Preclinical Assessment of Stem Cell Therapies for Neurological Diseases

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Abstract

Stem cells, as subjects of study for use in treating neurological diseases, are envisioned as a replacement for lost neurons and glia, a means of trophic support, a therapeutic vehicle, and, more recently, a tool for in vitro modeling to understand disease and to screen and personalize treatments. In this review we analyze the requirements of stem cell–based therapy for clinical translation, advances in stem cell research toward clinical application for neurological disorders, and different animal models used for analysis of these potential therapies. We focus on Parkinson's disease (typically defined by the progressive loss of dopaminergic nigral neurons), stroke (neurodegeneration associated with decreased blood perfusion in the brain), and multiple sclerosis (an autoimmune disorder that generates demyelination, axonal damage, astrocytic scarring, and neurodegeneration in the brain and spinal cord). We chose these disorders for their diversity and the number of people affected by them. An additional important consideration was the availability of multiple animal models in which to test stem cell applications for these diseases. We also discuss the relationship between the limited number of systematic stem cell studies performed in animals, in particular nonhuman primates and the delayed progress in advancing stem cell therapies to clinical success.

Key Words: cell-based therapies; grafting; multiple sclerosis; neurodegeneration; nonhuman primate (NHP); Parkinson's disease; preclinical evaluation; stem cells; stroke; transplantation

Introduction

Stem cells (SCs[1]) are typically defined as cells that are able to renew themselves through mitotic division and differentiation. SCs have been envisioned as a resource for regenerative medicine to treat neurological disorders—as a means to replace lost neurons and glia, as a source of trophic support, as a therapeutic vehicle, and, more recently, as a tool for in vitro modeling to understand specific diseases and to screen and personalize treatments (Table 1).

The clinical translation of SC-based therapies requires preclinical experimentation to assess their feasibility, safety, and efficacy. Depending on the stage of therapy development different animal species are tested before clinical translation. Most studies use rodent models of disease to assess SC properties. Nonhuman primates (NHPs[1]) are the next essential platform for assessing first-in-class and invasive therapies for neurological diseases, because their brain size and complexity allow for the evaluation of surgical targets and cell distribution. Additionally, because monkeys are outbred their immunological response to foreign cells is not compromised compared to other highly inbred species, such as rats and mice (Capitanio and Emborg 2008).

In this paper we focus on the preclinical evaluation of SC-based therapies for Parkinson's disease (PD[1]), stroke, and multiple sclerosis (MS[1]). We chose these disorders for their diversity and the number of people affected by them. An additional important consideration was the availability of multiple animal models that facilitate in vivo studies on SC applications for these diseases. Our goal is to survey the results of in vivo experimentation in order to overcome the challenges to successful clinical application of SC-based therapies for neurological disorders.

Preclinical Requirements for Clinical Translation of SC-Based Therapies

The original goal of cell-based therapies drives most SC research: to obtain cells that can replace those lost to disease. Although there has been much progress toward this goal, there remain concerns about the safety and efficacy of transplanted SCs as well as the ability of animal studies to predict clinical outcomes. In this context, it is important to consider, When is

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[1]Abbreviations used in this article: 6-OHDA, 6-hydroxydopamine; CNS, central nervous system; DA, dopamine; EAE, experimental autoimmune encephalomyelitis; ERC, endometrial regenerative cell; ES, embryonic stem; hNP, human neuroprogenitor; iPS, induced pluripotent stem; IV, intravenous; MCA, middle cerebral artery; MOG, myelin oligodendrocyte glycoprotein; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MS, multiple sclerosis; NHP, nonhuman primate; NP, neuroprogenitor; PD, Parkinson's disease; SC, stem cell; UCB, umbilical cord blood

Table 1 Possible applications of stem cells (SCs).
The different applications depend on the type of cell and the method used.

Cell grafting methods	Cell replacement Circuit reconstruction Neurotransmitter delivery Trophic support Vehicles of therapies via ex vivo gene transfer
Recruiting endogenous SCs	Cell replacement Circuit reconstruction
In vitro studies	Disease modeling Drug screening (toxicology, personalized medicine)

an SC-based therapy ready for clinical translation? The answer should take into account interrelated evaluations in vitro, in normal animals, and in animal models of disease, in each case considering the safety and/or antidisease properties of the proposed SC therapy. The US Food and Drug Administration (FDA), which regulates the development of SC-based therapies intended for transplantation in humans (Gould et al. 1999; Halme and Kessler 2006), requires the following:

- The donor cells are free of infectious or genetic disease.
- The processing of the cells does not damage or contaminate them.
- Only the intended cells (purity) with specific potency (efficacy) are present.
- The cells are safe and effective in vivo.

Although in vitro analysis provides evidence of pretransplantation safety, in vivo studies are necessary to demonstrate proof of concept in animal models of disease and predict potential problems with clinical application. Different neurological disorders require different types of cells with specific characteristics. The paradox in SC technology is that many of the properties that make it attractive for therapeutic purposes can affect the safety of the treatment. For example, the capacity of stem cells to multiply facilitates not only the generation of cell lines but also the possible development of tumors in the receiving host. Some SCs have shown migratory capacity, mainly toward an injured site (e.g., Aboody et al. 2000), indicating that preclinical evaluation should include on- and off-target distribution of the cells in case of side effects.

Overall, an efficacious SC treatment for neurological disorders needs SC lines that

- can differentiate into a desired phenotype (e.g., dopamine [DA[1]] neuron, gamma-aminobutyric acid [GABA] neuron, oligodendrocyte),
- are stable (no dedifferentiation or generation of tumors),
- have low immunogenicity (for grafting), and
- are susceptible to transfection (for vector-directed differentiation or ex vivo gene therapy).

One challenge of SC therapy development is the need to test SCs of human origin in rats and monkeys in accordance with the FDA requirement that a product proposed for use in humans first be tested in appropriate animal models. These animal models rely on immunosuppression to decrease immune response to xenografts, but significant immune reaction is still present.

Demonstration of efficacy of grafted SCs in animal models is usually defined as proof that the selected SCs reverse signs and, in the case of protective strategies, slow or stop cell death. This goal is complicated by the selection of the appropriate model, experimental design, and outcome measures to assess the therapy. The FDA has been working with the research community to generate guidelines for efficacy studies in animal models (www.fda.gov/cder/guidance), including recommendations regarding endpoints, timing of intervention, route of administration, and dosing regime.

Ideally, the same agent that induces the disease should be used to generate the model, but the etiology of many neurological disorders (e.g., PD, MS) has not yet been identified. The best alternative is to mimic the disease condition by administering an agent that recapitulates the ongoing degeneration and symptoms; rigorous experimental design can minimize the limitations of this approach (Kimmelman et al. 2009). Investigators are ultimately responsible for identifying the appropriate model and outcome measures that will provide meaningful data. As with other treatments, justification of the clinical translation of SC-based therapies requires the demonstration of efficacy and safety through statistically powered studies in several models and different species, replication of the results by independent research teams, and clear evidence that the benefits of the therapy outweigh its risks and cost compared to other treatments either currently available or in the pipeline.

Types of Stem Cells Proposed for Cell-Based Therapies of Neurological Diseases

The production of SC lines, thanks in part to the identification of new cell sources, has stimulated the development of novel experimental protocols. The types of SCs proposed for cell-based therapies of neurological diseases include neuroprogenitor, mesenchymal-derived, umbilical cord blood–derived, hematopoietic, adipose-derived stromal, endometrial regenerative, embryonic, embryonic-like, and induced pluripotent stem cells.

Neuroprogenitor Cells

Neuroprogenitor (NP[1]) cells, in the germinal layers of the brain, are generally suitable for regenerative medicine as they have the capacity both to multiply and to differentiate into a specific cell type, although these properties are limited compared to strictly defined SCs. They are multipotent and capable of differentiation into a variety of phenotypes in vitro (Sanchez-Pernaute et al. 2001).[2] Astrocytes in the

[2]However, most NP cell lines are of human fetal origin (hNP), which has raised ethical concerns and limited their use in research.

subventricular zone of the adult human brain appear to have the potential to generate NP cells (Sanai et al. 2004), indicating the possibility of brain self-repair through the recruitment of endogenous NP cells. Neurogenesis has also been observed in the brain of the adult rodent (Gage et al. 1995), nonhuman primate (Gould et al. 1999), and human (Eriksson et al. 1998). But the properties of NP cells differ between cell lines and according to the species into which they are transplanted, possibly explaining why migration does not always occur (e.g., Emborg et al. 2008).

Mesenchymal-Derived SCs

Mesenchymal (or marrow stromal) cells have been isolated from the nonhematopoietic fraction of bone marrow as well as other adult mesoderm tissue (muscle, joint synovial fluid) and infant dental pulp (Gregory 2008). Under the appropriate conditions, these multipotent cells proliferate extensively in vitro and differentiate into a variety of mesenchymal tissues, such as bone, adipose, and cartilage. Bone marrow–derived mesenchymal cells can give rise to neurons and glial-like cells in vitro by chemical or growth factor induction (Bossolasco et al. 2005; Levy et al. 2008; Woodbury et al. 2000). After implantation into the lateral ventricle in neonatal mice these cells have also differentiated into mature astrocytes (Kopen et al. 1999). Because mesenchymal cells can be obtained from an autologous source, rapidly expand in culture, have self-renewal properties, and have the potential for neural plasticity, they are appealing candidates for application in human neurodegenerative diseases (Westwood and Clements 2008).

Umbilical Cord Blood–Derived SCs

Umbilical cord blood (UCB[1])–derived cells may be an alternative cell source due to their ease of collection, greater availability, and relatively low immunogenic properties, which reduce the likelihood of rejection and may allow intravenous (IV[1]) delivery (Chen et al. 2001b). UCB-derived SCs, which can differentiate into hematopoietic and nonhematopoietic cells, are composed of immature lymphocytes and monocytes with approximately 1% $CD34^+$ cells, a marker for hematopoietic and endothelial progenitor cells (Newcomb et al. 2007). The immaturity of the cells enhances the possibility of their transdifferentiation to other cell types (Fallahi-Sichani et al. 2007; Stamm and Ma 2008) and their use in allogeneic or autologous transplantation. In addition, UCB cells yield mesenchymal cells, which have shown extensive proliferation in vitro but have a restricted lineage as they do not differentiate into adipocytes (Stamm and Ma 2008). Endothelial progenitor cells, which secrete angiogenic factors and promote neurogenesis (Taguchi et al. 2004), have also been isolated from UCB-derived $CD34^+$ cells and used in vitro to support embryonic stem (ES[1]) cell growth (Zhan et al. 2008). UCB cells are proposed for the treatment of neurological disorders because they can differentiate into glia and neural-like cells (Sanchez-Ramos et al. 2001).

Hematopoietic SCs

Hematopoietic SCs are present in circulating peripheral blood and bone marrow. Their potential to differentiate into other phenotypes has not yet been proven, but research indicates that autologous transplantation of these SCs may modulate immune responses in advanced neurodegenerative disorders with an autoimmune component (Burt et al. 2005; Mancardi and Saccardi 2008).

Adipose-Derived Stromal Cell (ADSC)–Derived SCs

ADSCs are an alternative source for mesenchymal cells and can differentiate into adipocytes, bone, and muscle. These multipotent cells are easily cultured and expanded for use in autologous transplantation. However, there is not a lot of research demonstrating their ability to differentiate into nonmesodermal tissue lineages such as neurons (Schaffler and Buchler 2007). Furthermore, they have not yet shown therapeutic benefit in animal models of neurodegeneration (McCoy et al. 2008).

Endometrial Regenerative Cells

Endometrial regenerative cells (ERCs[1]) are mesenchymal-like cells that have the ability to differentiate into various mesodermal, ectodermal, and endodermal tissues (Meng et al. 2007). They do not express the mesenchymal marker STRO-1 (which is characteristic of endometrial cells), but instead express nonhematopoietic phenotypes and an ES cell marker Oct-4. Cell expansion has demonstrated a lack of tumorogenicity and no karyotypic or functional abnormalities. Initial studies reveal that ERCs provide some immunomodulatory properties, which are imperative for allogeneic administration (Murphy et al. 2008). ERCs are an attractive new alternative source of stem cells as they are noninvasively collected from menstrual blood, demonstrate an abundant proliferative capacity, and have shown therapeutic effect in models of limb ischemia and infarcts (Hida et al. 2008; Meng et al. 2007; Murphy et al. 2008).

Embryonic Stem Cells

ES cells are obtained from blastocysts and have the capacity to differentiate to any cell type in the body (pluripotency). Researchers isolated the first ES cells in 1995 from rhesus monkeys (*Macaca mulatta*; Thomson et al. 1995), followed a few years later by marmoset (*Callithrix jacchus*; Thomson and Marshall 1998) and human cells (Thomson et al. 1998). The development of human ES (hES) cell lines represents an unlimited source for studies in developmental biology, drug

discovery, transplantation, and regenerative medicine.[3] But before their potential can be realized, ES cells require much more evaluation and assessment to overcome their inherent hurdles to application—lack of genetic stability, risk of teratoma formation, and lack of long-term survival.

ES-like Cells Derived by Parthenogenesis, Somatic, and Altered Somatic Cell Nuclear Transfer

Because of the limitations on research with ES cells, investigators looked for alternate means of creating cell lines with similar properties, using parthenogenesis, somatic cell nuclear transfer, and altered somatic cell nuclear transfer to bypass the ethical dilemma of destroying a human embryo to obtain hES cell lines (Kastenberg and Odorico 2008).

Parthenogenesis is an asexual form of reproduction through which an embryo develops without fertilization by a male; the absence of paternal DNA means mammalian eggs cannot develop fully but can provide blastocysts to generate ES cell lines for differentiation to a desired phenotype (e.g., Perrier et al. 2004). This method enables the generation of an immunological match to a female donor. Although at least one study has reported the achievement of somatic cell nuclear transfer (also called therapeutic cloning) in mammalian species (Wilmut et al. 1997), this method has a very low success rate and is still associated with the ethical issue of being able to produce an implantable embryo capable of fully developing. Altered nuclear transfer has evolved as a more acceptable alternative to cloning, as the cells are genetically engineered to generate defective trophoblasts and thus inhibit embryo implantation (Hurlbut 2005).

Induced Pluripotent Stem Cells

Induced pluripotent stem (iPS[1]) cells are generated from skin fibroblasts through the activation of a combination of genes that are capable of dedifferentiating the fibroblasts into a pluripotent cell (Takahashi et al. 2007; Yu et al. 2007). This technology bypasses ethical concerns associated with the destruction of embryos, cloning technologies, and the availability of oocytes. Investigators have successfully generated iPS cells from rodents (Okita et al. 2008; Takahashi and Yamanaka 2006), rhesus macaques (Liu et al. 2008), and humans (Takahashi et al. 2007; Yu et al. 2007), and they have also obtained iPS cells from the fibroblasts of patients with amyotrophic lateral sclerosis (Dimos et al. 2008), spinal muscular atrophy (Ebert et al. 2009), and PD (Soldner et al. 2009). iPS cells from sick donors may facilitate personalized medicine (e.g., drug screening) as well as in vitro modeling of disease. Furthermore, iPS cell–derived transplants allow perfect immunological matching of donor and host (Wernig et al. 2008), although the clinical feasibility of such transplants requires resolution of the major hurdles associated with other grafts—survival, long-term differentiation, and genetic stability.

Parkinson's Disease

Parkinson's disease is a progressive neurodegenerative disorder characterized by resting tremor, hypokinesia (decreased movement), bradykinesia (slowing of voluntary movements), altered gait, muscular rigidity, postural instability, and flat facial expression. The typical pathological features of PD are dopaminergic neuronal loss in the substantia nigra pars compacta (SNpc) and the presence of intracytoplasmic inclusions mainly formed by alpha-synuclein and ubiquitin (Lewy bodies). The loss of dopaminergic neurons in the SNpc results in decreased levels of DA in the striatum (which comprises the caudate nucleus and putamen), the primary area of projection of the SNpc DA neurons and part of the network that modulates motor function (Kumar et al. 2005).

PD affects 1-2% of the population over 55 years of age, and the number of 30- to 40-year-old patients is rising. In the United States alone 1.7 million people have the disease and approximately 70,000 new cases are diagnosed each year (www.apdawest.org). Only 1-5% of PD cases are familial, linked to genetic mutations (e.g., A53T alpha-synuclein); most cases are sporadic and their cause is unknown; possible risk factors include old age, exposure to environmental toxins, and head injury (Korell and Tanner 2005).

As the movement disorder typically associated with PD is related to decreased DA in the nigrostriatal system, most therapeutic approaches aim to restore function by replacing this neurotransmitter; thus administration of levodopa (L-dopa) and DA agonists is the mainstay of pharmaceutical treatment. But although DA replacement therapy attenuates the motor syndrome, it does not prevent the ongoing neurodegeneration; as the disease progresses, the efficacy of the treatment decreases and new symptoms appear, many of which do not respond to DA replacement (Braak et al. 2003; Langston 2006). In addition, long-term use of L-dopa has demonstrated serious side effects, such as abnormal movements (dyskinesias) and motor fluctuations (Bezard et al. 2001).

Deep brain stimulation (DBS) of the globus pallidus interna (Gpi) or subthalamic nucleus is an alternative surgical treatment that restores functional balance by disrupting the motor neural network. While DBS has provided significant relief from PD symptoms for numerous patients, there are reports of surgical and nonsurgical complications associated with its application (Videnovic and Metman 2008).

[3]However, because of controversy about their origin, from 2001 to 2009 human ES cell research supported by federal funds was limited to the original cell lines authorized by the US government. On March 9, 2009, President Barack Obama signed the "Stem Cell Executive Order and Scientific Integrity Presidential Memorandum" that lifted the 8-year ban in an effort to promote and expand the possibilities of this field (http://www.whitehouse.gov/the_press_office/Remarks-of-the-President-As-Prepared-for-Delivery-Signing-of-Stem-Cell-Executive-Order-and-Scientific-Integrity-Presidential-Memorandum/).

Preclinical Models of PD

Because the cause of PD is unknown, risk factors such as old age, genetic mutations, and exposure to environmental toxins are the basis of models of DA nigrostriatal degeneration (Emborg 2004). There are no comprehensive models of PD that allow for evaluation of non-DA symptoms (such as dysautonomias and depression), but there are multiple animal models of PD that are effective for studying different aspects of the disease. Age-related and genetic models of PD are especially useful in efforts to understand mechanisms of dopaminergic cell loss, but the most commonly used models for assessment of SC-based therapies depend on administration of the neurotoxins 6-hydroxydopamine (6-OHDA[1]) or 1-methy-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP[1]).

6-OHDA

The advantages of the 6-OHDA PD model compared to other new models are its low cost, relatively low complexity of implementation, and, most importantly, its proven reproducibility and versatility. 6-OHDA has high affinity to the DA transporter, which carries the toxin into the cells, where it induces nigral cell death by intense oxidative stress mainly due to autooxidation. The toxin, mainly administered to rats (usually Sprague-Dawley), cannot cross the blood brain barrier and requires direct injection into the brain (the medial forebrain bundle, substantia nigra, or striatum) to induce its deleterious effects (Blandini et al. 2008); the intensity of the syndrome depends on the dose, location, and number of injections (the administration of neuroprotective strategies before or during dosing prevents toxin-induced cell death). 6-OHDA-treated rats are responsive to DA replacement therapy whereas after chronic administration of L-dopa they develop dyskinesias (Cenci et al. 2007). A delay in SC replacement therapy until several weeks after 6-OHDA injection ensures the depth and stability of the lesion.

MPTP

MPTP is the most used neurotoxin to model PD in C57/BL6 mice and monkeys. Unlike 6-OHDA, MPTP can cross the blood brain barrier. In the brain, the enzyme monoamine oxidase B (MAO B) transforms MPTP to its toxic metabolite MPP^+. Both C57/BL6 and monkeys, like humans, have MAO B; rats do not, making them resistant to MPTP. MPP^+ is a mitochondrial complex I inhibitor and induces nigral cell death by affecting cell energy metabolism and increasing oxidative stress.

MPTP is usually given to mice intraperitoneally, in an acute (several injections in 1 day) or subchronic (daily injections for 5 days) regime (Przedborski et al. 2001). In monkeys, MPTP is administered as a single dose via the intracarotid artery or as multiple IV or intramuscular (IM) doses over several days or months (Emborg 2007).

Compared to rodents, the motor and motor planning abilities of monkeys are an advantage in the assessment of PD treatments and particularly in intracerebral transplantation studies. Monkeys have not only bigger brains, allowing an assessment of intracerebral volume distribution that more closely resembles that of humans, but also, most importantly, a caudate and putamen nucleus (the usual grafting targets), which are clearly separated by the internal capsule, affecting cell distribution and cell migration patterns. MPTP-treated NHPs develop a syndrome that closely resembles the sporadic form of PD in humans.

There are several approaches to injecting MPTP in monkeys: the intracarotid artery, systemic (intravenously or subcutaneously), or a combination of intracarotid artery plus systemic injections. The characteristics of the syndrome depend on the route of administration and dosing (Emborg 2007). Similar to humans, MPTP-treated monkeys respond to DA replacement therapy (Bezard et al. 2001). Interestingly, only monkeys with advanced bilateral MPTP-induced parkinsonism clearly develop L-dopa-induced dyskinesias (Emborg 2007). Spontaneous recovery after neurotoxin administration can be confused with therapy-induced positive improvements. To prevent misinterpretation of the results for the evaluation of cell replacement strategies, it is advisable to evaluate the monkeys for 2 to 3 months after the neurotoxin challenge and before grafting in order to identify recovering animals, remove animals from the study, or repeat neurotoxin administration. For neuroprotective studies, in which the animals receive treatment before or soon after toxin administration, the best strategy is appropriate statistical powering to account for subjects with spontaneous recovery.

Studies Assessing SC Treatments for PD

PD researchers have led the way in grafting procedures for neural repair, testing a variety of cell sources, including mesencephalic fetal tissue, adrenal medulla, retinal pigmented cells, and carotid glomus (Fitzpatrick et al. 2009). The first scientists to test the concept of cell replacement as a therapy for PD were Bjorklund and Stenevi (1979), who proposed the use of striatal grafts of DA-producing cells to replace the loss of DA nigral neurons. They transplanted mesencephalic fetal tissue into 6-OHDA-treated rats and showed that the grafted cells were able to survive and attenuate neurotoxin-induced behaviors. Mesencephalic fetal tissue grafts were first reported in parkinsonian MPTP-treated monkeys in the mid-1980s (Bakay et al. 1985; Redmond et al. 1986). Although preclinical and phase I clinical transplantation studies have reported promising results, double-blind placebo-control clinical trials have revealed poor effects and generated intense controversy, especially as follow-up studies uncovered complications, including the effect of disease progression on transplanted cells (Fitzpatrick et al. 2009). Although SCs have emerged as alternative cell sources to replace DA neurons or provide trophic support, investigators and the public alike share a cautious optimism regarding their clinical use.

NP Cells

Studies in PD rodent models have demonstrated that NP cells of rodent (O'Keeffe et al. 2008; Parish et al. 2008; Studer et al. 1998) or human (Sanchez-Pernaute et al. 2001) origin can differentiate into a DA phenotype in vitro and survive grafting. 6-OHDA-lesioned rats injected with mesencephalic NP cells expressed higher levels of differentiated tyrosine hydroxylase (TH; a dopaminergic cell marker)–positive neurons in the DA-depleted striatum than in intact striatum (Nishino et al. 2000), suggesting that the condition of the host may direct the phenotype of the grafted cells. Human neuroprogenitor (hNP[1]) transplants (which usually entail the use of an immunosuppressive treatment) in rodent models demonstrated graft survival and cell migration to the injured areas (Dziewczapolski et al. 2003; Svendsen et al. 1997), and intrastriatal transplants in 6-OHDA-lesioned rats survived for up to 20 weeks and expressed human neurons and glia throughout the striatum (Svendsen et al. 1997). Studies have shown behavioral improvements, cell survival, and migration in MPTP-treated monkeys, as well as the ability of transplanted hNP cell progeny to migrate and differentiate to glial and DA phenotypes in vivo (Bjugstad et al. 2005, 2008; Redmond et al. 2007). Properties of hNP cells seem to differ between cell lines and the species into which they are transplanted, so it is essential to study their effects in models that closely resemble human disease.

The confirmation of neurogenesis in the adult primate brain combined with the discovery of glial cell line–derived neurotrophic factor (GDNF), a potent dopaminergic trophic factor for DA neurons, established a possible treatment that assists brain self-repair rather than the replacement of dying cells (Gash et al. 1996; Lin et al. 1993). The option of recruiting hNP cells already present in the human brain is a possibility, but its utility has not been proven (Rakic 2004). An interesting alternative application of this research is the use of adult cadaveric sources to obtain NP cells; one study using this method has reported the successful derivation of DA neurons from NP cells that originated in the subventricular zone of adult rats (Shim et al. 2007).

An alternative role for hNP cells is that of therapeutic vehicle, based on their ability to be transfected to deliver a molecule of interest, such as GDNF or insulin growth factor (IGF). This concept has been successfully tested in immunosuppressed 6-OHDA-treated rats (Behrstock et al. 2006; Ebert et al. 2008) and immunosuppressed MPTP-treated monkeys (Emborg et al. 2008).

Mesenchymal-, UCB-, and ADSC-Derived SCs

Neural-induced human bone marrow–derived mesenchymal stromal cells have been transplanted into the striatum of immunosuppressed hemiparkinsonian rats. The cells induced significant behavioral improvement when compared to naïve bone marrow cells, and dopaminergic precursors were present in the grafted sites (Levy et al. 2008). ADAS-derived SCs have been differentiated in vitro toward a neuronal phenotype for transplantation. The differentiated cells compared to naïve grafts of ADAS cells had DA neuroprotective effects in hemiparkinsonian rats (McCoy et al. 2008). Further investigation and preclinical assessment in an NHP model are necessary to demonstrate the safety and efficacy of these cell types for PD treatment.

ES Cells

While some studies have suggested that ES cells can differentiate into DA neurons when transplanted into the denervated striatum (Bjorklund et al. 2002), most have used ES-derived DA neurons of rodent (Kawasaki et al. 2000; Kim et al. 2002; Rodriguez et al. 2007) and human (Cho et al. 2008; Roy et al. 2006) origin. These studies found that the cells survived after intrastriatal grafting into immunosuppressed PD rodent models. Human DA neurons can be generated in coculture with PA6 cells to express the appropriate markers and display the functional properties of mature DA neurons (Buytaert-Hoefen et al. 2004; Zeng et al. 2004). Transplantation of differentiated hES cells into immunosuppressed MPTP-treated monkeys resulted in limited cell survival, likely due to immune response in the host brain to the xenograft (Emborg, unpublished data). Allographic transplantation of monkey ES cell–derived DA neurons into the same species produced a better outcome, though similarly associated with limited cell survival (Takagi et al. 2005).

Advances in ES cell technology (e.g., the development of feeder-independent cultures and the addition of trophic support) are overcoming the obstacles to successful grafts. By applying transcription factors and then using a sorting process, investigators can select midbrain-specific DA neurons. As these neurons are the primary cells lost in PD, there are multiple benefits to their specific selection from ES cell culture, including decreased risk of tumor or teratoma formation and the possibility that grafts containing large portions of these neurons reduce PD symptoms. The transcription factor Pitx3 has been used successfully to select for midbrain DA neurons (Hedlund et al. 2008). In conjunction with fluorescence-activated cell sorting (FACS), a differentiating ES cell culture is an effective way to achieve specificity. Researchers have also investigated the role of transcription factors Lmx1a and Msx1 in the generation of midbrain-specific DA neurons from ES cells in culture (Andersson et al. 2006; Cai et al. 2009).

ES-like Cells Derived by Parthenogenesis and Somatic Cell Nuclear Transfer

Parthenogenesis-derived ES cell lines can be differentiated into desired phenotypes, as has been demonstrated with cynomolgus monkey (*Macaca fascicularis*) cells. Using the cyno-1 cell line, investigators differentiated the ES cells into dopaminergic neurons and successfully transplanted them in parkinsonian rats (Sanchez-Pernaute et al. 2005).

Despite the low success rate of somatic cell nuclear transfer, it has been used to generate ES cell–derived DA neurons that were then grafted into parkinsonian mice with the advantage of a perfect immunological match to the host (Tabar et al. 2008).

iPS Cells

In two separate studies researchers have recently isolated iPS cells from a PD patient (using viral reprogramming factor–free methods) and differentiated them into DA neurons (Soldner et al. 2009), and successfully grafted iPS-derived mouse DA neurons into PD rats (Wernig et al. 2008). These results suggest that iPS cells can be used for cell repair in PD. But despite the enormous potential of iPS cells, there remain hurdles inherent to cell grafts (long-term survival, persistent differentiation, and genetic stability). By using iPS cell–derived transplants, investigators will be able to assess the impact of immunological matching on cell survival, function, circuit integration, and synaptogenesis. Considerations such as genetic background (identification of patients with familial PD) may lead to the development of "healthy" iPS cell lines with major histocompatibility complex characteristics to match individual patient needs. The true advantage of iPS cell technologies, though, may be the ability to assess the behavior of cells in culture. The production of neurons and glia from PD patients for culture could promote the understanding of PD mechanisms of cell death and of the relative roles of environmental factors and genetics in disease occurrence and progression. It would also enable the screening of potential drugs and the tailoring of patient-specific therapies to treat PD (Chamberlain et al. 2008).

Stroke

Stroke, also called "brain attack" (as an equivalent to "heart attack"), is classically defined as "rapidly developed signs of focal (or global) disturbance of cerebral function lasting longer than 24 hours (unless interrupted by death), with no apparent nonvascular cause" (Hatano 1976). It is classified as ischemic or hemorrhagic depending on its nature.

Ischemic stroke results from a blocked artery that decreases or stops blood supply to the brain. The cause of the blockage may be thrombosis (a blood clot in the cerebral vasculature) or an embolus (a circulating blood clot that lands in a brain vessel). Hemorrhagic stroke results from the rupture of a blood vessel into the intracranial or subarachnoid space. Either type of stroke can lead to irreversible neurological damage from the interruption of blood supply to the brain. Deficits that resolve within 24 hours are called transient ischemic attacks (TIAs).[4] Both types of stroke present a central affected area that may develop extensive cell loss and necrosis, and, in the surrounding area, a penumbra that experiences less extreme cerebral blood flow loss and that has the most potential for regenerative treatments. In the hypoperfused area, the depletion of oxygen and glucose causes the production of free radicals, dysfunction of calcium channels, inflammatory changes, the release of glutamate, and apoptosis (Donnan et al. 2008).

Stroke is the second leading cause of death in the United States—every 40 seconds someone experiences a stroke—and a primary cause of long-term disability (Lloyd-Jones et al. 2009). Hypertension, hyperlipidemia, obesity, diabetes, smoking, and a previous stroke are all risk factors (Lloyd-Jones et al. 2009). Awareness and education are the first steps to reducing the incidence, as a recent survey of 163 stroke patients made clear: 43% of them could not cite a single risk factor (Lloyd-Jones et al. 2009).

The only FDA-approved acute stroke therapy is the IV delivery of a thrombolytic agent, tissue plasminogen activator; however, it is safe and effective only if administered to ischemic stroke cases within 3 hours of the onset of symptoms. Antiplatelet drugs, fibrinogen-depleting agents, and antithrombolitic agents are other pharmacological approaches to manage acute ischemia and maintain blood circulation (Hacke et al. 2008; Higgins and Lees 2009). Hemorrhagic stroke may require surgical intervention to stop bleeding.

Clinical management requires computed tomography or magnetic resonance imaging (MRI) to differentiate between ischemic and hemorrhagic cases in order to provide safe treatment. Several neuroprotective therapies (e.g., progesterone; Sayeed et al. 2007) to prevent neuronal cell death are under evaluation but no medications are approved for clinical use. Post-stroke rehabilitation through intense physical, occupational, and speech and language therapies is important to stimulate brain plasticity and to overcome disabilities associated with stroke damage. Secondary prevention strategies include the administration of aspirin or other anticoagulant medications (Donnan et al. 2008).

Preclinical Models of Stroke

Stroke modeling is based on interruption of the brain's blood supply, usually by occlusion of the middle cerebral artery (MCA[1]). Rats are the most commonly used species for stroke modeling, while transgenic mice are usually preferred to analyze stroke physiopathology and risk factors. Thromboembolic models entail the introduction of homologous blood clot fragments (Kaneko et al. 1985; Kudo et al. 1982; Zhang et al. 1997) or human blood clots or clot fragments (Papadopoulos et al. 1987) into the carotid arteries to occlude the MCA. Nonclot embolic models inject artificial materials (most commonly microspheres) into the internal carotid artery to induce ischemia (Fukuchi et al. 1999; Siegel et al 1972; Takeo et al. 1992). Occlusion/reperfusion models contribute to knowledge of reperfusion injury in areas of the ischemic core and the ischemic penumbra (Durukan and

[4]Information about TIAs is available at the National Institutes of Health National Institute of Neurological Disorders and Stroke (NINDS) Transient Ischemic Attack Information Page (www.ninds.nih.gov/disorders/tia/tia.htm).

Tatlisumak 2007). A popular technique in rodent models is the permanent or transient MCA occlusion induced by the insertion of a suture through the internal carotid artery and its advancement into the lumen of the MCA (Belayev et al. 1996; Koizumi et al. 1986; Longa et al. 1989). Direct occlusion of the MCA requires a surgical procedure to place microclips or snare ligatures (Buchan et al. 1992; Longa et al. 1989; Robinson et al. 1975; Shigeno et al. 1985). With all methods, the duration of the occlusion determines the extent of injury. Permanent models restrict the reperfusion in the ischemic territory by electrocauterization, coagulation, ligation, clamping of the MCA, or the placement of a permanent intraluminal suture (Buchan et al. 1992; Burns et al. 2009; Shigeno et al. 1985; Tamura et al. 1981).

The use of NHP models has the benefit of closer approximation to the functional and anatomical effects in humans of stroke and potential treatments, thanks to NHP similarities to humans in motor and cognitive abilities, cerebrovascular anatomy, and the ratio of white and grey matter (Traystman 2003; West et al. 2009). The most commonly used NHP ischemic models rely on occlusion of the M1 segment of the MCA, using retroorbital (Yonas et al. 1990), intracranial (Crowell et al. 1970, 1981), and transorbital (Branston et al. 1974; Hudgins and Garcia 1970; Spetzler et al. 1980) approaches together with clips (Crowell et al. 1970; Frazee et al. 1998; Hudgins and Garcia 1970; Symon 1975), balloon occluder (Spetzler et al. 1980), ligation (Crowell et al. 1981), or coagulation (Yonas et al. 1990). Blood clot embolization approaches have also been used to avoid the damage from surgical approaches that require craniotomies (Bremer et al. 1975; Watanabe et al. 1977). A global ischemic monkey model involved clamping both the innominate and left subclavian arteries for 18 to 20 minutes (Tsukada et al. 2001; Yamashima et al. 1996).

Prolonged observations after stroke induction are needed for proper assessment of transplantation and neuroprotective strategies. But due to the critical care needed after a stroke, most studies limit NHP survival to a few days postischemic induction. One study has reported a chronic stroke NHP model in which cynomolgus monkeys were evaluated for 8 months after permanent occlusion, using a battery of motor and cognitive tests, MRI, and, finally, extensive postmortem morphology (Roitberg et al. 2003).

Studies Assessing SC Treatments for Stroke

Cell-based therapies are proposed as a method to replace neurons lost due to stroke and to provide localized trophic support. Most preclinical studies testing cell replacement strategies for stroke have used rodent models. As with PD, early transplantation experiments for stroke used allogeneic fetal neocortical grafts (Elsayed et al. 1996; Grabowski et al. 1992, 1993; Jansen et al. 1997) and fetal porcine striatal cells (Savitz et al. 2002; Vora et al. 2006) implanted in ischemic stroke rat models. Clinical trials using fetal porcine striatal cells have produced variable results (Savitz et al. 2005). To decrease the number of failed clinical translations, the stroke community has designed Stroke Therapy Academic Industry Roundtable (STAIR) guidelines addressing the use of animal models, study design, and cross-species physiological differences in order to standardize the preclinical steps of stroke therapeutics (Gawrylewski 2007). Investigators have also established the Preclinical STEPS (Stem Cell Therapeutics as an Emerging Paradigm for Stroke) Consortium to focus on the reproducibility, reliability, safety, and efficacy of cell therapy and, with NINDS support, are developing Preclinical Stroke Consortia (Borlongan 2009).

NP Cells

The committed neuronal phenotype of NP cells and the ability to populate CNS regions suggest the potential for cell replacement after ischemic injury. Human neurospheres derived from fetal CNS were transplanted into Sprague-Dawley rats to reveal cell survival. After cautery of the distal MCA and occlusion of the common carotid arteries for 1 hour, cells were transplanted into nonischemic areas and migrated toward the ischemic area (Kelly et al. 2004). The only reported hNP cell transplantation in an intracerebral hemorrhage model (also in Sprague-Dawley rats) showed improvement in behavior testing 5 weeks after delivery without immunosuppressive therapy. hNP cells (transfected to express the marker gene LacZ) entered the brain and differentiated into astrocytes and neurons in areas of the lesion, but the presence of the hNP cells did not significantly reduce striatal atrophy between the treated and control groups (Jeong et al. 2003).

Although investigators have used cells of NHP origin in rats, there have been few preclinical studies in NHP models of stroke, probably because of the cost of the animals and the intensive, specialized care required for long-term maintenance of a stroke monkey. The only report available refers to the transplant of hNP cells in three cynomolgus monkeys with a permanent MCA stroke (Roitberg et al. 2006). The monkeys were followed for 45, 75, or 105 days and treated with cyclosporine twice daily (10 mg/kg). Postmortem analysis confirmed cell survival in grafting areas adjacent to the infarct. Colocalization of BrdU (used to label the hNP cells) and nestin or BIII tubulin denoted neural cell development. Migration of cells was not as widespread as in rodents (Qu et al. 2001).

Human neuroteratocarcinoma neurons (hNT or NT2-N), derived from a human embryonal teratocarcinoma cell line treated with retinoic acid, have been used in various preclinical postischemic models (Kleppner et al. 1995; Pleasure et al. 1992; Saporta et al. 1999) and showed enhanced survival compared to fetal grafts in the brain (Saporta et al. 1999). Considered to function as neuroprogenitor cells, hNT cells have survived and acquired the phenotype of fully mature in vivo neurons 1 year after transplantation in a nude mouse brain without conforming to a neoplastic state (Klepper et al. 1995) and survived 3 months after transplantation in rats with MCA stroke (Saporta et al. 1999). Behavioral deficits

produced by ischemia reversed as early as 1 month after transplantation and this correction persisted through the study (Borlongan et al. 1998; Saporta et al. 1999).

Based on the information from these studies an open-label phase I clinical trial was performed to evaluate safety in 12 patients with a basal ganglia infarct that occurred 6 months to 6 years before the transplantation. The patients received transplants of LBS neurons (Layton BioScience, Inc.) produced from postmitotic hNT cells and received immunosuppressant treatment 1 week before surgery and for 8 weeks after (Kondziolka et al. 2000). The investigators reported positive safety outcomes. In vivo imaging, using fluorodeoxyglucose positron emission tomography, showed an increase in radioligand uptake 6 months after the transplant, probably due to an increase in viable neuronal cells. The phase II randomized, blinded, open-label trial further verified the safety and feasibility of LBS neuron transplantation, but did not demonstrate statistically significant improvement (Kondziolka et al. 2005; Vora et al. 2006).

UCB-Derived SCs

Human UCB (hUCB)–derived SCs have been administered as a treatment for rats with a permanent MCA occlusion (Chen et al. 2001b; Willing et al. 2003), but reports have described differing rates of survival and expression of neuronal phenotypes of hUCB cells after grafting. One study in Wistar rats found hUCB survival after IV delivery either at 24 hours or 7 days after a transient MCA occlusion; cells were observed mostly surrounding the ischemic boundary zone, with some cells expressing specific neuronal markers (Chen et al. 2001b). Other reports describe low to undetectable numbers of intracerebral hUCB cells after IV injections in rodent models of stroke (Borlongan et al. 2004; Xiao et al. 2005). To assess the role of blood brain barrier penetration, investigators compared the effects of delivering hUCB cells alone versus their coadministration with the blood brain barrier permeabilizer mannitol in Sprague-Dawley rats subjected to MCA occlusion for 60 minutes; they found that the mannitol coadministration reduced infarct size, indicating that a permeable blood brain barrier is necessary for the mobilization of hUCB cells into the brain (Borlongan et al. 2004).

Even with mixed reports on cell survival, the use of hUCB cell therapy has yielded consistent reports of behavioral recovery (Borlongan et al. 2004; Chen et al. 2001b; Taguchi et al. 2004; Vendrame et al. 2004; Willing et al. 2003). Rats that underwent a permanent MCA occlusion showed significant improvement in various behavioral measures including the step test and spontaneous activity after a high-dose injection of 10^6 or more hUCB cells 24 hours after embolitic occlusion. High doses of 3 to 5×10^7 hUCB cells did not produce any further significant behavioral results but did significantly reduce infarct size (Vendrame et al. 2004). Another report suggested increased behavioral recovery 2 months after hUCB cells were transplanted intravenously compared to intrastriatally (Park et al. 2009).

Although functional recovery has been demonstrated with the transplant of hUCB cells, the mechanism involved is not yet defined. It is possible that cell protection results from the effect on the microenvironment and is not strictly due to cell replacement or integration. Trophic support may be a means of neuroprotection or recovery in ischemic brains (Chen et al. 2001b). It is not clear whether these effects are due to the direct release of growth factors by the injected UCB cells or by the host brain. Intravenously administered hUCB cells demonstrated elevated levels of trophic factors including GDNF, vascular endothelial growth factor (VEGF), and brain-derived neurotrophic factor (BDNF) in the ischemic brain (Borlongan et al. 2004; Taguchi et al. 2004). The delivery of hUCB cells along with antibodies against these neurotrophic factors did not lead to any detectable neuroprotection or behavioral recovery, suggesting that the trophic factors released by hUCB cells were partly responsible for the reduction in stroke damage (Borlongan et al. 2004). Further studies have demonstrated the effect of a favorable environment on angiogenesis and neurogenesis. The IV administration of human cord blood–derived $CD34^+$ cells to immunocompromised mice subjected to stroke 48 hours earlier promoted neovascularization, endogenous neuronal regeneration, and functional recovery (Taguchi et al. 2004).

Although UCB cells present certain advantages as a treatment for stroke, their clinical translation will require further research to improve survival rate, identify the mechanisms by which these cells seem to induce protection (Newman et al. 2005) and express neuronal markers, assess overall migration and crossing of the blood brain barrier, define the optimal time for transplantation, and determine effective and safe dosing parameters.

Mesenchymal-Derived SCs

The availability of mesenchymal cells, their potential for autologous intervention, and their ability to cross the blood brain barrier and migrate into the brain make them attractive for noninvasive stroke therapy. Bone marrow–derived mesenchymal cells have not only generated neurons in vitro and in vivo (Brazelton et al. 2000; Sanchez-Ramos et al. 2000) but also reduced ischemic injury and promoted functional improvement in animal stroke models (Chen et al. 2001a; Li et al. 2002).

Rats that underwent an interluminal vascular occlusion received IV injections of autologous mesenchymal cells 12 hours after infarct; improvement in functional outcomes (assessed by the Morris water maze and the treadmill test) and reduced infarction volume (determined by MRI) indicated the potential of mesenchymal cells for stroke recovery. However, a limited number of cells that reached the lesion demonstrated neuronal characteristics, suggesting that the reduction in infarct volume was not attributable to neurogenesis but rather to a neuroprotective effect mediated by the

release of growth factors and cytokines by the donor cells (Honma et al. 2006). Repeat studies have verified functional recovery from IV delivery of mesenchymal cells, possibly due to an increase in surrounding growth factors or the reduction of apoptotic mechanisms (Chen et al. 2001a; Li et al. 2002; Zhao et al. 2002). Functional recovery after intrastriatal delivery of mesenchymal cells in mice 4 days after MCA occlusion suggested the survival of the transplanted cells in the ischemic brain even though there was no significant change in infarct volume (Li et al. 2000). In a small clinical trial stroke patients received infusions of autologous mesenchymal cells within 7 days of the onset of symptoms; although no toxicity was observed, there was no significant improvement of neurological deficits (Bang et al. 2005).

ES Cells

Most stroke studies of the therapeutic potential of ES cells for transplantation predifferentiate the cells toward neurons or neural precursors. Investigators have tested ES-derived neuroprogenitors from mice (Buhnemann et al. 2006; Erdo et al. 2003; Takagi et al. 2005; Wei et al. 2005) and monkeys (Hayashi et al. 2006) as replacement therapies in animal models of stroke damage. Immature ES cells migrated extensively after contralateral transplantation in a focal ischemic rat model (Hoehn et al. 2002). In contrast, mouse ES-derived neural precursors demonstrated very low contralateral migration and their survival declined over time (Buhnemann et al. 2006). Other mouse predifferentiated ES cell grafts have associated survival and the presence of neuronal and glial markers with behavioral improvement 2 weeks after transplantation in Wistar rats (Wei et al. 2005). Cynomolgus monkey ES-derived NP cells transplanted into mice, 1 day after a striatal lesion induced by 30-minute intraluminal occlusion, formed a neuronal network and survived for 28 days. Furthermore, xenografts spread throughout the ischemic environment and occupied an area larger than in the striatum of sham control animals. However, this study did not prove to have an effect on the lesion size (Hayashi et al. 2006). Transplantation of ES-derived cells of various sources indicates electrophysiological neuronal maturation and synaptic connectivity in the host environment, yet an apparent connection to improved functional recovery must be still be made.

Multiple Sclerosis

Multiple sclerosis, an autoimmune disease that affects the central nervous system (CNS[1]), afflicts more than 2 million people worldwide, typically beginning in early adulthood and predominantly affecting females (Flachenecker and Stuke 2008).

No test can conclusively verify an MS diagnosis. Clinical diagnosis depends on the observation of neurological events or lesions overtime. Besides documentation of neurologic dysfunction, diagnostic tools include neuroimaging for lesions or plaques, analysis of the cerebrospinal fluid (CSF) for inflammation of the CNS, and visual evoked potential testing. The pathological hallmark of MS originates from the infiltration of autoreactive T cells across the blood brain barrier, resulting in a cascade of inflammatory reactions that develop demyelinating plaques in the brain and spinal cord, a loss of oligodendrocytes (myelin-generating cells), axonal damage, neurodegeneration, and astrocytic scarring (Courtney et al. 2009; Noseworthy et al. 2000). Although the pathogenic mechanisms of disease are reasonably understood the cause remains unknown; it seems likely that a combination of genetic susceptibility and environmental factors contribute to MS development.

MS forms have been characterized as relapsing or progressive subtypes. About 80% of patients experience relapsing-remitting MS, a partially reversible form that begins with sensory disturbances, diplopia (double vision), unilateral optic neuritis, Lhermitte's sign (trunk and limb sensations from bending the neck), problems with speech and swallowing, gait ataxia, and limb weakness (Noseworthy et al. 2000). This subtype experiences unpredictable attacks followed by periods of remission with no symptoms. Without therapeutic intervention most patients transition to a secondary progressive form of MS, an irreversible course of gradual neurological decline between attacks that causes an acceleration in disability (Courtney et al. 2009). Primary progressive MS occurs in 20% of affected patients as a steady clinical decline largely with myelopathic symptoms and no periods of remission (Noseworthy et al. 2000). Progressive relapsing MS, the rarest form of the disease, is characterized by progressive neurological decline with marked attacks that increase the severity of symptoms.

Corticosteroids, such as methylprednisolone, are a common therapeutic treatment for acute relapses to hasten clinical recovery. Other immunomodulatory agents, such as interferons or glatiramer acetate, tend to reduce both the frequency of clinical relapse and to some degree the development of lesions for relapsing-remitting or secondary progressive MS. No therapies have demonstrated any benefit for primary progressive MS (Noseworthy et al. 2000). Unfortunately, current therapies are only partially effective due to their inability to promote growth factors, signal mobilization of host CNS stem cells, and immunomodulation in CNS lesions (Karussis and Kassis 2008).

Preclinical Models of MS

Experimental autoimmune encephalomyelitis (EAE[1]) is an autoimmune inflammatory disease of the CNS that shares significant similarities—in particular, demyelination and chronic disability—with MS. Various EAE animal models have been used to research the etiology and therapeutic features of human MS, but none mimics the full pathology of the disease. The first EAE animal model was developed in macaque monkeys, but inbred laboratory animals such as

mice, rats, and guinea pigs are typically used to reliably produce susceptibility to EAE. The most common forms of EAE are active and adoptive transfer. In a number of species, including rats, mice, and monkeys, active EAE is induced from the direct antigen exposure of CNS tissue or myelin proteins such as myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG[1]), or myelin proteolipid protein. Adoptive transfer EAE models are generated by the IV delivery of myelin-specific T cells such as reactive $CD4^+$ Th1 lymphocytes (Mix et al. 2008).

The creation of variable courses of disease depends on the myelin proteins introduced and the animal species. Acute models experience spontaneous recovery and are most useful in studies of remission phases of the disease and induction mechanisms (Swanborg 1995). The acute EAE Lewis rat model is induced by immunization with MBP in complete Freund's adjuvant (CFA; a mycobacteria compound that stimulates cell-mediated immunity and is commonly administered with myelin proteins). In the Lewis rat transferred MBP-specific T cell lines develop an adoptive transfer EAE model that is limited by its monophasic properties and deficient CNS demyelination pathology (Gold et al. 2006). The development of mouse EAE models requires specific strains of mice—SJL/J, C57/BL6, Biozzi ABH, and PL/J—that are susceptible to reinduction of disease. Compared to rat models, mouse models feature demyelination leading to more extensive tissue and axonal damage characteristic of MS. However, the inconsistent incidence and the unpredictability of disease onset are limitations of murine models. Transgenic or knockout mice can also be used in MS studies of immune tolerance and disease pathogenesis (Gold et al. 2006; Swanborg 1995).

Compared to rodent models (which are usually inbred), monkey models offer larger and more complex anatomical structures, more defined MRI analysis of lesions, and greater resemblance to human immunological and genetic features. Macaques are partially susceptible to EAE because of a genetic link (*Mamu-DP1*01*) when EAE is induced with bovine MBP or human myelin ('t Hart et al. 2000). But because EAE in macaques presents clinical and pathological features that are more closely associated with postinfection encephalomyelitis than chronic MS, these models are not commonly used.

New World monkeys such as the common marmoset consistently develop clinical courses that resemble relapsing-remitting or primary progressive MS defined by widespread primary demyelination ('t Hart et al. 2000). Marmosets give birth to genetically nonidentical twins or triplets. The chimeric condition at birth creates a unique advantage as individual animals can tolerate the adoptive transfer of fraternal siblings' T or B cells without producing an alloresponse (Uccelli et al. 2003). A relapsing-remitting form of EAE was developed in the common marmoset after immunization with human white matter (Massacesi et al. 1995), MOG (Brok et al. 2000; Genain et al. 1995), or MBP. Some models have demonstrated hemorrhagic-necrotic lesions up to 5 weeks after immunizations that included CFA (Genain et al. 1996). EAE characteristics in marmoset models vary by immunological responses to the encephalitogenic protein injected and the dosing regime. The additional IV administration of *Bordetella pertussis* avoids resistance to actively induced EAE (Massacesi et al. 1995); however, other investigators have been able to develop an EAE marmoset model without the infusion of *Bordetella* particles (Brok et al. 2000). Monitoring of EAE in marmosets typically involves MRI examinations, clinical ratings, and measurement of CSF pleocytosis to gauge the severity of inflammation (Genain et al. 1996). Investigators are using these models to test new experimental therapies that transplant autologous green fluorescent protein (GFP)–labeled Schwann cells (Ben-Hur and Einstein 2006) and anti-inflammatory, tolerance-based, and costimulation-targeted therapies ('t Hart et al. 2000).

Studies Assessing SC Treatments for MS

The success of cell-based remyelinating therapies for multifocal and chronic diseases depends on an unlimited supply of cells and access to the various CNS lesion sites. Stem cells are a promising alternative to achieve remyelination, neuroregeneration, and restored nerve function. Myelin-deficient animal models have demonstrated the functional effect of transplanted ES cells in damaged CNS areas. These cell-based therapies have been evaluated in clinics, but early results only suggest the feasibility of managing MS; further randomized trials are necessary. Because not all patients experience a life-threatening form of the disease, it is always appropriate to weigh the risks of SC transplantation against its benefits.

NP Cells

Adult mouse NP cells intravenously or intracerebroventricularly delivered to MOG-induced EAE mice migrate to CNS lesions and differentiate into brain cells that undergo active remyelination. Researchers have observed a fivefold increase in oligodendrocyte progenitors in areas of demyelination, a notable decline in demyelination and axonal loss, and amelioration of clinical signs 10 to 15 days after neural SC delivery (Pluchino et al. 2003). Other reports have described reductions in both brain inflammatory processes and clinical deficiencies after the injection of neural precursor cells into the white matter of EAE rats (Einstein et al. 2003). Specifically, no astrocytes were present in areas of intraventricular delivery, suggesting that the transplant of NP cells mediates the reduction of T cell infiltration and markers of active inflammation (Einstein et al. 2003, 2006). The results of intraventricular transplantation of newborn rat NP cell spheres in an EAE rat model suggest an association between the migration of cells to inflamed white matter and signals of the inflammatory process of EAE, as the same cells transplanted in intact rats did not migrate to the targeted areas. Two weeks after the transplant of spheres, specific markers of

astroglial and oligodendroglial lineages were identified in the host white matter (Ben-Hur et al. 2003). Investigators have intravenously or intracerebrally injected hNP cells in immunosuppressed marmosets with EAE but the results have not been reported (Ben-Hur and Einstein 2006).

Hematopoietic SCs

Hematopoietic SCs can be collected from both bone marrow and peripheral blood sources. Immune reconstitution is possible with the transplantation of autologous hematopoietic SCs after intense immunosuppression to eradicate self-reactive immune cells. Since 1995 more than 400 patients have been treated with autologous hematopoietic SCs and their mortality rate has dropped from 5-6% in 1995-2000 to 1-2% in 2008 (Mancardi and Saccardi 2008).

Allogeneic bone marrow–derived hematopoietic SCs have been used to successfully treat EAE rats, only 5% of which experienced spontaneous relapses (van Gelder and van Bekkum 1996). A nonrandomized clinical study assessed the safety, tolerability, and clinical outcome of transplanted autologous nonmyeloablative hematopoietic SCs in relapsing-remitting MS patients, and found that the patients demonstrated no progression of disease and significant improvements in neurological disability after a mean of 37 months (Burt et al. 2009). In a phase I/II trial involving injections of either bone marrow– or peripheral blood–derived hematopoietic SCs in progressive MS patients (mean of 7 years after disease onset), the SC grafts provided some support in the management of MS, but high mortality rates associated with infection and toxicity complications compromised the overall results (Fassas et al. 2002). Nonetheless, long-lasting suppression of inflammation and decreased axonal injury (measured by MRI) demonstrated that the transplant of hematopoietic SCs may reset the immune system in MS patients (Roccatagliata et al. 2007). For these reasons, it is necessary to conduct large controlled clinical trials of patients treated with hematopoietic SCs and compare the outcomes with those of an already approved therapy (Karussis et al. 1993; van Gelder and van Bekkum 1996).

Mesenchymal-Derived SCs

Preclinical studies have shown that mesenchymal-derived cells have immunomodulatory properties such as suppression of T and B cell function in vitro and in vivo. Another advantageous property for MS therapy is the ability of these cells to differentiate into neural and glial cell lineages (Bartholomew et al. 2002; Di Nicola et al. 2002; Karussis and Kassis 2008). These features have prompted investigators to evaluate their effects in EAE animal models of MS.

In two recent studies transplanted syngeneic mouse mesenchymal cells downregulated myelin-specific T cells and prevented axonal damage (Gerdoni et al. 2007; Kassis et al. 2008). In addition, mice with chronic EAE received IV and intraventricular transplants of mouse mesenchymal cells that migrated to the white matter lesions and produced significant improvements in the clinical course of disease (Karussis et al. 2008). Similarly, human mesenchymal cells have modulated disease progression after IV delivery in mouse models of MS after the onset of disease (Bai et al. 2009; Zhang et al. 2005).

The time of treatment is a factor in effective treatment. Functional recovery occurred after mesenchymal cell transplants both at the peak of disease onset and, in MOG-induced EAE mice, before the onset of disease. Importantly, EAE mice that received murine mesenchymal cells after disease stabilization did not show any therapeutic effect (Zappia et al. 2005). However, human mesenchymal cells transplanted after the onset of disease migrated and increased oligodendrocytes in demyelinated lesions of a remitting-relapsing EAE mouse model (Bai et al. 2009). As noted in other cell transplant studies for MS therapy, the appropriate time of transplant is dependent on the cells and the animal model.

Successful clinical studies using mesenchymal cells as treatment for other medical conditions (Giordano et al. 2007) have influenced clinical trials in MS and amyotrophic lateral sclerosis (ALS) patients. Initial reports demonstrated no significant adverse side effects 1 year after intrathecal and IV administration. Early improvement in some patients has prompted a phase I/II clinical protocol using IV administration of mesenchymal cells in MS patients (Karussis et al. 2008; Karussis and Kassis 2008).

ERCs

Limited research on ERCs has been conducted on animal models (mainly peripheral limb ischemia) (Hida et al. 2008; Murphy et al. 2008) and there are no reports of research on animal models of MS. In humans, four MS patients whose standard treatment was unsuccessful enrolled in a clinical trial as part of a compassionate-use, physician-initiated program; they received IV and/or intrathecal injections of clinical-grade allogeneic ERCs and showed no notable immunological reaction or disease progression 5 to 7 months after the injections (Zhong et al. 2009). Further preclinical studies are necessary to verify the safety and efficacy of ERCs before their general clinical application.

ES Cells

ES cells can be differentiated into oligodendrocytes and therefore are appealing for therapeutic use in demyelinating diseases (Brüstle et al. 1999). hES-derived NP cells were transplanted in the cerebral ventricles of EAE mice 7 days after MOG immunization, and a neuroprotective mechanism resulted in a reduction of both axonal injury and demyelination and the attenuation of clinical signs measured by daily clinical scores. Inasmuch as MOG EAE mouse models create extensive axonal injury and fewer than 1% of transplanted

hES-derived NP cells differentiate into oligodendroglial progenitors, neuroprogenitors, or astrocytes, donor remyelination is an unlikely explanation for the therapeutic effect; it is more likely that the grafts' immunosuppressive effects, observed as a significant reduction of infiltrating T cells, modulated the immune response (Aharonowiz et al. 2008). ES cell transplants in other demyelinating disease animal models (Izrael et al. 2007; Keirstead et al. 2005; Nistor et al. 2005) may provide additional evidence for the use of hES cells in clinical trials.

Conclusion

SC-based therapies have potential as powerful tools to treat neurological disorders, but a review of the current state of the field reveals how much work is still necessary to successfully and safely implement these treatments in the clinical setting. The evidence of transplantation studies in support of clinical translation with different cell types emphasizes the need for systematic analysis in multiple models to assess positive as well as negative effects.

A number of factors warrant consideration in studies of SC-based treatments. For example, it is important to remember that the SCs will be transplanted in a living organism with a disease condition—and may be affected by the host as much as or more than they affect the host. Reports have documented different survival and migration properties in transplanted cells based on the disease model or lesion severity (Behrstock et al. 2008; Shindo et al. 2006; Watts and Dunnett 1998). In addition, clinical grafting in non-SC PD treatments has clearly illustrated the importance of the microenvironment in which the cells are placed (Fitzpatrick et al. 2009; Lindvall and Kokaia 2009). Modification of the graft microenvironment by combining, for example, cell replacement and neuroprotective strategies may be needed to ensure therapy success. A true advantage of new SC technologies, such as iPS, is the opportunity to assess cells' behavior in culture. Last, careful evaluation of patient selection and cotreatments is essential before initiating new transplant trials.

Further insight into patient-specific disease processes at the molecular, cellular, and system levels will enable the development of customized therapies and, hopefully, clear the path toward curing neurological diseases.

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