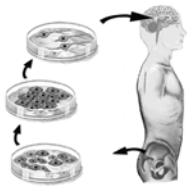

Human Embryonic Stem Cells for Neuronal Repair

Tamir Ben-Hur MD PhD

Department of Neurology, Center for Human Neurogenetics, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Key words: human embryonic stem cells, neurologic diseases

Abstract

Human embryonic stem cells may serve as a potentially endeless source of transplantable cells to treat various neurologic disorders. Accumulating data have shown the therapeutic value of various neural precursor cell types in experimental models of neurologic diseases. Tailoring cell therapy for specific disorders requires the generation of cells that are committed to specific neural lineages. To this end, protocols were recently developed for the derivation of dopaminergic neurons, spinal motor neurons and oligodendrocytes from hESC. These protocols recapitulate normal development in culture conditions. However, a novel concept emerging from these studies is that the beneficial effect of transplanted stem cells is not only via cell replacement in damaged host tissue, but also by trophic and protective effects, as well as by an immunomodulatory effect that down-regulates detrimental brain inflammation.

IMAJ 2006;8:122–126

The central nervous system is composed of three main neural lineages – neurons, astrocytes and oligodendrocytes. Extensive research on the hirarchy of development of these neural lineages led to the emerging concept of the neural stem cell as a common cellular precursor from which all neural lineages develop. Multipotential NSCs support neurogenesis and gliogenesis within specific areas of the CNS during development and throughout adulthood and can be isolated from fetal and adult brains [1]. NSC can be expanded *in vitro*, maintain their capacity for self-renewal, and generate progeny of the three neural cell lineages. While NSC were initially identified only by the expression of the intermediate filament protein nestin, recent studies have recognized several factors that play a role in maintaining their functions as stem cells, including *Musashi*, *Sox*2, and *b*HLH genes. However, a stem cell is still first and foremost defined and characterized functionally by its ability for continuous self-renewal and its multipotentiality, i.e., the generation of daughter cells that differentiate along different lineages, all arising from the same clone.

Embryonic stem cells are derived from the inner cell mass of blastocyst-stage embryos and are the totipotent stem cells that generate the entire repertoire of cells in the body. The growth of ESC from mouse embryos, established for the first time over 20 years ago, formed the basis of transgenic and knock-out mice technology. Embryonic stem cell lines have now been established from many other mammals and can be banked and propagated *in vitro* almost indefinitely, with maintenance of a normal karyotype and totipotency, as was shown by the culturing of mouse ESC lines in the presence of leukemia inhibitory factor [2]. The isolation of human ESC [3,4] introduced an endless source of transplantable hESC-derived neural precursors [5,6]. Currently, there are over 150 lines of hESC available.

Targeted differentiation into specific neural lineages

The notion that all cells in the organism arise from the common, totipotent ESC led to early studies that tried to direct the specification of ESC into neural precursors. Mouse ESC can be induced to differentiate *in vitro* into neurons [7]. For hESC, the generation of cultures that are highly enriched with neural precursors was performed initially by picking up from the culture dish cell clumps from areas that differentiated spontaneously [5]. It has now become possible to generate highly enriched hESC-derived neural precursors in a controlled manner. The BMP antagonist noggin is used to convert undifferentiated hESC into spheres that are highly enriched for neural precursors either from adherent cultures in the presence of feeders and serum [8] or in suspension and serum-free defined culture conditions, on human foreskin feeders [9,10]. The key importance of this advancement is that growing hESC-derived cell populations in a defined environment makes them an appropriate (safe) candidate for transplantation into human patients. The neural spheres are propagated and expanded in the presence of growth factors (20 ng/ml basic fibroblast growth factor with or without 20 ng/ml human recombinant epidermal growth factor). The potential of hESC-derived neural precursors to differentiate into the neural lineages is examined by plating them on laminin-coated dishes and withdrawing the growth factors [5,10], leading to formation of multiple neurons of different types and glial cells. The developmental potential of these cells may also be revealed after transplantation *in vivo*. When hESC-derived neural precursors

hESC = human embryonic stem cells
NSC = neural stem cells
CNS = central nervous system
ESC = embryonic stem cells

were transplanted into the lateral ventricles of newborn mice they responded to the normal developmental cues. Transplanted cells migrated in regions where gliogenesis and neuronogenesis continue after birth (such as the corpus callosum, rostral migratory stream, and dentate gyrus of the hippocampus) and differentiated into neurons, astrocytes and oligodendrocytes [5,6].

Considerable efforts have been made to direct the differentiation of hESC to specific neural lineages. This is potentially relevant for cell-specific neural diseases, such as Parkinson's disease (where there is loss of midbrain dopaminergic neurons), amyotrophic lateral sclerosis (with loss of spinal and cortical motor neurons), and multiple sclerosis (with loss of oligodendrocytes). Such specific populations were generated first from mouse ESC and proved to be functional after transplantation *in vivo*. For example, the sequential use of growth factors, such as basic fibroblast growth factor, epidermal growth factor, and platelet-derived growth factor, in a program that mimics embryonic development, has been successful in deriving glial precursors from mouse ESC [11]. These cells formed normal-appearing, compact myelin after transplantation into the CNS of the myelin-deficient (*md*) rat, an animal model for the human dysmyelinating Pelizaeus-Merzbacher disease. Genetic modification of mouse ESC to over-express Nurr1, a transcription factor that is intimately involved in differentiation into dopaminegic neurons, forced the cells into this lineage. These mouse ESC-derived dopaminergic progenitors fully corrected the behavioral deficit in an animal model of Parkinson's disease [12]. Similarly, motoneuron-committed mouse ESC that were transplanted into the spinal cord of adult rats with motoneuron injury survived and differentiated into motoneurons. For functional recovery, these motor neurons should grow axons through the surrounding white matter. Here, inhibition of axonal growth by myelin was partially overcome by intrathecal administration of dibutyryl cAMP that facilitated motoneuron axonal growth into the ventral roots [13].

Recently, protocols have been established to generate these specific neural populations from hESC. The functional properties of such cell populations were exemplified by transplanting hESC-derived high purity oligodendroglial lineage cells into the spinal cord of the shiverer (*shi*) mouse where they integrated, differentiated into oligodendrocytes and formed compact myelin [14]. The basic principle in the protocols that were established to generate specific neural lineages from hESC is to recapitulate the normal sequence of development in the culture dish. For example, hESC may be directed to differentiate into functional motor neurons *in vitro* in response to retinoic acid and sonic hedgehog [13,15]. Retinoic acid directs ESC-derived early neuroectodermal cells towards caudal specification and sonic hedgehog is required for ventralization. In the human system, it has been demonstrated in adherent cultures that early neural precursors, which express Pax6 but not Sox1, are responsive to retinoic acid-induced motoneuron specification, while later neuroectodermal precursors, which express both Pax6 and Sox1, are not posteriorized by retinoic acid [15].

Transplantation of NSC and ESC in experimental CNS diseases

The main advantage of stem cells is that they are non-transformed precursors that are potentially able to self-renew indefinitely, allowing their expansion in large quantities. These uncommitted NSC can integrate and repair the damaged CNS and thus might represent a renewable source of cells that can be used for transplantation procedures.

Transplanted neural precursor cells of various origins improved the clinical outcome in several experimental models of brain disease, including stroke [16], MS [17,18] and spinal cord trauma [19]. The myelinogenic properties of neural precursors has been demonstrated extensively. Early studies showed the remyelinating properties of oligodendrocyte progenitor cells. As these cells are limited in number and proliferative capacity, later studies focused on the myelinogenic properties of earlier neural precursors. Neural precursors that were isolated from the subventricular zone (a major source of glial precursors in the postnatal brain) and propagated in culture myelinated the CNS after transplantation in various models of genetic dymyelination, such as the *md* rat, the shaking (*sh*) pup and *shi* mouse. Intraventricularly transplanted clonal NSC in the newborn *shi* mouse disseminated in widespread brain areas and participated in myelination [20]. Some recipient animals showed a decrease in their symptomatic tremor. Expression of the polysialylated form of neural cell adhesion molecule on the cell membrane has been associated with stem cell commitment to neuronal or glial fate, depending on the time and place in the development process [21], and with especially good migratory and regenerative properties [22]. Such PSA-NCAM-positive glial-committed precursors, growing as neurospheres, remyelinated 95–100% of the axons in the dorsal columns of rats [23], compared to only 70% remyelinated axons, as expected from oligodendrocyte progenitors. PSA-NCAM-enriched oligodendrocyte progenitor cells from fetal or adult human brains, xenografted to newborn *shi* mouse brains, dispersed as well throughout the white matter, differentiated into oligodendrocytes and remyelinated nude axons [24]. The migratory capacity of PSA-NCAM+ precursors was demonstrated in the adult CNS, when transplanted cells migrated efficiently along the inflamed white matter tracts of rats with experimental autoimmune encephalomyelitis [25,26]. Recently, it was also shown that intraventricular as well as intravenous transplantation of NSC into mice with EAE resulted in clinical improvement [17,18]. This was correlated by graft-derived and endogenous remyelination and by a reduction in axonal pathology. These data suggest that cell transplantation in de- and dysmyelinated human disease may have therapeutic potential for functional restoration.

Given the extensive experience in transplantation of embryonic and adult brain-derived neural precursors, one may wonder what the specific role of ESC will be in future cell therapy. A major advantage of ESC is in their potential to generate an

MS = multiple sclerosis
PSA-NCAM = polysialylated form of neural cell adhesion molecule
EAE = experimental autoimmune encephalomyelitis

endless supply of specific neural poulations. For example, the ability to generate highly enriched oligodendroglial lineage cultures from ESC provides them with an advantage over other sources of transplantable oligodendrocyte lineage cells. The myelinogenic potential of mouse embryonic stem-derived oligodendrocyte progenitors, which were expanded *in vitro*, was demonstrated in embryonic *md* rat brains, when these cells extensively myelinated the brain and spinal cord [11]. When transplanted in a rodent model of chemically induced demyelination and in the spinal cords of *shi* mice, mouse embryonic stem-derived progenitor cells were also able to differentiate into glial cells and remyelinate demyelinated axons *in vivo*.

The therapeutic value of transplanted dopaminergic cells in an animal model of Parkinson's disease is very well established. This has led to clinical trials in which human fetal mesencephalic dopamine neurons were transplanted into patients with this disease. Although there were motor complications in these patients, these studies provided the proof of principle, supporting the continuation of research in therapeutic cell transplantation. However, attempts to generate highly enriched dopaminergic cell cultures from banked NSC lines, which were prepared from embryonic or adult forebrains, were unsuccessful. ESC, however, can be directed to generate highly enriched midbrain dopaminergic neurons, by recapitulating their developmental sequence *in vitro*, as described above. We recently demonstrated, for the first time, the therapeutic potential of hESC-derived neural precursors that partially corrected motor and behavioral deficits in an animal model of Parkinson's disease after transplantation into the rat striatum [8]. The spontaneous differentiation of hESC-derived neural progenitors into dopaminergic neurons was very low both *in vitro* and *in vivo*, resulting in small numbers of transplant-derived dopaminergic neurons and, therefore, partial recovery [8]. This indicates that hESC indeed should be directed to a midbrain dopaminergic fate prior to transplantation in order to obtain better clinical results.

Mechanisms of therapeutic effects by neural stem cells

The mechanisms by which transplanted stem cells affect brain recovery are not fully understood. Until recently, restorative neurotransplantation research has focused mainly on the potential of the neural graft to replace damaged or missing cell populations. Although transplanted stem cells might exert their therapeutic effect by differentiating into lineage-restricted daughter cells and directly replacing missing cells, it was suggested recently that transplanted stem cells also enhance endogenous brain repair systems [27]. Accumulating evidence, particularly in models of MS [17], Parkinson's disease [27], spinal cord trauma [28], brain ischemia [29], and ALS [30], support the hypothesis that neural cell grafts can enhance regeneration of the host CNS by their inherent capacity to induce protective and restorative mechanisms within the host.

Fetal mesencephalic tissue and dopaminergic cell suspension grafts resulted in impressive anatomic, biochemical repair and signifcant amelioration of behavioral symptoms in Parkinson's disease models, in correlation to generation of transplant-derived dopaminergic neurons [31]. Ourednik et al. [27] suggested that behavioral recovery after cell transplantation may also be related to a trophic effect of transplanted stem cells to "rescue" dysfunctional and dying host dopaminergic neurons. In their study, unilateral implantation of murine NSC into the midbrains of aged mice, in which the presence of impaired but non-apoptotic dopaminergic neurons was increased by treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, was associated with bilateral reconstitution of the mesostriatal system. Although conversion of donor NSC to tyrosine hydroxylase-positive cells contributed to nigral reconstitution in dopaminergic depleted areas, the majority of dopaminergic neurons in the mesostriatal system were "rescued" host cells.

It has also been demonstrated that NSC, seeded on a synthetic biodegradable scaffold and grafted into the hemisectioned adult rat spinal cord, induced a significant improvement in animal movement by reduction of necrosis in the surrounding parenchyma, and prevention of extensive secondary cell loss, inflammation, and formation of a glial scar [28]. Here again, the host displayed regenerated neurites that were not derived from donor NSC but were of recipient origin. Substantial reconstitution of brain parenchyma and structural connectivity was reported when NSC were transferred by biodegradable scaffolds into regions of extensive brain degeneration caused by hypoxia [29]. The injured brain interacted reciprocally with NSC supported by scaffolds to reconstitute lost tissue.

ALS, a neurodegenerative disease affecting adult motor neurons, has also been suggested as a candidate for stem cell therapy. Mouse embryonic stem cells can be directed to differentate into progenitors of spinal motor neurons *in vitro* and into mature motor neurons after transplantation into embryonic [32] and adult rats [13] *in vivo*, which can populate, extend axons, and form synapses with target muscles. It is still questionable how transplanted cells will repopulate the missing motor neurons in the entire spinal cord and motor cortex. However, recent studies, using the Cu-Zn superoxide dismutase model of familial ALS, have shifted the therapeutic target of cell transplantation in ALS from a neuron-replacement approach, and focused instead on the potential of these cells to change the environment to support motor neuron survival [33]. The neuroadapted Sinbdis virus, a neuronotropic, single stranded RNA virus, specifically targets motor neurons in the spinal cord and, although it is cleared from animals 7–10 days after infection, it triggers progressive motor neuron death, resulting in permanent hindlimb paralysis. Cells derived from embryonic germ cells, termed embryoid body-derived cells, were transplanted into the cerebrospinal fluid of rats with motor neuropathy induced by neuroadapted Sinbdis virus [30]. Transplanted EBDs were distributed over the rostrocaudal length of the spinal cord and migrated into the spinal cord parenchyma. Paralyzed animals transplanted with EBD cells partially recovered. It was found

ALS = amyotrophic lateral sclerosis
EBD = embryoid body-derived cell

that the efficacy of neuronal differentiation and the extension of neurites could not account for the functional recovery. It was suggested that EBD cells protected host neurons from death and facilitated reafferentation of motor neuron cell bodies, thereby restoring neurologic function via enhancment of host neuron survival and function [30].

An important step toward the future application of stem cell therapy in MS was recently made with the observation that stem cell transplantation attenuates the clinical course of EAE, a reliable model of multiple sclerosis [17,34]. In accordance with the above findings, in NSC-transplanted EAE mice, only 20% of remyelination was derived from donor cells [17].

Thus, a neurotrophic effect may underline at least part of the beneficial action of transplanted NSC. Neural precursors produce a variety of neurotrophic factors that may protect the brain from injury [35]. It has been documented that undifferentiated donor progenitors spontaneously express neuroprotective substances such as FGF2, glial cell line-derived neurotrophic factor, neurotrophin-3, brain-derived neurotrophic factor, stem cell-derived neural stem/progenitor cell supporting factor, nerve growth factor, insulin-like growth factor-1, ciliary neurotrophic factor, leukemia inhibitory factor, and erythropoietin [34,35]. The spectrum of donor cell-released trophic factors may influence specific host cell populations, as shown in targeting the regeneration of either motor or sensory fibers in the lesioned spinal cord [35]. Similarly, glial cell line-derived neurotrophic factor, which is neuroprotective for ventrally located neurons (such as mesencephalic dopaminergic neurons and spinal ventral horn motor neurons) was found expressed in undifferentiated donor cells and glia adjacent to rescued host dopaminergic neurons in the MPTP-treated aged mice [27]. I*n vitro*, EBD cells secrete transforming growth factor-β and BDNF [30]. Indeed, neutralizing antibodies to TGFβ and to BDNF abrogated the ability of EBD-conditioned media to sustain motor neuron survival in culture [30]. Thus, transplanted stem cell-derived trophic factors may shift the balance between a permissive and non-permissive milieu, to favor reacquisition of host CNS tissue integrity and function.

Our recent studies suggest an additional mechanism of stem cell action in the EAE brain. We showed that intraventricular transplantation of newborn rat-derived neural spheres inhibited the clinical and pathologic features of acute EAE, an experimental model for brain inflammation, with a minor demyelinating component [18]. Specifically, neurosphere transplantation reduced the inflammatory process in the brain, as determined by several pathologic criteria. Similarly, the immune-mediated demyelination and axonal injury were reduced in neurosphere-transplanted chronic EAE mice [17,34]. Cell transplantation inhibited the cellular inflammatory process in the brain and there was a decrease in local cellular inflammatory-associated axonal injury. These findings suggest that the beneficial clinical and pathologic effects of neural precursor cell transplantation were related, in part, to their immunomodulatory and anti-inflammatory properties. As the autoimmune process is a major determinant of tissue injury in EAE and MS, its local suppression by cell transplantation decreases the pathologic and clinical consequences of disease. This has major importance for a transplantation approach in immune-mediated diseases, since the down-regulation of the inflammatory process may protect the graft from future immune attacks. An immunosuppressive effect has also been described for ESC-derived lines [36]. Bi-directional interactions between NSC and the immune system are largely unknown. NSC can directly inhibit the specific response of lymph node cells to a myelin antigen [18]. In addition, neurotrophins that may be released by stem cells inhibit EAE not only by enhancing oligodendrocyte survival but also by decreasing neuroinflammation [37,38].

Thus, stem cells may exert their therapeutic effects via different, interacting mechanisms, including their own regenerative potential, their neurotrophic and neuroprotective properties, and their immune regulatory functions.

Future prospects of ESC transplantation

What is the roadmap to a clinical trial of hESC transplantation into the CNS? First, there is the issue of safety. New hESC lines need to be generated under tightly controlled and documented conditions of a good manufacturing practice facility to be appropriate for human transplantation. The most important potential hazard in stem cell (and especially embryonic cell) transplantation is the risk of tumor formation. By definition, embryonic stem cells are capable of forming teratomas after transplantation. When undifferentiated mouse ES cells are transplanted into the brain, they can generate brain cells, but at the expense of teratoma formation. Clearly, the commitment to a restricted neural lineage should be complete prior to transplantation in order to eliminate this problem. Indeed, transplantation of mouse and human embryonic cell-derived neural precursors, obtained after multiple *in vitro* passages and exposure to various growth factors, did not result in teratoma formation. Another potential problem is the possibility of graft rejection. In most studies of xeno-transplantation of human cells into rodents, graft rejection did not pose a significant problem. However, there is no long-term systematic follow-up of the survival of these cells in the brain. If graft rejection proves to be a major obstacle in the future, then nuclear transfer from somatic cells to oocyte or stem cells may generate the patient's own ESC-like line for syngeneic transplantation. This has recently been accomplished for human cells. Finally, the transplanted cells need to survive in the brain for a prolonged period in a hostile environment. We have shown that neurospheres create a trophic microenvironment that supports the survival of transplanted cells in an otherwise growth factor-poor environment [39].

MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
BDNF = brain-derived neurotrophic factor
TGFβ = transforming growth factor-β

References

1. Gage FH. Mammalian neural stem cells. S*cience* 2000:287:1433–8.
2. Smith AG, Heath JK, Donaldson DD, et al. Inhibition of pluripo-

tential embryonic stem cell differentiation by purified polypeptides. *Nature* 1988;336(6200):688–90.
3. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. *Science* 1998;282(5391):1145–7.
4. Reubinoff BE, Pera MF, Fong CY, Trounson A, Bongso A. Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. *Nature Biotechnol* 2000;18(4):399–404.
5. Reubinoff BE, Itsykson P, Turetsky T, et al. Neural progenitors from human embryonic stem cells. *Nature Biotechnol* 2001;19(12):1134–40.
6. Zhang SC, Wernig M, Duncan ID, Brustle O, Thomson JA. In vitro differentiation of transplantable neural precursors from human embryonic stem cells. *Nature Biotechnol* 2001;19(12):1129–33.
7. Xian HQ, Gottlieb DI. Peering into early neurogenesis with embryonic stem cells. *Trends Neurosci* 2001;24(12):685–6.
8. Ben-Hur T, Idelson M, Khaner H, et al. Transplantation of human embryonic stem cell-derived neural progenitors improves behavioral deficit in Parkinsonian rats. *Stem Cells* 2004;22:1246–55.
9. Amit M, Shariki C, Margulets V, Itskovitz-Eldor J. Feeder layer- and serum-free culture of human embryonic stem cells. *Biol Reprod* 2004;70(3):837–45.
10. Itsykson P, Ilouz N, Turetsky T, et al. Derivation of neural precursors from human embryonic stem cells in the presence of noggin. *Mol Cell Neurosci* 2005;30(1):24–36.
11. Brustle O, Jones KN, Learish RD, et al. Embryonic stem cell-derived glial precursors: a source of myelinating transplants. *Science* 1999;285(5428):754–6.
12. Kim JH, Auerbach JM, Rodriguez-Gomez JA, et al. Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. *Nature* 2002;418(6893):50–6.
13. Harper JM, Krishnan C, Darman JS, et al. Axonal growth of embryonic stem cell-derived motoneurons in vitro and in motoneuron-injured adult rats. *Proc Natl Acad Sci* USA 2004;101:7123–8.
14. Nistor GI, Totoiu MO, Haque N, Carpenter MK, Keirstead HS. Human embryonic stem cells differentiate into oligodendrocytes in high purity and myelinate after spinal cord transplantation. *Glia* 2004;49(3):385–96.
15. Li XJ, Du ZW, Zarnowska ED, et al. Specification of motoneurons from human embryonic stem cells. *Nature Biotechnol* 2005;23:215–21.
16. Veizovic T, Beech JS, Stroemer RP, Watson WP, Hodges H. Resolution of stroke deficits following contralateral grafts of conditionally immortal neuroepithelial stem cells. *Stroke* 2001;32(4):1012–19.
17. Pluchino S, Quattrini A, Brambilla E, et al. Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. *Nature* 2003;422:688–94.
18. Einstein O, Karussis D, Grigoriadis N, et al. Intraventricular transplantation of neural precursor cell spheres attenuates acute experimental allergic encephalomyelitis. *Mol Cell Neurosci* 2003;24(4):1074–82.
19. McDonald JW, Liu XZ, Qu Y, et al. Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. *Nature Med* 1999;5(12):1410–12.
20. Learish RD, Brustle O, Zhang SC, Duncan ID. Intraventricular transplantation of oligodendrocyte progenitors into a fetal myelin mutant results in widespread formation of myelin. *Ann Neurol* 1999;46(5):716–22.
21. Ben-Hur T, Rogister B, Murray K, Rougon G, Dubois-Dalcq M. Growth and fate of PSA-NCAM+ precursors of the postnatal brain. *J Neurosci* 1998;18(15):5777–88.
22. Kleene R, Schachner M. Glycans and neural cell interactions. *Nature Rev Neurosci* 2004;5(3):195–208.
23. Keirstead HS, Ben-Hur T, Rogister B, O'Leary MT, Dubois-Dalcq M, Blakemore WF. Polysialylated neural cell adhesion molecule-positive CNS precursors generate both oligodendrocytes and Schwann cells to remyelinate the CNS after transplantation. *J Neurosci* 1999;19(17):7529–36.
24. Windrem MS, Nunes MC, Rashbaum WK, et al. Fetal and adult human oligodendrocyte progenitor cell isolates myelinate the congenitally dysmyelinated brain. *Nature Med* 2004;10(1):9397.
25. Ben-Hur T, Einstein O, Mizrachi-Kol R, et al. Transplanted multipotential neural precursor cells migrate into the inflamed white matter in response to experimental autoimmune encephalomyelitis. *Glia* 2003;41(1):73–80.
26. Bulte JW, Ben-Hur T, Miller BR, et al. MR microscopy of magnetically labeled neurospheres transplanted into the Lewis EAE rat brain. *Magn Reson Med* 2003;50(1):201–5.
27. Ourednik J, Ourednik V, Lynch WP, Schachner M, Snyder EY. Neural stem cells display an inherent mechanism for rescuing dysfunctional neurons. *Nature Biotechnol* 2002;20(11):1103–10.
28. Teng YD, Lavik EB, Qu X, et al. Functional recovery following traumatic spinal cord injury mediated by a unique polymer scaffold seeded with neural stem cells. *Proc Natl Acad Sci* USA 2002;99(5):3024–29.
29. Park KI, Teng YD, Snyder EY. The injured brain interacts reciprocally with neural stem cells supported by scaffolds to reconstitute lost tissue. *Nature Biotechnol* 2002;20(11):1111–17.
30. Kerr DA, Llado J, Shamblott MJ, et al. Human embryonic germ cell derivatives facilitate motor recovery of rats with diffuse motor neuron injury. *J Neurosci* 2003;23(12):5131–40.
31. Bjorklund A, Lindvall O. Cell replacement therapies for central nervous system disorders. *Nature Neurosci* 2000;3(6):537–44.
32. Wichterle H, Lieberam I, Porter JA, Jessell TM. Directed differentiation of embryonic stem cells into motor neurons. *Cell* 2002;110(3):385–97.
33. Clement AM, Nguyen MD, Roberts EA, et al. Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. *Science* 2003;302:113–17.
34. Einstein O, Grigoriadis N, Mizrachi-Kol R, et al. Transplanted neural precursor cells reduce brain inflammation to attenuate chronic experimental autoimmune encephalomyelitis. *Exp Neurol* 2006 (in press).
35. Lu P, Jones LL, Snyder EY, Tuszynski MH. Neural stem cells constitutively secrete neurotrophic factors and promote extensive host axonal growth after spinal cord injury. *Exp Neurol* 2003;181(2):115–29.
36. Fandrich F, Lin X, Chai GX, et al. Preimplantation-stage stem cells induce long-term allogeneic graft acceptance without supplementary host conditioning. *Nature Med* 2002;8(2):171–8.
37. Linker RA, Maurer M, Gaupp S, et al. CNTF is a major protective factor in demyelinating CNS disease: a neurotrophic cytokine as modulator in neuroinflammation. *Nature Med* 2002;8(6):620–4.
38. Villoslada P, Hauser SL, Bartke I, et al. Human nerve growth factor protects common marmosets against autoimmune encephalomyelitis by switching the balance of T helper cell type 1 and 2 cytokines within the central nervous system. *J Exp Med* 2000;191(10):1799–806.
39. Einstein O, Ben-Menachem-Tzidon O, Mizrachi-Kol R, Reinhartz E, Grigoriadis N, Ben-Hur T. Survival of neural precursor cells in growth factor poor environment: implications for transplantation in chronic disease. *Glia* 2006;53(4):449–55.

Correspondence: Dr. T. Ben-Hur, Dept. of Neurology, Hadassah Hebrew University Medical Center, P.O. Box 12000, Jerusalem 91120, Israel.
Phone: (972-2) 677-6944
Fax: (972-2) 643-7782
email: tamir@hadassah.org.il