

Repair of the Injured Spinal Cord and the Potential of Embryonic Stem Cell Transplantation

JOHN W. MCDONALD,^{1,2} DANIEL BECKER,¹ TERRENCE F. HOLEKAMP,¹
MICHAEL HOWARD,¹ SU LIU,¹ AIWU LU,¹ JAMES LU,² MARINA M. PLATIK,¹ YUN QU,¹
TODD STEWART,² and SUDHAKAR VADIVELU¹

ABSTRACT

Traditionally, treatment of spinal cord injury seemed frustrating and hopeless because of the remarkable morbidity and mortality, and restricted therapeutic options. Recent advances in neural injury and repair, and the progress towards development of neuroprotective and regenerative interventions are basis for increased optimism. Neural stem cells have opened a new arena of discovery for the field of regenerative science and medicine. Embryonic stem (ES) cells can give rise to all neural progenitors and they represent an important scientific tool for approaching neural repair. The growing number of dedicated regeneration centers worldwide exemplifies the changing perception towards the do-ability of spinal cord repair and this review was born from a presentation at one such leading center, the Kentucky Spinal Cord Injury Research Center. Current concepts of the pathophysiology, repair, and restoration of function in the damaged spinal cord are presented with an overlay of how neural stem cells, particularly ES cells, fit into the picture as important scientific tools and therapeutic targets. We focus on the use of genetically tagged and selectable ES cell lines for neural induction and transplantation. Unique features of ES cells, including indefinite replication, pluripotency, and genetic flexibility, provide strong tools to address questions of neural repair. Selective marker expression in transplanted ES cell derived neural cells is providing new insights into transplantation and repair not possible previously. These features of ES cells will produce a predictable and explosive growth in scientific tools that will translate into discoveries and rapid progress in neural repair.

Key words: embryonic stem cell; myelination; neural progenitor; regeneration; restoration of function; spinal cord injury; transplantation

INTRODUCTION

SPINAL CORD INJURY occurs when fragments of broken vertebrae and ligaments compress the very soft cord (which is no thicker than the diameter of the thumb). This

mechanical trauma initiates a cascade of secondary events that rapidly enlarge the damaged area creating ultimately a fluid-filled cyst (Figure 1; Schwab and Bartholdi, 1996; McDonald and Sadowsky, 2002). Most of the affected cells die within 12 hours, however a delayed phase of

Center for the Study of Nervous System Injury and the Restorative Treatment and Research Program, Department of ¹Neurology and ²Neurological Surgery, Washington University School of Medicine, St. Louis, Missouri.

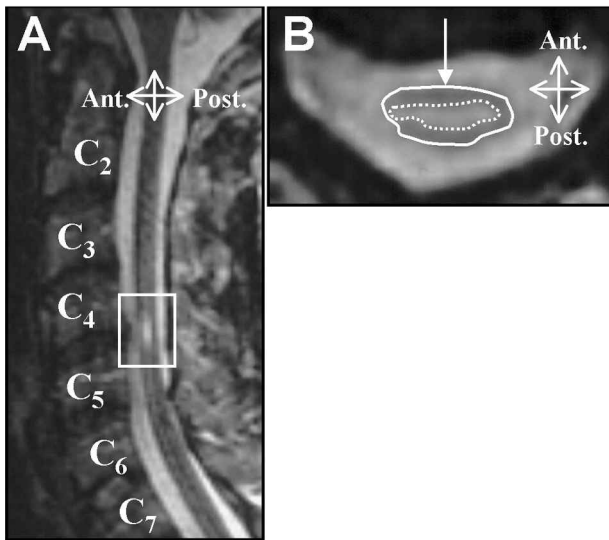


FIG. 1. Magnetic resonance image of a human spinal cord injured between levels C2 and C7; (C2 through C7). **(A)** Sagittal view of a T2 weighted MRI. **(B)** Cross-sectional view of the spinal cord at the lesion epicenter (C4/5 level). The outer continuous line marks the cord's periphery. The inner dotted line outlines the cyst that formed when gray matter died. Reproduced with permission (McDonald and Sadowsky, 2002).

cell death persists for several days or weeks, but its relative contribution to spinal cord dysfunction has not been determined (Beattie et al., 2000).

Cell death occurs in the center of the cord first, where the bodies of nerve cells—gray matter—reside. This region is exquisitely vulnerable because swelling of the cord within the confines of the spinal canal creates far greater pressure than venous blood pressure, which is only 40 to 50 mm of mercury. Therefore, blood flow ceases, and the venous infarct deprives the cord interior of oxygen and nutrients.

Except when the cord is completely transected (which is rare), the damaged region retains a donut-like rim of living tissue (Fig. 1; Bunge et al., 1993; Kakulas et al., 1998; Kakulas, 1999). This rim, which varies in size, contains mostly white matter—and neuronal axons that run to and from the brain. A sheath of a fatty myelin gives axons their typical white color and enables them to conduct nerve impulses, like the insulation around an electric wire. Left in the outer rim are damaged axons and some intact but not functioning properly because of damaged to their myelin and the myelinating oligodendrocytes.

RESTORATION OF FUNCTION

Except for certain areas in the neck, loss of gray matter from a few spinal segments is not very detrimental to

cord function. Losing all the gray matter from a spinal segment in your upper back would not affect your ability to walk or control bowel and/or bladder function, or in the case of rodents affect the Basso-Beattie-Bresnahan (BBB) locomotor score (Basso et al., 1995)—used to test hindlimb walking abilities—because gray matter communicates only with muscles near the injury site, like a local phone service (Hadi et al., 2000; Reier et al., 2002). In contrast, white matter is the long-distance carrier that transmits messages between the brain and distant parts of the body. Consequently, most of the animal models we use in our research involve contusion injury to white matter in the thoracic region of the spine. Our rationale is that the injured spinal cord would not need to be repaired completely to regain function; repairing damaged white matter most likely would be sufficient. Indeed, the person whose MRI is pictured in Figure 1 was injured in an automobile accident 20 years ago. His cord was severely damaged between vertebrae C4 and C5. To date, he has been able to compete in one of the world's most grueling events, the "Iron Man Triathlon," because the white matter running between C4 and C5 now allows sufficient messages from his brain to transverse that region.

Although most people with spinal cord injury do not aspire to compete in triathlons, many would like to regain some normal functions. Researchers therefore have two primary goals (McDonald and Sadowsky, 2002). Most investigators are looking for ways to prevent the secondary damage that enlarges the injured area of the cord, hoping to preserve more function. The second, and more difficult, goal is partial regeneration. A cure is not required, and it cannot be accomplished in the foreseeable future due to the inability to insert new cells into their previous configurations in the cord. Fortunately, partial repair can provide disproportionately large gains in clinical function. Most importantly, it will improve quality of life. If asked to name one function they would most like to regain, most people with spinal cord injury would choose bowel and bladder control. This improvement should be achievable because the nerve fibers that communicate with the bowel and bladder lie at the outer edge of the cord. Other patients, such as tetraplegic actor Christopher Reeve, would like to breathe by themselves, whereas others would like to grasp objects with their hands or enhance their sexual function. Walking is not the top goal of most patients' lists (McDonald et al., 2002).

BASIC SCIENCE OF SPINAL CORD REPAIR

This emphasis on partial repair has fixed our group's focus on oligodendrocytes, the cells that wrap axons in

myelin and allow them to function. Our research, and that of others, has shown that oligodendrocytes disappear during the delayed phase of death that follows spinal cord injury in rodents (Liu et al., 1997; Crowe et al., 1997; Shuman et al., 1997; Li et al., 1999; Abe et al., 1999; Springer et al., 1999; Dong et al., 2003) and possibly humans (Emery et al., 1998). To their demise, each oligodendrocyte myelinates 10 to 20 different axons, and the resulting loss of conductance through that segment has an exponential effect on the loss of function.

Our studies, and those of others, suggest that oligodendrocytes in degenerating regions of the cord die by a suicidal process called apoptosis (Dong et al., 2003; Beattie et al., 2000). Uncovering the details of this type of death and finding ways to prevent it should help preserve these cells, and presumably function, in the injured spinal cord. One of the biggest hurdles is the lack of suitable rodent models for studying remyelination. The variable loss of axons that occurs in current models makes it difficult to correlate axonal loss with physiological changes and, more importantly, with loss of function. Fortunately, recent approaches are likely to produce better animal models. Moreover, many laboratories are using multiple cell types and multiple models to show that remyelination is possible, particularly if myelinating cells can be transplanted into the cord (Kocsis et al., 2002; Blakemore and Franklin, 2000; Bartolomei and Greer, 2000; Ramon-Cueto and Santos-Benito, 2001; Bunge et al., 2002). Harnessing the myelinating potential of endogenous progenitor cells might also be possible (McTigue et al., 1998, 2001; Horner et al., 2000).

Ultimately, many types of restorative strategies will be needed for optimal recovery, but the feasibility of each approach differs. Although we can now make axons sprout from surviving but damaged neurons, it is not possible to make long axons grow and form appropriate reconnections, even in an animal model. Experiments on the brain by Jeffrey Macklis, M.D., Ph.D., at Harvard University are moving us in that direction (Gates et al., 2000).

One area of progress has been the use of molecular approaches that encourage spinal cord cells to secrete substances that promote repair. Such substances might, for example, stimulate the birth of neuronal cells. Although no one has detected birth of neurons in the normal cord (Horner et al., 2000), spinal cord neural precursors are capable of neuronal differentiation in culture or when transplanted into the rat hippocampus (Shihabuddin et al., 2000). It is also clear that the injured cord can promote neurogenesis by transplanted embryonic stem (ES) cell derived neural precursors (McDonald et al., 1999). Understanding the molecular switches governing neuronal differentiation will be important to control and optimize

differentiation of neurons in the injured cord (Wichterle et al., 2002).

Optimizing the function of neuronal circuits that survive spinal cord injury is probably the most achievable strategy. Without losing sight of long-term goals, we should focus on interventions that could be quickly translated into human therapies.

EMBRYONIC STEM CELLS

One of the many possible approaches to spinal cord injury is to use stem cells for cord repair. With this goal in mind, we are focusing our studies on one type of stem cell, embryonic stem (ES) cells, which are obtained from animal embryos (Fig. 2). ES cells have revolutionized biology by permitting the creation of transgenic animals. Neuroscientists are now harvesting the potential of ES cells to obtain cultured cells with added or deleted genes (McDonald, 2001). In the end, the primary importance of ES cells will be as scientific tools rather than therapeutics.

ES cells have several unique features (McDonald, 2001; Wobus 2001; Gottlieb, 2002). First, they can replicate indefinitely without aging. Second, they are pluripotent, being able to give rise to all the different types of cells in the body, as when an embryo becomes a fetus. Third, they are more likely than other types of dividing cells to give rise to genetically normal cells. Fourth, and probably most importantly, ES cells can be easily manipulated genetically (Li et al., 1998).

In most cases, ES cells are obtained from an embryo derived through *in vitro* fertilization. This has been accomplished in most vertebrate species including humans (Evans and Kaufman, 1981; Martin, 1981; Thomson et al., 1995, 1998; Shamblo et al., 1998). Alternatively, the blastocyst stage of an embryo—an inner cell mass surrounded by a trophoblast layer—can be flushed out of fallopian tubes before it implants into the uterus. The stem cell mass can be recognized immunochemically and dissected out. After two decades of work, we know how to grow these cells and expand them in culture to obtain an unlimited source of cells. One flask of cells will give rise to 625 flasks by the end of a week; each containing 5 million undifferentiated ES cells.

Through all their cell divisions, ES cells remain genetically stable (Suda et al., 1987). However, it is important to demonstrate karyotypes stability for each cell line passaged *in vitro* as karyotypes instability can occur. Transplanting them can pose problems, however, because pluripotent cells can deposit normal tissue in the wrong places. If, for example, ES cells are injected into immunosuppressed mice, they form early bone and chon-

drocytes in muscle, which are inappropriate in the nervous system. Transplanted ES cells can also generate teratomas—tumors made of more than one tissue. The best way to avoid such outcomes is to transplant progenitors restricted to making neural cells instead of unselected ES cells.

LABELED NEURAL PROGENITORS

To select neural progenitors, we use a simple technique called the 4-/-4+ retinoic acid induction protocol, which was developed at Washington University in St. Louis by David Gottlieb, Ph.D. (Bain et al., 1995; Liu et al., 2000; for review see McDonald, 2001). During this 8-day procedure, ES cells are passaged with leukemia inhibitory factor (LIF), which prevents their differentiation. When the LIF is withdrawn, the cells differentiate. They can then be plated into nonstick dishes, where they tend to aggregate into small clusters called embryoid bodies. After the first four days, retinoic acid is added to the culture dish. Retinoic acid is a powerful stimulus for the formation of neural tissue; it destines the majority of the ES cells to become progenitor cells of neurons and glia, which can be recognized because they stain with nestin (Table 1). By

cloning cells that have been treated with retinoic acid, we have demonstrated that they are neural progenitors that can give rise to all three types of nerve cells: neurons, astrocytes, and oligodendrocytes (Liu et al., 2000; McDonald, 2001). Moreover, embryoid bodies often contain a structure that resembles the neural tube, the precursor of the central nervous system (Liu et al., 2000). As the cells in retinoic acid treated embryoid bodies get ready to divide, they pull back down towards to tube, divide symmetrically or asymmetrically, and then move back out. When embryoid bodies are stained with various markers of differentiated cells, very few cells stain because most of the cells are nestin-positive neural precursors.

Most of our early studies used the 4-/-4+ protocol to generate cells for culture or transplantation. During 9 days in culture, ES cell derived neural progenitors give rise to several different neuronal phenotypes, type I and type II astrocytes, as well as all stages of the oligodendrocyte lineage. We can also obtain several types of neurons that have axons and other processes. Moreover, Jim Huettner, Ph.D., at Washington University in St. Louis, has shown that these neurons form both inhibitory and excitatory synapses (Bain et al., 1995; Finley et al., 1996; Gottlieb and Huettner, 1999). The synapses form between axons and also between axons and dendrites.

TABLE 1. ANTIBODIES USED TO CHARACTERIZE PHENOTYPES

<i>Antibody against</i>	<i>Source</i>	<i>Marker for</i>
A2B5	Boehringer Mannheim	Oligodendrocytes, astrocytes, and glial progenitors
APC CC-1	Oncogene, Cambridge, MA	Oligodendrocytes
CNPase	Chemicon, Temecula, CA	Oligodendrocytes
Glial fibrillary protein (GFAP)	Diasorin, Stillwater, MN	Astrocytes
MAP-2	Chemicon, Temecula, CA	Neurons
Myelin basic protein (MBP)	Chemicon, Temecula, CA	Oligodendrocytes
Nestin	DSHB	Tripotential CNS progenitor cells, and reactive astrocytes
Neuronal nuclei (NeuN)	Chemicon, Temecula, CA	Neurons
NG2 chondroitin sulfate proteoglycan	Chemicon, Temecula, CA	Bipotential glial progenitors
O4	DSHB	Immature oligodendrocytes
O1	DSHB	Mature oligodendrocytes
Rip	DSHB	Mature oligodendrocytes
Tubulin beta III (TUB-1)	Babco, Richmond, CA	Neurons

DSHB, Developmental Studies Hybridoma Bank.

No marker is specific for any one progenitor or cell stage and the classifications noted above accepted within the field.

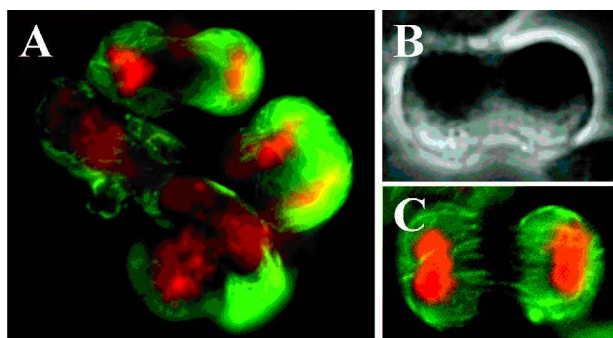


FIG. 2. (A–C) Undifferentiated embryonic stem (ES) cells dividing in a culture dish. (A,C) Immunofluorescence images demonstrating anti-B-tubulin (green) and anti-DNA (Hoechst; red) The phase image (B) of identical field corresponding to the immunofluorescence image (C) is shown in B.

After demonstrating that neural cells derived from ES cells behave in a rather normal manner, we evaluated their suitability for transplantation. For this purpose, we used the New York University rat model of contusion injury (Gruner, 1992), dropping a weight on the spinal cord

between thoracic vertebrae 8 and 10. We began with a 25-mm weight drop injury, which inflicts a rather severe injury but produces animals whose BBB score stabilizes at about 8. Such animals are unable to support their body weight, and the difference between bearing weight and not bearing weight is both clinically relevant and easy to determine.

Nine days after injury, we transplanted 4–/4+ ES cells dissociated from embryoid bodies (McDonald et al., 1999). We chose this time point because we are interested in the cells' regenerative potential. By 9 days, the blood–brain barrier is beginning to reseal and the inflammatory cascade that promotes secondary injury is abating (Popovich et al., 1996, 1997).

During the transplantation procedure, we placed about 1 million cells directly into the cyst that had formed in the center of the cord. To exclude the possibility that outcomes would be specific to one cell line, we initially used two cell lines (D3 and Rosa 26) and have experimented with additional ones since that time. Because we were transplanting male mouse ES cells into immunosuppressed rats, we used adult mouse cortical cells as a control. These cultures contained mainly dead cells plus

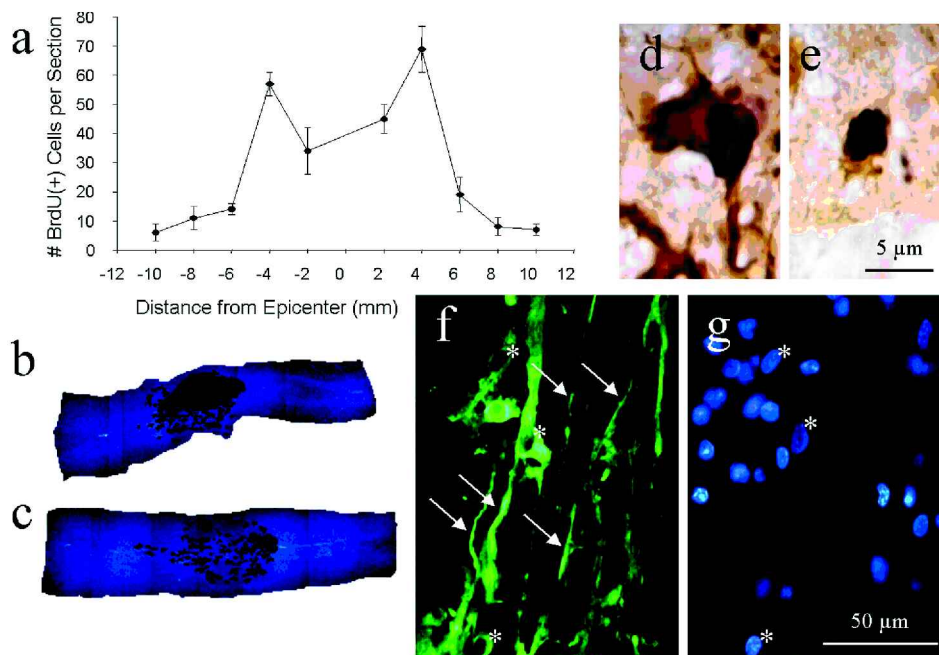


FIG. 3. BrdU-labeled ES cell-derived cells 2 weeks after transplantation. Mean \pm SEM BrdU-labeled nuclei per 1-mm segment in longitudinal sections ($n = 11$ rats, 3 sections per rat) (A). Hoechst 33342-labeled sections 42 days after injury, transplanted with vehicle (B) or ES cells (C) 9 days after injury. BrdU-positive (purple, long arrow) cell co-labeled with GFAP (brown, short arrows) (D). BrdU-labeled (purple and long arrow) co-labeled with APC CC-1 (brown, short arrow) (E). The mouse-specific marker EMA showed processes (arrows) emanating from ES cells (F). Corresponding nuclei are marked by asterisks (G). (Modified from McDonald et al., 1999.)

some astrocytes; if both they and the transplanted neural progenitors had produced an effect, we would have attributed the outcome to inflammation rather than ES cell transplantation.

In order to track the cells after transplantation, we pre-labeled them with three different markers. One was the genetic marker lacZ or green fluorescent protein (GFP; Friedrich and Soriano, 1991; Hadjantonakis et al., 1998, 2000); another was mouse-specific antibodies [M2, EMA (M6)](Lagenaur and Schachner, 1981; Baumrind et al., 1992). We also could use the Y chromosome because we were transplanting cells from male mice into female rats. Hoechst33342 prelabeling was also used but with the understanding of its limitations in marking transplanted cells (Baron-Van Evercooren et al., 1991). Triple labeling is important because of the need to identify the phenotype of transplanted cells and to determine whether they have sprouted axons or other process. Simply staining the cells with a dye specific to neural cells is insufficient.

We also needed to determine whether the transplanted cells would colonize the spinal cord (Fig. 3). Labeling cells before transplantation can be misleading because dying cells can exhibit the markers, as often happens with lacZ. Spontaneous GFP expression is a good solution to this problem because adequate expression requires living cells and diffusible small molecules like GFP are rapidly metabolized when released from dead cells.

Many ES cells die after transplantation, but others divided until they completely fill the cyst (McDonald and Howard, 2002). Then a small percentage of the new cells migrate away from the epicenter of the injury. They can move as far as a centimeter within 12 h of the transplant. Instead of flowing in cerebrospinal fluid, they walk in both directions through the central canal. For guidance, they use radial glia, the barrier between gray and white matter, and the surface of the cord.

We determined how far transplanted cells traveled by tagging them with bromodeoxyuridine (BrdU), which becomes incorporated into DNA when cells divide. Pinpointing BrdU in the cord told us how far the descendants of transplanted cells had moved. By 9 days, ES cells occupied much of the cyst (Fig. 3). Other parts of the cyst were filled with extracellular matrix. Unlike neural progenitors, that are endogenous to the central nervous system, progenitors derived from ES cells produce considerable quantities of laminin and fibronectin (Liu and McDonald, data not shown).

Our more recent experiments have shown that most of the cells that colonize the spinal cord test positive for NG2, a proteoglycan found on oligodendrocyte precursors (Table 1). These cells were not restricted to the oligodendrocyte lineage but developed into the three types of neural cells. Using anti-mouse antibodies, we also showed that the cells that migrate away from the lesion

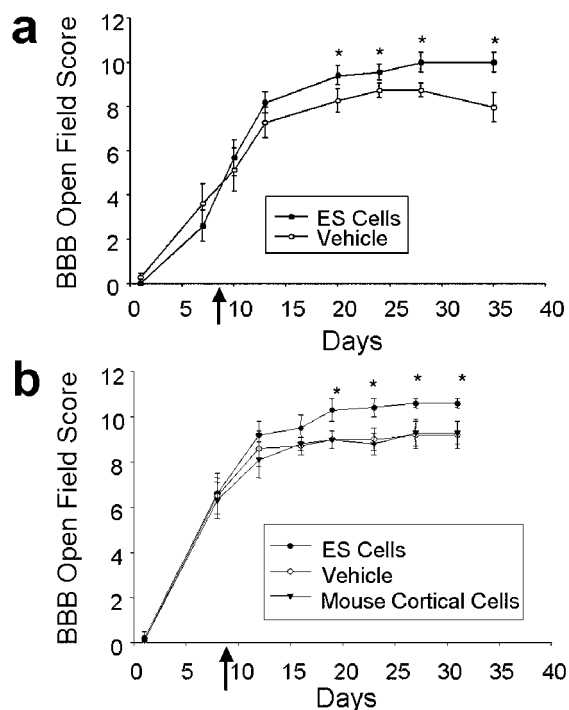


FIG. 4. ES cell-derived cell transplantation improved behavioral recovery. **(A)** Closed circles, ES cell transplant group; open circles, vehicle treated group ($n = 11$ per group, mean \pm SEM). *Difference at $p < 0.05$ vs. control at same time point (repeated measures ANOVA with Tukey's test). **(B)** Similar experiment comparing transplantation of ES cells (closed circles), vehicle (open circles), or adult mouse neocortical cells (closed diamonds) ($n = 6$ per group). The ES cell transplantation group differed from both control groups at the $p < 0.05$ level. Arrows indicate transplantation. Reproduced with permission (McDonald et al., 1999).

resembled neurons. They had a cell body with a prominent nucleolus and processes that were identifiable with GFP.

Approximately 60% of the identifiable transplanted ES cells became oligodendrocytes two weeks after transplantation; most traveled away from the lesion. A few (10%) remained at the lesion level, turning into neurons, and the rest of the surviving cells became astrocytes (about 30%). We have not seen abnormal tissue in transplanted cords, in contrast with our brain experiments. As long as two years after transplantation, the only abnormality has been chondrocyte formation, which can occur in the normal spinal cord. Thus, abnormal tissue formation might depend on where cells are transplanted and on their inherent rate of neurogenesis.

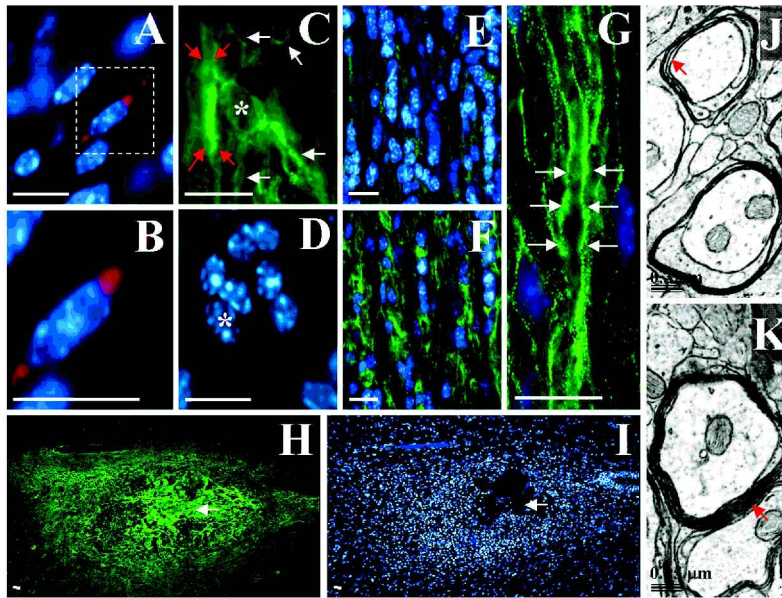


FIG. 5. ES oligosphere-derived cells can migrate and myelinate axons when transplanted into dysmyelinated spinal cords of adult *shiverer* mice, which lack the gene to produce myelin basic protein (MBP) (Dupouey et al., 1979; Gumpel et al., 1983; Molineaux et al., 1986). Transplanted cells were identified by Cell Tracker Orange (CTO) epifluorescence (red) or immunoreactivity for MBP (green). Hoechst 33342 (blue). CTO-labeled cells were found to align with native intrafascicular oligodendrocytes in white matter (A,B). An ES cell-derived (MBP+) oligodendrocyte (asterisk) with longitudinally oriented processes (white arrows) is shown in panels C and D. Red arrows mark probable myelination around an adjacent axon (C). Little MBP immunoreactivity is present in white matter of a longitudinal spinal cord section from a mouse that received sham transplantation (E). A gradient of MBP immunoreactivity is centered on the site of ES cell transplantation (F). Panel G (high magnification) shows intrafascicular oligodendrocyte nuclei (blue) and MBP immunoreactivity (green) characteristic of axonal myelination (white arrows; McTigue et al., 1998; Demerens et al., 1999) in white matter from a mouse that received ES cell transplantation. The spatial distribution of MBP immunoreactivity, one month after ES cell transplantation, is shown at low magnification (H) with corresponding Hoechst 33342 counterstaining (I). White arrows indicate the center of the transplant. Transmission EM shows four loose wraps of myelin, which represents the maximal number of layers typically seen around axons in control animals (red arrow, J), and 9 or greater compact wraps around axons from the area of the transplant (red arrow, K). *Shiverer* mutant mice lack a functional MBP gene that is required to form mature compact myelin, therefore the presence of mature compact myelin is a gold standard for transplant oligodendrocyte associated myelin. Bars = 10 μm (A-I), 0.3 μm (J,K). Reproduced with permission (Liu et al., 2000).

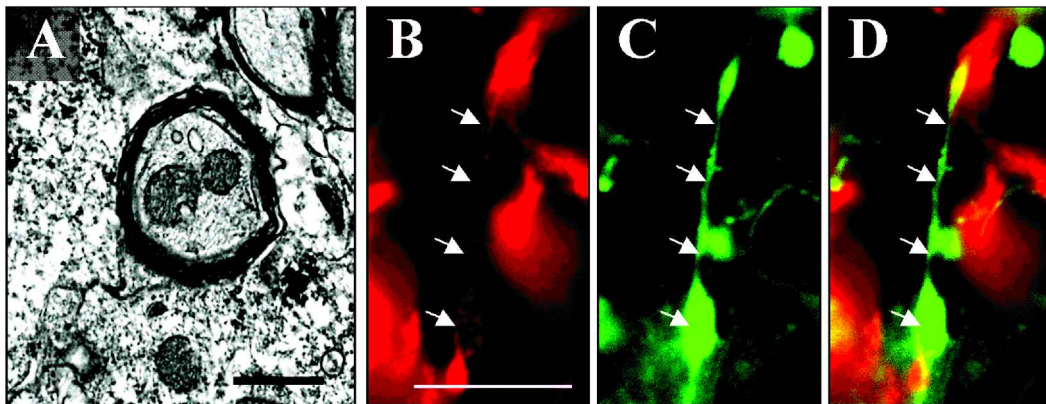


FIG. 6. ES cells can myelinate axons when transplanted into the injured spinal cords of rats. (A) TEM of ES cell derived myelin sheaths surrounding an axon following spinal cord injury. (B-D) Transplanted ES cells were identified by expression of green fluorescent protein (GFP; green), and immunoreactivity for MBP (red). Arrows mark probable myelination around an ES cell derived axon. Bar = 1 μm (A), 10 μm (B-D).

FUNCTIONAL IMPROVEMENT

These early experiments did not involve genetically enhanced cells. Therefore, we did not expect transplantation to improve function. Surprisingly, the score on the BBB scale rose from 8 to 10 after transplantation (McDonald et al., 1999).

The score of sham-transplanted animals typically reaches a plateau at 8 (on the BBB scale), which signifies an inability to bear weight. These animals have about approximately 5–10% of axons remaining in the outer rim of white matter with this lesion, based on white matter area measurements (Basso et al., 1996). In many repetitions of the 25-mm weight-drop protocol, animals transplanted with ES cells have obtained a BBB score of 10 by 6 weeks after transplantation.

The two-point difference is very significant clinically. A typical sham-transplanted animal can move its hind limbs in an uncoordinated fashion but not support the weight of its body or lift its tail (Fig. 4A). A rat transplanted with ES cells can support its body weight while walking, partially lift its tail and stand on its hind limbs (Fig. 4B). This is not normal walking, and we have not identified the underlying mechanism. One possibility is that transplantation allows naked axons in the spinal cord to become remyelinated, given that the majority of transplanted neural progenitors become oligodendrocytes. In the future, we hope to use genetically manipulated oligodendrocytes for transplantation so we can selectively destroy them after function improves. Simultaneous disappearance of the recovered function would strongly implicate remyelination in functional improvement.

MYELINATION IN THE INJURED ADULT SPINAL CORD

Brustle et al. (1999) were the first to demonstrate that ES cell-derived oligodendrocytes could myelinate in the normal immature nervous system. However, remyelination in the adult and injured adult nervous system is a much more difficult task because the developmental sequence of appropriate differentiation signals is lost. To study the myelinating potential of transplanted cells in the injured adult spinal cord, we devised a way to make our 4–/4+ cultures produce a mixture of cells that was 90% oligodendrocytes and 10% neurons and astrocytes (Liu et al., 2000). To obtain this mixture, we dissociated 4–/4+ cultures and grew the cells for 9 days under conditions that promoted neural differentiation. During this time, the neural progenitors differentiated into the three cell types, which then interacted with each other. One of

the early interactions was axon wrapping by oligodendrocytes.

Transmission electron microscopy images of the same cultures revealed axons being loosely wrapped with myelin (Liu et al., 2000). When we repeated this experiment with *shiverer* mice, which cannot make their own myelin (Dupouey et al., 1979; Gumpel et al., 1983; Mo-lineaux et al., 1986), we detected anti-myelin basic protein 1 month after we transplanted 125,000 ES cells into the spinal cord. Thus, myelination must have resulted from the transplant. Hoechst nuclear labeling revealed a higher-than-normal cell density at 1 month and a sub-normal density subsequently, which suggests that some of the transplanted cells either migrated or died. Using immunolabeling for GFP expressing ES cells we have replicated the observation of ES cell derived myelination at the ultrastructural level in the contusion injury model (data not shown).

Although the migration of transplanted cells is encouraging, it does not replenish the spinal cord in desired locations. In the experiment illustrated in Figure 5, we pre-labeled the cytoplasm of ES cells with cell tracker orange. A month after transplantation, the labeled cells were lined up in white matter and co-labeled for mature oligodendrocytes. Therefore, in part, oligodendrocytes from ES cells are instructed to largely perform appropriate repair.

Figures 5 and 6 show ES cells labeled with anti-myelin basic protein. In the high-power image (Fig. 5A), an axon appears to have been wrapped. The lower-power image (Fig. 5B) compares the distribution of immunoreactivity to myelin basic protein in sham-transplanted and ES-transplanted *shiverer* mice. In the latter, transmission electron microscopy (Fig. 5C) detected loosely wrapped myelin that was beginning to compact at this 1-month time point. Although myelin was distributed over several millimeters, the migration of myelinating cells was obviously limited.

We also observed myelination in adult rat spinal cords damaged with ethidium bromide (Liu et al., 2000). In those experiments, we used anti-mouse immunoreactivity to conclusively demonstrate that the myelinating cells came from the transplant rather than from the rat itself. In collaboration with Jeff Bulte, Ph.D., at the National Institutes of Health, we also pre-labeled embryoid bodies with a paramagnetic marker. Two weeks after transplantation, we looked at the marker's distribution.

Unfortunately, MRI studies with paramagnetic markers are complicated by the endogenous deposition of iron that occurs after contusion injury. The paramagnetic approach will probably be more useful for models of chronic spinal cord damage, which lack the hemosiderin and do not deposit iron in the extracellular space.

STEM CELLS AND THEIR PROGENY— IS IT REALLY ONE BIG FAMILY?

Are phenotypically similar neural cells derived from different sources of stem cells similar functionally? Growing evidence cautions such a naive acceptance of linkage of phenotype to function. Particularly since the methods available to subclassify neural cells (e.g., marker expression, electron microscopy, electrophysiology, and genetic selection) are limited at best. Why do ES cell derived neural precursors give rise to neurons but not similar spinal cord derived precursors? Work from multiple labs is suggesting that the cells microenvironment is important in establishing the sequence of what cells will and will not do, particularly in vitro systems. For example, our data suggest that ES cell derived oligodendrocyte progenitors readily myelinate in the injured cord whereas the phenotypically similar endogenous derived precursor appears to be very limited in myelination capacity despite a marked increase in number of oligodendrocyte progenitors following injury. Answers to these questions are just beginning to be unraveled. Understanding these regulator systems will be a key to unlocking the capacity of endogenous and transplanted stem cells.

TOWARDS THE FUTURE

It is impossible to predict what treatments will be effective in restoring the damaged spinal cord as such cures are too futuristic. Medical history has taught us that major treatment breakthroughs were generally not even thought of or even conceived of 20 years earlier. For example, manipulation of the genome was not even a concept until restriction enzymes were discovered. However, it is possible to move forward in a step-wise fashion focusing on small improvements in function. Since we are at the beginning of the field of spinal cord injury repair, even small steps forward can translate into giant leaps for the field.

MARRIAGE OF BASIC AND CLINICAL SCIENCE

To help translate our basic science studies into clinical therapies, Washington University recently erected a building that houses both a 40-bed clinical spinal cord injury unit and 10,000 square feet of research space. This proximity will allow researchers and clinicians to interact on a daily basis to integrate all aspects of research on spinal cord injury. The program has an Acute Spinal Cord

Injury Spinal Trauma Assessment Treatment Team, which includes a physician from each of the eight key clinical services involved in spinal cord injury care. This team sees patients from the time of admission, and its physiatrist and rehabilitation specialists are often the first to arrive in the emergency room, where they immediately initiate rehab. The rehabilitation team also contains four physicians dedicated to spinal cord injury, and the outpatient program offers the full spectrum of neuro-rehabilitation. With this combination of expertise, and with important links to national resources, we hope to quickly translate basic science studies into clinical trials, avoiding the usual roadblock between the two areas of endeavor.

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Address reprint requests to:

John W. McDonald, M.D., Ph.D.

Department of Neurology

Restorative Treatment and Research Program

Campus Box 8518

Washington University School of Medicine

4444 Forest Park Ave.

St. Louis, MO 63108

E-mail: mcdonald@neuro.wustl.edu