CHAPTER

30

Stem Cells in the Nervous System

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INTRODUCTION/OVERVIEW

During development of the embryo, tissues are built from a few cells that generate highly proliferative committed progenitor or precursor cells, which go on to differentiate into the cells of the mature tissues. In the adult, the majority of cells are non-proliferative and the proportion of precursor and stem cells is dramatically reduced. Only a few tissues such as the skin and blood retain high levels of regular cell turnover in the adult, and they rely upon a continued source of stem cells for replenishment. By contrast, in the nervous system the majority of cells are generated during development, and cell division and morphogenesis are largely complete in the mammalian brain soon after birth. Although there were hints that some new neurons and glia were generated in the adult brain, the recognition that neural stem cells remained in the adult CNS was very surprising and suggested new insights into development and opportunities for neural repair. The role of these stem cells in building the brain in response to injury and their potential for repair is an area of intense study and progress.

STEM CELLS ARE MULTIPOTENT AND SELF-RENEWING

The term *stem cell* has erroneously become widely used to describe cells that have a high proliferative capacity and that can generate more than one cell type. These characteristics do not, however, distinguish stem cells from precursors or progenitors. A stem cell differs from other cells in two essential ways: ***stem cells are multipotent***, with the ability to give rise to multiple differentiated cell types, and ***stem cells are self-renewing,*** with the virtually unlimited ability to make more of themselves. In general, stem cells proliferate relatively slowly

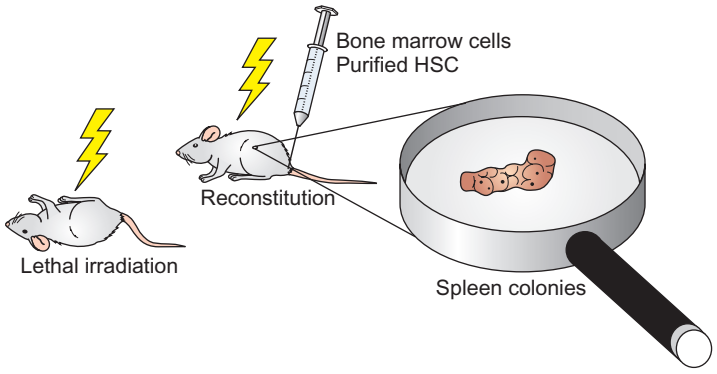

FIGURE 30-1 **Hematopoietic reconstitution by stem cells.** Studies by Till & McCulloch, 1961, established that cells in the bone marrow could reconstitute the immune lineage. Animals exposed to high levels of irradiation die, but can be rescued by infusion of bone marrow cells or purified hematopoietic stem cells. The entire blood-forming system could be restored by injecting bone marrow cells from a compatible donor. After about a week, the spleens of the injected mice contain colonies of cells, each from a single hematopoietic stem cell.

and the precursors/progenitors they generate proliferate rapidly. Within this broad definition, there are various types of stem cells normally found at different stages of development and in different tissues.

Embryonic stem (ES) cells are derived from the inner cell mass of embryos

Embryonic stem (ES) cells are derived from the inner cell mass of embryos, generally of 32 cells or fewer. Because they are isolated from very early embryonic tissues, they are the least restricted type and can give rise to the widest range of cell derivatives in the body, including germ cells. ES cells can be directed *in vitro* to acquire characteristics of neuronal or glial progenitors. In the embryo, ES cells generate all tissue types that comprise the body, and in so doing, they disappear as development proceeds. Because of their source and the potential that these cells can give rise to all cell types in the body and thus could be used for "cloning," the use of these cells for research or therapy has been associated with significant ethical concerns.

Hematopoietic stem cells (HSC) in bone marrow reconstitute the blood

As development proceeds, stem cells become increasingly restricted in the cell types they generate and are converted into tissue-restricted stem cells. One of the best-understood tissues that harbors stem cells in the adult is the blood or hematopoietic system. In healthy individuals, circulating red and white cells in the blood are replaced every few weeks from new cells generated in the bone marrow. The bone marrow contains the hematopoietic stem (HS) cells that give rise to blood and immune cells as well as bone marrow stromal cells. Hematopoietic stem cells are rare in bone marrow (perhaps 1 in 2000 cells) but can be prospectively isolated from bone marrow, peripheral blood and umbilical cord by virtue of specific cell antigens expressed on the surface of HS cells. Due to their self-renewing capability, these cells can be greatly expanded in number with specific growth factors. A key breakthrough in our understanding of blood stem cells came from Till and McCulloch's reconstitution bioassay (Fig. 30-1). In this approach, animals that received lethal irradiation died, but those that received just a few purified hematopoietic stem cells had a completely restored immune system, which demonstrated the multipotency of the transplanted cells (Becker et al., 1963). Indeed, during the reconstitution of the immune system, clusters of hematopoietic cells infiltrated the spleen and formed colonies in numbers that reflected the original stem cells transplanted, and dissociation of those clusters could also reconstitute the immune system, demonstrating self-renewal. Thus far, no similarly powerful functional repopulation assay is possible with neural cells, but this may reflect the challenges of integrating neurons into existing circuits rather than the lack of reparative cells. The early studies led to successful allogeneic (from a genetically different person) hematopoietic stem cell transplants in the 1960s. E. Donnal Thomas, who subsequently won the Nobel Prize in 1990, led this work. Today, thousands of patients have received HS cell bone marrow transplants to treat blood diseases.

NEURAL STEM CELLS CONTRIBUTE TO NEURONS AND GLIA DURING NORMAL DEVELOPMENT

Unlike the hematopoietic system, the nervous system is not characterized by high levels of cell turnover in the adult, and for many years it was not considered to have regenerative capacity (Conti & Cattaeneo, 2010). Indeed, the majority of the neurons in the CNS are generated in embryogenesis. They do not divide again, which leads to the concept of a neuronal birthdate, and they are not turned over, so that any loss of neurons or glia in the adult is associated with loss of function. Classical studies by Altman and Das (1965; Altman, 1963) demonstrated the presence of new neurons in the adult mammalian hippocampus and olfactory bulb. Other studies revealed seasonal generation of new neurons in adult songbirds. Although at first imagined to be peculiarities limited to the dentate gyrus of the hippocampus and subventricular zone leading to the olfactory bulb,

neurogenesis has now been observed in many parts of the adult mammalian brain. The origin of many of these new neurons has been found to be among adult neural stem cells.

Neural stem cells (NSCs)

Neural stem cells (NSCs) have been identified in the embryonic rodent and human brain, spinal cord, and neural retina (McKay, 1997; Gage, 2000). In the mammal, neural development begins with induction and formation of the *neural tube* at about E 7.5 in mice and fourth week of gestation in humans. The walls of the neural tube contain neuroepithelial cells oriented like spokes on a wheel that will eventually divide dramatically to give rise to each of the major brain regions, and on a cellular level, to all the neurons and glial cells of the entire nervous system (see Development, Chapter 28). (Fig. 30-2) Elegant live-cell imaging of embryonic cortical slices reveals the relationships between dividing neuroepithelial cells and their daughter cell neurons and glia (Kosodo et al., 2008).

Radial glia are stem cells

Radial glial cells make up one of the earliest classes of cells to emerge from the neuroepithelium. These cells arise during the expansion of the neural tube (about E9.5 in mice) and may be characterized by expression of specific antigens including GLAST, BLBP and GFAP. Originally, radial glia were thought to be simply scaffolds that maintained the cytoarchitecture of the nervous system. However, several lines of evidence suggest that early radial glial cells have stem cell-like properties. In the developing cortex, initial retroviral lineage tracing identified "clones" of cells with a radial orientation and containing both neurons and glia. This observation suggested that these clones contribute to functional columns in the brain. Yet the key studies to demonstrate that particular stem cells give rise to particular progeny or derivates in situ remain incomplete. One example of a good critical approach has been to use Cre/*loxP* lineage tracing with different radial glial promoters, which reveals that many, if not most, neurons in the brain/ventral telencephalon are derived from radial glia (Anthony & Heintz, 2008). More extensive genetic lineage analysis of not only radial glia but also their derivatives *in vivo* are needed to understand the quantitative contribution of stem cells to the adult brain and to understand key regulatory factors that direct the fate of their progeny.

The neural tube rapidly acquires regional distinctions as a result of soluble inductive cues. For example, in presumptive caudal ventral regions of the neural tube, local signaling by retinoic acid and sonic hedgehog induces a series of transcription factors that result in the generation of motor neurons and oligodendrocytes, while local signaling by Wnt and BMP in dorsal regions generates neural crest and sensory neuron precursors (Fig. 30-3). Although the vast majority of early neural stem cells become patterned and committed to generate specific neural fates, in particular regions of the CNS, some cells retain stem-like properties and contribute to replacement cells in the adult (see below).

Numerous studies have isolated stem cells from a variety of brain regions at various ages and have shown that these

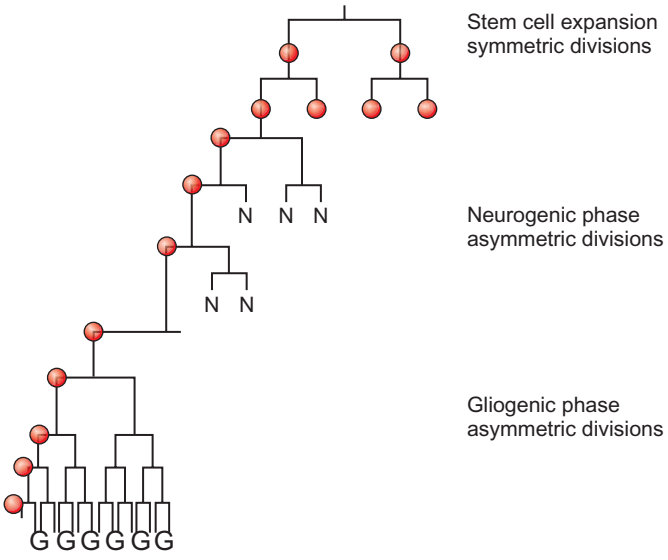

FIGURE 30-2 **Asymmetric cell divisions give rise first to neurons and later to glia as development proceeds.** Neural stem cells expand early in development, and then give rise to neurogenic and then gliogenic lineages. While these events occur, it is not yet clear how the lineages are regulated or whether they follow similar patterns in each region of the CNS.

cells give rise to particular derivatives *in vitro* (see Temple, 2001). However, it is also clear that the progeny of neural stem cells can migrate long distances. Not only are the properties of neural stem cells regionally specified; in addition, the specification of their progeny may change during development. For example in most regions of the CNS, the first cell populations to be generated during development are neurons, and this is followed by the development of glia. Stem cells isolated during the period of neurogenesis preferentially generate neurons *in vitro* while those isolated later in development during gliogenesis preferentially generate glia.

The peripheral nervous system (PNS) is derived from neural crest stem cells

Neural crest stem cells also give rise to peripheral nervous system neurons and glia (Morrson et al, 1999). Early in development, some neural tube cells in dorsal regions delaminate or migrate away from the neural tube as neural crest cells. Neural crest cells are transient embryonic cells that give rise to sensory neurons, autonomic neurons, and the Schwann glial cells of the PNS, but also to non-nervous tissues such as face and neck bone and cartilage, the outflow tract of the heart, and pigment cells of the skin. Lovely chick–quail transplantation studies by LeDouarin [see video ref] led to the understanding that, as a population, neural crest cells are multipotent, but it took *in vitro* studies to finally prove that neural crest cells are also self-renewing and are, indeed, stem cells. Neural crest cells can be prospectively isolated with the use of antigenic tools. Continuing work in this field has revealed that inductive cues including BMPs also specify neural crest derivatives, and that adult peripheral tissues continue to harbor some neural crest

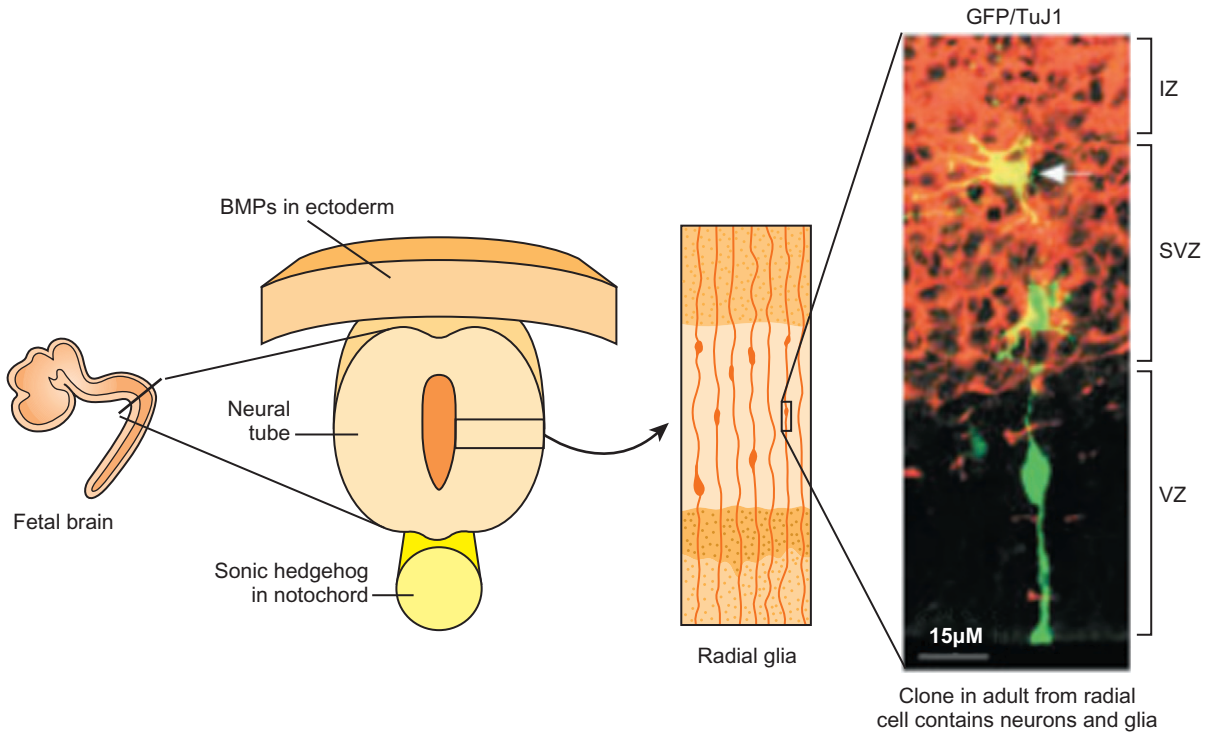

FIGURE 30-3 **Inductive cues affect regional development of the CNS.** Signals include dorsalizing BMPs and ventralizing sonic hedgehog alter cell fate in the neural tube. Radial glial cells that initially span the wall of the neural tube give rise to clones of cells that include both neurons and glial cells (note green and yellow cells).

cells. Given their common embryological origins, it is not surprising that adult skin cells can be stimulated to give rise to neurons (Toma et al., 2001).

Taken all together, this body of research results demonstrates the presence of neural stem cells that become increasing patterned as development proceeds. The molecular basis of this increasing restriction in the fate of cellular progeny during development and maturation is an area of current investigative interest.

STEM CELLS CAN BE IDENTIFIED ANTIGENICALLY AND FUNCTIONALLY

Stem cell markers in the nervous system

Stem cells are generally relatively rare in mature tissues, and their identification and efficient isolation require specific tools. Estimates suggest that HSCs represent perhaps 1 stem cell to 2,000 cells in bone marrow or 1: 200,000 cells in circulating blood. Even in embryonic tissues where they may be more plentiful, stem cells are intermingled with numerous progenitor and mature cells. To identify stem cells antigenically for isolation, investigators have worked to distinguish selective stem cell markers, as well as differentiated neural cell markers. Some stem cell markers reflect transcription factors present only at certain stages of differentiation, while other markers expressed on the cell surface are useful for prospective isolation. Commonly used stem cell markers in the nervous system as well as differentiated neural cell markers are described in Table 30-1. While these moieties are useful in isolated applications, there is not yet consensus that any one marker identifies all neural stem cells, and only neural stem cells, in every region of the CNS. Several markers are expressed on stem cells from several tissues (for example, CD133 or Sox2; see Suh et al., 2007), but in many cases, a combination of multiple markers must be used to identify a particular stem cell.

The neurosphere functional assay

The strongest functional data for the existence of a self-renewing, multipotent neural stem cell comes from *in vitro* studies. The neurosphere assay first developed by Reynolds and Weiss (1992) has emerged as a useful approach to expand and study neural stem cells and progenitors. In essence, dispersed neural cells are grown in suspension in Fibroblast Growth Factor 2 and/or Epidermal Growth Factor, during which stem cells proliferate and form floating cell aggregates called "neurospheres" while the vast majority of precursors and differentiated cells die. When these clumps are re-dissociated and plated on an adherent substrate, single cells from neurospheres again proliferate to form clumps of cells that contain neurons and glial cells, thusly demonstrating the multipotency of the original stem cells. Cells from such "monolayer" clumps can again be dissociated, after which they will re-form neurospheres upon addition of growth factor, thusly indicating self-renewal of stem cells. Although the extended passaging capability of these cells is not known, they have been maintained for at least 20 passages (Fig. 30-4). Remarkably,

TABLE 30-1 Neural Markers

Stem-like or precursor cells

Sox2 is a transcription factor expressed in the neuroepithelium and is centrally important for neural stem cell proliferation and differentiation.

Nestin is an intermediate filament present predominantly in stem cells of the CNS, radial glial cells; its expression is absent from nearly all mature CNS cells.

CD31/PECAM (Cluster of Differentiation 31/Platelet-Endothelial Cell Adhesion Molecule-1)

CD133 (also known as AC133 or prominin 1) is a cell surface protein found on stem cells of many tissues including the nervous system, as well as some cancer cells.

SSEA 4 (Stage-specific embryonic antigen 4)

GLAST is a glutamate transporter expressed in the ventricular zone and radial glia.

BLBP (Brain lipid binding protein) is a marker for radial glia.

Astrocytes

Glial fibrillary acidic protein is present in astrocytes, although expression may be low in the mature intact CNS.

Neurons

Neuronal tubulin (beta 3 tubulin) is a cytoskeletal element found in most neurons and neuronal processes.

Microtubule-associated protein 2 is expressed in dendrites.

Tau is a tubulin-binding protein enriched in axons.

Neurofilament proteins

Oligodendrocytes

O4 (immature), O1 (more mature), myelin basic protein

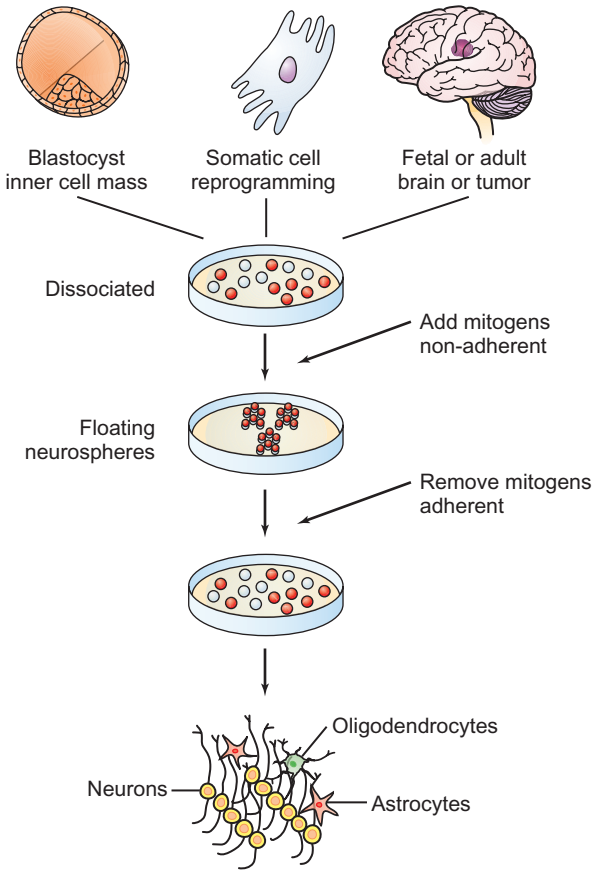

FIGURE 30-4 The neurosphere assay in vitro has been widely used to identify neural stem cells. Dissociated cells grown in EGF/FGF form clusters of floating cells called neurospheres. When plated on adherent substrate, these cells differentiate into neurons and glial cells as well as stem cells.

neurospheres can be generated not only from embryonic brain, but also from adult brain and spinal cord. Neural stem cells derived from various brain regions have regional identity and give rise to site-specific derivatives, at least initially. Emerging data now suggest that propagating neurospheres from different regions in response to mitogenic factors may de-regulate their distinct characters and result in more homogeneity *in vitro*. Despite these concerns, the neurosphere "functional assay" of floating, multicellular clusters with differentiative capacity has remained an important tool in neural stem cell biology.

Is there a brain neoplasm stem cell?

Brain neoplasms, like those from other organs, are composed of a heterogeneous mixture of cells including progenitor-like and differentiated cells. Recently, cells with characteristics of stem cells—multipotency and self-renewal—have been identified in several glial brain tumors including glioblastoma multiforme (Hemmati et al., 2003; Singh et al., 2004; Piccrillo et al., 2009). These stem-like cells can self-replicate, form neurospheres in culture, give rise to differentiated neuronal and glial cells *in vitro* and can respond to typical differentiative cues. Interestingly, transplantation of stem-like cells derived from human brain tumors (defined by CD133 expression) in animal models results in highly invasive tumors that resemble the original human GBM.

The role of a cancer stem cell in the generation of neoplasia remains unclear. For example, it is unclear whether cancer stem cells are ultimately responsible for tumor initiation, metastasis and resistance to treatments, or are instead products of de-differentiation in the tumor environment (Rosen et al., 2009). Like normal neural stem cells, cancer stem cells are often closely associated with the vasculature. In several cases, the expression of markers in GBM stem-like cells differs from those found in NSCs, and the proliferative capacity of the GBM stem-like cells is robust even without mitogenic control. It remains to be learned if these stem-like cells have acquired mutations leading to or resulting from transformation.

Induced pluripotent stem cells, reprogramming and directed differentiation

Advances in cell biology promise new approaches in regenerative medicine but also call into question our views of stem cells and their progeny. Initially, pluripotent cells were derived

from ES cells and much work was focused on how to appropriately stimulate differentiation to derive the desired mature cell type. Then with "therapeutic cloning" or somatic cell nuclear transfer, it was clear that the replacement of the egg nucleus with the nucleus of a somatic cell, like a fibroblast or mammary cell, would reprogram the host cell. This technically laborious approach was used in the generation of "Dolly" the sheep (Wilmut et al., 1997). The advent of induced pluripotent stem cell (iPS) technology suggests we may be able to use a new stem cell model to dissect the mechanisms of neural cell fate determination and understand the etiology of many neural diseases.

Induced pluripotent stem cells can be generated from somatic cells like fibroblasts with a cocktail of transcription factors, rather than a nuclear transfer, to force genetic reprogramming. In 2006, Yamanaka reported that the insertion of four genes—Oct 3/4, Sox2, c-Myc, and Klf4—into fibroblasts converted these cells into "induced pluripotent" stem cells with characteristics of ES cells (Takahashi & Yamanaka, 2006). It has been shown that the oncogene c-Myc is not needed. Thompson and colleagues used a different set of factors—Oct4, Sox2, Nanog and Lin28—with similar results. Overall, the method is thought to reverse differentiation back to a stem cell fate. The initial proof of principle was based on approaches in which the genes were delivered by viruses. An attractive alternative may be a "piggyback transposable element" that allows transfection but is also reversible and that avoids the complication of residual viral gene influences.

An iPS-based cell therapy opens the door to using the patient's own skin cells or other somatic cells as a source for neural stem cells. iPS cells may allow comparison of genes and proteins between normal and affected individuals to reveal disease-related traits, or may allow for use in high-throughput-candidate drug screening. iPS cells also represent a potential cellular source of cells for regenerative therapy, either as a source of expanded normal cells, or as a target for gene therapy to repair the disorder.

iPS cell models appear to reflect disease-related phenotypes of developmental disorders, but their fidelity in adult-onset disorders is less clear. For example, iPS-derived neurons from a child with spinal muscle atrophy reflected characteristics of this fatal disorder, including reduced expression of smn protein (Ebert et al., 2009). By contrast, iPS cells from patients with ALS or PD do not seem to reflect disease phenotypes (Dimos et al., 2008). It is not clear if these cells require stressors like toxins or proinflammatory factors or other culture conditions to correctly reflect the disease state. Technical aspects of optimal isolation or expansion of stem cells, as well as determination of which cells are appropriate for neural treatments, continue to be addressed and this field is moving very rapidly. In practice, while appropriate cells can be generated, the process is not simple and must address proliferation of cells, appropriate differentiation, isolation and removal of any inappropriate cells as well as identification of effective cells for transplantation. iPS technology clearly highlights new questions about what it means to be a terminally differentiated cell. In part due to the devastation cause by neural disease and the otherwise modest treatments available, a number of studies using various stem cells for neural treatments have been initiated. These will provide an initial glimpse of both the promise and the challenges ahead.

STEM CELLS OFFER POTENTIAL FOR REPAIR IN THE ADULT NERVOUS SYSTEM

The discovery that parts of the adult mammalian brain continue to generate new neurons led to the identification of adult stem cells in the mature brain. Adult stem cells were initially found in areas that undergo adult neurogenesis, including the dentate gyrus of the hippocampus and the anterior region of the subventricular zone (SVZ). A remarkable example is found in the subventricular origin of neurons in the olfactory bulb (Alvarez Buylla & Garcia-Verdugo, 2002). The SVZ near the walls of the lateral ventricles contains numerous neuronal precursors. Many newly born neurons in this area migrate long distances in "migratory chains" through the forebrain to become integrated with cellular components in the olfactory bulb. Cells in this region can be antigenically and morphologically discriminated as A, young neuron cells; B, astrocytic cells with a cilium; C, proliferative cells; and E, non-neural ependymal cells. In an interesting approach, when anti-mitotic agents were used to kill dividing cells in the SVZ, B cells were shown to be precursors for new neurons; other studies suggest they are neural stem cells (Doetsch et al., 1999).

Studies tracing the fate of newly generated cells in the adult brain suggest that at least some of the dividing precursors are multipotent and may be stem cells. Indeed, neural stem cells have been isolated from many brain regions as well as the spinal cord in mammals, including humans. The presence of stem cells in the adult brain raises the question as to why, after trauma or onset of neurodegenerative disease, neural repair is ineffective. At least three possible explanations have been posed: (1) there are too few endogenous stem cells to be effective; (2) stem cells don't "home" to the injured area; and (3) the adult molecular environment does not promote appropriate differentiation. Resolving the relative contribution of each of these potential inhibitors of repair will be critical to developing the most effective approaches to stem cell therapies.

Stem cells to replace depleted neurochemicals: Parkinson's disease

Many neuroscientists feel that cell replacement therapy to treat Parkinson's disease is a reasonable goal in our lifetimes. Parkinson's disease is a progressive movement disorder characterized by resting tremor, bradykinesia with cog-wheel rigidity, and difficulty initiating voluntary movement (see Ch. 49). The symptoms result from the death of dopaminergic neurons in the substantia nigra, leading to decreased dopamine in the striatal circuits that are responsible for regulating motor movements. In many cases, symptoms may be ameliorated by administration of dopaminergic agents. Therapeutic approaches have been designed to (1) prevent neuronal death in the first place; (2) replace the missing neurons; and (3) replace the missing dopamine.

In the initial cell implantation studies, primary fetal brain tissue was employed to increase dopamine function. Transplanting fetal mesencephalic tissue containing the developing nigro-striatal regions into brains of rodents or monkeys modeling Parkinson's disease seemed to reverse the

symptoms. These studies led to human trials in which human fetal tissue was transplanted into patients (Lindvall et al., 1988; 1990). While not all patients improved, in those that did, dopamine function increased, measured by positron emission tomography (PET). Autopsy of a number of patients who died from other causes revealed survival of the grafted neurons and outgrowth into the striatum. These initial studies were then extended in two double-blind, sham surgery controlled clinical trials. In the first, patients who received fetal tissue transplant did not have obvious improvement in quality of life measures over controls. More worrisome, 5 of 33 treated individuals developed dyskinesia or uncontrolled increased motor movements (Freed et al., 2001). The dyskinesia reflects dysregulation of increased dopamine levels. The second trial incorporated changes in the tissue source, the cells implanted, immunosuppression and outcome measures. Again, however, the average condition of the 23 patients who received transplants did not differ from the 11 who did not when symptomatic outcomes were assessed, and about half the patients in this trial had dyskinesias (Olanow et al., 2003, see also Olanow et al., 2009). While these studies provide hope that transplantation may offer benefit, they also point to the need for more standardized cells for transplantation.

Stem cell approaches offer the promise of cell standardization, but current technology has not yet routinely delivered it. One approach uses a cocktail of factors to direct differentiation of embryonic stem cells into dopaminergic neurons over several weeks, while in a second approach, suspension culture in growth media has produced large numbers of neurons with the appropriate characteristics. In either case, however, the resulting neurons are not homogenous, and contaminating stem cell and non-DA neurons remain unacceptable transplantation risks.

A second idea has been to transplant less-differentiated cells and allow the brain environment to direct their maturation using signals that may be present following acute injury. Undifferentiated human NSCs transplanted into brains of primates with a Parkinsonian syndrome—caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)—survived and induced behavioral recovery (Redmond et al., 2007). These cells were directly isolated from the ventricular zone of second-trimester human cadavers, maintained as cell lines, and tracked after transplantation by pre-labeling with BrdU. A small number of hNSC progeny differentiated into tyrosine hydroxylase–immunoreactive or dopaminergic cells, and a large number of the transplanted cells survived but did not express neuronal markers. The authors speculated that the cells may have provided glial derived neurotrophic factor (see Growth Factors, Chapter 29) to support dopaminergic neurons, but did not demonstrate this connection. Despite these setbacks, much has been learned about the use and regulation of replacement cells in Parkinson's disease, and these observations are being rapidly translated into human therapy trials.

Stem cell treatment to deliver missing enzymes or proteins: leukodystrophies

A group of lysosomal storage disorders called the *neuronal ceroid lipofuscinoses* result in intracellular accumulation of an autofluorescent lipopigment storage material, progressive neurodegeneration and dementia, loss of motor abilities, visual loss and seizures (see Ch. 43). The biochemical defects that underlie several of these disorders have been discovered. For example, low activity of the enzyme palmitoyl-protein thioesterase-1 (PPT1) contributes to the infantile form of Batten disease (now referred to as CLN1). In the late infantile form (CLN2), a deficiency of an acid protease (TPP1, tripeptidyl-peptidase-1) has been found as the cause. Mice deficient in PPT1 have some symptoms similar to those caused by the human disorder. In a promising approach, human NSCs grown as neurospheres were transplanted into the brains of immunodeficient PPT1(-/-) mice where they engrafted, migrated and produced sufficient levels of PPT1 to alter host neuropathology and to delay loss of motor coordination (Tamaki et al., 2009).

In 2005, the Food and Drug Administration approved a phase I clinical trial of a therapy developed by Stem Cells, Inc., using neural stem cells to treat infantile and late infantile Batten disease, and as of this writing, a Phase 1A trial has been completed and a Phase 1B trial is recruiting. In February 2010, Stem Cells, Inc., announced that its proprietary HuCNS-SC® human neural stem cells have been used to treat the first patient enrolled in its Phase I clinical trial in Pelizaeus-Merzbacher Disease (PMD), a hypomyelinative disorder with deficiency of proteolipid protein 1 that afflicts male children. The stem cells were administered by direct injection into the brain of a patient with connatal PMD, the most severe form of the disease.

Still other disorders due to gene deficiencies may be addressed with stem cell therapy. Pediatric leukodystrophies are manifested by deficiencies in myelin, and appear to be due to hereditary gene defects (see Box 30-1). For example, in adrenoleukodystrophy, patients lack a transporter protein needed to break down very-long-chain fatty acids in the diet, so that these fatty acids build up in the adrenal gland and the nervous system, producing damage. While there are several forms, the most common is X-linked, in which boys are most affected. Hematopoietic stem cell or umbilical cord transplantation may slow progression of the disease if performed early in the course of the disease (Cartier et al., 2010). While these approaches may still be "experimental," the rational cell-based approach and clear improvement in function suggest that stem cell-based therapies hold great promise for this class of neural diseases.

Stem cells for cell replacement therapy: myelin

Deficiencies in glial cells, particularly in oligodendrocytes that myelinate the CNS, lead to significant functional deficits (see Chs 10, 39). The *shiverer* mouse isolated several decades ago has proven an important model for the assay of myelinating capacity *in vivo*. These mice are globally deficient in myelin basic protein (MBP) and in structural myelin. They exhibit functional abnormalities and die by 20–22 weeks. Transplantation of neonatal brain tissue, primary oligodendrocytes or adult NS fragments generates compact myelin in these animals (see Lachapelle, et al. 1983; Blakemore & Crang, 1988). In particular, Goldman demonstrated that human oligodendrocyte precursors could "rescue" the shiverer phenotype (Windrem et al., 2008), and more importantly,

STEM CELL THERAPIES FOR DYSMYELINATING DISEASES

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Leukodystrophies are a family of diseases that result from defects in lysosomal enzymes (see Chapter 43). Some 40 known lysosomal storage diseases cumulatively affect 1 in 5,000 births. Much of the pathology associated with the disorders is related to the degree of substrate accumulation, as well as the sensitivity of the cell to the stored substrate. A general principle behind stem cell or gene therapies is that successful engraftment will provide a source of the missing enzyme for the life of a patient.

Hematopoietic stem cell transfer (HCT) has been used for several decades to cure or arrest several neurodegenerative lysosomal storage diseases. Disease phenotype and extent of the disease at the time of treatment have proven to be important variables:

- Krabbe disease is caused by a deficiency of galactocerebrosidase, an essential enzyme for myelin metabolism. Results of a very small clinical trial of patients with infantile Krabbe disease found that children who received umbilical cord blood stem cells from unrelated donors prior to symptom onset developed with little neurological impairment. Disease progression stabilized faster in patients who received cord blood compared to those who received adult bone marrow. Bone marrow transplantation has been shown to benefit mild cases early in the course of the disease.
- Cerebral X-linked adrenoleukodystrophy (X-ALD) is a clinically heterogeneous X- linked leukodystrophy affecting fatty acid metabolism. The majority of affected boys also have Addison's disease, and neurological deterioration includes visual, hearing and motor deficits. While HCT is the only potentially effective treatment, it is recommended only when cerebral disease is present. The first HCT for X-ALD was performed in 1982, and long-term HCT results have been reported. Typically, boys with very early stage disease are the best candidates. Recently, insertion of the corrected gene in autologous hematopoietic stem cells has shown clinical outcomes similar to allogeneic HCT, suggesting that gene therapy of the patient's own stem cells may prove practical.
- Globoid cell leukodystrophy (GLD) is a recessive lysosomal disorder due to deficiency of galactocerebroside GalC. When manifested with early onset, this is classical Krabbe disease, and can lead to death. HCT for GLD was reported in 1998 in five cases, and additional information on umbilical cord transplants is accumulating.
- Metachromatic leukodystrophy (MLD) is a recessive lysosomal storage disorder due to deficiency of arylsulfatase A, and can present at various stages, with the earliest infantile forms leading to death within years. All forms of the disease involve progressive deterioration of motor and neurocognitive function. HCT for MLD has proven challenging, with rather disappointing results for infantile forms due to rapid progression of the disease itself. HCT may be useful in stabilizing CNS disease in late-onset patients, while having little benefit for the PNS components.

Despite significant progress, HCT is still associated with significant risks of graft failure or graft-versus-host-disease, and there is strong interest in identifying optimal treatment variables. Stem cell sources for transplantation are bone marrow, peripheral blood and cord blood. Preparative conditioning with supralethal chemoradiotherapy is needed for donor cell engraftment, and immunosuppressive agents are given for months as prophylaxis against graft-versus-host disease (GVHD).

some of the mice lived much longer and appeared functionally "cured." Key aspects of this approach were to inject many cells in many locations, as predicted for a cell therapy to remyelinate many parts of the CNS. In a second more complex model of demyelination, experimental allergic encephalomyelitis (EAE), syngeneic adult neural stem cells expanded *in vitro* were used to repair animals with EAE (Pluchino et al., 2003). These cells clearly promoted multifocal remyelination and functional recovery, although we now interpret at least some of the effects to be due to immunomodulation (see below) rather than frank cellular replacement. There is strong interest in optimizing the cell types most efficient for potential therapy. One intriguing approach might be to develop a bank of cells reprogrammed to an appropriate stage of oligodendrocyte precursor differentiation.

The first human clinical trial of an embryonic stem cell-based therapy for neural cell repair was authorized in 2009. A company based on this technology, NeuralStem, Inc., used its spinal cord stem cells in 18 patients with ALS at the Emory ALS Center in safety trials. In 2010, the U.S. Food and Drug Administration gave approval to Geron Corporation to begin the first clinical trial of hESC therapy aimed at regenerating myelin in patients with spinal cord lesions. Subsequently, NeuralStem, Inc., was approved to test stem cell therapy in patients with amyotrophic lateral sclerosis (see Ch. 45).

While these approaches might be effective after acute injury, treatment of demyelinating diseases such as multiple sclerosis is likely to be more complex, and require additional strategies that address the immune etiology of the disease. Active disease will likely destroy transplanted glial cells as well as host oligodendrocytes.

Stem cells as a source of growth factors and guidance cues

Stem cells may serve as a source of neurotrophic factors to support neuronal survival or enhance their growth. An interesting example of this action was identified in studies of Alzheimer's disease. Alzheimer's disease is characterized

histologically by an accumulation of amyloid plaques and neurofibrillary tangles, gliosis, and neuronal and synaptic loss (see Ch. 46). Behaviorally, the disease manifests in progressive loss of memory and cognitive function. Transplantation of cholinergic enhancers or growth factors that support basal cholinergic neurons seems to improve function. These observations have led to cell or gene transfer approaches to rescue or retain cholinergic neurons.

A number of the hallmarks of Alzheimer's disease can be modeled in the "triple-transgenic AD" mouse (3xTg-AD) that expresses pathogenic forms of amyloid precursor protein, presenillin and tau. 3xTg-AD mice at 18 months recapitulate histological and behavioral signs of Alzheimer's disease. In the study by Blurton-Jones, et al., NSCs were isolated from haplotype-matched GFP-expressing neonatal mouse brains and injected bilaterally into the hippocampus. The host animals were tested one month later. Injection of wild-type NSCs into 3xTg-AD mice rescued learning and memory impairments as tested by the Morris Water Maze and novel object recognition tests (Blurton-Jones et al., 2009). In these animals, only about 6% of the stem cells turned into neurons, while the majority became astrocytes and oligodendrocytes. These stem cells did not improve memory by becoming new neurons nor did they reduce hallmark plaques and tangles of Alzheimer's disease. Instead, the stem cells produced BDNF, a neurotrophic factor that increased connections between the neurons. Most importantly, gain-of-function studies showed BDNF injection mimics the beneficial actions of NSCs, while loss-of-function studies in which BDNF was depleted from NSCs showed a reduction in benefit.

Others have engineered fetal neural stem cells to produce quantities of GDNF, another growth factor. When injected into the spinal cords of rats suffering from an ALS-like disease, these cells survived well and continued to secrete GDNF. Similarly, neural stem cells taken from mouse fetuses secrete GDNF and promote recovery in mouse models of Parkinson's disease.

Neural precursors also appear to produce trophic factors for glial cells. In the chronic cuprizone model in aged rats extensive demyelination occurs, particularly of long tracts in the corpus callosum, and is followed by slow remyelination. When GFP-labeled neural precursor/neurospheres were administered into the lateral ventricle of cuprizone treated mice, they remained relatively undifferentiated in the periventricular area. One week after treatment with stem cells, significant remyelination of the corpus callosum occurred, and all remyelination came from host OPCs. *In vitro* studies suggest that mitogenic effects *in vitro* of neural precursor cells on OPCs were due to PDGF and FGF (Einstein et al., 2009).

Stem cells for immunomodulation: multiple sclerosis

Stem cell transplantation may suppress the inflammatory process, allowing any native repair systems to restore function. Such immunomodulation may be most apparent in chronic inflammatory disorders such as multiple sclerosis (MS). Multiple sclerosis is an immune-mediated demyelinating disease of the central nervous system in which the insulating myelin is lost and axons are damaged. In early stages of disease, remyelination by resident oligodendrocyte progenitor cells and functional recovery are possible, but this eventually fails with prolonged disease. Current therapeutic treatments for MS are designed to suppress the immune response and to allow remyelination to occur. Experimental models using allergic/autoimmune encephalomyelitis to model chronic disease have been useful in identifying roles for stem cells in promoting functional recovery. Other approaches like lysolethicin or cuprizone treatment to induce acute focal demyelination have been used to assess remyelination in the CNS.

Neural precursor cells or neurospheres transplanted into the CNS of rodents with EAE reduce inflammation and promote functional recovery (Einstein et al., 2007; Ben-Hur 2008). It is interesting that both intraventricular and intravenous administration of neurospheres reduced inflammation in EAE by actions in lymph nodes and spleen. *In vitro*, neurospheres reduced activation and proliferation of T cells, suggesting that neurospheres acted on T cells by peripheral immunosuppression (Einstein et al., 2007; Ben-Hur, 2008). Similarly, human embryonic stem cell-derived neurospheres were effective in mouse EAE (Aharonowiz et al., 2008).

Immunosuppression may be a feature of many types of stem cells (Uccelli et al., 2007). Along with hematopoietic, blood-forming stem cells, the bone marrow also contains stromal or mesenchymal stem cells (MSCs) that can proliferate extensively *in vitro* and differentiate under appropriate conditions into bone, cartilage and other tissues. MSC injection intravenously also reduces EAE, particularly when delivered early in the disease process (Zappia et al., 2005). MSCs can inhibit T cell receptor-dependent and -independent proliferation. Studies *in vitro* suggest that when co-cultured with MSCs, T cells are viable, but in anergy, and unreactive to foreign substances. The beneficial effects of MSCs in immune-mediated demyelination may reflect not only immunosuppression but also promotion of neuroprotection or neural repair.

Human MSCs have similar immunosuppressive characteristics (Bai et al., 2009), and the general observation that MSC transplants may provide relief in chronic immune responses such as those seen in MS is very exciting. Based on these observations, a number of early clinical trials have been initiated using MSCs and peripheral blood stem cells. In one recent trial in which peripheral blood stem cells were used, Xu and colleagues showed that of 36 patients with relapsing remitting disease, 22 showed no relapses over a 48-month period and 20 had continuous neurological improvement.

Common challenges for stem cell therapy in the nervous system

Stem cell therapies have some challenges in common. In addition to having proven efficacy via various mechanisms, any cell therapy for human disorders must also be safe. A cell source that is expandable and "bankable" without change over generations will be important. Purity will be best defined using specific prospective tools; however, unambiguous identification of neural stem cells is not currently feasible. Stem cells are highly proliferative and unrestricted in potential.

Therefore, approaches to either pre-differentiate or limit their proliferation remain crucial. It is clear that transplants of undifferentiated ESCs form teratomas (Thompson et al., 1998), while there is less evidence that NSCs are tumorogenic.

Unlike the hematopoietic system, in which stem cells produce mature cells that integrate into a dispersed adult system (the blood), stem cell therapy in the nervous system includes spatial challenges for effectiveness, in addition to issues concerning selection of the appropriate stem cell itself. Depending on the mechanism of action, it may be necessary to surgically place or biologically "home" the transplanted cells to the site of injury or disease. Because in the adult nervous system normal neurons and glia do not undergo significant turnover, it is also likely that specific growth factors for induction of cell types and their survival are not present in the adult nervous system, so an adjunct growth factor therapy is required. Further, neural networks of cells in functional circuits developed and were reinforced over time, making integration of a new cell challenging. Despite these *a priori* concerns, methodology for isolation and propagation of neural stem cells and for their direction to specific lineages to replace neurons has moved at an extraordinary pace, and the promise of stem cells as therapeutic agents in neural repair remains enormous.

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