3. REPAIRING THE NERVOUS SYSTEM WITH STEM CELLS

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Diseases of the nervous system, including congenital disorders, cancers, and degenerative diseases, affect millions of people of all ages. Congenital disorders occur when the brain or spinal cord does not form correctly during development. Cancers of the nervous system result from the uncontrolled spread of aberrant cells. Degenerative diseases occur when the nervous system loses functioning of nerve cells. Most of the advances in stem cell research have been directed at treating degenerative diseases. While many treatments aim to limit the damage of these diseases, in some cases scientists believe that damage can be reversed by replacing lost cells with new ones derived from cells that can mature into nerve cells, called neural stem cells. Research that uses stem cells to treat nervous system disorders remains an area of great promise and challenge to demonstrate that cell-replacement therapy can restore lost function.

STRATEGIES TO REPAIR THE NERVOUS SYSTEM

The nervous system is a complex organ made up of nerve cells (also called neurons) and glial cells, which surround and support neurons *(see Figure 3.1)*. Neurons send signals that affect numerous functions including thought processes and movement. One type of glial cell, the oligodendrocyte, acts to speed up the signals of neurons that extend over long distances, such as in the spinal cord. The loss of any of these cell types may have catastrophic results on brain function.

Although reports dating back as early as the 1960s pointed towards the possibility that new nerve cells are formed in adult mammalian brains, this knowledge was not applied in the context of curing devastating brain diseases until the 1990s. While earlier medical research focused on limiting damage once it had occurred, in recent years researchers have been working hard to find out if the cells that can give rise to new neurons can be coaxed to restore brain function. New neurons in the adult brain arise from slowly-dividing cells that appear to be the remnants of stem cells that existed during fetal brain development. Since some of these adult cells still retain the ability to generate both neurons and glia, they are referred to as adult neural stem cells.

These findings are exciting because they suggest that the brain may contain a built-in mechanism to repair itself. Unfortunately, these new neurons are only generated in a few sites in the brain and turn into only a few specialized types of nerve cells. Although there are many different neuronal cell types in the brain, we now know that these new neurons can "plug in" correctly to assist brain function.[1] The discovery of these cells has spurred further research into the characteristics of neural stem cells from the fetus and the adult, mostly using rodents and primates as model species. The hope is that these cells may be able to replenish those that are functionally lost in human degenerative diseases such as Parkinson's Disease, Huntington's Disease, and amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig's disease), as well as from brain and spinal cord injuries that result from stroke or trauma.

Scientists are applying these new stem cell discoveries in two ways in their experiments. First, they are using current knowledge of normal brain development to modulate stem cells that are harvested and grown in culture. Researchers can then transplant these cultured cells into the brain of an animal model and allow the brain's own signals to differentiate the stem cells into neurons or glia. Alternatively, the stem cells

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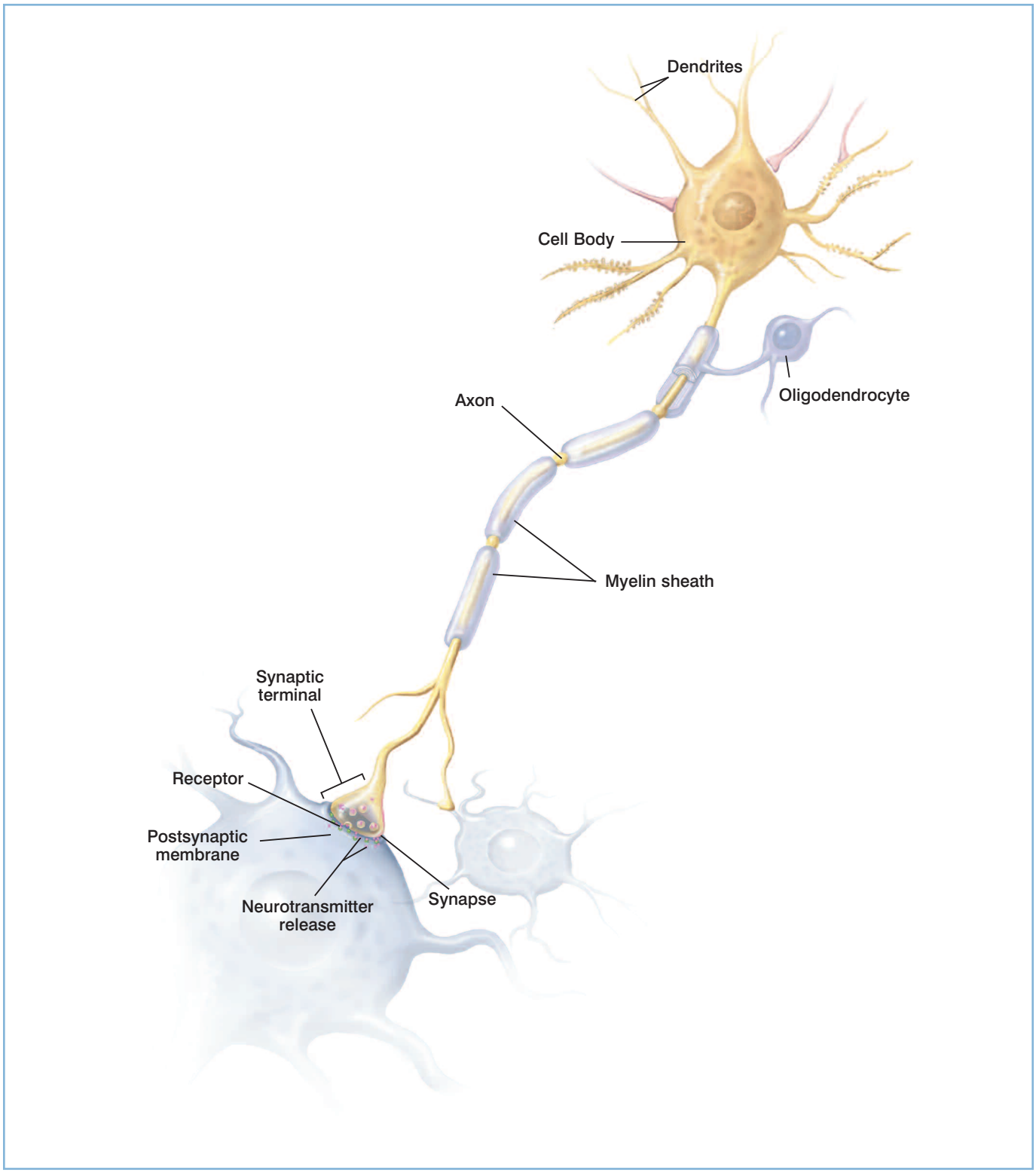

Figure 3.1. The Neuron.
When sufficient neurotransmitters cross synapses and bind receptors on the neuronal cell body and dendrites, the neuron sends an electrical signal down its axon to synaptic terminals, which in turn release neurotransmitters into the synapse that affects the following neuron. The brain neurons that die in Parkinson's Disease release the transmitter dopamine. Oligodendrocytes supply the axon with an insulating myelin sheath.

can be induced to differentiate into neurons and glia while in the culture dish, before being transplanted into the brain. Much progress has been made the last several years with human embryonic stem (ES) cells that can differentiate into all cell types in the body. While ES cells can be maintained in culture for relatively long periods of time without differentiating, they usually must be coaxed through many more steps of differentiation to produce the desired cell types. Recent studies, however, suggest that ES cells may differentiate into neurons in a more straightforward manner than may other cell types.

Second, scientists are identifying growth (trophic) factors that are normally produced and used by the developing and adult brain. They are using these factors to minimize damage to the brain and to activate the patient's own stem cells to repair damage that has occurred. Each of these strategies is being aggressively pursued to identify the most effective treatments for degenerative diseases. Most of these studies have been carried out initially with animal stem cells and recipients to determine their likelihood of success. Still, much more research is necessary to develop stem cell therapies that will be useful for treating brain and spinal cord disease in the same way that hematopoietic stem cell therapies are routinely used for immune system replacement (see Chapter 2).

The majority of stem cell studies of neurological disease have used rats and mice, since these models are convenient to use and are well-characterized biologically. If preliminary studies with rodent stem cells are successful, scientists will attempt to transplant human stem cells into rodents. Studies may then be carried out in primates (*e.g.,* monkeys) to offer insight into how humans might respond to neurological treatment. Human studies are rarely undertaken until these other experiments have shown promising results. While human transplant studies have been carried out for decades in the case of Parkinson's disease, animal research continues to provide improved strategies to generate an abundant supply of transplantable cells.

PARKINSON'S DISEASE — A MAJOR TARGET FOR STEM CELL RESEARCH

The intensive research aiming at curing Parkinson's disease with stem cells is a good example for the various strategies, successful results, and remaining challenges of stem cell-based brain repair. Parkinson's disease is a progressive disorder of motor control that affects roughly 2% of persons 65 years and older. Triggered by the death of neurons in a brain region called the substantia nigra, Parkinson's disease begins with minor tremors that progress to limb and bodily rigidity and difficulty initiating movement. These neurons connect via long axons to another region called the striatum, composed of subregions called the caudate nucleus and the putamen. These neurons that reach from the substantia nigra to the striatum release the chemical transmitter dopamine onto their target neurons in the striatum. One of dopamine's major roles is to regulate the nerves that control body movement. As these cells die, less dopamine is produced, leading to the movement difficulties characteristic of Parkinson's disease. Currently, the causes of death of these neurons are not well understood.

For many years, doctors have treated Parkinson's disease patients with the drug levodopa (L-dopa), which the brain converts into dopamine. Although the drug works well initially, levodopa eventually loses its effectiveness, and side-effects increase. Ultimately, many doctors and patients find themselves fighting a losing battle. For this reason, a huge effort is underway to develop new treatments, including growth factors that help the remaining dopamine neurons survive and transplantation procedures to replace those that have died.

RESEARCH ON FETAL TISSUE TRANSPLANTS IN PARKINSON'S DISEASE

The strategy to use new cells to replace lost ones is not new. Surgeons first attempted to transplant dopamine-releasing cells from a patient's own adrenal glands in the 1980s.[2,3] Although one of these studies reported a dramatic improvement in the patients' conditions, U.S. surgeons were only able to achieve modest and temporary improvement, insufficient to outweigh the risks of such a procedure. As a result, these human studies were not pursued further.

Another strategy was attempted in the 1970s, in which cells derived from fetal tissue from the mouse substantia nigra was transplanted into the adult rat eye and found to develop into mature dopamine neurons.[4] In the 1980s, several groups showed that transplantation of this type of tissue could reverse Parkinson's-like symptoms in rats and monkeys when placed in the damaged areas.The success of the animal studies led to

several human trials beginning in the mid-1980s.[5,6] In some cases, patients showed a lessening of their symptoms. Also, researchers could measure an increase in dopamine neuron function in the striatum of these patients by using a brain-imaging method called positron emission tomography (PET) *(see Figure 3.2).*[7]

The NIH has funded two large and well-controlled clinical trials in the past 15 years in which researchers transplanted tissue from aborted fetuses into the striatum of patients with Parkinson's disease.[7,8] These studies, performed in Colorado and New York, included controls where patients received "sham" surgery (no tissue was implanted), and neither the patients nor the scientists who evaluated their progress knew which patients received the implants. The patients' progress was followed for up to eight years. Unfortunately, both studies showed that the transplants offered little benefit to the patients as a group. While some patients showed improvement, others began to suffer from dyskinesias, jerky involuntary movements that are often side effects of long-term L-dopa treatment. This effect occurred in 15% of the patients in the Colorado study.[7] and more than half of the patients in the New York study.[8] Additionally, the New York study showed evidence that some patients' immune systems were attacking the grafts.

However, promising findings emerged from these studies as well. Younger and milder Parkinson's patients responded relatively well to the grafts, and PET scans of patients showed that some of the transplanted dopamine neurons survived and matured. Additionally, autopsies on three patients who died of unrelated causes, years after the surgeries, indicated the presence of dopamine neurons from the graft. These cells appeared to have matured in the same way as normal dopamine neurons, which suggested that they were acting normally in the brain.

Researchers in Sweden followed the severity of dyskinesia in patients for eleven years after neural transplantation and found that the severity was

Dopamine-Neuron Transplantation

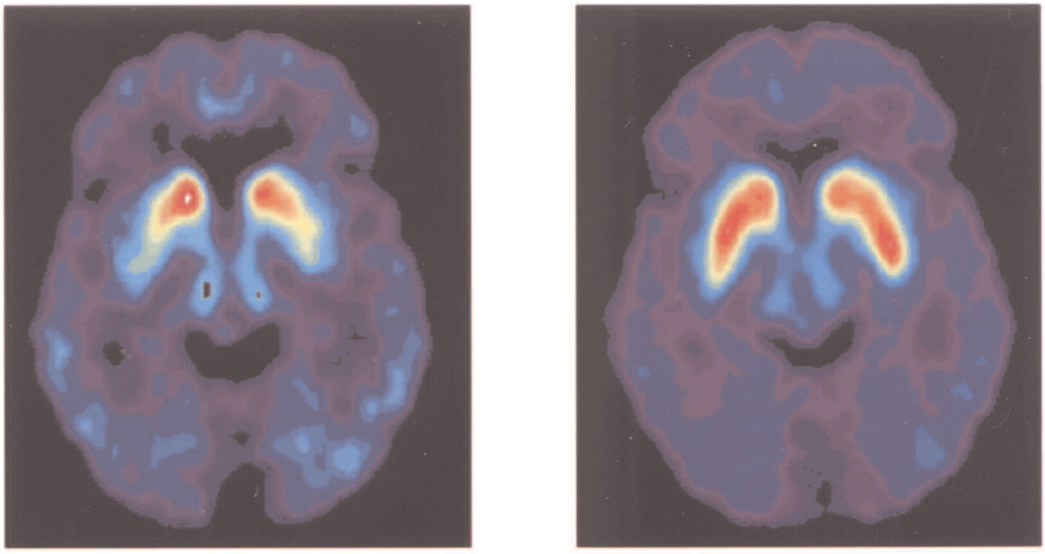

Figure 3.2. Positron Emission Tomography (PET) images from a Parkinson's patient before and after fetal tissue transplantation. The image taken before surgery (left) shows uptake of a radioactive form of dopamine (red) only in the caudate nucleus, indicating that dopamine neurons have degenerated. Twelve months after surgery, an image from the same patient (right) reveals increased dopamine function, especially in the putamen. (Reprinted with permission from N Eng J Med 2001;344 (10) p. 710.)

typically mild or moderate. These results suggested that dyskinesias were due to effects that were distinct from the beneficial effects of the grafts.[9] Dyskinesias may therefore be related to the ways that transplantation disturbs other cells in the brain and so may be minimized by future improvements in therapy. Another study that involved the grafting of cells both into the striatum (the target of dopamine neurons) and the substantia nigra (where dopamine neurons normally reside) of three patients showed no adverse effects and some modest improvement in patient movement.[10] To determine the full extent of therapeutic benefits from such a procedure and confirm the reliability of these results, this study will need to be repeated with a larger patient population that includes the appropriate controls.

The limited success of these studies may reflect variations in the fetal tissue used for transplantation, which is of limited quantity and can not be standardized or well-characterized. The full complement of cells in these fetal tissue samples is not known at present. As a result, the tissue remains the greatest source of uncertainty in patient outcome following transplantation.

STEM CELLS AS A SOURCE OF NEURONS FOR TRANSPLANTATION IN PARKINSON'S DISEASE

The major goal for Parkinson's investigators is to generate a source of cells that can be grown in large supply, maintained indefinitely in the laboratory, and differentiated efficiently into dopamine neurons that work when transplanted into the brain of a Parkinson's patient. Scientists have investigated the behavior of stem cells in culture and the mechanisms that govern dopamine neuron production during development in their attempts to identify optimal culture conditions that allow stem cells to turn into dopamine-producing neurons.

Preliminary studies have been carried out using immature stem cell-like precursors from the rodent ventral midbrain, the region that normally gives rise to these dopamine neurons. In one study these precursors were turned into functional dopamine neurons, which were then grafted into rats previously treated with 6-hydroxy-dopamine (6-OHDA) to kill the dopamine neurons in their substantia nigra and induce Parkinson's-like symptoms. Even though the percentage of surviving dopamine neurons was low following transplantation, it was sufficient to relieve the Parkinson's-like symptoms.[11] Unfortunately, these fetal cells cannot be maintained in culture for very long before they lose the ability to differentiate into dopamine neurons.

Cells with features of neural stem cells have been derived from ES-cells, fetal brain tissue, brain tissue from neurosurgery, and brain tissue that was obtained after a person's death. There is controversy about whether other organ stem cell populations, such as hematopoietic stem cells, either contain or give rise to neural stem cells

Many researchers believe that the more primitive ES cells may be an excellent source of dopamine neurons because ES-cells can be grown indefinitely in a laboratory dish and can differentiate into any cell type, even after long periods in culture. Mouse ES cells injected directly into 6-OHDA-treated rat brains led to relief of Parkinson-like symptoms. Further investigation showed that these ES cells had differentiated into both dopamine and serotonin neurons.[12] This latter type of neuron is generated in an adjacent region of the brain and may complicate the response to transplantation. Since ES cells can generate all cell types in the body, unwanted cell types such as muscle or bone could theoretically also be introduced into the brain. As a result, a great deal of effort is being currently put into finding the right "recipe" for turning ES cells into dopamine neurons — and only this cell type — to treat Parkinson's disease. Researchers strive to learn more about normal brain development to help emulate the natural progression of ES cells toward dopamine neurons in the culture dish.

The recent availability of human ES cells has led to further studies to examine their potential for differentiation into dopamine neurons. Recently, dopamine neurons from human embryonic stem cells have been generated.[13] One research group used a special type of companion cell, along with specific growth factors, to promote the differentiation of the ES cells through several stages into dopamine neurons. These neurons showed many of the characteristic properties of normal dopamine neurons.[13] Furthermore, recent evidence of more direct neuronal differentiation methods from mouse ES cells fuels hope that scientists can refine and streamline the production of transplantable human dopamine neurons.

One method with great therapeutic potential is nuclear transfer. This method fuses the genetic material from one individual donor with a recipient egg cell that has had its nucleus removed. The early embryo that develops from this fusion is a genetic match for the donor. This process is sometimes called "therapeutic cloning" and is regarded by some to be ethically questionable. However, mouse ES cells have been differentiated successfully in this way into dopamine neurons that corrected Parkinsonian symptoms when transplanted into 6-OHDA-treated rats.[14] Similar results have been obtained using parthenogenetic primate stem cells, which are cells that are genetic matches from a female donor with no contribution from a male donor.[15] These approaches may offer the possibility of treating patients with genetically-matched cells, thereby eliminating the possibility of graft rejection.

ACTIVATING THE BRAIN'S OWN STEM CELLS TO REPAIR PARKINSON'S DISEASE

Scientists are also studying the possibility that the brain may be able to repair itself with therapeutic support. This avenue of study is in its early stages but may involve administering drugs that stimulate the birth of new neurons from the brain's own stem cells. The concept is based on research showing that new nerve cells are born in the adult brains of humans. The phenomenon occurs in a brain region called the dentate gyrus of the hippocampus. While it is not yet clear how these new neurons contribute to normal brain function, their presence suggests that stem cells in the adult brain may have the potential to re-wire dysfunctional neuronal circuitry.

The adult brain's capacity for self-repair has been studied by investigating how the adult rat brain responds to transforming growth factor alpha (TGFα), a protein important for early brain development that is expressed in limited quantities in adults.[16] Injection of TGFα into a healthy rat brain causes stem cells to divide for several days before ceasing division. In 6-OHDA-treated (Parkinsonian) rats, however, the cells proliferated and migrated to the damaged areas. Surprisingly, the TGFα-treated rats showed few of the behavioral problems associated with untreated Parkinsonian rats.[16] Additionally, in 2002 and 2003, two research groups isolated small numbers of dividing cells in the substantia nigra of adult rodents.[17,18] These findings suggest that the brain can repair itself, as long as the repair process is triggered sufficiently. It is not clear, though, whether stem cells are responsible for this repair or if the TGFα activates a different repair mechanism.

POSSIBILITIES FOR STEM CELLS IN THE TREATMENT OF OTHER NERVOUS SYSTEM DISORDERS

Many other diseases that affect the nervous system hold the potential for being treated with stem cells. Experimental therapies for chronic diseases of the nervous system, such as Alzheimer's disease, Lou Gehrig's disease, or Huntington's disease, and for acute injuries, such as spinal cord and brain trauma or stoke, are being currently developed and tested. These diverse disorders must be investigated within the contexts of their unique disease processes and treated accordingly with highly adapted cell-based approaches.

Although severe spinal cord injury is an area of intense research, the therapeutic targets are not as clear-cut as in Parkinson's disease. Spinal cord trauma destroys numerous cell types, including the neurons that carry messages between the brain and the rest of the body. In many spinal injuries, the cord is not actually severed, and at least some of the signal-carrying neuronal axons remain intact. However, the surviving axons no longer carry messages because oligodendrocytes, which make the axons' insulating myelin sheath, are lost. Researchers have recently made progress to replenish these lost myelin-producing cells. In one study, scientists cultured human ES cells through several steps to make mixed cultures that contained oligodendrocytes. When they injected these cells into the spinal cords of chemically-demyelinated rats, the treated rats regained limited use of their hind limbs compared with un-grafted rats.[19] Researchers are not certain, however, whether the limited increase in function observed in rats is actually due to the remyelination or to an unidentified trophic effect of the treatment.

Getting neurons to grow new axons through the injury site to reconnect with their targets is even more challenging. While myelin promotes normal neuronal function, it also inhibits the growth of new axons following spinal injury. In a recent study to attempt post-trauma axonal growth, Harper and colleagues

treated ES cells with a combination of factors that are known to promote motor neuron differentiation.[20] The researchers then transplanted these cells into adult rats that had received spinal cord injuries. While many of these cells survived and differentiated into neurons, they did not send out axons unless the researchers also added drugs that interfered with the inhibitory effects of myelin. The growth effect was modest, and the researchers have not yet seen evidence of functional neuron connections. However, their results raise the possibility that signals can be turned on and off in the correct order to allow neurons to reconnect and function properly. Spinal injury researchers emphasize that additional basic and preclinical research must be completed before attempting human trials using stem cell therapies to repair the trauma-damaged nervous system.

Since myelin loss is at the heart of many other degenerative diseases, oligodendrocytes made from ES cells may be useful to treat these conditions as well. For example, scientists recently cultured human ES cells with a combination of growth factors to generate a highly enriched population of myelinating oligodendrocyte precursors.[21,22] The researchers then tested these cells in a genetically-mutated mouse that does not produce myelin properly. When the growth factor-cultured ES cells were transplanted into affected mice, the cells migrated and differentiated into mature oligodendrocytes that made myelin sheaths around neighboring axons. These researchers subsequently showed that these cells matured and improved movement when grafted in rats with spinal cord injury.[23] Improved movement only occurred when grafting was completed soon after injury, suggesting that some post-injury responses may interfere with the grafted cells. However, these results are sufficiently encouraging to plan clinical trials to test whether replacement of myelinating glia can treat spinal cord injury.

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is characterized by a progressive destruction of motor neurons in the spinal cord. Patients with ALS develop increasing muscle weakness over time, which ultimately leads to paralysis and death. The cause of ALS is largely unknown, and there are no effective treatments. Researchers recently have used different sources of stem cells to test in rat models of ALS to test for possible nerve cell-restoring properties. In one study, researchers injected cell clusters made from embryonic germ (EG) cells into the spinal cord fluid of the partially-paralyzed rats.[24] Three months after the injections, many of the treated rats were able to move their hind limbs and walk with difficulty, while the rats that did not receive cell injections remained paralyzed. Moreover, the transplanted cells had migrated throughout the spinal fluid and developed into cells that displayed molecular characteristics of mature motor neurons. However, too few cells matured in this way to account for the recovery, and there was no evidence that the transplanted cells formed functional connections with muscles. The researchers suggest that the transplanted cells may be promoting recovery in some other way, such as by producing trophic factors.

This possibility was addressed in a second study in which scientists grew human fetal CNS stem cells in culture and genetically modified them to produce a trophic factor that promotes the survival of cells that are lost in ALS. When grafted into the spinal cords of the ALS-like rats, these cells secreted the desired growth factor and promoted the survival of the neurons that are normally lost in the ALS-like rats.[25] While promising, these results highlight the need for additional basic research into functional recovery in ALS disease models.

Stroke affects about 750,000 patients per year in the U.S. and is the most common cause of disability in adults. A stroke occurs when blood flow to the brain is disrupted. As a consequence, cells in affected brain regions die from insufficient amounts of oxygen. The treatment of stroke with anti-clotting drugs has dramatically improved the odds of patient recovery. However, in many patients the damage cannot be prevented, and the patient may permanently lose the functions of affected areas of the brain. For these patients, researchers are now considering stem cells as a way to repair the damaged brain regions. This problem is made more challenging because the damage in stroke may be widespread and may affect many cell types and connections.

However, researchers from Sweden recently observed that strokes in rats cause the brain's own stem cells to divide and give rise to new neurons.[26] However, these neurons, which survived only a couple of weeks, are few in number compared to the extent of damage caused. A group from the University of Tokyo added a growth factor, bFGF, into the brains of rats after stroke and showed that the hippocampus was able to generate

large numbers of new neurons.[27] The researchers found evidence that these new neurons were actually making connections with other neurons. These and other results suggest that future stroke treatments may be able to coax the brain's own stem cells to make replacement neurons.

Taking an alternative approach, another group attempted transplantation as a means to treat the loss of brain mass after a severe stroke. By adding stem cells onto a polymer scaffold that they implanted into the stroke-damaged brains of mice, the researchers demonstrated that the seeded stem cells differentiated into neurons and that the polymer scaffold reduced scarring.[28] Two groups transplanted human fetal stem cells in independent studies into the brains of stroke-affected rodents; these stem cells not only survived but migrated to the damaged areas of the brain.[29,30] These studies increase our knowledge of how stem cells are attracted to diseased areas of the brain.

There is also increasing evidence from numerous animal disease models that stem cells are actively drawn to brain damage. Once they reach these damaged areas, they have been shown to exert beneficial effects such as reducing brain inflammation or supporting nerve cells. It is hoped that, once these mechanisms are better understood, this stem cell recruitment can potentially be exploited to mobilize a patient's own stem cells.

Similar lines of research are being considered with other disorders such as Huntington's Disease and certain congenital defects. While much attention has been called to the treatment of Alzheimer's Disease, it is still not clear if stem cells hold the key to its treatment. But despite the fact that much basic work remains and many fundamental questions are yet to be answered, researchers are hopeful that repair for once-incurable nervous system disorders may be amenable to stem cell based therapies.

Considerable progress has been made the last few years in our understanding of stem cell biology and devising sources of cells for transplantation. New methods are also being developed for cell delivery and targeting to affected areas of the body. These advances have fueled optimism that new treatments will come for millions of persons who suffer from neurological disorders. But it is the current task of scientists to bring these methods from the laboratory bench to the clinic in a scientifically sound and ethically acceptable fashion.

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