Cell Therapy, Stem Cells, and Brain Repair

Contemporary Neuroscience

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Preface

As our world continues to evolve, the field of regenerative medicine follows suit. Although many modern day therapies focus on synthetic and natural medicinal treatments for brain repair, many of these treatments and prescriptions lack adequate results or only have the ability to slow the progression of neurological disease or injury.

Cell therapy, however, remains the most compelling treatment for neurodegenerative diseases, disorders, and injuries, including Parkinson's disease, Huntington's disease, traumatic brain injury, and stroke, which is expanded upon in more detail in Chapter 1 by Snyder and colleagues. Cell therapy is also unique in that it is the only therapeutic strategy that strives to replace lost, damaged, or dysfunctional cells with healthy ones. This repair and replacement may be due to an administration of exogenous cells itself or the activation of the body's own endogenous reparative cells by a trophic, immune, or inflammatory response to cell transplantation. However, the precise mechanism of how cell therapy works remains elusive and is continuing to be investigated in terms of molecular and cellular responses, in particular. Moreover, Chapter 11 by Emerich and associates, discusses some of the possibilities of cell immunoisolation and the potential for treating central nervous system diseases.

During the past 20 years most investigations have utilized cells derived from fetal tissue as a source of transplantable cells for cell therapy, which have demonstrated an underlying proof of principle for current cell transplants for a treatment of a variety of neurological diseases and injuries, including Huntington's disease which are discussed in Chapter 4 by Dunnett and colleagues. Chapter 4 also reviews challenges in harvesting the tissue, the analogy of developmental stages between species, clinical trials, alternative tissue sources, as well as specific xenogenic issues. In addition, stem cells have emerged as the leading topic regarding cell therapy. According to the National Institutes of Health, "a stem cell is a cell that has the ability to divide (self replicate) for indefinite periods-often throughout the life of the organism. Under the right conditions, or given the right signals, stem cells can give rise (differentiate) to the many different cell types that make up the organism. That is, stem cells have the potential to develop into mature cells that have characteristic shapes and specialized functions, such as heart cells, skin cells, or nerve cells."

Previous studies in fetal tissue also contributed a great deal to the discovery of neural stem cells. Neural stem cells are derived from fetal and adult brain and have the ability to divide and give rise to more stem cells or to several types of precursor cells, which can then become neurons and glia. Neural stem cells in the mammalian fetal brain have been located in the subventricular zone, ventricular zone, hippocampus, olfactory bulb, cerebellum, and cerebral cortex. Chapter 1 by Snyder and colleagues describes the promising potential of neural stem cells for therapeutic use. In addition, studies using neurospheres also help to identify the subependymal zone as another source of stem cells in the brain. Another source of neural stem cells, which are quite different than the fetal neural stem cells, is neural crest cells. During development, the neural crest cells migrate from the sides of the neural tube as it closes, and the cells differentiate into a variety of tissues, which are not all part of the nervous system. Many neural crest cells are responsible for comprising most of the peripheral nervous system, including hormone-producing glands, as well as skin, cartilage, bone, and many connective tissues within the body. Neural stem cells and neural transplantation in primates are discussed in Chapter 3 by Bjugstad and Sladek. This chapter also elaborates on direct comparisons between successful rodent studies and marginal human studies and the limitations of a rodent Parkinson's disease (PD) model; thereby, demonstrating the proof of principle for a primate PD model.

A new era in stem cell research began in 1998 with the derivation of embryonic stem cells. Techniques involving embryonic stem cells have developed greatly since 1998, when James Thomson and his colleagues reported methods for deriving and maintaining these cells. Stem cells derived from embryos have also been extensively studied and have demonstrated the remarkable ability to differentiate into neurons, glia, and numerous cell types in animals, which is summarized in Chapter 1. Despite current negative views regarding the use of these tissue types, this previous research has paved the way for many new types of stem cell research which follow similar experimental paths of the original embryonic stem cell research. This proof of principle involving embryonic stem cells, as well as an overview of various types of stem cells suitable for transplantation, particularly in Parkinson's disease, is reviewed in Chapter 2 by Brundin and colleagues.

Owing to the heightened ethical concerns and governmental issues regarding embryonic and fetal tissue research, cellular research has continued to expand its search for alternative sources of stem cells. More recently, adult stem cells, which are cells obtained post-birth, have made a breakthrough in the field of stem cell research. Adult stem cells make identical

copies of themselves for long periods of time (self-renewal), and can produce mature cell types that have specific morphologies and functions. Their primary functions are to maintain the steady state of a cell and to replace cells that die due to injury or disease. Adult stem cells usually generate an intermediate cell type or types before they become fully differentiated. The intermediate cell type is commonly called a precursor or progenitor cell. This progenitor cell has the capacity to produce cells of the original tissue or organ (multipotent). For instance, stem cells isolated from the brain will give rise to neural cells, stem cells from the heart will give rise to cardiac cells, or stem cells from the bone marrow will give rise to blood cells. In addition, the adult stem cells also have the capacity to produce cells giving rise to many different cell types, tissues, and organs regardless of the origin of the stem cell (pluripotent). For example, stem cells from umbilical cord blood may give rise to neural cells, cardiac muscles, or other blood cells depending upon the condition or environment of the stem cells themselves. The ability of the adult stem cells to display pluripotency is quite similar to embryonic stem cells, thus expanding our resources for stem cells for cell therapy. Adult stem cells may be obtained from many different types of tissues, however, they retain the ability to produce many tissue types as well. These cells can be harvested from donors and isolated within the laboratory, where scientists culture and grow these cells for transplantation.

Bone marrow has also been found to be rich in adult stem cells. This idea, however, is not novel; hematopoietic stem cells were recognized as stem cells more than 40 years ago. However, more recent research has shown that these stem cells have exercised enormous potential for cellular therapy by demonstrating the capability of neuronal and astrocytic differentiation following transplantation. Thus, studies in bone marrow have advanced cell therapy to now include brain repair as well. Bone marrow actually contains three specific stem cell populations-hematopoietic stem cells, stromal cells, and endothelial progenitor cells, although more specifics on bone marrow stem cell types and classifications are discussed in Chapter 7 by Low and colleagues. In addition, Chapter 7 also includes a review of the experimental progress toward a therapeutic for each type of bone marrow stem cell, as well as the concepts and studies necessary to translate bone marrow stem cell research into clinic. Moreover, Chapter 10 by Emerich and colleagues covers the therapeutic potential of transplanted bone marrow stem cells into the choroids plexus (CP), in particular, as well as the future potential for using transplantable CP cells as a means of delivering neurotrophic factors to the brain and spinal cord. Chapter 5 by Dunbar and associates elaborates upon the specific use of autologous whole bone mar-

row and mesenchymal stem cell transplants in a model of Huntington's disease, and a comprehensive comparison between autologous and heterologous marrow stem cell transplants.

Another hematopoietic source that is rich in adult stem cells includes umbilical cord blood. The umbilical cord which supports the fetus during pregnancy, is delivered with the baby, and is typically discarded. Since the first successful umbilical cord blood transplants in children with Fanconi anemia, the collection of cord blood and cellular therapeutic use has grown rapidly. Moreover, there are none of the ethical issues regarding the use of cord blood stem cells compared to embryonic stem cells, and the method of harvesting the stem cells from the umbilical vein poses no risk to the mother or baby, since the cord is removed and set aside prior to the blood collection. From a cellular therapeutic perspective, umbilical cord blood offers many advantages. Like bone marrow it is rich in stem cells, but is much easier to obtain than bone marrow. Fortunately, both bone marrow and umbilical cord blood stem cells have been shown to migrate and engraft to neurological sites of injury, following non-invasive intravenous injection, and amazingly produce recovery of function resulting from stroke and other forms of neurological injury, which offers an extreme advantage for cell therapy with these cells. Chapter 13 by Vendrame and Willing, comprises an overview of human cord blood cells, their phenotype, functional characteristics, and potential as a therapy for neurodegenerative diseases and disorders. This chapter also discusses other hematopoietic stem cells, including G-CSF stimulated peripheral blood, and its therapeutic potential for brain repair.

Although the field of stem cell research has evolved into a promising therapy for brain repair many challenges still exist. The process of identifying the desired type of stem cell in culture will involve tedious research, while developing the right biochemical environment or media is essential to ensure that the stem cell differentiates into the desired cell type. Also, once the stem cells have been transplanted the cells must be integrated within the body's own tissue and organs and function correctly. Yet another challenge is tissue rejection. The body's immune system must not recognize the transplanted cells as foreign. Fortunately, cord blood-derived stem cells are considered to be more immune immature cells, thus making the incidence of tissue rejection much less than other types of transplantable cells. In addition, Sertoli cells are described in Chapter 9 in more detail for their potential role in immune system modulation and their capability to reduce rejection for cell transplants. Another concern is the possible risk of cancer. Cancer results when the cells continue to proliferate and keep further dividing beyond the desired point. This point is a delicate balance once the cells have

been transplanted, fostering the growth of the new cells without them dividing out of control. Interestingly, however, much evidence has been presented that cells isolated from a specific human neuroteratocarcinoma (NT2N cells) have the ability to generate neurons once transplanted into stroke patients, which is outlined in Chapter 6 by Borlongan and associates. Thus, continued efforts are being made to address the positive and negative issues in order for these cell therapies to complete human clinical trials.

However, with these challenges in mind, stem cell therapy remains one of the best "natural" candidates to help heal the human body. Despite the many challenges, many scientists believe that cell therapy will revolutionize medicine. These cell therapies may one day offer cures for cancer, Parkinson's disease, diabetes, kidney disease, multiple sclerosis, cardiovascular disease, and symptoms of stroke. Cell therapy may also fill a tremendous need for chronic pain management and traumatic brain injury (TBI), which is examined in more detail using several intervention strategies in Chapter 8 by Eaton and Sagen. A variety of potential cell sources for chronic pain and TBI are elaborated upon in Chapter 8 as well. Stem cell therapies have also shown encouraging results in helping to repair spinal cord injuries, and helping to regain movement resulting from paralysis. It is also possible that the human life span could be increased due to the regeneration and repair of tissue and organs by stem cells. Stem cells also seem to be in the forefront in providing a treatment for brain repair, in general, as the incidence of neurological injuries and disease increase in our world today. While our knowledge of cell therapy continues to develop, so does our revolutionary precision in how to design a better therapy to treat disease. Chapter 12 by Polgar, identifies recent developments in health research methodology that may be useful for ensuring progress in cellular therapy for brain repair, goals for cellular therapy, best practices, and some critical analysis and ethics. In addition, the commercial and pharmaceutical implications of stem cells and their role in regenerative medicine are discussed in Chapter 14 by Cruz and Azevedo.

This compilation attempts to explicate previous cornerstones and milestones of neurological cellular therapy, which have provided a foundation for modern stem cell research. Ongoing challenges are discussed, as well as many obstacles that have been overcome already. The current direction of cellular research is described, and modern techniques involving certain subsets of cell populations explained. In addition, the ongoing discovery of stem cell sources for cell therapy is discussed, while expounding upon clinical applications for cell therapeutic brain repair as they become increasingly promising. The clinical applications include potential cell therapy for

Parkinson's disease, traumatic brain injury, and ischemic stroke. We hope to provide a good understanding of the stem cell research field by presenting literature from renowned scientists and clinicians in the field of cell therapy today, and share their data, conclusions and future investigations, and the challenges that they overcame to reach their results. Also, varying methods of cell transplantation are revealed and how the method or route of administration affects the behavioral outcome in animal injury models.

Scientists have begun to recognize the amazing versatility of these primitive cells, which exist for only a short period of time prior to differentiating into other cell types and tissues within the body. Since cells are the basic building blocks of the human body, it would only stand to reason that we should harness the power of these stem cells to sustain and repair the body's tissues and organs and with the appropriate research, as demonstrated here, the many obstacles of stem cell research that can be overcome. It is by sharing knowledge with reputable scientists and clinicians that enables the field of cellular research to continue to thrive and move forward.

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1

Current Views of the Embryonic and Neural Stem Cell

Cell Replacement or Molecular Repair?

Roya Sabetrasekh, Yang D. Teng, Jitka Ourednik, Kook In Park, and Evan Y. Snyder

ABSTRACT

Stem cell biology, construed in its broadest sense, has forced Medicine to view development and disease, and subsequent potential therapies, from an entirely different perspective *(1–3)*. We have learned that there is an inborn plasticity and flexibility "programmed" into the organism and its organ systems *(1)*. The repository of this plasticity is thought to be the stem cell—the most primordial cell in the body and in any given structure. Nearly two decades ago, investigators began to identify cells with surprising plasticity and a propensity for dynamically shifting their fates within cultures obtained from developing and mature organs *(1)*. The existence of such cells challenged the prevailing dogma that organs were rigidly and immutably constructed. Stem cells, as these plastic cells came to be termed, began to garner the interest of the developmental community, as well as that of the repair, gene therapy, and transplant communities. This interest arose when it was recognized that stem cells could be expanded in number and reimplanted into organs, where they would reintegrate appropriately and seamlessly, shift their fate in response to local cues to compensate for the absence of cells, express new genes, and in some cases, help promote functional improvement in disease models *(4–13)* (Fig. 1). Exploiting the power of a cell that presumably had a pivotal role in development for repair purposes is somewhat analogous to rebooting a computer or reseeding a lawn. Optimizing these natural processes is the primary focus of today's regenerative medicine. There have been a wide range of compelling studies conducted in animal models using various stem cells, including models of aging, spinal cord injury, stroke, parkinsonism, amyotrophic lateral sclerosis (ALS), cancer, multiple sclerosis, blood

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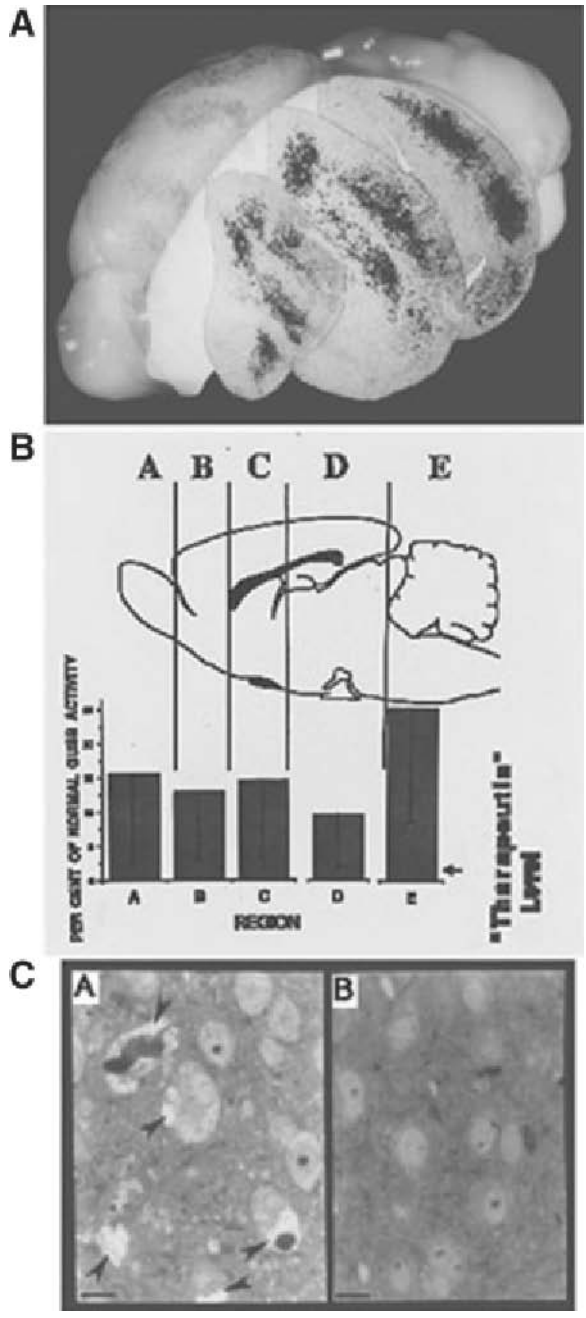
A
B
A B C D E
PER CENT OF NORMAL GLIAL ACTIVITY
A B C D E
REGION
"Therapeutic" Level
C
A
B

Fig. 1.

diseases, immunodeficiencies, enzyme deficiencies, myocardial infarcts, and diabetes.

This chapter outlines investigations that are capturing the most attention and considers the current state of scientific understanding and controversy regarding the properties of embryonic and neural stem cells. Its objective is to provide a framework to appreciate the medical promise of this research and also to describe the challenges of translating fundamental stem cell biology into novel clinical therapies.

Key Words: Embryonic; neural stem cells; MPS, tumor.

THERAPEUTIC POTENTIAL OF EMBRYONIC STEM CELLS

Most of the enthusiasm relating to embryonic stem (ES) cells results directly from the perceived need for cell replacement therapy for a host of degenerative diseases. Indeed, disorders of organ failure are not reversible, and organ transplantation cannot meet the needs of an ever-aging population. Primary pump failure in the heart, alcoholic or viral liver failure, β-cell–deficient type 1 diabetes, and Parkinson's disease (PD) are frequently cited as examples of monocellular deficiency states that might be amenable

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Fig. 1. *(previous page)* Widespread engraftment of NSCs expressing GUSB throughout the brain of the MPS VII mouse. (Adapted from ref. *6*). **(A)** Brain of a mature MPS VII mouse after receiving a neonatal intraventricular transplant of murine NSCs expressing GUSB. Donor NSC-derived cells, identified by their X-gal histochemical reaction (blue precipitate) for expression of the LacZ marker gene, have engrafted throughout the recipient mutant brain. Representative coronal sections (placed at their appropriate level by computer) show these cells to span the rostral-caudal expanse of the brain. **(B)** Distribution of GUSB enzymatic activity throughout the brains of MPS VII NSC transplant recipients. Serial sections were collected throughout the brains of transplant recipients and assayed for GUSB activity. Sections were pooled to reflect the activity present within the regions demarcated in the scheme. The regions were defined by anatomical landmarks in the anterior-to-posterior plane to permit comparison among animals. The mean levels of GUSB activity (n = 17) are presented as the percentage of average normal levels for each region. Untreated MPS VII mice show no GUSB activity biochemically or histochemically. Enzyme activity 2% of normal is corrective based on data from the liver and spleen. **(C)** Decreased lysosomal storage in a treated MPS VII mouse brain at 8 mo of age. (A) Extensive vacuolation representing distended lysosomes (arrowheads) in both neurons and glia in the neocortex of an 8-mo-old, untransplanted control MPS VII mouse. (B) Decrease in lysosomal storage in the cortex of an MPS VII mouse treated at birth from a region analogous to the untreated control section in part A. The other regions of this animal's brain showed a similar decrease in storage, compared to untreated, age-matched mutants in regions where GUSB was expressed. Scale bars: 21 μm.

to cell replacement strategies if a suitable and inexhaustible cell source could be found. Human ES cells may represent such a source, but the overriding challenge is to achieve efficient, directed differentiation of ES cells into therapeutically relevant cells, followed by proof-of-principle for effective restoration of tissue function in animal models. Once success is achieved in these areas of cell engineering many obstacles will remain, especially the immune barrier to tissue transplantation. Current strategies for confronting these challenges are outlined below.

ES cells are derived from the inner cell mass of the preimplantation embryo. When placed in culture, ES cells proliferate indefinitely and yet retain their potential to form all the tissues of the developing organism. Murine ES cells have been intensively studied for over 20 yr; yet, the first derivation of ES cells from the human embryo was reported in 1998 *(14)*. Although advances in ES cell biology have revolutionized the creation of mouse disease models, generating and breeding mice is time-consuming and costly. To address questions in cell and developmental biology, ES cells represent an excellent in vitro model system.

To maintain their undifferentiated, pristine state, ES cells are typically grown on feeder cell layers of mouse embryonic fibroblasts, and cultures are supplemented by the antidifferentiation cytokine leukemia inhibitory factor. When ES cells are removed from these conditions, they undergo spontaneous differentiation and initiate the diverse programs of tissue and cell specification *(15)*. ES cells recapitulate many developmental programs of the early embryo in culture, including cell generation from all three classical embryonic germ layers: ectoderm, mesoderm, and endoderm.

Because the mouse embryo remains microscopic and inaccessible during most of the first gestation week—a time when many central developmental programs are laid down—in vitro differentiation of ES cells provides a tractable model system to investigate the genetic and cell biological regulation of early development. Moreover, genes can be altered in ES cells through ectopic transgene expression or homologous recombination (HR), a process enabling specific sites in the genome to be altered directly. HR can be designed to produce gene deletion, mutation, or substitution. Using these techniques in ES cells, the genetic programs responsible for directing the development of blood, neurons, hepatocytes, cardiomyocytes, and a host of other tissues have been extensively explored.

Although ES cells have been touted as an inexhaustible resource for cell replacement therapies, they have already been proven as highly valuable research and discovery tools. By analyzing the effect of targeted gene deletions on the formation of specific cell lineages, ES cells can help validate

potential therapeutic targets for small-molecule drug development. ES cells are emerging as a platform technology, around which chemical screens can be built, leading to the identification of compounds that promote or block cell differentiation. Schultz and colleagues *(16)* recently performed a chemical screen to identify agents that induce neurogenesis in ES cells, thereby establishing the proof-of-principle for using stem cell differentiation in assays for drug discovery. Kamp and colleagues *(17)* have shown that human ES cells differentiate into a number of cardiomyocyte classes, including embryonic atrial, ventricular, and nodal subtypes, each recapitulating their respective electrophysiologic properties and pharmacologic responses. Gauging the effects of compounds on the differentiation of specific cell populations from ES cells would provide a screen for potential drug toxicities prior to clinical development. The assembly of a genetically diverse bank of human ES cells, together with detailed knowledge of human genetic variation from the international haplotype mapping project ("the hap map"), could translate into a discovery platform for pharmacogenomics.

ES Cells as a Source of Neurons for Neurodegenerative Diseases

Despite inadequate knowledge about disease etiology and pathogenesis, neurodegenerative diseases (e.g., PD, Alzheimer's, Huntington's, amyotrophic lateral sclerosis, stroke, anoxic brain injury, and a host of lysosomal storage diseases with central nervous system (CNS) pathology) represent poorly managed diseases that are worthy targets for cell replacement therapy. The hippocampus and olfactory bulb maintain self-renewing populations of neural stem/progenitor cells, but there is scant evidence for cell renewal beyond these limited regions of the CNS. Given the likelihood that many classes of highly specialized neurons develop only during critical periods of embryogenesis, ES cells might, in principle, be directed to differentiate into specialized neuronal subtypes for use in cell replacement therapy. Several groups have reported success in differentiating specific neuronal subtypes from mouse and human ES cells, and some have reported positive data from transplantation of such cells into animal models of disease.

The most compelling reports of directed ES cell differentiation have derived from the laboratories of Jessell *(18)* and McKay *(19)*. Both have exploited knowledge of the morphogens and transcription factors that program neuronal development during embryogenesis and recapitulated the timing and sequence of exposure to direct neuronal patterning during in vitro ES cell differentiation. They initially used retinoic acid to program ectodermal commitment, then followed with exposure to the morphogen sonic hedgehog, which acts to "ventralize" neuronal subtypes. With these meth-

ods, Jessell and colleagues showed that they could pattern the formation of spinal motor neurons that successfully engrafted the embryonic spinal cord of the chick, extended axons, and formed synapses with target muscles. McKay's group exploited the instructive effects of sonic hedgehog and FGF8 to drive the commitment of ES cells to ventral midbrain fates and, ultimately, to tyrosine hydroxylase–positive dopaminergic neurons. These cells functioned after transplantation into a rodent model of PD. Isacson and colleagues *(20)* have similarly demonstrated improvement in a rodent model of PD from transplantation of undifferentiated ES cells into the striatum, suggesting that the local environment is capable of inducing proper development of dopaminergic neurons. Introduction of neuronal populations of differentiated murine ES cells into a rat model of spinal cord injury has shown improved motor function *(21)*, but it is by no means clear that the mechanism was direct neuronal reconstitution, as opposed to modulation of the repair process in the host through remyelination. Several research groups have demonstrated neuron formation from human ES cells *(22–25)*, presaging future human applications.

Despite these apparent successes, there may be as many as 200 distinct neuronal subtypes in the adult brain, and even within a given subtype, neurons show remarkable degrees of regional specificity. A great leap of faith is required to believe that neurons produced from ES cells in culture will recapitulate the differentiated features of specific neuronal subtypes and reestablish relevant neural networks produced during the formation of the embryonic brain. An alternative strategy is to differentiate ES cells into neural stem cells (NSC) and progenitors in vitro, then coax local environments in the diseased region of the brain or spinal cord to direct further differentiation and accommodation of neural cells to their new niche. Whether this will occur is a matter of pure speculation and is subject to hyperbolic claims. The applications of NSCs are described in detail below.

THERAPEUTIC POTENTIAL OF NSCS

The recognition that NSCs propagated in culture could be reimplanted into mammalian brain, where they could reintegrate appropriately and stably express foreign genes *(4,26)* made this strategy an appealing alternative for CNS gene therapy and repair. Numerous subsequent studies over the past decade *(27)* reaffirmed that neural progenitors from many regions and developmental stages could be maintained, perpetuated, and passaged in vitro by epigenetic, and genetic methods. Examples include the transduction of genes interacting with cell cycle proteins (e.g., *vmyc*) and by mitogen stimulation (e.g., epidermal growth factor and/or basic fibroblast growth

factor; *(4,26,28–32)*. Some of these methods may operate through common cellular mechanisms. This speculation is supported by the observation that many progenitor cell lines behave similarly in their ability to reintegrate into the CNS, despite that they were generated by different methods, obtained from various locations, and reimplanted into various CNS regions. Some NSC lines appear sufficiently plastic to participate in normal CNS development from germinal zones of multiple regions along the neuraxis and at multiple stages of development from embryo to old age *(4,6,9,33–37)*. In addition, they appear to model the in vitro and in vivo behavior of some primary fetal and adult neural cells *(38–43)*, suggesting that insights gleaned from these NSC lines may legitimately reflect the potential of CNS progenitor or stem cells.

The inherent biologic properties of NSCs may circumvent limitations of other techniques for treating metabolic, degenerative, or other widespread lesions in the brain. They are easy to administer (often directly into the cerebral ventricles), are readily engraftable, and circumvent the blood–brain barrier. Unlike BMT, a preconditioning regime is not required before administration (e.g., total-body irradiation). One important property of NSCs is their apparent ability to develop into integral cytoarchitectural components *(47)* of many regions throughout the host brain as neurons, astrocytes, oligodendrocytes, and even incompletely differentiated, but quiescent, progenitors. Therefore, they may be able to replace a range of missing or dysfunctional neural cell types. A given NSC clone can give rise to multiple cell types within the same region. This is important in the likely situation where return of function may require the reconstitution of the whole milieu of a given region, e.g., not just the neurons but also the glia, and support cells required to nurture, detoxify, and/or myelinate the neurons. They appear to respond in vivo to neurogenic signals not only when they occur appropriately during development, but even when induced at later stages by certain neurodegenerative processes, like during apoptosis *(4,45)*. NSCs may be attracted to regions of neurodegeneration in the young, as well as in the elderly *(12,46–48*; Fig. 2).

NSCs also appear to accommodate to the engraftment region, perhaps obviating the necessity to obtain donor cells from many specific CNS regions or the imperative for precise targeting during reimplantation. The cells might express certain genes of interest intrinsically (e.g., many neurotrophic factors), or they can be engineered ex vivo to do so because they are readily transduced by gene transfer vectors. These gene products can be delivered to the host CNS in a direct, immediate, and stable manner *(6,7,47,49)*. Although NSCs can migrate and integrate widely throughout the brain

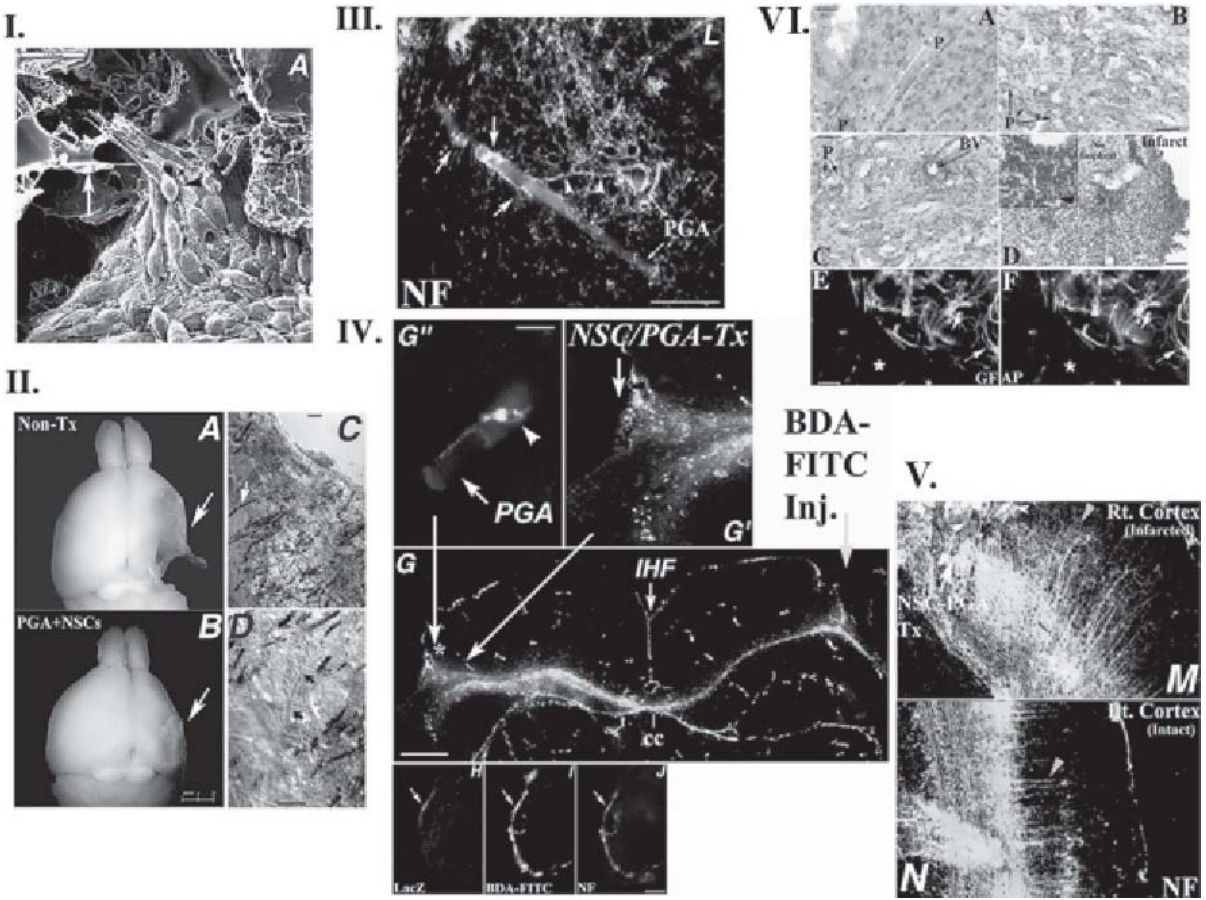
I.
A
II.
Non-Tx
A
C
PGA+NSCs
B
D
III.
L
PGA
NF
IV.
G''
PGA
NSC/PGA-Tx
G'
G
IHF
cc
H
I
J
LacZ
BDA-FITC
NF
BDA-
FITC
Inj.
VI.
A
B
P
P
P
BV
Infarct
C
D
E
F
GF
AP
V.
Rt. Cortex
(Infarcted)
Tx
M
Rt. Cortex
(Intact)
N
NF

Fig. 2. The injured brain interacts reciprocally with NSCs supported by scaffolds to reconstitute lost tissue—evidence from hypoxic-ischemic (HI) injury. (Modified from ref. 8.)

(**I**) Characterization of NSCs in vitro when seeded upon a PGA scaffold. Cells seen with scanning electron microscopy at 5 d after seeding were able to attach to, impregnate, and migrate throughout a highly porous PGA matrix (arrow). The NSCs differentiated primarily into neurons (>90%) that sent out long, complex processes that adhered to, enwrapped, and interconnected the PGA fibers.

(II) Implantation of NSC–PGA complexes into a region of cavity formation following extensive HI brain injury and necrosis. (A) Brain of an untransplanted (non-Tx) mouse subjected to right-HI injury with extensive infarction and cavitation of the ipsilateral right cortex, striatum, thalamus, and hippocampus (arrow). In contrast with part B, the brain of a similarly injured mouse implanted with an NSC–PGA complex (PGA+NSCs) (generated in vitro as per part I. into the infarction cavity 7 d after the induction of HI (arrow) (n = 60). At maturity (age-matched to the animal pictured in part A), the NSC–scaffold complex appears in this whole-mount to have filled the cavity (arrow) and become incorporated into the infracted cerebrum. Representative coronal sections through that region are seen at higher magnification in parts C and D, in which parenchyma appears to have filled in spaces between the dissolving black polymer fibers (white arrow in part C) and, as seen in part D, even to support neovascularization by host tissues. (Blood vessel is indicated by closed black arrow in part D; open arrow in part D points to degrading black polymer fiber.) Scale bars (C and D): 100 μm.

(III) Characterization in vivo of the neural composition of NSC–PGA complexes within the HI-injured brain. At 2 wk following transplantation of the NSC–PGA complex into the infarction cavity, donor-derived cells showed robust engraftment within the injured region. An intricate network of multiple long, branching NF+ (green) processes were present within the NSC–PGA complex and its parenchyma enwrapping the PGA fibers (orange autofluorescent tube-like structures under a Texas Red filter), adherent to and running along the length of the fibers (arrows), often interconnecting and bridging the fibers (arrowheads). Those NF+ processes were of both host and donor derivation. In other words, not only were donor-derived neural cells present, but host-derived cells also seemed to have entered the NSC–PGA complex, migrating and becoming adherent to the PGA matrix. In a reciprocal manner, donor-derived (*lacZ*+) neurons (NF+ cells) within the complex appeared to send processes along the PGA fibers out of the matrix into host parenchyma, as seen in part IV. Scale bars: 100 μm. *(Figure 2 caption is continued on the next page.)*

Fig. 2. *(continued)* **(IV)** Long-distance neuronal connections extend from the transplanted NSC–PGA complexes in the HI-injured brain toward presumptive target regions in the intact contralateral hemisphere. By 6 wk following engraftment, donor-derived *lacZ*+ cells appeared to extend many exceedingly long, complex NF+ processes along the length of the disappearing matrix, apparently extending into host parenchyma. To confirm the suggestion that long-distance processes projected from the injured cortex into host parenchyma, a series of tract-tracing studies were performed. [G-G"] BDA-FITC was injected (G) into the contralateral intact cortex and external capsule (green arrow) at 8 wk following implantation of the NSC–PGA complex into the infarction cavity (NSC/PGA-Tx). Axonal projections (labeled green with fluorescein under an FITC filter) are visualized (via the retrograde transport of BDA), leading back to (across the interhemispheric fissure (IHF) via the corpus callosum ["cc"]), and emanating from, cells in the NSC–PGA complex within the damaged contralateral cortex and penumbra (seen at progressively higher magnification in parts G' (region indicated by arrow to part G) and G" (region indicated by an arrow and asterisk in part G). In part G", the retrogradely BDA-FITC–labeled perikaryon of a representative neuron adherent to a dissolving PGA fiber is well visualized. The fact that such cells are neurons of donor derivation is supported by their triple labeling (H–J) for *lacZ* (H) (βgal), BDA-FITC (I), and the neuronal marker NF (J); arrow in (H–J), indicates the same cell in all three panels). Such neuronal clusters were never seen under control conditions, i.e., in untransplanted cases or when the vehicle, or even an NSC suspension unsupported by scaffolds, was injected into the infarction cavity. Scale bars: (G) 500 μm; (G") 20 μm; (H–J) 30 μm.

Fig. 2. *(continued)* **(V)** Adverse secondary events that typically follow injury (e.g., monocyte infiltration and astroglial scar formation) are minimized by and within the NSC–PGA complex. (A–D) Photomicrographs of H&E-stained sections prepared to visualize the degree of monocyte infiltration in relation to the NSC–PGA complex and the injured cortex 3 wk following implantation into the infarction cavity. Monocytes are classically recognized under H&E as very small cells with small round nuclei and scanty cytoplasm (e.g., inset in part D, arrowhead). Although some localized monocyte infiltration was present immediately surrounding a blood vessel (BV in part C, arrow) that grew into the NSC–PGA complex from the host parenchyma, there was little or no monocyte infiltration either in the center of the NSC–PGA complex (B) or at the interface between the NSC–PGA complex and host cortical penumbra (A). This is in stark contrast to the excessive monocyte infiltration seen in an untransplanted infarct of equal duration, age, and extent (D), the typical histopathologic picture otherwise seen following HI brain injury (see inset, a higher magnification of the region indicated by the asterisk in part D; a typical monocyte is indicated by the arrowhead). Whereas neural cells (nuclei of which are seen in A–C) adhere exuberantly to the many polymer fibers (P in parts A–C), monocyte infiltration was minimal, compared to that in part D. (E,F) Astroglial scarring (another pathological condition confounding recovery from ischemic CNS injury) is also much constrained and diminished following implantation of the NSC–PGA complex. While GFAP+ cells (astrocytes) were among the cell types into which NSCs differentiated when in contact with the PGA fibers, there was minimal astroglial presence either of donor or host origin *away* from the fibers (*). (E) GFAP immunostaining that is recognized by a fluorescein-conjugated secondary antibody (green) is observed. Note little scarring in the regions indicated by the asterisk. Under a Texas red filter (F) (merged with the fluorescein filter image), the tube-like PGA fibers (arrowhead in both panels) become evident (as autofluorescent orange), and most of the donor-derived astrocytes (arrows) (yellow because of their dual *lacZ* and GFAP immunoreactivity) are seen to be associated with these fibers, again leaving most regions of the infarct (*) astroglial scar-free. (Arrows in parts E and F point to the same cells.) Far from creating a barrier to the migration of host- or donor-origin cells, or to the ingrowth/outgrowth of axons of host- or donor-origin neurons (as per parts III and IV), NSC-derived astrocytes may have helped provide a facilitating bridge. Scale bars: (A) 10 μm; (C,D) and (E,F) 20 μm.

particularly well when implanted into germinal zones, allowing reconstitution of enzyme or cellular deficiencies in a global manner *(6,7,47)*, this extensive migratory ability is present even in the parenchyma of the diseased adult and aged brain *(6,7,50)*. Despite their extensive plasticity, NSCs never give rise to cell types inappropriate to the brain, such as muscle, bone, teeth, or yield neoplasms.

These attributes of NSCs may provide multiple strategies to treat CNS dysfunction. As proof of principle, they were first tested experimentally in mouse models of genetically based neurodegeneration. Their ability to mediate gene therapy was affirmed in a model of the neurogenetic lysosomal storage disease (LSD)—mucopolysaccharidosis type VII (MPS VII; *6*). Mice homozygous for a frameshift mutation in the β-glucuronidase gene are devoid of the secreted enzyme β-glucuronidase (GUSB). The enzymatic deficiency results in lysosomal accumulation of undegraded glycosaminoglycans in the brain and other tissues, causing a fatal progressive degenerative disorder. Treatments for MPS VII and most other LSDs are designed to provide a source of normal enzymes for uptake by diseased cells—a process termed *cross-correction (51)*. The goal of ex vivo gene therapy is to engineer donor cells to express the normal GUSB protein for export to other host cells. The engraftment and integration of GUSB overexpressing NSCs throughout the newborn MPS VII mutant brain succeeded in providing a sustained, lifelong, widespread source of cross-correcting enzyme in a manner not previously achieved *(6)*.

A rapid intraventricular injection technique was devised for the diffuse engraftment of the NSCs. Injecting the progenitors into the cerebral ventricles presumably allowed them to gain access to most of the subventricular germinal zone (SVZ), as well as to networks of cerebral vasculature, along the surface of which they would also migrate. This approach worked equally well in the fetus, where donor NSCs gained access to the ventricular germinal zone *(47)*, migrating into the parenchyma within 24–48 h. This engraftment technique, exploiting many of the inherent properties of NSCs, permitted missing gene products to be delivered without disturbing other neurobiological processes and was a potential strategy for gene therapy of a class of neurogenetic diseases that had not been adequately treated thus far (Fig. 3A). Although MPS VII may be regarded as "uncommon," the broad category of diseases that it models (neurogenetic conditions) afflicts as many as 1 in 1500 children and serves as a model for many adult neurodegenerative processes of genetic origin. (Alzheimer's disease could broadly fall into this category.) Therapy instituted early in life might arrest disease progression

and prevent irreversible CNS alterations. Even in the adult brain, there are routes of relatively extensive migration followed by both endogenous and transplanted NSCs *(52,53)*. If injected into the cerebral ventricles of normal adult mice, NSCs (including those expressing transgenes) will integrate into the SVZ and migrate long distances, e.g., to the olfactory bulb, where they differentiate into interneurons, and occasionally into subcortical parenchyma, where they become glia *(9,34,35,54,55)*. These migratory paths are still relatively restricted and stereotyped, compared to that seen in the fetal or newborn brain. However, in the degenerating, abnormal, or injured adult brain (as discussed below), migration by foreign gene-expressing NSCs can be extensive and directed specifically to regions of pathology—a phenomenon observed with stroke, head injury, dopaminergic dysfunction, brain tumors, and amyloid plaques.

The therapeutic paradigm described above can be extended to other untreatable neurodegenerative diseases that are characterized by an absence of gene products and/or the accumulation of toxic metabolites. In almost all cases, NSCs, because they are normal cells, constitutively express normal amounts of the particular enzyme in question. The extent to which this amount needs to be augmented may vary from model to model and enzyme to enzyme. Reassuringly, in most inherited metabolic diseases, the amount of enzyme required to restore normal metabolism and forestall CNS disease may be quite small. It is significant to note that while the histograms in Fig. 1B illustrate the widespread distribution of a lysosomal enzyme, they could similarly reflect the NSC-mediated distribution of other diffusible (e.g., synthetic enzymes, neurotrophins, viral vectors; *56,57*) and nondiffusible (e.g., myelin, extracellular matrix) factors, as well as the distribution of "replacement" neural cells (see the following section). For example, neural progenitors and stem cells have been used for the local expression of NT-3 within the rat spinal cord, nerve growth factor and brain-derived neurotrophic factor within the septum, and tyrosine hydroxylase, Bcl-2, and glial cell–derived neurotrophic factor to the striatum *(58–65)*. These earlier studies helped to advance the idea that NSCs, as a prototype for stem cells from any solid organ, might aid in reconstructing both the molecules, along with the cells of a maldeveloped or damaged organ. A further complexity, however, is the recognition that the same NSC may not be able to be engineered to express certain neurotrophic agents simultaneously, because they may be processed antagonistically within the cell and/or within the environment. Therefore, a greater knowledge of the NSC processing of certain molecules is a prerequisite *(66)*.

I.
II.
intact
non-grafted
MPTP-treated
mock-grafted
Grafted 1 wk after MPTP
analysed 1 wk later
Grafted 1 wk after MPTP
analysed 3 wks later
TH/β-gal
TH
β-gal
ectopic
SN / VTA
NeuN/β-gal
GFAP/β-gal
CNPase/β-gal
DAT
intact
MPTP-sham
MPTP grafted
TH / BrdU
GFAP / BrdU
CNPase / BrdU