype choices, migration, axonal elongation, guidance and path finding, synapse elimination, and myelination. The red graph demonstrates the hypothesized level of regeneration with increasing activity. Increased neural activity in the cord (eg, mediated by FES) would likely increase regeneration. Decreased neural activity (eg, mediated by baclofen) in the spinal cord may decrease regeneration. The curve is bell shaped, reflecting the idea that excessive activity might be deleterious to regeneration. (*From* McDonald JW. Repairing the damaged spinal cord: from stem cells to activity-based restoration therapies. Am J Phy Med Rehabil 2003;82(10 Suppl):S44; with permission.)

individualized lifelong neurorestoration therapy program for a home-based program, (4) implementation of an ABRT home-based program, and (5) rapid advancement of physical conditioning and functional recovery by means of an intensive day-treatment ABRT program (typically 4 hours per day for 3–5 days per week, with a duration of 1–6 weeks). Multiple studies in animals and human beings are compatible with the idea that optimizing patterned neural activity is able to promote recovery of function. In stroke, constraint-induced force use studies have demonstrated recovery in rodents with stroke as well as in human beings [145,146]. Studies using "enriched environment" paradigms have shown promise in functional recovery in rodent models of stroke and SCI [147–151]. Additionally, experimental rodent studies using running wheels have shown that availability of exercise (ie, activity) is associated with better recovery [110,147,149]. For example, functional gait is improved with PBWSW [110,114,143] and is further enhanced when locomotor training (PBWSW) is combined with FES through epidural spinal cord stimulation [152]. In the authors' clinical experience at the ICSCI, FES bicycle ergometry allows those who are paralyzed to exercise adequately for the first time. Stimulation of the hamstring, quadriceps, and gluteus muscles through superficial electrodes induces substantial plasticity and recovery, even in chronic SCI [1,41,43]. In addition, resistance FES ergometry provides the predicted benefits of exercise in terms of the physical integrity of the body (see Table 2) [3,43]. Therefore, rehabilitative therapy needs to precede any molecular or cellular therapeutic intervention to recover function after SCI to optimize curative efforts. The molecular mechanisms through which FES induces neuronal plasticity are an active area of investigation. Developmental data suggest that cell birth, cell survival, differentiation, myelination, and axonal outgrowth are activity-dependent and key mechanisms malleable by activity-based approaches. To investigate the effects of ABRT and optimizing neural activity further, the authors have pioneered a powerful animal model using FES to stimulate the peroneal nerves to induce a gait-like pattern in the paralyzed hind limbs of animals paralyzed by SCI (Fig. 11) [155]. These studies demonstrate that in chronic SCI rats, FES induces a regionally selective and robust enhancement of progenitor proliferation, survival, and incorporation of new oligodendrocytes [1]. This represents the first demonstration that patterned activity can cause regeneration. The authors have performed similar studies evaluating axonal regeneration, graft survival, and myelination; activity optimized each process.

Multiple studies in rodents are compatible with the interpretation that optimizing neural activity can stimulate regeneration. For example, running wheel activity is associated with enhanced proliferation and incorporation of new neurons in the dentate gyrus of the hippocampus [153]. In additional studies, similar running wheel activity has shown that this activity elevates BDNF levels in the brain and spinal cord [154]. These results are predictable, because BDNF is released from neurons in an activity-dependent manner. Using activity in the endogenous remaining circuitry after injury to elevate BDNF levels is an advanced idea. BDNF is important for cell proliferation, survival, and differentiation as well as for oligodendrocyte myelination.

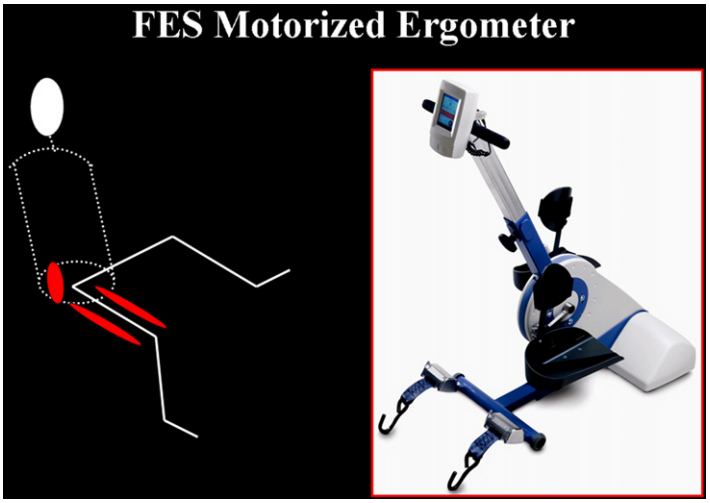

Fig. 10. FES-motorized cycle ergometry for ABRT. Computer-controlled surface stimulation of three muscle groups in each leg (quadriceps, hamstrings, and gluteal muscles) allows paralyzed patients to rotate the wheels of a bicycle under their own muscle power despite lack of volitional control of muscles. FES ergometry is the only method by which paralyzed individuals are able to obtain the benefits of exercise. An hour of FES ergometry is the equivalent of 6000 steps. Such patterned stimulation activates the lumbar gait CPG, sending a normal pattern of neural activity up the cord. In addition to the benefits of physical reconditioning, FES ergometry is done to replicate normal levels of patterned neural activity in the spinal cord below the lesion level in an effort to optimize spontaneous regeneration.

This discussion raises important queries in the current treatment of SCI. Three quarters of individuals with SCI are treated chronically with antispasmodic drugs like baclofen, diazepam, and related drugs [1–3,41]. Baclofen is a γ-aminobutyric acid (GABA) agonist and is known to reduce patterned neural activity in the brain and spinal cord dramatically [1–3,41,43]. Based on this reduction in activity, the authors' hypothesis would suggest that reduced activity would inhibit spontaneous regeneration and recovery (see Fig. 9). Recent studies from our laboratory confirm this hypothesis (unpublished data). Baclofen profoundly inhibits cell proliferation, survival, and differentiation, particularly myelination. Furthermore, baclofen produces an irreversible loss of function. Therefore, considerable thought should now go into treating spasticity. Baclofen is not an extremely effective oral agent, and there is now evidence that it can harm by inhibiting regeneration and recovery. Furthermore, the authors have shown that patterned activity, such as FES bicycling (three times per week for 1 hour each time) is a better way to control spasticity than medications (see Fig. 10). A growing number of groups are now putting their shoulders to this wheel to determine the mechanisms and to optimize ABRT approaches.

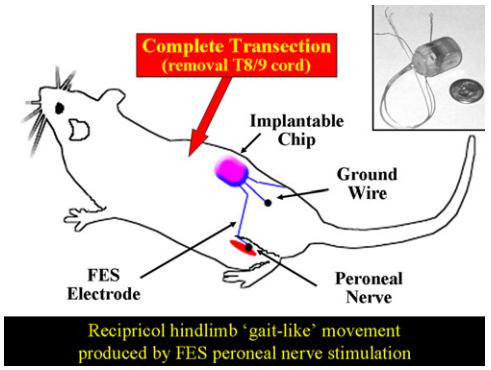

Fig. 11. Experimental FES paradigm. A two-channel FES system can be implanted in rats with SCI to create patterned neural activity that results in leg movements. When connected to the peroneal nerves, 1 second of alternating activation produces a stepping-like movement in the legs. (*From* McDonald JW, Becker D. Spinal cord injury: promising interventions and realistic goals. Am J Phys Med Rehabil 2003;82(10 Suppl):S44; with permission.)

Summary

SCI is a debilitating injury of the CNS that evolves in several stages: development, acute

injury (primary and secondary injury), subchronic injury, chronic injury, and aging. Individuals with chronic SCI are known to lose motor and sensory function below the injury level over a matter of years, and they frequently experience pain, bladder and bowel dysfunction, sexual dysfunction, and autonomic dysreflexia. Several molecular and cellular therapeutic approaches are showing great promise for restoring meaningful function after SCI, however. Additionally, the development of important assessment tools, such as high-resolution advanced MRI, promises to offer superior methods for injury characterization, assessment, classification, prognostication, and treatment tailoring. Advanced structural MRI should enable us to measure the extent of SCI and to tailor treatments according to the microscopic anatomy of an injury. Such treatments might include transplantation of neuronal or oligodendrocyte progenitors derived from hES cells. ES cells are much less likely than somatic cells to be rejected by the host's immune system because of their low alloantigen expression. Also, the CNS offers the benefit of transplantation into an immunologically privileged site. Animal experiments have already shown that transplanted ES cells can remyelinate damaged axons, integrate themselves into the host's motor and sensory circuits, and induce functional improvements. Although cell transplantation alone is unlikely to be sufficient, it could be combined with new approaches to optimize spontaneous regeneration, such as ABRT therapies, including FES cycling and PBWSW therapy, which simulate the electrical stimuli that are absent below the injury level, inducing spontaneous plasticity and regeneration. Thus, a combination of treatments is likely to offer the best chance of ameliorating symptoms and restoring useful function to patients with SCI. The next decade promises great excitement in the field of regenerative medicine and probably effective treatments for optimizing spontaneous regeneration and recovery of function in chronic SCI.

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References

[1] Becker D, Sadowsky CL, McDonald JW. Restoring function after spinal cord injury. Neurologist 2003;9(1):1–15.
[2] Kelly DF, Becker DP. Advances in management of neurosurgical trauma: USA and Canada. World J Surg 2001;25(9):1179–85.
[3] McDonald JW, Sadowsky C. Spinal-cord injury. Lancet 2002;359(9304):417–25.
[4] Muraszko KM. What's new in neurological surgery. J Am Coll Surg 2003;196(6):919–25.
[5] Tator CH. Strategies for recovery and regeneration after brain and spinal cord injury. Inj Prev 2002; 8(Suppl 4):IV33–6.
[6] Penrod LE, Hegde SK, Ditunno JF Jr. Age effect on prognosis for functional recovery in acute, traumatic central cord syndrome. Arch Phys Med Rehabil 1990;71(12):963–8.
[7] Bregman BS, Kunkel-Bagden E, Reier PJ, et al. Recovery of function after spinal cord injury: mechanisms underlying transplant-mediated recovery of function differ after spinal cord injury in newborn and adult rats. Exp Neurol 1993;123(1):3–16.
[8] Bregman BS, Goldberger ME. Infant lesion effect: II. Sparing and recovery of function after spinal cord damage in newborn and adult cats. Brain Res 1983;285(2):119–35.
[9] Hagg T, Oudega M. Degenerative and spontaneous regenerative processes after spinal cord injury. J Neurotrauma 2006;23(3–4):264–80.
[10] Atkinson PP, Atkinson JL. Spinal shock. Mayo Clin Proc 1996;71(4):384–9.
[11] McDonald JW. Repairing the damaged spinal cord. Sci Am 1999;281(3):64–73.
[12] McDonald JW, Becker D. Spinal cord injury: promising interventions and realistic goals. Am J Phys Med Rehabil 2003;82(10 Suppl):S38–49.
[13] Park E, Velumian AA, Fehlings MG. The role of excitotoxicity in secondary mechanisms of spinal cord injury: a review with an emphasis on the implications for white matter degeneration. J Neurotrauma 2004;21(6):754–74.
[14] McDonald JW, Levine JM, Qu Y. Multiple classes of the oligodendrocyte lineage are highly vulnerable to excitotoxicity. Neuroreport 1998;9(12):2757–62.
[15] McDonald JW, Liu XZ, Qu Y, et al. Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. Nat Med 1999;5(12):1410–2.
[16] McDonald JW, Bhattacharyya T, Sensi SL, et al. Extracellular acidity potentiates AMPA receptor-mediated cortical neuronal death. J Neurosci 1998; 18(16):6290–9.
[17] Casha S, Yu WR, Fehlings MG. Oligodendroglial apoptosis occurs along degenerating axons and is associated with FAS and p75 expression following spinal cord injury in the rat. Neuroscience 2001; 103(1):203–18.

[18] Schnell L, Fearn S, Klassen H, et al. Acute inflammatory responses to mechanical lesions in the CNS: differences between brain and spinal cord. Eur J Neurosci 1999;11(10):3648–58.
[19] Bareyre FM, Schwab ME. Inflammation, degeneration and regeneration in the injured spinal cord: insights from DNA microarrays. Trends Neurosci 2003;26(10):555–63.
[20] Jones TB, McDaniel EE, Popovich PG. Inflammatory-mediated injury and repair in the traumatically injured spinal cord. Curr Pharm Des 2005; 11(10):1223–36.
[21] Popovich PG, Horner PJ, Mullin BB, et al. A quantitative spatial analysis of the blood-spinal cord barrier. I. Permeability changes after experimental spinal contusion injury. Exp Neurol 1996;142(2): 258–75.
[22] Popovich PG, Wei P, Stokes BT. Cellular inflammatory response after spinal cord injury in Sprague-Dawley and Lewis rats. J Comp Neurol 1997;377(3):443–64.
[23] McDonald JW, Belegu V. Demyelination and remyelination after spinal cord injury. J Neurotrauma 2006;23(3–4):345–59.
[24] Fawcett JW, Asher RA. The glial scar and central nervous system repair. Brain Res Bull 1999;49(6): 377–91.
[25] Carulli D, Rhodes KE, Brown DJ, et al. Composition of perineuronal nets in the adult rat cerebellum and the cellular origin of their components. J Comp Neurol 2006;494(4):559–77.
[26] Sandvig A, Berry M, Barrett LB, et al. Myelin-, reactive glia-, and scar-derived CNS axon growth inhibitors: expression, receptor signaling, and correlation with axon regeneration. Glia 2004; 46(3):225–51.
[27] Tang X, Davies JE, Davies SJ. Changes in distribution, cell associations, and protein expression levels of NG2, neurocan, phosphacan, brevican, versican V2, and tenascin-C during acute to chronic maturation of spinal cord scar tissue. J Neurosci Res 2003; 71(3):427–44.
[28] Buchli AD, Schwab ME. Inhibition of Nogo: a key strategy to increase regeneration, plasticity and functional recovery of the lesioned central nervous system. Ann Med 2005;37(8):556–67.
[29] Zhang Y, Winterbottom JK, Schachner M, et al. Tenascin-C expression and axonal sprouting following injury to the spinal dorsal columns in the adult rat. J Neurosci Res 1997;49(4):433–50.
[30] Pasterkamp RJ, Giger RJ, Baker RE, et al. Ectopic adenoviral vector-directed expression of Sema3A in organotypic spinal cord explants inhibits growth of primary sensory afferents. Dev Biol 2000;220(2): 129–41.
[31] Benson MD, Romero MI, Lush ME, et al. Ephrin-B3 is a myelin-based inhibitor of neurite outgrowth. Proc Natl Acad Sci USA 2005;102(30): 10694–9.
[32] GrandPre T, Nakamura F, Vartanian T, et al. Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. Nature 2000; 403(6768):439–44.
[33] Mukhopadhyay G, Doherty P, Walsh FS, et al. A novel role for myelin-associated glycoprotein as an inhibitor of axonal regeneration. Neuron 1994; 13(3):757–67.
[34] Moreau-Fauvarque C, Kumanogoh A, Camand E, et al. The transmembrane semaphorin Sema4D/CD100, an inhibitor of axonal growth, is expressed on oligodendrocytes and upregulated after CNS lesion. J Neurosci 2003;23(27):9229–39.
[35] Faulkner JR, Herrmann JE, Woo MJ, et al. Reactive astrocytes protect tissue and preserve function after spinal cord injury. J Neurosci 2004;24(9): 2143–55.
[36] Sadowsky C, Volshteyn O, Schultz L, et al. Spinal cord injury. Disabil Rehabil 2002;24(13):680–7.
[37] Guizar-Sahagun G, Velasco-Hernandez L, Martinez-Cruz A, et al. Systemic microcirculation after complete high and low thoracic spinal cord section in rats. J Neurotrauma 2004;21(11):1614–23.
[38] McKinley WO, Jackson AB, Cardenas DD, et al. Long-term medical complications after traumatic spinal cord injury: a regional model systems analysis. Arch Phys Med Rehabil 1999;80(11):1402–10.
[39] Vaidyanathan S, Soni BM, Gopalan L, et al. A review of the readmissions of patients with tetraplegia to the Regional Spinal Injuries Centre, Southport, United Kingdom, between January 1994 and December 1995. Spinal Cord 1998; 36(12):838–46.
[40] Corbetta M, Burton H, Sinclair RJ, et al. Functional reorganization and stability of somatosensory-motor cortical topography in a tetraplegic subject with late recovery. Proc Natl Acad Sci USA 2002;99(26):17066–71.
[41] McDonald JW, Becker D, Sadowsky CL, et al. Late recovery following spinal cord injury. Case report and review of the literature. J Neurosurg 2002; 97(2 Suppl):252–65.
[42] Myckatyn TM, Mackinnon SE, McDonald JW. Stem cell transplantation and other novel techniques for promoting recovery from spinal cord injury. Transpl Immunol 2004;12(3–4):343–58.
[43] McDonald JW. Repairing the damaged spinal cord: from stem cells to activity-based restoration therapies. Clin Neurosurg 2004;51:207–27.
[44] Stauffer ES. Neurologic recovery following injuries to the cervical spinal cord and nerve roots. Spine 1984;9(5):532–4.
[45] Bedbrook GM, Sakae T. A review of cervical spine injuries with neurological dysfunction. Paraplegia 1982;20(6):321–33.
[46] Frankel HL, Hancock DO, Hyslop G, et al. The value of postural reduction in the initial management of closed injuries of the spine with paraplegia and tetraplegia. I. Paraplegia 1969;7(3):179–92.

[47] Marino RJ, Rider-Foster D, Maissel G, et al. Superiority of motor level over single neurological level in categorizing tetraplegia. Paraplegia 1995;33(9): 510–3.
[48] Maynard FM, Reynolds GG, Fountain S, et al. Neurological prognosis after traumatic quadriplegia. Three-year experience of California Regional Spinal Cord Injury Care System. J Neurosurg 1979;50(5):611–6.
[49] Stover SL, Witten DM, Kuhlemeier KV, et al. Iatrogenic dilatation of the upper urinary tract during radiographic evaluation of patients with spinal cord injury. J Urol 1986;135(1):78–82.
[50] Wu L, Marino RJ, Herbison GJ, et al. Recovery of zero-grade muscles in the zone of partial preservation in motor complete quadriplegia. Arch Phys Med Rehabil 1992;73(1):40–3.
[51] Waters RL, Adkins RH, Yakura JS. Definition of complete spinal cord injury. Paraplegia 1991; 29(9):573–81.
[52] Waters RL, Adkins RH, Yakura JS, et al. Motor and sensory recovery following incomplete tetraplegia. Arch Phys Med Rehabil 1994;75(3): 306–11.
[53] Crozier KS, Graziani V, Ditunno JF Jr, et al. Spinal cord injury: prognosis for ambulation based on sensory examination in patients who are initially motor complete. Arch Phys Med Rehabil 1991; 72(2):119–21.
[54] Folman Y, el Masri W. Spinal cord injury: prognostic indicators. Injury 1989;20(2):92–3.
[55] Foo D, Rossier AB. Preoperative neurological status in predicting surgical outcome of spinal epidural hematomas. Surg Neurol 1981;15(5):389–401.
[56] Katoh S, el Masry WS. Motor recovery of patients presenting with motor paralysis and sensory sparing following cervical spinal cord injuries. Paraplegia 1995;33(9):506–9.
[57] Foo D. Spinal cord injury in forty-four patients with cervical spondylosis. Paraplegia 1986;24(5): 301–6.
[58] Merriam WF, Taylor TK, Ruff SJ, et al. A reappraisal of acute traumatic central cord syndrome. J Bone Joint Surg Br 1986;68(5):708–13.
[59] Roth EJ, Lawler MH, Yarkony GM. Traumatic central cord syndrome: clinical features and functional outcomes. Arch Phys Med Rehabil 1990; 71(1):18–23.
[60] Mori S, van Zijl PCM. Diffusion weighting by the trace of the diffusion tensor within a single scan. Magn Reson Med 1995;33:41–52.
[61] Silberstein M, Tress BM, Hennessy O. Prediction of neurologic outcome in acute spinal cord injury: the role of CT and MR. AJNR Am J Neuroradiol 1992;13(6):1597–608.
[62] Flanders AE, Schaefer DM, Doan HT, et al. Acute cervical spine trauma: correlation of MR imaging findings with degree of neurologic deficit. Radiology 1990;177(1):25–33.
[63] Flanders AE, Spettell CM, Tartaglino LM, et al. Forecasting motor recovery after cervical spinal cord injury: value of MR imaging. Radiology 1996; 201(3):649–55.
[64] Kulkarni MV, Bondurant FJ, Rose SL, et al. 1.5 Tesla magnetic resonance imaging of acute spinal trauma. Radiographics 1988;8(6):1059–82.
[65] O'Beirne J, Cassidy N, Raza K, et al. Role of magnetic resonance imaging in the assessment of spinal injuries. Injury 1993;24(3):149–54.
[66] Ramon S, Dominguez R, Ramirez L, et al. Clinical and magnetic resonance imaging correlation in acute spinal cord injury. Spinal Cord 1997;35(10): 664–73.
[67] Tewari MK, Gifti DS, Singh P, et al. Diagnosis and prognostication of adult spinal cord injury without radiographic abnormality using magnetic resonance imaging: analysis of 40 patients. Surg Neurol 2005;63(3):204–9 [discussion: 209].
[68] Bondurant FJ, Cotler HB, Kulkarni MV, et al. Acute spinal cord injury. A study using physical examination and magnetic resonance imaging. Spine 1990;15(3):161–8.
[69] Marciello MA, Flanders AE, Herbison GJ, et al. Magnetic resonance imaging related to neurologic outcome in cervical spinal cord injury. Arch Phys Med Rehabil 1993;74(9):940–6.
[70] Schaefer DM, Flanders AE, Osterholm JL, et al. Prognostic significance of magnetic resonance imaging in the acute phase of cervical spine injury. J Neurosurg 1992;76(2):218–23.
[71] Yamashita Y, Takahashi M, Matsuno Y, et al. Acute spinal cord injury: magnetic resonance imaging correlated with myelopathy. Br J Radiol 1991; 64(759):201–9.
[72] Flanders AE, Spettell CM, Friedman DP, et al. The relationship between the functional abilities of patients with cervical spinal cord injury and the severity of damage revealed by MR imaging. AJNR Am J Neuroradiol 1999;20(5):926–34.
[73] Yamazaki T, Yanaka K, Fujita K, et al. Traumatic central cord syndrome: analysis of factors affecting the outcome. Surg Neurol 2005;63(2):95–9 [discussion: 99–100].
[74] Yamashita Y, Takahashi M, Matsuno Y, et al. Chronic injuries of the spinal cord: assessment with MR imaging. Radiology 1990;175(3): 849–54.
[75] Basser PJ, Mattiello J, Le Bihan D. MR diffusion tensor spectroscopy and imaging. Biophys J 1994; 66:259–67.
[76] Pierpaoli C, Jezzard P, Basser PJ, et al. Diffusion tensor MR imaging of human brain. Radiology 1996;201:637–48.
[77] Hsu EW, Mori S. Analytical interpretations of NMR diffusion measurements in an anisotropic medium and a simplified method for determining fiber orientation. Magn Reson Med 1995;34: 194–200.

[78] Moseley ME, Cohen Y, Kucharczyk J, et al. Diffusion-weighted MR imaging of anisotropic water diffusion in cat central nervous system. Radiology 1990;176:439–45.
[79] Mori S, Itoh R, Zhang J, et al. Diffusion tensor imaging of the developing mouse brain. Magn Reson Med 2001;46:18–23.
[80] Zhang J, van Zijl PC, Mori S. Three dimensional diffusion tensor magnetic resonance micro-imaging of adult mouse brain and hippocampus. Neuroimage 2002;15:892–901.
[81] Bammer R, Fazekas F, Augustin M, et al. Diffusion-weighted MR imaging of the spinal cord. AJNR Am J Neuroradiol 2000;21(3):587–91.
[82] Cercignani M, Horsfield MA, Agosta F, et al. Sensitivity-encoded diffusion tensor MR imaging of the cervical cord. AJNR Am J Neuroradiol 2003; 24(6):1254–6.
[83] Facon D, Ozanne A, Fillard P, et al. MR diffusion tensor imaging and fiber tracking in spinal cord compression. AJNR Am J Neuroradiol 2005; 26(6):1587–94.
[84] Holder CA, Muthupillai R, Mukundan S Jr, et al. Diffusion-weighted MR imaging of the normal human spinal cord in vivo. AJNR Am J Neuroradiol 2000;21(10):1799–806.
[85] Ries M, Jones RA, Dousset V, et al. Diffusion tensor MRI of the spinal cord. Magn Reson Med 2000; 44(6):884–92.
[86] Tsuchiya K, Fujikawa A, Suzuki Y. Diffusion tractography of the cervical spinal cord by using parallel imaging. AJNR Am J Neuroradiol 2005;26(2): 398–400.
[87] Demir A, Ries M, Moonen CT, et al. Diffusion-weighted MR imaging with apparent diffusion coefficient and apparent diffusion tensor maps in cervical spondylotic myelopathy. Radiology 2003; 229(1):37–43.
[88] Tsuchiya K, Katase S, Fujikawa A, et al. Diffusion-weighted MRI of the cervical spinal cord using a single-shot fast spin-echo technique: findings in normal subjects and in myelomalacia. Neuroradiology 2003;45(2):90–4.
[89] Fatemi A, Smith SA, Dubey P, et al. Magnetization transfer MRI demonstrates spinal cord abnormalities in adrenomyeloneuropathy. Neurology 2005; 64(10):1739–45.
[90] Smith SA, Golay X, Fatemi A, et al. Magnetization transfer weighted imaging in the upper cervical spinal cord using cerebrospinal fluid as intersubject normalization reference (MTCSF imaging). Magn Reson Med 2005;54(1):201–6.
[91] Azari MF, Profyris C, Zang DW, et al. Induction of endogenous neural precursors in mouse models of spinal cord injury and disease. Eur J Neurol 2005; 12(8):638–48.
[92] Horky LL, Galimi F, Gage FH, et al. Fate of endogenous stem/progenitor cells following spinal cord injury. J Comp Neurol 2006;498(4):525–38.
[93] Yang H, Lu P, McKay HM, et al. Endogenous neurogenesis replaces oligodendrocytes and astrocytes after primate spinal cord injury. J Neurosci 2006; 26(8):2157–66.
[94] Bulsara KR, Iskandar BJ, Villavicencio AT, et al. A new millennium for spinal cord regeneration: growth-associated genes. Spine 2002;27(17): 1946–9.
[95] Skene JH, Willard M. Changes in axonally transported proteins during axon regeneration in toad retinal ganglion cells. J Cell Biol 1981;89(1):86–95.
[96] Kwon BK, Liu J, Messerer C, et al. Survival and regeneration of rubrospinal neurons 1 year after spinal cord injury. Proc Natl Acad Sci USA 2002; 99(5):3246–51.
[97] Storer PD, Dolbeare D, Houle JD. Treatment of chronically injured spinal cord with neurotrophic factors stimulates betaII-tubulin and GAP-43 expression in rubrospinal tract neurons. J Neurosci Res 2003;74(4):502–11.
[98] Chaisuksunt V, Zhang Y, Anderson PN, et al. Axonal regeneration from CNS neurons in the cerebellum and brainstem of adult rats: correlation with the patterns of expression and distribution of messenger RNAs for L1, CHL1, c-jun and growth-associated protein-43. Neuroscience 2000;100(1): 87–108.
[99] Zai LJ, Yoo S, Wrathall JR. Increased growth factor expression and cell proliferation after contusive spinal cord injury. Brain Res 2005;1052(2):147–55.
[100] Hill CE, Beattie MS, Bresnahan JC. Degeneration and sprouting of identified descending supraspinal axons after contusive spinal cord injury in the rat. Exp Neurol 2001;171(1):153–69.
[101] Inman DM, Steward O. Ascending sensory, but not other long-tract axons, regenerate into the connective tissue matrix that forms at the site of a spinal cord injury in mice. J Comp Neurol 2003;462(4): 431–49.
[102] Li Y, Raisman G. Sprouts from cut corticospinal axons persist in the presence of astrocytic scarring in long-term lesions of the adult rat spinal cord. Exp Neurol 1995;134(1):102–11.
[103] Gilson JM, Blakemore WF. Schwann cell remyelination is not replaced by oligodendrocyte remyelination following ethidium bromide induced demyelination. Neuroreport 2002;13(9):1205–8.
[104] Blight AR. Cellular morphology of chronic spinal cord injury in the cat: analysis of myelinated axons by line-sampling. Neuroscience 1983;10(2):521–43.
[105] Windle WF, Smart JO, Beers JJ. Residual function after subtotal spinal cord transection in adult cats. Neurology 1958;8(7):518–21.
[106] Eidelberg E, Walden JG, Nguyen LH. Locomotor control in macaque monkeys. Brain 1981;104(Pt 4):647–63.
[107] Nathan PW. Effects on movement of surgical incisions into the human spinal cord. Brain 1994; 117(Pt 2):337–46.

[108] Vining EP, Freeman JM, Pillas DJ, et al. Why would you remove half a brain? The outcome of 58 children after hemispherectomy—the Johns Hopkins experience: 1968 to 1996. Pediatrics 1997;100(2 Pt 1):163–71.
[109] Dietz V. Spinal cord pattern generators for locomotion. Clin Neurophysiol 2003;114(8):1379–89.
[110] Edgerton VR, Tillakaratne NJ, Bigbee AJ, et al. Plasticity of the spinal neural circuitry after injury. Annu Rev Neurosci 2004;27:145–67.
[111] Rossignol S, Chau C, Brustein E, et al. Pharmacological activation and modulation of the central pattern generator for locomotion in the cat. Ann NY Acad Sci 1998;860:346–59.
[112] Barbeau H, Norman K, Fung J, et al. Does neurorehabilitation play a role in the recovery of walking in neurological populations? Ann NY Acad Sci 1998;860:377–92.
[113] Dietz V, Harkema SJ. Locomotor activity in spinal cord-injured persons. J Appl Physiol 2004;96(5): 1954–60.
[114] Edgerton VR, Kim SJ, Ichiyama RM, et al. Rehabilitative therapies after spinal cord injury. J Neurotrauma 2006;23(3–4):560–70.
[115] Dobkin B, Apple D, Barbeau H, et al. Weight-supported treadmill vs over-ground training for walking after acute incomplete SCI. Neurology 2006; 66(4):484–93.
[116] Wirz M, Zemon DH, Rupp R, et al. Effectiveness of automated locomotor training in patients with chronic incomplete spinal cord injury: a multicenter trial. Arch Phys Med Rehabil 2005;86(4):672–80.
[117] Christopher Reeve Foundation. NeuroRecovery Network. Available at: http://www.christopherreeve.org. Accessed December 10, 2006.
[118] Enzmann GU, Benton RL, Talbott JF, et al. Functional considerations of stem cell transplantation therapy for spinal cord repair. J Neurotrauma 2006;23(3–4):479–95.
[119] Reier PJ. Cellular transplantation strategies for spinal cord injury and translational neurobiology. NeuroRx 2004;1(4):424–51.
[120] Li L, Baroja ML, Majumdar A, et al. Human embryonic stem cells possess immune-privileged properties. Stem Cells 2004;22(4):448–56.
[121] Keirstead HS, Nistor G, Bernal G, et al. Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. J Neurosci 2005; 25(19):4694–705.
[122] Cummings BJ, Uchida N, Tamaki SJ, et al. Human neural stem cells differentiate and promote locomotor recovery in spinal cord-injured mice. Proc Natl Acad Sci USA 2005;102(39):14069–74.
[123] Liu S, Qu Y, Stewart TJ, et al. Embryonic stem cells differentiate into oligodendrocytes and myelinate in culture and after spinal cord transplantation. Proc Natl Acad Sci USA 2000;97(11):6126–31.
[124] Muotri AR, Nakashima K, Toni N, et al. Development of functional human embryonic stem cell-derived neurons in mouse brain. Proc Natl Acad Sci USA 2005;102(51):18644–8.
[125] Wu P, Tarasenko YI, Gu Y, et al. Region-specific generation of cholinergic neurons from fetal human neural stem cells grafted in adult rat. Nat Neurosci 2002;5(12):1271–8.
[126] Gao J, Coggeshall RE, Tarasenko YI, et al. Human neural stem cell-derived cholinergic neurons innervate muscle in motoneuron deficient adult rats. Neuroscience 2005;131(2):257–62.
[127] Deshpande DM, Kim YS, Martinez T, et al. Recovery from paralysis in adult rats using embryonic stem cells. Ann Neurol 2006;60(1):32–44.
[128] Vadivelu S, Platik MM, Choi L, et al. Multi-germ layer lineage central nervous system repair: nerve and vascular cell generation by embryonic stem cells transplanted in the injured brain. J Neurosurg 2005;103(1):124–35.
[129] Vadivelu S, Becker D, McDonald JW. Generating chimeric spinal cord: a novel model for transplantable oligodendrocyte progenitors derived from embryonic stem cells. Neurosurg Focus 2005; 19(3):E3.
[130] Drukker M. Immunogenicity of human embryonic stem cells: can we achieve tolerance? Springer Semin Immunopathol 2004;26(1–2):201–13.
[131] Housset D, Malissen B. What do TCR-pMHC crystal structures teach us about MHC restriction and alloreactivity? Trends Immunol 2003;24(8): 429–37.
[132] Rogers NJ, Lechler RI. Allorecognition. Am J Transplant 2001;1(2):97–102.
[133] Jurisicova A, Casper RF, MacLusky NJ, et al. HLA-G expression during preimplantation human embryo development. Proc Natl Acad Sci USA 1996;93(1):161–5.
[134] Drukker M, Katchman H, Katz G, et al. Human embryonic stem cells and their differentiated derivatives are less susceptible to immune rejection than adult cells. Stem Cells 2006;24(2):221–9.
[135] Draper JS, Pigott C, Thomson JA, et al. Surface antigens of human embryonic stem cells: changes upon differentiation in culture. J Anat 2002; 200(Pt 3):249–58.
[136] Drukker M, Katz G, Urbach A, et al. Characterization of the expression of MHC proteins in human embryonic stem cells. Proc Natl Acad Sci USA 2002;99(15):9864–9.
[137] Tian L, Catt JW, O'Neill C, et al. Expression of immunoglobulin superfamily cell adhesion molecules on murine embryonic stem cells. Biol Reprod 1997;57(3):561–8.
[138] Taylor AL, Watson CJ, Bradley JA. Immunosuppressive agents in solid organ transplantation: mechanisms of action and therapeutic efficacy. Crit Rev Oncol Hematol 2005;56(1):23–46.

[139] Ferguson TA, Green DR, Griffith TS. Cell death and immune privilege. Int Rev Immunol 2002; 21(2–3):153–72.
[140] Freed CR, Greene PE, Breeze RE, et al. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. N Engl J Med 2001;344(10): 710–9.
[141] Hicks AL, Adams MM, Martin Ginis K, et al. Long-term body-weight-supported treadmill training and subsequent follow-up in persons with chronic SCI: effects on functional walking ability and measures of subjective well-being. Spinal Cord 2005;43(5):291–8.
[142] Nash MS. Exercise as a health-promoting activity following spinal cord injury. J Neurol Phys Ther 2005;29(2):87–103, 106.
[143] Raineteau O, Schwab ME. Plasticity of motor systems after incomplete spinal cord injury. Nat Rev Neurosci 2001;2(4):263–73.
[144] Mahoney ET, Bickel CS, Elder C, et al. Changes in skeletal muscle size and glucose tolerance with electrically stimulated resistance training in subjects with chronic spinal cord injury. Arch Phys Med Rehabil 2005;86(7):1502–4.
[145] Dobkin BH. Strategies for stroke rehabilitation. Lancet Neurol 2004;3(9):528–36.
[146] Taub E, Uswatte G. Constraint-induced movement therapy: bridging from the primate laboratory to the stroke rehabilitation laboratory. J Rehabil Med 2003;(41 Suppl):34–40.
[147] Engesser-Cesar C, Anderson AJ, Basso DM, et al. Voluntary wheel running improves recovery from a moderate spinal cord injury. J Neurotrauma 2005;22(1):157–71.
[148] Will B, Galani R, Kelche C, et al. Recovery from brain injury in animals: relative efficacy of environmental enrichment, physical exercise or formal training (1990–2002). Prog Neurobiol 2004;72(3): 167–82.
[149] Van Meeteren NL, Eggers R, Lankhorst AJ, et al. Locomotor recovery after spinal cord contusion injury in rats is improved by spontaneous exercise. J Neurotrauma 2003;20(10):1029–37.
[150] Dobrossy MD, Dunnett SB. Optimising plasticity: environmental and training associated factors in transplant-mediated brain repair. Rev Neurosci 2005;16(1):1–21.
[151] Maegele M, Lippert-Gruener M, Ester-Bode T, et al. Multimodal early onset stimulation combined with enriched environment is associated with reduced CNS lesion volume and enhanced reversal of neuromotor dysfunction after traumatic brain injury in rats. Eur J Neurosci 2005;21(9):2406–18.
[152] Herman R, He J, D'Luzansky S, et al. Spinal cord stimulation facilitates functional walking in a chronic, incomplete spinal cord injured. Spinal Cord 2002;40(2):65–8.
[153] van Praag H, Kempermann G, Gage FH. Neural consequences of environmental enrichment. Nat Rev Neurosci 2000;1(3):191–8.
[154] Oliff HS, Berchtold NC, Isackson P, et al. Exercise-induced regulation of brain-derived neurotrophic factor (BDNF) transcripts in the rat hippocampus. Brain Res Mol Brain Res 1998;61(1–2):147–53.
[155] McDonald JW, Becker D, Holekamp TF, et al. Repair of the injured spinal cord and the potential of embryonic stem cell transplantation. J Neurotrauma 2004;21(4):383–93.