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Autotransplantation of Bone Marrow-Derived Stem Cells as a Therapy for Neurodegenerative Diseases

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Abstract Neurodegenerative diseases are characterized by a progressive degeneration of selective neural populations. This selective hallmark pathology and the lack of effective treatment modalities make these diseases appropriate candidates for cell therapy. Bone marrow-derived mesenchymal stem cells (MSCs) are self-renewing precursors that reside in the bone marrow and may further be exploited for autologous transplantation. Autologous transplantation of MSCs entirely circumvents the problem of immune rejection, does not cause the formation of teratomas, and raises very few ethical or political concerns. More than a few studies showed that transplantation of MSCs resulted in clinical improvement. However, the exact mechanisms responsible for the beneficial outcome have yet to be defined. Possible rationalizations include cell replacement, trophic factors delivery, and immunomodulation. Cell replacement theory is based on the idea that replacement of degenerated neural cells with alternative functioning cells induces long-lasting clinical improvement. It is reasoned that the transplanted cells survive, integrate into the endogenous neural network, and lead to functional improvement. Trophic factor delivery presents a more practical short-term approach. According to this approach, MSC effectiveness may be credited to the production of neurotrophic factors that support neuronal cell survival, induce endogenous cell proliferation, and promote nerve fiber regeneration at sites of injury. The third potential mechanism of action is supported by the recent reports claiming that neuroinflammatory mechanisms play an important role in the pathogenesis of neurodegenerative disorders. Thus, inhibiting chronic inflammatory stress might explain the beneficial

effects induced by MSC transplantation. Here, we assemble evidence that supports each theory and review the latest studies that have placed MSC transplantation into the spotlight of biomedical research.

Keywords Neurodegenerative disease · Autologous transplantation · Adult stem cells · Mesenchymal stem cells

1 Introduction

Many of the neurodegenerative disorders are attributed to the degeneration of specific neurons with subsequent functional loss. The susceptibility of specific neuronal populations has yet to be solved. The same uncertainty applies to the underling causes of progressive neurodegeneration. Nevertheless, the sporadic occurrence of these disorders counters the theory of programmed cell degeneration and supports the existence of multifactorial stimulating factors. Accumulating evidence implies that the physiological process of aging has an important role in the occurrence of such diseases (Mattson and Magnus 2006). During the process of aging the occurrence of neurodegenerative disorder is determined by genetic and environmental factors that counteract or facilitate fundamental mechanisms of aging. Therapeutic modalities of neurodegenerative diseases are limited, and although several treatments have been shown to modify the course of the disease, no treatments have proved to halt the degeneration.

Mounting evidence now suggests that cell therapy may be of functional benefit in many neurodegenerative diseases. Each condition has specific requirements for the phenotype, developmental stage, and number of cells required. The ideal cells for universal application in cell therapy would possess several key properties. First, they have to be highly proliferative, allowing optimal exploitation of donor material. Second, the cells must have wide differentiation potential, allowing differentiation into appropriate neural and glial cell types. Importantly, both proliferation and differentiation would be controllable. Stem cells are feasible candidates for cell therapy of neurodegenerative diseases. Different therapeutic strategies based on stem cells have been developed and studied. Several strategies support cell replacement of the damaged tissue while others rely on therapeutic effects induced by cell transplantation.

The most primitive of all stem cell populations, offering the most potential, are embryonic stem cells (ESCs) obtained from the inner cell mass of developing blastocysts. ESCs are pluripotent stem cells that can give rise to cells of the three germ layers found in the implanted embryo, fetus, or developed organism, but not to embryonic components of the trophoblast and placenta. Application of the embryonic pluripotent stem cells to clinical studies have been impeded due to: potential immune rejection in allogeneic transplantation (Vogel 2002), formation of teratomas (Reubinoff et al. 2000), lack of their availability, and serious ethical and political issues (Perin et al. 2003). In view

of the latter, autologous cell sources may prove to be more beneficial and acceptable as a therapeutic tool in the future.

Adult stem cells remain in an undifferentiated, or unspecialized, state in different tissues of the adult organism and may be exploited in autologous transplantation. They possess the ability to self-renew, and can differentiate into at least one mature, specialized cell type. In the traditional developmental paradigm, adult stem cells are able to differentiate only to the tissue in which they reside. Recent data challenge the committed fate of the adult stem cells and present evidence for their plasticity. Thus, adult stem cell therapy may offer an accessible, therapeutic tool for damaged tissue replacement and tissue engineering that is free of ethical debate. Stem cell therapy in general and adult stem cell exploitation in particular are predominantly relevant in tissues and organs that have little capacity for self-repair. One such organ is the brain.

Traditionally, the mammalian central nervous system (CNS) was considered to be a nonrenewable tissue, but this principle has been challenged in the past decade. Studies have demonstrated that neural stem cells (NSCs) exist not only in the developing mammalian nervous system but also in the adult nervous system of all mammalian organisms, including humans (Gage 2000; Rakic 2002). NSCs are capable of undergoing expansion and differentiation into neurons, astrocytes, and oligodendrocytes in vitro (Reynolds and Weiss 1992; Gage et al. 1995) and after transplantation in vivo (Svendsen et al. 1997). These studies imply that NSCs may further be utilized for treatment of severe brain disorders. However, because of the inaccessibility of NSC sources deep in the brain, stimulation of endogenous neurogenesis may provide a more applicable treatment modality. During the past few years several groups studied the ongoing process of neurogenesis in the intact and diseased adult nervous system. Zhao et al. (2003) have provided evidence for the continuous turnover of dopaminergic cells in the adult substantia nigra pars compacta (SNpc). Moreover, several studies showed a selective increase in the production of dopaminergic neurons in the adult olfactory bulb in response to dopaminergic deficiency (Yamada et al. 2004; Winner et al. 2006). A recent report of Tande et al. (2006), however, was unable to obtain evidence for the existence of neurogenesis in the striatum of normal or the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced monkey model of Parkinson's disease (PD). The process of augmented neurogenesis has also been observed in animal models of Alzheimer's disease (AD) (Jin et al. 2004a) and in patients with AD (Jin et al. 2004b). Chi et al. (2006) showed that motor neuron degeneration promotes neural progenitor cell proliferation, migration, and neuronal differentiation in the spinal cords of amyotrophic lateral sclerosis (ALS) mice. However, the amount of neuronal cell replacement by endogenous stem cells without additional manipulation is minimal and does not allow significant functional recovery of the damaged tissue. Nevertheless, stimulation with molecules that govern proliferation and differentiation might dramatically change the therapeutic spectrum of neurodegenerative diseases (Lie et al. 2004).

2
Selective Pattern of Neurodegeneration

Selective vulnerability is most readily appreciated in the context of neurodegenerative disorders such as PD, AD, and ALS. Each disease is characterized by its own, unique pattern of degeneration.

In PD, whether sporadic or inherited, dopaminergic neurons of the SNpc (A9) progressively degenerate. Interestingly, other dopaminergic populations are relatively spared, including the adjacent ventral tegmental area (VTA or A10) neurons (Chung et al. 2005).

In AD the earliest and the most consistent degeneration occurs in the forebrain cholinergic projection system particularly in a structure called nucleus basalis of Meynert (Whitehouse et al. 1981, 1982). West et al. (1994) showed that although neuronal and synaptic loss occurs diffusely across the brain, neurons in layer II of the entorhinal cortex and hippocampal CA1 neurons are particularly vulnerable.

ALS (or Lou Gehrig's disease) involves the loss of upper and lower motor neurons. However, in ALS motor neurons do not display universal vulnerability. Some motor nuclei (III, IV, VI, and Onuf's nucleus) remain relatively intact during terminal stages of the disease, while others (V, VII, XII, and most of the spinal nuclei) usually degenerate (Laslo et al. 2000).

Understanding the basis of the selective vulnerability that characterizes many neurodegenerative diseases might generate a more refined therapeutic approaches in which transplanted cells more closely reflect a lost neuronal subtype.

3
Bone Marrow Stem Cells: Cell Candidates for Autologous Transplantation

Autologous cell transplantation is a promising strategy for treatment of several CNS pathologies and offers the prospect of permanent cure. Deriving cells from an adult patient's own tissues entirely circumvents the problem of immune rejection. In addition, adult stem cells do not form teratomas, and their application raises very few ethical or political issues associated with the use of human embryos.

Postnatal bone marrow (BM) is a readily accessible source of adult stem cells for autologous transplantation and contains two major stem cell populations—hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs).

3.1
Hematopoietic Stem Cells

Human HSCs—characterized as being $CD34^+$, c-Kit^+, Thy-1^{low}, $CD10^-$, $CD14^-$, $CD15^-$, $CD16^-$, $CD19^-$, and $CD20^-$ (Shizuru et al. 2005)—differentiate into

progenitor cells and mature blood cells of all hematopoietic lineages. Krause et al. (2001) demonstrated that a single HSCs was not only able to repopulate the hematopoietic system in irradiated mice, but differentiated into lung epithelium, skin, liver, and the gastrointestinal tract. In addition, several studies have reported that HSCs may give rise to neurons in vitro and in vivo (Koshizuka et al. 2004; Sigurjonsson et al. 2005; Reali et al. 2006). These studies suggest that transdifferentiation of HSCs into neurons is a rare event in the intact adult brain, but can be induced by a particular microenvironment present in the injured tissues. Other experiments dispute these findings and claim that HSCs and their progeny maintain lineage fidelity in the brain and do not adopt neural cell fates with any measurable frequency (Massengale et al. 2005).

3.2 Mesenchymal Stem Cells

Over 30 years ago Friedenstein et al. (1966, 1974, 1976) defined another BM cell population, fibroblast-colony-forming cells, which adhere to cell culture plastic surfaces and can differentiate into osteoblasts, adipocytes, and chondrocytes—mesenchymal cell types, ex vivo and in vivo. In later experiments, fibroblast-colony forming-cells were termed MSCs or BM stromal cells (BMSC) (Prockop 1997; Pittenger et al. 1999). Subsequently, most of the research concentrated on the BMSC-created microenvironment, which is essential for lineage commitment and differentiation of HSCs and has regulatory roles in hematopoiesis (Cherry et al. 1994; Moreau et al. 1993). MSCs represent a very small fraction of BM cells, only 0.001%–0.01% of the total population of nucleated cells in the BM. Although millions of cells could be harvested after several passages of the cells in culture (Pittenger et al. 1999), prolonged culture might change MSC characteristics, thus reducing their therapeutic potential. Previous studies revealed that during culture expansion MSCs undergo an aging process in which their early progenitor properties, proliferation, and homing capability are gradually lost (Banfi et al. 2000; DiGirolamo et al. 1999; Rombouts and Ploemacher 2003). This negative effect of in vitro expansion has been correlated with the rate of their telomere loss. Current culture protocols that involve population expansion stimulate rapid aging of MSCs, with large telomere shortening and little remaining proliferative capacity. Surprisingly, some MSC cultures were able to maintain telomere length for over 40 population doublings after an initial telomere shortening. Telomere length preservation may point toward a selection of more primitive MSCs, a small population that may not always present in all samples due to the small volume of the BM primary sample (Baxter et al. 2004). Several studies report the existence of rare BM-derived multipotent adult progenitor cells (MAPCs) that can be cultured for more than 70 doublings and have long telomeres that do not shorten during culture (Jiang et al. 2002; Reyes and Verfaillie 2001). MAPCs, selected from the BM mononuclear cells by depletion of CD45 and

glycophorin-A (Gly-A) cells, can be identified in the adherent cultured MSCs only after the cells undergo approximately 30 or more population doublings. The MAPC population is characterized by rapid replication and can differentiate into multiple mesenchymal cell types as well as hematopoietic lines, representing a primitive progenitor cell. To date, no research has determined whether MAPCs constitute a small rare subpopulation of MSCs or a new cell population developed under unique in vitro cell culture conditions. Several researchers have tried to characterize MSCs. Minguell et al. (2001) reviewed these studies and concluded that the antigenic phenotype of MSCs is not unique, but borrows features of mesenchymal, endothelial, epithelial, and muscle cells, and does not include the typical hematopoietic antigens CD45, CD34, and CD14. In addition, the constitutive or stimulated production of growth factors, interleukins, chemokines, matrix molecules, and the expression of their receptors provide evidence that MSCs contribute to the formation and function of a stromal microenvironment, responsible for the inductive/regulatory signals for MSCs, hematopoietic progenitor cells, and other nonmesenchymal stromal cells present in the BM.

In this chapter we will assess the reported benefits and mechanisms of action of MSC transplantation for treatment of neurodegenerative diseases concentrating on AD, PD and ALS.

4
Autologous Transplantation: Mechanisms of Action

4.1
Neuroregeneration: Cell Replacement

Cell replacement is a single application of cell therapy for the treatment of neurodegenerative diseases. It aims to replace the degenerated neural cells with alternative functioning cells. It is reasoned that these cells will survive, integrate into the endogenous neural network, and lead to significant clinical improvement.

4.1.1
The Concept of Cell Replacement

The clinical trials with cell therapy in PD patients are based on the idea that restoration of striatal dopaminergic transmission by grafted dopaminergic neurons will induce long-lasting clinical improvement in PD symptoms. In support, open-label clinical transplantation trials of embryonic dopaminergic neurons show that intrastriatal transplantation can give substantial symptomatic relief in advanced PD patients and provide the proof-of-principle for the cell replacement strategy in PD (Freed et al. 1992; Lindvall et al. 1990).

In these studies clinical improvement correlated with graft survival and host reinnervation. Two recent NIH-sponsored placebo-controlled trials, however, have given disappointing results, which included the development of graft-induced dyskinesias (Freed et al. 2001; Olanow et al. 2003). These studies stressed that issues such as graft standardization, slowly developing inflammatory responses, and dyskinesias need to be addressed before the application of fetal dopaminergic tissues for treatment of PD. In addition, ethical issues associated with the use of tissue from aborted fetuses make it necessary to develop alternative sources of cells for transplantation.

In AD, long projections and the expression of nerve growth factor receptors led some researchers to declare basal forebrain cholinergic neurons irreplaceable (Sugaya 2003). Although there are no reports at the moment of clinical use of embryonic transplants in AD patients, several works have demonstrated that cholinergic-rich cells of fetal origin can improve the performance in animal models of cholinergic depletion (Gage and Bjorklund 1986; Hodges et al. 1991a, b; Muir et al. 1992; Grigoryan et al. 2000).

In ALS, if stem cells could be used to generate large projection neurons that would appropriately connect to their targets upon transplantation, there is a prospect for replacement and a possible cure. Wichterle et al. (2002) reported that motor neurons derived from mouse embryonic stem cells could populate the embryonic spinal cord, extend axons, and form synapses with target muscles. A later study by Deshpande et al. (2006) administered ESC-derived motor neurons along with phosphodiesterase type 4 inhibitor and dibutyryl cyclic AMP (dbcAMP) to overcome myelin-mediated repulsion into paralyzed adult rats. In addition, the researchers applied a focal attractant, glial cell-derived neurotrophic factor (GDNF), within the peripheral nervous system (PNS) to direct the transplanted embryonic stem cell-derived axons toward skeletal muscle targets. This well-designed study showed that transplant-derived axons reached muscle, formed neuromuscular junctions, were physiologically active and mediated partial recovery from paralysis. In animal models of ALS, several studies showed that transplantation of cells derived from the human teratocarcinoma cell line or human umbilical cord blood resulted in beneficial effects. However, neuroprotection was suggested to be the main cause of the observed benefits rather than the direct motor neuron replacement (Ende et al. 2000; Garbuzova-Davis et al. 2002, 2003).

4.1.2 Mesenchymal Stem Cell Transplantation

Several recent studies report the generation of dopaminergic neurons from BM-derived MSCs. Woodbury et al. (2002) reported that rat MSCs might be induced to differentiate into neuron-like cells expressing neurotransmission-related genes. Moreover, a small portion of the differentiated cells expressed tyrosine hydroxylase (TH), a rate-limiting enzyme in the synthesis of dopamine.

Hermann et al. (2004) showed that human MSCs could be converted into a clonogenic neural stem cell-like population growing in neurosphere-like structures. In addition, following neuronal differentiation, these cells demonstrated dopamine production and potassium-dependent release. Our laboratory demonstrated that following induced neuronal differentiation, human MSCs displayed neuron-like morphology, neuronal markers, and transcription factors that characterize midbrain dopaminergic neurons and secreted dopamine in response to depolarization (Blondheim et al. 2006; I. Kan, Y. Barhum, T. Ben-Zur, T. Charlow, Y. Levy, A. Burstein, B. Bulvik, E. Melamed, and D. Offen, submitted).

Schwarz et al. (1999, 2001) utilized rat MSCs, genetically engineered by transgene to express human TH and guanosine triphosphate cyclohydrolase I (GTPCH), an enzyme required for the biosynthesis of tetrahydropterin cofactor for TH (BH4). These cells released L-3,4-dihydroxyphenylalanine (L-DOPA), a precursor of dopamine, and induced behavioral recovery following the transplantation into the rat model of PD. However, the ameliorative effect of the transplanted rat MSCs was short-lived (up to 9 days), presumably due to inactivation of the transgenes. Recently, Dezawa et al. (2004) demonstrated a highly efficient and specific induction of rat and human MSCs using gene transfer of Notch intracellular domain (NICD) and subsequent treatment with basic fibroblast growth factor (bFGF), forskolin, ciliary neurotrophic factor (CNF), brain-derived growth factor (BDNF) and nerve growth factor (NGF). Following the transfection, MSCs expressed neural stem cell markers such as microtubule-associated protein 2 (MAP-2), neurofilament M (NF-M) and β-tubulin III. Following subsequent trophic factor administration, action potentials, compatible with characteristics of functional neurons, were recorded. Further treatment of the induced neuronal cells with GDNF increased the proportion of TH-positive and dopamine-producing cells. Transplantation of these GDNF-treated cells showed functional improvement when grafted into a 6-hydroxydopamine (6-OHDA) rat model of PD. Li et al. (2001) showed in vivo differentiation of mouse MSCs, prelabeled with bromo-deoxyuridine (BrdU) and grafted into the striatum of the MPTP mouse model of PD. The transplanted mice exhibited significant motor improvement 35 days after transplantation. At least 4 weeks after the transplantation BrdU-reactive cells were revealed in the grafted mice striatum and approximately 0.8% of these cells expressed TH. These studies show that engraftment of MSCs, naive or following in vitro induction, may result in a significant clinical improvement in animal models of PD. However, whether or not cell replacement underlies the recovery mechanism has yet to be determined.

The evidence for cholinergic-induced differentiation of MSCs is limited. As mentioned, Woodbury et al. (2002) showed that MSCs were induced into neuron-like cells and expressed neurotransmitter-related genes. Precise analysis showed that a large population of MSC-derived neurons expressed choline acetyltransferase (ChAT), which catalyzes the synthesis of the excitatory trans-

mitter acetylcholine and a smaller subpopulation expressed TH. Chen et al. (2006) demonstrated that the α-secretase-cleaved fragment of the amyloid precursor protein (sAPPα), a potent neurotrophic factor, potentiates the NGF/retinoic acid (RA)-induced transdifferentiation of MAPCs into neural progenitor cells and, more specifically, enhances their terminal differentiation into a cholinergic-like neuronal phenotype. In addition, after the intravenous transplantation of sAPPα-transfected MAPCs into transgenic PS/APP mice, sAPPα-immunopositive MAPCs were identified within the septohippocampal system and found in close proximity to the cerebral vasculature.

In ALS, Mazzini et al. (2003, 2004) have evaluated the feasibility and safety of intraspinal cord implantation of autologous MSCs in 7 patients affected by ALS. Following in vitro expansion, the cells were suspended in the autologous cerebrospinal fluid and directly injected into the surgically exposed spinal cord at the T7–T9 levels. No patients manifested severe adverse effects and the researchers noticed a significant slowing down in the linear decline of the forced vital capacity of 4 out of 7 patients 24 months after transplantation of MSCs. This effect was correlated with the number of implanted cells. To date, the specific replacement of motor neurons poses a formidable challenge that might require multisegmental delivery to sites of need, reestablishment of appropriate afferent innervations, and the long-distance extension of their axons through often degenerating nerve roots to specific loci in the distant musculature. Our poor understanding of the biology underlying these processes suggests that despite the recent progress described in generating and isolating motor neurons, cell replacement treatment of the motor neuronopathies remains a difficult goal. Clement et al. (2003) showed that in SOD1G93A chimeric mice, motor neuron degeneration requires damage from mutant SOD1 acting in nonneuronal cells. Wild-type nonneuronal (glial) cells could delay degeneration and extend survival of mutant-expressing motor neurons. This theory implies that replacing the degenerated neurons might be insufficient and the administration of supportive nonneuronal cells might be required. These nonneuronal cells might protect the grafted cells from the progressive damage and might even prolong the survival of endogenous degenerating neural cells.

4.2 Trophic Factor Delivery

Using stem cells to generate new neurons and replace those lost in neurodegenerative diseases would be a major breakthrough. A more practical short-term approach may be to use stem cells to protect neurons dying in these diseases.

4.2.1 The Concept of Trophic Factor Delivery

Trophic factors are believed to have the capability of providing neuroprotective or restorative effects in PD. Collier and Sortwell (1999) summarized the effects of 29 molecules that promote survival and growth of dopaminergic neurons. These molecules are either naturally occurring factors or synthetic molecules that access endogenous receptors. The authors concluded that the single greatest challenge to neurotrophic therapies is the development of techniques for targeted delivery of these potential materials. Among these factors GDNF, a powerful neuroprotective agent, became the subject of extensive studies. Its neuroprotective properties coupled to its reduced levels in the basal ganglia in PD (Chauhan et al. 2001) made it the first trophic factor to be tested in PD patients. The research of Nutt et al. (2003) revealed that the drug was not efficacious when given via an intracerebroventricular catheter. Researchers speculated that GDNF did not reach the target tissues—namely putamen and substantia nigra—and therefore did not improve Parkinsonism. In later trials GDNF was administered directly into the site of greatest dopamine loss in PD—the posterior putamen. In a small open-label study GDNF delivery showed clear positive clinical effects, with evidence of dopaminergic-fiber sprouting at the site of GDNF delivery, using both fluorine-18-labeled-dopa positron emission tomography (PET) scanning and postmortem analysis of a single case (Love et al. 2005; Gill et al. 2003). By contrast, Lang and colleagues' (2006) double-blind, placebo-controlled trial showed no beneficial clinical effect. In this study, 34 patients with moderately advanced PD were randomly assigned intraputaminal placebo or GDNF, in equal numbers, with the primary endpoint being the unified PD rating scale (UPDRS) "off" motor score at 6 months. No significant difference was seen in this or any other measures, apart from ^{18}F-dopa scanning of the posterior putamen, but not of the whole striatum. Additionally, device-related adverse events (infection and catheter misplacement) and anti-GDNF antibodies were reported in 3 patients. However, the change in the outcome of open-label and double-blind, placebo-controlled studies should not discourage further research but set a challenge to researchers to improve and refine the GDNF delivery mode. Other neurotrophic factors that have been extensively studied in PD are neurturin (NTN), BDNF, and sonic hedgehog (SHH). Several studies showed the protective effect of NTN, a trophic factor of the GDNF family of ligands, on dopaminergic neurons in substantia nigra (Fjord-Larsen et al. 2005; Li et al. 2003; Oiwa et al. 2002). These neuroprotective effects have led to the initiation of a phase I clinical study of NTN delivery via an adeno-associated viral vector delivery system. SHH has also displayed neuroprotective effects protecting nigral dopaminergic neurons from 6-OHDA-induced toxicity following adenoviral delivery (Dass et al. 2005; Hurtado-Lorenzo et al. 2004). BDNF, however, failed to protect dopaminergic neurons from 6-OHDA-induced death but did block

the amphetamine-induced, ipsiversive, turning-behavior caused by the partial unilateral lesion of the nigrostriatal pathway (Klein et al. 1999). Researchers suggested that exogenous BDNF exerts a modulatory influence on the remaining dopaminergic or other types of neurons, perhaps leading to an enhanced release of dopamine in the striatum.

In AD, the influence of NGF, which specifically targets basal forebrain cholinergic neurons, nociceptive dorsal root ganglion neurons, and some third-order sympathetic neurons (Levi-Montalcini 1987) has been the subject of extensive research. Various studies showed that NGF increases the synthesis of ChAT and prevents basal forebrain cholinergic neurons atrophy caused by experimental injury or associated with physiological aging, suggesting that NGF might reduce cholinergic cell loss in AD (Hefti et al. 1984; Fischer et al. 1987; Koliatsos et al. 1993; Tuszynski et al. 1991; Markowska et al. 1994, 1996). However, development of an effective NGF delivery mode to AD patients encounters several challenges. First, NGF does not cross the blood–brain barrier when administered peripherally. Second, adverse side effects were revealed following direct infusion of NGF into the brain ventricular system (Tuszynski 2002). Consequently, several alternative methods have been proposed for NGF delivery. Tuszynski et al. (2005) showed that autologous fibroblasts, obtained from small skin biopsies, genetically modified to produce and secrete human NGF, ameliorated cognitive deficits of AD patients after stereotaxic injections. Capsoni et al. (2002) proposed another way of NGF administration—intranasal delivery. By using the intranasal route of administration, this group showed that NGF could rescue all the histological hallmarks characterizing the AD-like neurodegeneration in AD11 mice.

In ALS, several growth factors have been proposed and tested. In a transgenic mouse model of ALS, intrathecal infusion of insulin-like growth factor (IGF)-1 improved motor performance, delayed the onset of clinical disease, and extended survival (Nagano et al. 2005b). In order to evaluate the potential benefits of intrathecal IGF-1 administration in ALS patients a double-blind clinical trial was performed. However, only a modest beneficial effect was recorded (Nagano et al. 2005a). Beck et al. (2005) explored the effects of intrathecal administered BDNF on autonomic functions in patients with ALS. Unfortunately, the group concluded that autonomic nervous system function deteriorated along with motor performance independently from treatment with BDNF. In another study, human neural progenitor cells (hNPC) were isolated from the cortex, expanded in culture, and modified using lentivirus to secrete GDNF. These cells were transplanted into the lumbar spinal cord of a rat model of ALS. The cells survived up to 11 weeks following transplantation, integrated into both gray and white matter, and secreted GDNF within the region of cell survival, but not outside this area. However, no positive clinical effects were recorded (Klein et al. 2005). Additional clinical trials in ALS are being carried by a Chinese neurosurgeon Huang Hongyun. The procedure includes isolation of olfactory ensheathing cells (OECs) from the glomerular layer of olfactory bulbs inside

the noses of aborted fetuses, and in vitro expansion and injection into the atrophied area of the frontal lobes. The group claimed that this procedure could stabilize ALS in about half the patients treated. The beneficial effects were assigned to the secretion of growth factors, which encourage the regeneration of nerve axons, rather than to the cell replacement (Watts 2005). To date no double-blind, controlled study has been performed that can verify these results. As a final point, the safety issues of these procedures need to be addressed.

4.2.2 Mesenchymal Stem Cell Transplantation

Several recent studies suggest that MSC effectiveness may be credited to production of neurotrophic factors that support neuronal cell survival, induce endogenous cell proliferation, and promote nerve fiber regeneration at sites of injury (Li et al. 2002; Mahmood et al. 2004). Indeed, Crigler et al. (2006) demonstrated that MSCs express a variety of neuro-regulatory proteins in addition to BDNF and β-NGF, which promote survival and induce neurite formation in neuroblastoma cells and primary nerves from the lumbar spine. In addition, Arnhold et al. (2006) showed that naive MSCs express BDNF, NGF, and GDNF when cultivated in standard medium and elevate the expression levels after exposure to neural precursor selection medium or to differentiation medium. Synthesis and release of growth factors by the grafted cells or indirect stimulation of neurotrophic release from the host tissue may be in part accountable for the functional recovery induced by MSC transplantation. Moreover, MSCs may be well suited as vehicles for treating neurological deficits due to their propensity to home to sites of tissue injury (Ji et al. 2004), which may target neuroprotective agents to brain or spinal cord lesions. Part of the recovery mechanism may include elevated neurogenesis. Chopp and Li (2002) showed that the presence of MSCs promoted induction and migration of new cells from a primary source within the ventricular zone and the choroid plexus into the injured brain. The authors claim that these cells may contribute to functional repair after stroke, although the relation of the induction of neurogenesis and the migration of these cells to the restoration of function has not been directly tested. A recent study of Rivera et al. (2006) revealed that the interactions between adult MSCs and NSCs in vitro, mediated by soluble factors, induce oligodendrogenic fate decision in NSCs at the expense of astrogenesis. Thus, trophic factor delivery and/or elevated neurogenesis might underlie the beneficial effects of MSC transplantation in neurodegenerative diseases.

4.3 Immunomodulation

4.3.1 The Concept of Immunomodulation

Recent evidence clearly indicates that neuroinflammatory mechanisms may play an important role in the pathogenesis of PD (Hunot and Hirsch 2003; McGeer and McGeer 2004; Marchetti et al. 2005). Under normal conditions, astrocytes exert a fundamental protective function against oxidative stress, and the interaction between astrocytes and neurons has been variously demonstrated to exert striking neurotrophic, differentiation, and neuroprotective effects (Takeshima et al. 1994; McNaught and Jenner 1999; Marchetti et al. 2005). However, under conditions of chronic inflammatory stress, activated astrocytes and microglia may become dysfunctional and overexpress a variety of cytotoxic mediators eventually resulting in dopaminergic neuron death (Morale et al. 2006). Indeed, studies accumulated over the last two decades have clearly indicated the activation of microglia and astroglia in the nigrostriatal system of PD patients and animal models of PD (Hunot and Hirsch 2003; McGeer and McGeer 2004). Activated microglia release proinflammatory and neurotoxic factors that are thought to contribute to neuronal damage. These factors include proinflammatory cytokines such as tumor necrosis factor α (TNF-α) and interleukin 1 (IL-1), reactive nitrogen species, proteases, reactive oxygen species (ROS), eicosanoids, and excitatory amino acids (Liu and Hong 2003; Merrill and Benveniste 1996). The inflammatory process, induced by proinflammatory agents, can result in the degeneration of dopamine-containing neurons and may be an important contributor to the neuronal loss in PD. Moreover, the experimental evidence that inhibition of the inflammatory process correlates with less neuronal impairment supports the notion that inflammation plays a deleterious role in the neurodegeneration in PD. Thus, inflammatory inhibition might become a promising therapeutic intervention for PD (Gao et al. 2003).

Activated astrocytes and microglia (gliosis) have been documented in studies of AD (Giulian et al. 1995; Sasaki et al. 1997). As mentioned already, when activated, astrocytes and microglia produce several proinflammatory signal molecules, including cytokines, growth factors, complement molecules, and adhesion molecules. Of particular interest in AD are the cytokines S100β, which is mainly produced by astrocytes, and IL-10, which is mainly produced by activated microglia. A review article by Griffin (2006) showed that inflammation is a driving force in the neuropathology of AD, mediated by proinflammatory cytokines and creating a chronic and self-sustaining inflammatory interaction between activated microglia and astrocytes, stressed neurons and β-amyloid plaques. The author brings evidence that the key initiating factor appears to be overexpression of IL-1, which may be a result of any number of events, including

disease, trauma, genetic polymorphisms, or simply age-related wearing away. Through various pathways, IL-1 overexpression causes neuronal death, which activates more microglia, which in turn release more IL-1 in a self-amplifying fashion. Over the years, this inflammation destroys sufficient neurons to cause the clinical signs of AD. Interestingly, nearly all the cytokines and chemokines that have been studied in AD, including IL-1β, IL-6, TNF-α, IL-8, transforming growth factor β (TGF-β), and macrophage inflammatory protein-1α (MIP-1α) seem to be upregulated in AD compared with control individuals (Akiyama et al. 2000). Epidemiological studies have documented a beneficial effect of nonsteroidal antiinflammatory drugs (NSAIDs) in AD (McGeer et al. 1996; Stewart et al. 1997; Akiyama et al. 2000; In 't Veld et al. 2001; Szekely et al. 2004). Long-term NSAID therapy delayed the onset and the progression of the disease, reduced symptomatic severity, and significantly slowed the rate of cognitive impairment (Rich et al. 1995). The putative target of NSAID actions is thought to be microglia associated with the senile plaques (Sastre et al. 2006).

The evidence for involvement of microglial cells in ALS pathology is abundant and very strong. In a review of the inflammatory processes in ALS, McGeer and McGeer (2002) presented extensive evidence that activated microglia, astrocytes, and infiltrating leukocytes are present in affected tissues in ALS. These investigators noted that overreactive innate immune defenses, where microglia play a major part, could generate a neuroinflammatory environment that is harmful to the viable host tissue. This process, known as autotoxicity, could evolve from a persistent stimulation of microglia. Accumulation of microglia-activating signals next to the injured neurons in ALS could lead to the initiation of autotoxicity. The Sargsyan et al. (2005) review brings evidence that microglia are the likely glial cell type responsible for the secretion of microglia-activating signals and for propagation of glial activation and inflammation in ALS. Activated microglia and factors secreted by these cells appear to play a direct role in ALS pathology, although it is still unclear whether they contribute as initiators, propagators, or both initiators and propagators of motor neuron injury. Further evidence of microglial involvement in ALS pathology comes from studies with pharmacological suppression of microglial activation using antibiotics, such as minocycline, which has been reported to reduce or suppress microglial activation after neuronal injury (Kriz et al. 2002; Zhu et al. 2002; van den Bosch et al. 2002; Tikka et al. 2002; Zhang et al. 2003). These results led to phase I/II studies of minocycline in ALS patients (Gordon et al. 2004). Although no difference was observed between treated and untreated groups in these studies, pivotal phase III trials are ongoing. Cyclooxygenase-2 (COX-2) is a key molecule in the inflammatory pathway in ALS (Kiaei et al. 2005). Celecoxib (Celebrex) and rofecoxib are inhibitors of COX-2. Treatment with these COX-2 inhibitors combined with creatine increased survival by up to 30% in SOD1 mutant mice (Drachman et al. 2002; Pompl et al. 2003; Klivenyi et al. 2004). However, to date none of the COX inhibitors tested has shown efficacy in human ALS patients (Moisse and Strong 2006).

It seems that the brain's immune response during the neurodegenerative disease is a double-edged sword, simultaneously beneficial and detrimental. Inflammation seems to start as a time- and site-specific defense mechanism aimed at eliminating irreversibly damaged neurons and at favoring survival of cells that retain a chance for recovery. However, at later stages, inflammation can evolve as an uncontrolled chronic reaction. Therefore, inhibiting chronic inflammatory stress and persistent microglial stimulation would be an effective therapeutic approach to slow the progression of neurodegenerative diseases.

4.3.2 Mesenchymal Stem Cell Transplantation

Several studies have reported the in vitro and in vivo immunosuppressive properties of BM-derived MSCs (Bartholomew et al. 2002; Djouad et al. 2003; Le Blanc 2003; Zappia et al. 2005; Corcione et al. 2006). It is reasoned that the transplanted MSCs face a foreign, inflammatory environment and may induce immunomodulating processes that limit local inflammation in order to enhance their survival. The underling mechanism of action may include the secretion of soluble factors that create an immunosuppressive milieu (Ryan et al. 2005). An additional mechanism may be a reduction in infiltration of blood-borne inflammatory cells. This mechanism of action has been associated with the beneficial effect of MSC transplantation in the animal model of multiple sclerosis (MS) (Zhang et al. 2005). The proposed processes may profoundly change the inflammatory environment and promote partial recovery. However, additional studies are needed to determine the precise influence of MSC transplantation on innate chronic inflammation.

5 Conclusions

Several studies have demonstrated the neuroprotective and neuroregenerative effects that were associated with functional improvements following MSC transplantation. Functional recovery may be due to the replacement of degenerated neurons, neurotrophic factors delivery, and/or immunomodulation of the ongoing inflammatory reaction. These beneficial effects and the lack of ethical issues or immune rejections involved in autologous transplantation of MSCs have drawn the attention of biomedical research to this therapeutic approach. There are, however, many unresolved questions and problems regarding the safety and the feasibility of this approach. These issues are not obstacles but challenges to overcome in order to develop a permanent cure for neurodegenerative diseases.

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