

Chapter 6

Bone marrow stem cells: possible source for cell therapy in Parkinson's disease

Y S Levy, S Bulvik, A Burshtein, Y Barhum, E Melamed, D Offen

INTRODUCTION

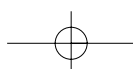
Clinical trials with transplantation of human embryonic mesencephalic tissue into the caudate and putamen (striatum) of Parkinson's disease (PD) patients were initiated in 1987. About 350 patients have been operated upon since then.¹ At that time, it was not known whether neuronal replacement could be effective in the diseased human brain. The main objective of scientific efforts in the past 15 years have been to provide proof-of-principle that: the grafted dopamine neurons can survive and form connections in the PD patient's brain; the patient's brain can integrate and use the grafted neurons; and the grafts can induce a measurable clinical improvement.² Dopaminergic neurons isolated from human fetuses were transplanted to the striatum of PD patients, on one or both sides. The researchers who conducted the open trials in several medical centers reported on significant clinical benefits and demonstrated that the transplanted cells survived for years and produced dopamine.^{1,2} However, practical and ethical issues such as the need for up to eight fetuses to provide sufficient numbers of dopaminergic neurons for one PD patients limited this specific treatment.³⁻⁵ Recent reports on double-blind controlled trails of fetal nigral

transplantation raised serious questions on the safety and the efficacy of this procedure. They reported that improvement was not significant in most of the patients, while high percentages of the treated patients developed severe uncontrolled movements (tardive dyskinesia).⁶⁻⁷ Moreover, Olanow's team found some evidence to suggest that an immunological reaction was destroying or disabling the tissue grafts.⁷

These observations add significantly to the other practical and ethical problematic issues concerning the use of up to eight human fetuses to provide sufficient numbers of dopaminergic neurons for one PD patient.^{1,2,6,7} However, the challenge of cell replacement in PD is great, and finding the best cell source is a high priority. Advanced methods for isolating stem cells, the progenitors of all body tissues, have increased the expectation of these cells to provide an unlimited source that might be induced to differentiate into mature and functional dopaminergic cells.

BONE MARROW STROMAL CELLS

The use of cells originating from the same patient for autologous transplantation avoids



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the introduction of foreign material and reduces the possible rejection complications and the need for immunosuppression. This autologous transplantation strategy by-passes many ethical, technical and logistical issues. In recent years, there has been an increasing interest in adult bone marrow-derived stromal stem cells that support hematopoiesis. These mesenchymal stem cells differentiate into connective tissue, muscle, bone, cartilage and fat cells.⁸⁻¹⁰ Evidence has accumulated that human, rat and mouse bone marrow stromal cells (BMSc) can also be induced to differentiate into neuron-like cells in culture.¹¹⁻¹⁸ Following induction, most (up to 80%) stromal cells may exhibit neuronal phenotypes. Moreover, it has been shown that the differentiated cells express neuronal protein markers such as neuron-specific enolase (NSE), neural nuclei protein (NeuN), neurofilament-M, and trkA. Other experiments with rodents demonstrated that transplanted bone marrow-derived cells might migrate into various brain regions and develop neuron-like features.¹⁹⁻²⁶ Furthermore, Mezey and co-investigators found Y-chromosomes in the human brains of females following transplantation of male bone marrow.²⁷ Donor cells were found in several brain regions, especially in the hippocampus and cerebral cortex. However, other researchers claim that bone-to-brain transdifferentiation may not be a general phenomenon but may reflect fusion with neurons or transient expression of many proteins, including neuronal markers.²⁸⁻³²

The evidence for differentiation of BMSc into dopaminergic neuron-like cells is limited. However, genes for human tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine biosynthesis, and GTP cyclohydrolase I (GC), the enzyme providing the tetrahydropterin (BH₄), cofactor for TH, were introduced into rat BMSc.^{33,34} The engineered rat BMSc indeed synthesized and released 3,4,-dihydroxy-

phenylalanine (L-dopa). When these rat BMSc were transplanted into the routinely used PD model, the striatum of 6-hydroxydopamine unilaterally lesioned rats, the synthesized L-dopa was converted into dopamine metabolites, and behavioral recovery was observed. However, the ameliorative effect of the modified BMSc transplantation was short-lived (up to 7 days), presumably owing to inactivation of transgenes introduced into the brain with retroviruses.

Woodbury and co-investigators developed a method for inducing rat BMSc to differentiate into neuron-like cells that express genes associated with neurotransmission.¹³ Rat BMSc maintained in the induction medium for 10 days expressed tau in levels that correlated with the degree of neuronal morphological differentiation. β -Tubulin III, an intermediate filament characteristic of mature neurons, was present in virtually all cells. Analysis by reverse transcriptase-polymerase chain reaction (RT-PCR) indicated that synaptophysin mRNA, which is associated with synaptic vesicles and transmission, was not present in undifferentiated BMSc but was detected after 24 h of neuronal differentiation and continued to increase thereafter. The synaptophysin protein was detected in cell bodies as well as in varicose, putative transmitter-release sites along processes. Moreover, at 10 days of rat BMSc differentiation, a large population of the neuron-like cells expressed choline acetyltransferase (ChAT), which catalyzes the synthesis of the excitatory transmitter acetylcholine. A smaller subpopulation of rat BMSc-derived neuron-like structures were reported to express TH. Nevertheless, Woodbury's group did not report on dopamine production or synthesis of other catecholamine neurotransmitters.

The search for a therapeutic potential of BMSc for the treatment of PD was stimulated by a publication from Li *et al.* in 2001.²⁰ Mouse

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BMSc prelabeled with bromodeoxyuridine (BrdU) were grafted into the striatum of the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease. The grafted MPTP-treated mice exhibited a significant improvement on the rotarod test at 35 days after transplant, compared to nongrafted controls. Immunohistochemistry revealed BrdU-reactive cells in the striatum of the grafted MPTP-treated mice at least 4 weeks after transplantation. Double staining showed that only 0.8% of BrdU-reactive cells expressed TH. Although the injected mouse BMSc survived, expressed TH and promoted some functional recovery, much more work is required to understand the mechanism of recovery. It is not known whether the grafted cells increase production of dopamine or whether other processes, such as the secretion of neurotrophic factors by the marrow-derived cells, mediate the improvement in motor function.

Park and co-investigators introduced the glial cell line-derived neurotrophic factor (GDNF) gene into the mouse bone marrow cells, injected the cells intravenously and found GDNF-expressing cells within the brain parenchyma.³⁵ Furthermore, this *ex-vivo* gene transfer strategy, performed six weeks prior to exposure to the dopaminergic neurotoxin, MPTP, provided protection of nigral neurons and their striatal terminals.

In our laboratory we have demonstrated that human BMSc might change their designations following induction in culture. The differentiation of human BMSc into neuron-like cells was associated with dramatic morphological changes. Before treatment, human BMSc displayed a flat, fibroblastic morphology (Figure 6.1a), whereas after 24 h of treatment the cells were rounded, exhibited highly retractile cell bodies and displayed prominent process-like extensions (Figure 6.1b). The neuron-like morphology of the cells was retained up to 26

days of culture (Figure 6.1c). The structural changes were accompanied by the expression of the tissue-specific neuronal marker, Neu-N, as indicated by nuclear immunostaining (Figure 6.1d). We also demonstrated, using RT-PCR methods, that the differentiated human BMSc expressed Nurr1, the transcription factor regulator of the midbrain dopamine neuron. Moreover, the dopamine-related genes; dopa-decarboxylase (*DDC*), D2 dopamine receptor (*D2DR*) and dopamine transporter (*DAT*) were increased during the induction of differentiation (Figure 6.2).

MULTIPOTENT ADULT PROGENITOR CELLS

Rare cells, termed the multipotent adult progenitor cell (MAPCs), were isolated from human and rat bone marrow mesenchymal stem cultures.³⁶⁻⁴¹ These cells can be expanded for more than 120 population doublings and differentiate into mesenchymal, endothelium^{28,29} and endoderm lineages.⁴⁰ It was also shown that mouse MAPCs injected into the blastocyst contributed to most, if not all, somatic cell lineages including the brain, similar to embryonic stem (ES) cells.⁴¹ Within the brain, region-specific appropriate differentiation occurred.⁴¹

Jiang and co-investigators cultured rodent MAPCs sequentially with basic fibroblast growth factor (bFGF) for 7 days, FGF-8 for 7 days and brain-derived neurotrophic factor (BDNF) for 7 days.³⁶ They demonstrated that the cells became polarized and expressed tTau and microtubule-associated protein 2 (MAP2). Moreover, 30% of cells expressed markers of dopaminergic neurons, DDC and TH, 20% of serotonergic neurons and 50% were γ -aminobutyric acid (GABA)-ergic neurons. A particularly useful approach would be if the

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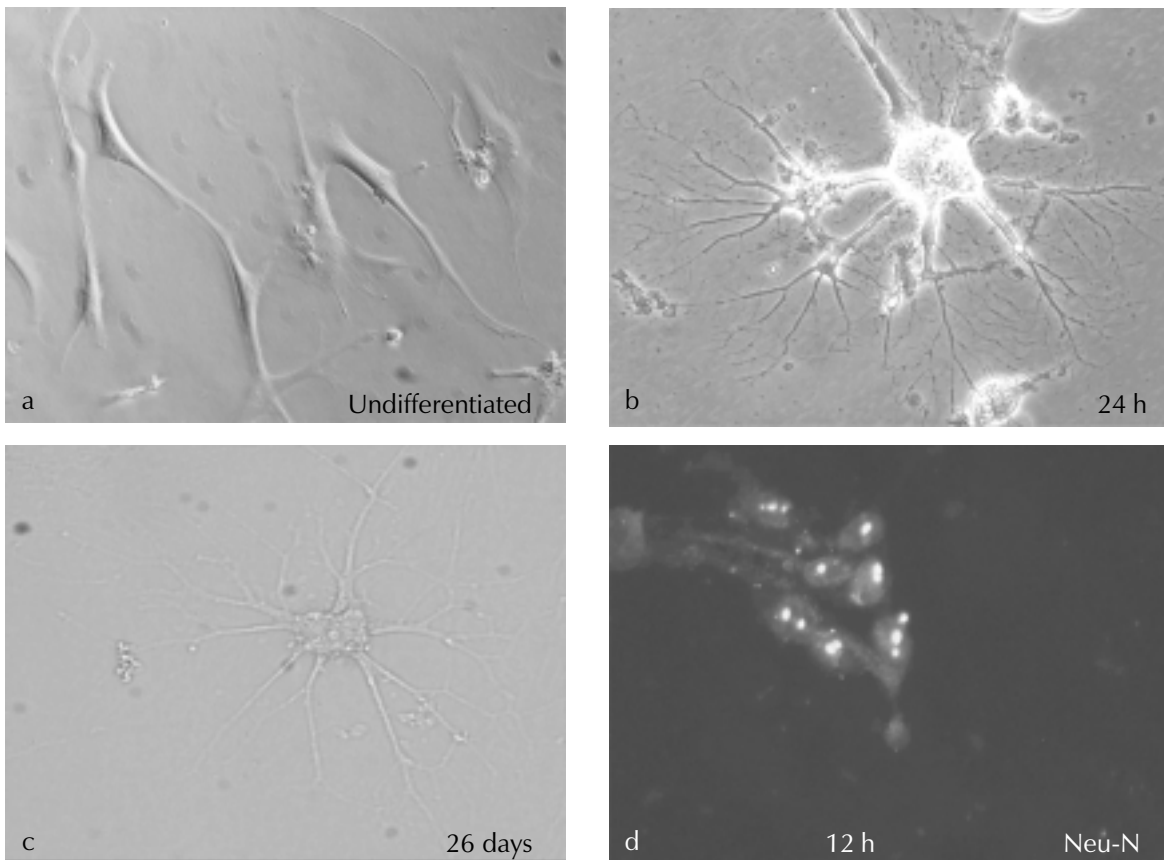


Figure 6.1 Formation of neural tissues by human bone marrow stromal cells (hBMSc). (a) hBMSc isolated from bone aspirate and grown as a sub-confluent monolayer. (b) Cultured hBMSc incubated in a combination of media supplements that induced significant morphological changes. During the first hours of exposure to induction medium, the cytoplasm in the adherent cells started to condense and retracted towards the nucleus, creating a spherical structure, and developed branches. (c) After 26 days of differentiation, hBMSc formed cells displaying a range of neuron-like morphologies. (d) Immunocytochemistry analysis confirms expression of nuclear nuclei (Neu N) protein after 12 h of differentiation

MAPCs could be administered systemically and could then find their way to the damaged central nervous system (CNS) region, where they would adopt the phenotype of the missing neuron. However, no significant engraftment of mouse MAPCs was seen in the brain after intravenous infusion, and rare donor cells found in the brain did not co-label with neuroectodermal markers.³⁶

Recently, Jiang and co-investigators reported that, similar to mouse ES cells, mouse MAPCs could be induced to differentiate *in vitro* into cells with biochemical, anatomical and electrophysiological characteristics of midbrain neuronal cells.⁴² Mouse MAPCs were cultured sequentially for 7 days with BFGF, FGF8 plus sonic hedgehog (SHH) and BDNF. They found that 23% of the cells were positive to nestin, a

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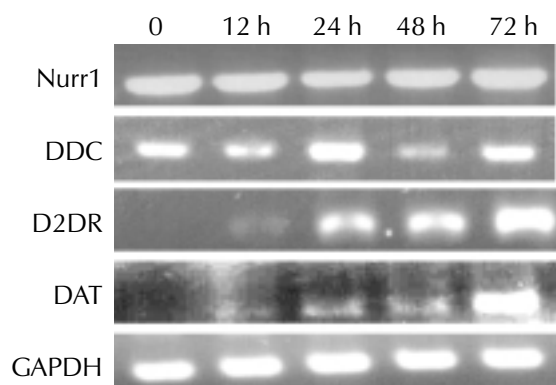


Figure 6.2 Differentiated human bone marrow stromal cells express genes associated with the dopaminergic lineage. mRNA isolated after 12–72 h of differentiation were analyzed by polymerase chain reaction with the primers of the genes found in dopaminergic neurons: nuclear receptor related 1 (Nurr1), dopa decarboxylase (DDC), D2 dopamine receptor (D2DR), dopamine transporter (DAT) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) a housekeeping gene used as a positive control

marker for neuronal progenitor, and expressed nuclear receptor related 1 (Nurr1), the key transcription factor for dopaminergic neuron development. Quantitative RT-PCR demonstrated that, on days 10 and 14, levels of GABA, TH and tryptophan hydroxylase (TPH) increased up to 120-fold. Immunophenotypic analysis on day 21 showed that 25% of cells expressed markers of dopaminergic neurons (DDC and TH), 18% expressed markers of serotonergic (TPH) neurons, and 52% expressed markers of GABAergic neurons. Double immunohistochemistry showed that GABA, TPH and TH were never detected in the same cell. They also demonstrated that mouse MAPC-derived neuron-like cells cultured in the presence of fetal brain astrocytes demonstrated much more mature neural morphology with more elaborate array axons.⁴²

FUTURE STRATEGIES

In establishing stem cells as an alternative graft source, logistical, ethical and political issues need to be resolved. There is disagreement over the feasibility of 'adult' stem cells compared with ES cells. Adult stem cells might be capable of developing into only a limited number of cell types as compared to ES cells. However, ES cells could retain their mitotic ability after transplantation, which could give rise to tumors. Furthermore, ethical concerns surrounding the use of fetal tissues and ES cells will not apply to adult stem cells.⁴³ Thus, safety and efficacy issues on the use of stem cells include the following questions: Do they maintain long-term stable neuronal phenotypes crucial for rescuing the degenerating brain? Are transplanted stem cells functional as a dopaminergic neuron and thus able to provide beneficial effects?

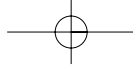
It seems clear that there is an urgent need for more basic research if the field is to progress beyond the level of clinical phenomenology. There are three main challenges. First, it will be necessary to learn much more about neuronal development, in order to define cell types that can be cultured in sufficient quantities and that can adopt appropriate fates when transplanted to different sites *in vivo*. Second, it will be necessary to establish better animal models – perhaps including genetically modified primates – in order to perform more realistic tests of cognitive recovery after transplantation. Third, it will be important to develop methods for testing whether transplanted neurons can become functionally integrated into brain circuitry: in other words, whether they can actually contribute to the restoration of normal information processing in the damaged brain. This will require the identification and electrophysiological characterization of transplanted neurons *in vivo*.

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