Bone marrow-derived stem cells in neurological diseases: stones or masons?

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In spite of the commonly held belief that 'the brain does not regenerate', it is now accepted that postnatal neurogenesis does occur. Thus, one wonders whether cellular-replacement therapy might be used to heal the brain in diseases caused by neuronal cell loss. The existence of neural stem cells has been demonstrated by many scientists and is now generally accepted. The exact role of these cells, how their numbers are regulated and how they participate in CNS and spinal cord regeneration in postnatal life are still not well known. There are many reviews summarizing work on these cells; consequently, I will focus instead on other cells that may participate in postnatal neurogenesis: bone marrow-derived stem cells. The possibility that bone marrow-derived stem cells populate the CNS and differentiate into various neural elements is certainly not universally accepted.

Neural stem cells are thought to be responsible for postnatal (adult) neurogenesis [1,2]. A far less accepted concept is the possibility that other cells that normally do not reside in the brain might also play a role in generating neural cells in certain circumstances. The most studied of these cells are those deriving from the bone marrow. There are three major populations of stem cells in the bone marrow (BM): endothelial, hematopoetic (HSC) and stromal (or mesenchymal [MSC]) cells. HSCs were the first stem cells used in humans to reconstitute the blood of patients who lacked viable stem cells or whose BM had been ablated to treat malignant diseases. In fact, administration of HSCs as an adjunct to cancer chemotherapy and radiation treatment has been used for many decades. It is remarkable that only a few HSCs are required to reconstitute the blood of patients or animals.

However, the MSC population is a subset of BM stromal cells that can regenerate bone, cartilage, myelosupportive stroma and adipocytes. While methods have been developed for purification of murine HSCs, prospective isolation of MSCs has yet to be reported. Consequently, it must be recognized that studies using BMSCs are, in fact, using a heterogeneous population of cells even if clonally derived; approximately 10–20% are multipotent, with the remaining representing more committed cell types [3]. In 1997, we suggested that circulating cells originating in the BM might enter the brain and differentiate into microglia and astrocytes in rodents [4]. Microglia have long been thought to be macrophage-like cells in the brain, therefore the idea that they could arise in the BM was not too radical. Macroglial cells (including astrocytes), however, were thought to derive from the neuroectoderm, just like other neural cells (oligodendrocytes and neurons). Thus, the demonstration that circulating BM cells (BMCs) might generate astrocytes clashed with accepted wisdom in the field of developmental biology and the work was not recognized. The possibility that BM-derived stem cells (BMSCs) might enter the brain and differentiate into neural cells received attention again when, in the summer of 2000 two independent groups demonstrated that BMSCs from both humans and rodents could differentiate *in vitro* into cells positive for neuronal markers [5,6]. In the same year, two additional groups reported a similar phenomenon *in vivo* in the mouse [7,8]. Two independent groups later confirmed these findings in the human, and Cogle and colleagues concluded that the donor-derived neuronal cells are not the result of fusion [9,10]. The debate initiated by these papers continues today. Although few people dispute the fact that BM derived stromal cells can adopt a neural phenotype, several groups failed to detect differentiation of HSCs in the CNS [11,12]. The techniques (e.g., tracking technique, animals, transplantation, surgeries) used by various groups to pursue these questions have differed and laboratory-to-laboratory inconsistencies are prevalent. Although the possibility of cell fusion (as opposed to transdifferentiation) was raised [13–15], it can certainly not account for the majority of the findings. In either case, due to the difficulty of studying neuronal function in these cells, the physiological importance of these observation and the mechanisms underlying it are still

Keywords: adult stem cells, bone marrow derived cells, cell therapy, neurological diseases, neuroprotection

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Table 1. Adult bone marrow-derived cells in ischemic brain disease

Experiment	Evaluation	Findings	Ref.
The authors gave GCSF/SCF to GFP chimeric mice after focal cerebral ischemia and injected BRDU to track cell proliferation	Function and histology	GCSF/SCF induces BM-derived cell proliferation, neural stem cell-derived proliferation and improves functional recovery	[41]
Authors used *in vivo* ischemic brain injury model in rats as well as *in vitro* neuronal cultures to study differentiation of cells. They also collected data from human autopsy tissues	Radiolabeled iodine for BBB studies, function ICC, ISHH	GCSF passes the intact BBB and reduces infarct volume in two different rat models of acute stroke. GCSF has anti-apoptotic activity in mature neurons and activates cell survival pathways. Both GCSF and GCSFr are widely expressed by neurons and are induced by ischemia. GCSFr is also expressed by adult neural stem cells; GCSF induced neuronal differentiation *in vitro*. GCSF treatment improved long-term behavioral outcome.	[42]
Administered GCSF and SCF to GFP chimeric mice after focal cerebral ischemia	Study apoptosis microglial migration and infarct size in stroke	GCSF exerts a neuroprotective effect through the direct activation of teh anti-apoptotic pathway and significantly decreases the infarct size	[43]
The authors used mice, induced transient MCAO; and at the same time administered GCSF	Function (behavior tests) and histology	GCSF reduces infarct volume and improves weight and motor recovery.	[44]
Control or injured brains of irradiated mice that had been reconstituted with whole BM or single c-kit$^+$, Thy1.1(lo) Lin$^-$, Sca-1$^+$ (KTLS) HSCs, and unirradiated parabionts with surgically anastomosed vasculature were studied	ICC	HSCs and their progeny maintain lineage fidelity – no neural cells among 20,000 cells examined	[45]
Old rats were exposed to MCAO. BMSC were injected iv at 7 days and tissues were examined 4 months later	Function ICC	Improve long-term functional outcome and decreases scaring; GAP-43; GFAP; RIP cells from BM	[29]
MCAO (transient 30 min) was induced in GFP chimeric mice. The animals were studied at 1, 2, 5, 7, 14 days following surgery	ICC	Local microglia are more effective in tissue clearance after ischemic damage compared with macrophages recruited from the circulation. A different role for these cells after cerebral ischemia is outlined.	[46]
Immortalized human MSC were administered intravenously into MCAO rats	MRI and spectroscopy, histology	Significant improvement	[47]
FGF-2 transfected MSC were administered intracerebrally 24 h following MCAO in rats	Function, histology	Significant improvement due to FGF production by MSCs – less cell death Reduction in lesion size	[36]
Rats that underwent MCAO were given GCSF intravenously	MRI	GCSF induces neurogenesis and angiogenesis and reduces infarct size	[48]
GFP$^+$ BMSC were transplanted into the striatum of mice that undergone MCAO 7 days before. CD34$^-$;Sca1$^+$;CD90$^+$ cells were separated based on adherence before administration	Imaging through skull 4–12 weeks postmortem ICC	Migration towards the ischemic boundary zone GFAP and MAP2$^+$ cells	[30]

BBB: Blood–brain barrier; BM: Bone marrow; BMSC: Bone marrow stromal cell; BRDU: Bromodeoxyuridine; FGF: Fibroblast growth factor; GCSF: Granulocyte colony-stimulating factor; GCSFr: GCSF receptor; GFAP: Glial fibrillary acidic protein; GFP: Green fluorescent protein; HSC: Hematopoetic stem cell; hUCB: Human umbilical cord blood; ICC: Immunocytochemistry; ISHH: In situ *hybridization histochemistry; i.v.: Intravenous; MCAO: Middle cerebral artery occlusion; MRI: Magnetic resonance imaging; SDF: Stromal cell-derived factor; MCP: Monocyte chemotactic protein; MIP: Macrophage-inflammatory protein; NGF: Nerve growth factor; RT-PCR: Reverse transcriptase polymerase chain reaction; SCF: Stem cell factor; VEGF: Vascular epidermal growth factor; VEGFR: VEGF receptors.*

Table 1. Adult bone marrow-derived cells in ischemic brain disease (cont.).

Experiment	Evaluation	Findings	Ref.
Rats with MCAO received 3 × 106 HuMSC intravenously and were examined 2–30 days later	Function IGF1 and IGF1r with RT-PCR ICC: DCX; BRDU	IGF-1 and IGF-1r improved recovery and increased neurogenesis	[37]
BDNF transfected human MSC were injected intracerebrally into rats with MCAO	Function ICC	Less apoptosis; NeuN and GFAP+ cells; functional improvement	[31]
MCAO was performed in GFP mice chimeras	ICC	SDF1 in vessels and astrocytes, which plays a role in homing of BMSC to the ischemic injury	[38]
Mouse BMSC were infused into the striatum of MCAO rats	Cerebral bloodflow and BBB studied	BMSC might exert effect through restoring CBF and BBB, elevate TGF-β and neurotrophic factor levels	[49]
Mouse GFP+ BMSCs were injected bilaterally into the striatum of rats with MCAO	Microscopy for GFP	More cells survived in the MCAO side Migration in the lesioned but not in the control striatum	[28]
Marrow SCs were isolated by adherence. Nuclei were labelled with biz-benzimide and injected into control and MCAO mice brain and spinal cord	ICC	Migration towards the lesion and differentiate into NeuN, DCX and GFAP cells	[32]
Mobilized peripheral SC or human UCB were injected i.v. into the femoral vein in rats with MCAO	Function	Significant improvement	[22]
UCB were injected i.v. into the femoral vein or intrastriatally in rats with MCAO	Function	i.v. seems more effective than local	[22]
MCAO was performed in GFP BM chimeric mice	ICC (MAP2 and Iba1)	Activation and distribution of BM-derived monocytes/macrophages in the ischemic brain	[50]
Male mice with MCAO were treated with injections of GCSF and killed at day 4 after surgery	Histology	Increased survival rate and decreased volume size	[51]
MCAO was performed in GFP BM chimeric mice and the mice were studied between 1–28 days after surgery	GFP staining	Hematogenic macrophages became ramified and microglia became macrophage. The vast majority of macrophages in the infarct area derive from local microglia, and local defense mechanisms dominate over immune cells arriving from the blood.	[52]
The neuroprotective effect of GCSF was studied in rats after MCAO and in culture conditions	ICC, immunoblotting and PCR	GCSF was neuroprotective in cell culture and after i.v. administration after stroke. STAT3 expression in the penumbra of GCSF-treated rats increase, suggesting mediation by GCSFr	[27]

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Experiment	Evaluation	Findings	Ref.
Human BM (10^6 i.v.) MSC were administered into MCAO rats	Image analysis, ELISA, ICC and tissue culture	Enhanced angiogenesis in the host brain and hMSC-enhanced angiogenesis is mediated by endogenous VEGF and VEGFR2	[21]
Male rat MSC were injected into female MCAO rats.	Behavior ICC and FISH (Y) tunnel	Reduces apoptosis, increase bFGF, promotes endogenous proliferation and improves functional recovery	[20]
GFP chimera mice underwent MCAO and were studied 6 weeks and 6 months later	ICC	After 6 weeks 90% were microglia and no neural cells were detected	[53]
Authors studied the effect of chemokines on migration of hMSC with and without the presence of MCAO injured rat tissue	Microchemotaxis assay in separated chambers	MCP-1, MIP-1α and IL-8 enhance the migration of hMSCs. Ischemic brain tissue extracts at 24 and 48 h and 1 week after ischemia significantly increased hMSC migration across the membrane compared with nonischemic tissue	[39]
Same as above using rat MSC	Microchemotaxis assay in separated chambers	MCP-1, MIP-1α and IL-8 enhance the migration of hMSCs. Ischemic brain tissue extracts at 24 and 48 h and 1 week after ischemia significantly increased hMSC migration across the membrane compared with nonischemic tissue	[54]
Gender mismatched GFP mice chimera underwent MCAO and GFP positive cells were studied.	Y chromosome ICC (GFP)	BM-derived cells are a source of endothelial cells and NeuN-expressing cells after cerebral infarction	[33]
hMSCs were cultured with normal and MCAO brain extracts	Measure BDNF, NGF, VEGF and HGF in supernatant	These neurotrophins and angiogenic GFs increase in a time-dependent manner. The transplanted human MSCs can increase expression of growth and trophic factors and, thus, may benefit the ischemic brain	[40]
BRDU labeled adult whole BM was transplanted into the ischemic site of rat brains	ICC for NeuN, GFAP, Nestin and BRDU	BM cells induced plasticity: 1% NeuN; 5% GFAP and proliferating SVZ	[18]
MSCs were cultured with or without NGF and then transplanted into the ischemic boundary zone of rats with MCAO	Function ICC for GFAP, MAP2 and NeuN	MSCs survive, migrate and differentiate into phenotypic neural cells. Significant functional recovery observed	[19]
MSCs were labelled with BRDU and injected intravenously into MCAO rats	Function ICC	Functional recovery and some cells with neural phenotype	[34]
BRDU labelled selected non-HSC were injected into the ischemic area of rat brains	Function ICC	$BRDU^+$ cells 1% NeuN and 8% GFAP. Intrastriatal MSC survived and improved recovery in stroke	[17]
Authors performed intracerebral implantation of BRDU labelled fresh whole BM with BDNF into the ischemic boundary zone of MCAO rats	Function ICC	Improves functional recovery. NeuN, GFAP coloc with BRDU	[16]

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unknown. Published data on the use of BMSCs for cellular replacement therapy are summarized in Tables 1–3 and the techniques used in the studies are listed, although the validation of these techniques is not commented on.

Most of the work performed to date has focused on fatal or extremely debilitating neurological diseases because of the belief that cell-replacement therapy might be most beneficial in these cases (e.g., stroke, traumatic brain and spinal cord injury and progressive diseases, including multiple sclerosis [MS], Parkinson's disease [PD], amyotrophic lateral sclerosis [ALS], Alzheimer's disease).

Stroke

In 2000 Chen and colleagues tested the hypothesis that fresh BMCs implanted into the boundary zone (part of the brain tissue affected by ischemia that could potentially recover) of stroke in rats can help the healing process. Growth factors were tested in parallel with the cells. Brain-derived neurotrophic factor (BDNF) [16] was shown to facilitate survival of BMSCs and improve functional recovery after middle cerebral artery occlusion (MCAO). When bromodeoxyuridine (BRDU)-labeled mesenchymal stem cells (MSC) were used instead of BM or HSCs, the same authors found that 1% of the donor cells in the brain expressed NeuN, a neuron-specific marker. In addition, a large number of astrocytes were found in the CNS [17]. Similar results were obtained with whole BM [18] or MSC's pretreated with growth factors, such as NGF, which appeared to improve the outcome [19]. Local MSC injections appeared to reduce apoptosis and promote proliferation of endogenous neural stem cells as well as improve behavioral and functional neurological scores [20].

While Chen examined implanted cells, a number of other workers studied MSCs that were injected intravenously into rats or mice following MCAO. Human MSCs injected into such rats induced angiogenesis by increasing the production of host-derived vascular epidermal growth factors (VEGF) and VEGF receptors (VEGFr) [21]. In fact, human cord blood cells were found to have a more beneficial effect on MCAO when they were administered intravenously than when they were injected locally [22]. Similarly, in rats, HSC-like stem cells isolated from peripheral blood after GMCSF treatment reduced stroke-induced hyperactivity and prevented the development of extensive motor asymmetry [23]. This suggested that mobilizing stem cells could improve outcome in stroke patients, and clinical trials are underway to test this hypothesis [24–26]. Another possible mechanism could be a direct neuroprotective effect of granulocyte colony-stimulating factor (GCSF), since GCSF receptors were demonstrated to be present in neurons and glial cells [27].

In the case of xenotransplantation (mouse cells into rat brain), data indicate that immunosuppression therapy can enhance graft survival and reduce the graft-induced immunological response, although BMSCs can migrate within the brain and differentiate even without immunosupression [28].

Although most groups agree that implanting BMSCs into an infracted area of the CNS or giving stem cells intravenously improves recovery, the mechanisms responsible for this are not known. Using a variety of methods to trace the lineage of their progeny, BMSCs have been shown to differentiate into neural cells [16–19,29–34]. In spite of this, it has been argued that the beneficial effects seen are not due to replenishment of lost cells, but are instead a result of the release of growth factors by the MSCs and a reduction in neuronal cell death. This could have a direct effect on neuronal survival or a secondary effect on induction of endothelial proliferation, improved blood supply and better oxygenization of the injured area. These neuroprotective effects could be caused by a variety of cytokines and growth factors [19,21,31,35–40].

Studies of stem cells have grown rapidly in number in the past 10 years, but it is still challenging to decide which models are most predictive of therapeutic utility. Given this, a variety of injuries, species, drugs, drug doses, survival times and lineage tracking methods have been employed. The last of these is of utmost importance. Even the best designed experiment can fail if the methods used to follow cell fate are faulty.

Traumatic brain & spinal cord injury

From early 2000 onwards, scientists have tried to determine whether BMSCs might be used to replace neurons lost to traumatic brain injury (TBI) or spinal cord injury (SCI). Mahmood and colleagues have used several experimental strategies to study the former. They have cultured MSCs with neurotrophic factors [55] and injected them intracerebrally; injected MSCs intravenously 24 h following TBI [56,57] and administered MSCs intracisternally [58]. In every case, they found functional improvement along with differentiation of MSCs into neuronal cells. A recent paper by Lu and colleagues exposed BMSCs to a cocktail of Iscove's Modified Dulbecco's Medium

Table 2. Adult bone marrow-derived cells in traumatic brain and spinal cord injury

Experiment	Evaluation	Findings	Ref.
Autologous MSCc or mononuclear BMSCs were injected i.v. into rats with spinal cord lesion. Hydrogel scaffolds were seeded with MSCs labeled with iron oxide nanoparticles and then these were implanted in the spinal cord lesion in rats. Clinical trial use autologous BMSCs iv or intraarterially	Functional tests (ASIA score) MRI Histology	Functional improvement was most pronounced in MSC-treated rats. Bridging the lesion with scaffold might be advantageous. There was improvement in patients – greater after intravertebral artery injections of MSCs.	[69]
Autologous stem cells were injected into SCI rats in three experimental groups: MSC injected i.v.;mononuclear BM cells injected i.v. or GCSF injected s.c. for 5 days. Evaluation was done at 7–11th days post injury	Neurological scores (BBB) Morphometric measurements of white matter MRI	All three treatments were beneficial. MSC injection gave the best outcome.	[68]
SCI was induced in Wistar rats and 3 months later BMSCs were injected either iv or locally in the cavity of the trauma	6 months later neurological score, tight perimeter, cold spray test	Both treatments induced significant functional improvement. Local application was more efficient	[70]
SCI was induced in Wistar rats and 3 months later BMSCs were added locally into the cavity	Function, ICC labeling of pyramidal axons	Significant functional recovery: 35% neurons; axons bridge the gap a year later	[66]
Authors cultured rat MSCs in induction medium towards neural lineage. These fluorescent-labelled cells were then injected intravenously at 72 h after TBI	Function ICC for MAP-2, CNPase, GFAP and OX-42	Differentiate into neural cells *in vivo* and significant improvement of neurological scores	[59]
Authors induced pneumatic SCI then locally injected mouse GFP BMSCs and observed their fate for 2-4 weeks in live rats	Function ICC for GFAP, NeuN, MAP2 live imaging	Improved scores; GFAP; MAP2 and NeuN doubles with GFP. More doubles in injury than control	[65]
MSCs release of BDNF GDNF,NT-3 and NGF were studied *in vitro*. MSC is injected intraventricularly in mice that suffered TBI	ICC ELISA	NGF is made by MSC in culture and in CSF. Helps in TBI by releasing NGF	[71]
Selected BMSC or HSC were injected into the epicenter of the lesion in mice with SCI.	Function ICC	3 weeks later, functional improvement. HSC becomes oligodendrocyte; MSC only fibronectin	[67]
BDNF and NGF expression was studied after injection of rat MSCs into the cisterna magna of adult rats subjected to TBI	Semiquantitative PCR	Cisternal injection of BMSCs induces expression of NGF and BNDF in local brain cells	[72]

ASIA: American Spinal Injury Association; BRDU: Bromodeoxyuridine; GCSF: Granulocyte colony-stimulating factor; GFAP: Glial fibrillary acidic protein; HSC: Hematopoetic stem cell; ICC: Immunocytochemistry; i.v.: Intravenous; MRI: Magnetic resonance imaging; s.c.: Subcutaneous; SCI: Spinal cord injury; TBI: Traumatic brain injury.

Table 2. Adult bone marrow-derived cells in traumatic brain and spinal cord injury (cont.).

Experiment	Evaluation	Findings	Ref.
1 week after SCI HSC from LacZ Rosa26 were transplant-ed into the lesion site. Authors studied function on a weekly basis, while histology was done 5 weeks later	Function ICC for NeuN; GFAP; O4 and nestin	HSC promotes functional recovery and differentiates into neural lineage	[62]
Rat MSCs were cultured and then given intracisternally to TBI rats	Function ICC for GFAP, BRDU, MAP2 NSS 2, 3 and 4 weeks;	MSCs migrated into the brain, survived and differentiated into neural cells. There was a significant functional improvement	[73]
Authors study the effects of i.v. administration of MSCs on the expression of growth factors in rat brain after traumatic brain injury (TBI) at 2, 5, 8 days	Function ICC for BRDU, TuJ, DCX and NeuN	There were newly generated cells in the SVZ and boundary zone after intracerebral and in the SVZ, hippocampus and boundary zone after intravenous administration. Functional improvement in both groups with MSC	[58]
Intravenous or intracerebral injection of MSCs were performed in rats following TBI. The rats received BRDU to enable the authors to track proliferation. The animals were perfused 15 days later	Function ICC NGF, BDNF and bFGF measured with ELISA	i.v. MSCs increased the expression of NGF and BDNF. There was significant functional improvement and more $BRDU^+$ in the injured side. Both MSC and intrinsic cells made growth factors	[58]
Authors sterotaxically transplanted bizbenzimide labeled BMSCs into SCI in mice	4 weeks later ICC: NeuN; MAP2 and DCX	Cells migrated and survived in the spinal cord and many had markers of astrocytes and some neurons	[32]
Human MSCs are administered iv in rats 24 hours following TBI	Function ICC for Tuj and GFAP	Scores improved and cells exhibited neuronal and astro markers	[56]
MSCs are cultured with neurotrophic factors and then injected intracerebrally into rats with TBI	Function	Injury. MSC injected – if cultured with BDNF and NGF – significant improvement. Migrate to injury. 8 Days after TBI	[55]
Human cord blood is injected intravenously into rats with TBI and tissues are examined 28 days later	Function ICC and laser microscopy	28 days later significant improvement. NeuN, MAP2 and GFAP expression in donor cells.	[60]
BMSCs are injected into rats spinal cord 1 week following SCI. Tissues are studied 28 days later	Function ICC Rip and NeuN and nestin	Very few cells; functional improvement	[61]

ASIA: American Spinal Injury Association; BRDU: Bromodeoxyuridine; GCSF: Granulocyte colony-stimulating factor; GFAP: Glial fibrillary acidic protein; HSC: Hematopoetic stem cell; ICC: Immunocytochemistry; i.v.: Intravenous; MRI: Magnetic resonance imaging; s.c.: Subcutaneous; SCI: Spinal cord injury; TBI: Traumatic brain injury.

Table 3. Adult bone marrow-derived cells in progressive neurological disorders.

Experiment	Evaluation	Findings	Ref.
WT BMSCs were injected into SOD mice	Life span and motor functions	Significant improvement	[96]
Using adenovirus, MSCs were transfected with tyrosine hydroxylase and transplanted into the striatum of PD rats	APO induced rotation	Significant improvement; TH expressed	[89]
Transfection of MSCs were performed with Notch intracellular domain (NICD) and cells were treated with bFGF, forskolin, and ciliary neurotrophic factor. Treatment with GDNF increased the number of TH and dopamine-producing cells that were then transplanted into the striatum of PD rats	Apomorphine-induced rotational behavior and adjusting step and paw-reaching test	Significant improvement	[88]
The authors compare implanting ventral mesencephalic and BM stromal cells into the striatum of hemi-Parkinsonian rats	Rotation and motor skills	Improvement on rotation with both types of cells; no improvement in motor skills	[87]
WT BM was transplanted into chimeric SOD mice	Life span	Improved	[91]
13 days following quinolinic acid lesions, BM was implanted in the damaged striatum of rats and examined 37 days later. Rats – Huntington	Radial Arm Water Maze	Transplants significantly reduced working memory deficits as seen 37 days later. Suggests the role of released growth factors. Using ICC very few differentiated cells were seen	[94]
Human EGFP MSCs were transplanted into the striatum of PD rats	Rotation, migration, differentiation ICC	Significant improvement and MSC are all over (striatum, CC, vascular wall) NF,NSE GFAP immunopositive MSC in the brain	[86]
HSCs are injected intracerebrally into the brain of newborn (myelinating) mouse	ICC	Oligodendrocytes, ependymal cells, astros and neurons can become	[78]
Parkinson's mice were given: (A) no treatment or (B) 5.6 x 10^6 congenic bone marrow mononuclear cells or (C) 100-110 x 10(6) HCB mononuclear cells intravenously	Survival	Survival curve of mice that had congenic marrow had a p-value of <.05; the survival curve of mice receiving cord blood mononuclear cells had a p-value <.001	[85]
BMSCs were labeled with GFP using a retrovirus prior to BM transplantation with irradiation or local injection into the mouse brain	ICC	After iv only microglia; after local injection oligodendrocytes and astrocytes as well	[79]
GDNF transduced in mesenchymal cells are injected iv into MTPT treated mice	Morphology (TH cells and terminals) and function	MSC can be used as gene delivery vehicle	[84]
Megadose of HCB cells were injected intravenously into transgenic Huntington mice	Life span	Lifespan significantly increased	[93]
Megadose of HCB cells were injected intravenously into transgenic SOD1 mice	Life span	130 days vs 187 days survival	[90]
Rat marrow stromal cells (rMSCs) and human marrow stromal cells (hMSCs) were genetically engineered by transduction with retroviruses encoding tyrosine hydroxylase (TH) and GTP cyclohydrolase. These transduced rMSCs were injected into the striatum of PD rats	Morphology and rotation	Cells survive but the transgene shuts off at 9 days	[83]
hMSC were transplanted into striatum of rats	Microscopy; ICC	Cells survive; downregulate fibronectin (change character)	[82]

ALS: Amyotrophic lateral sclerosis; bFGR: Basic fibroblast growth factor; BM: Bone Marrow; GCSF: Granulocyte-Colony Stimulating Factor; GFAP: Glial Fibrillary Acidic Protein; HCB: Hexachlorobenzene; HSC: Hematopoetic stem cell; QA: Quinolinic acid; WT: Wild type.

(IMDM) and 5 mM β-mercaptoethanol to induce neural differentiation. They then labeled the cells with a fluorescent marker and injected them intravenously into rats 3 days after TBI. The cells that entered the brain expressed neural markers (nestin, glial fibrillary acidic protein [GFAP],

neurofilament and β-tubulin III) 4 months after the injury and the rats demonstrated significant functional improvement when compared with uninjected controls [59]. Similar results were achieved following intravenous injections of human cord blood into rats with TBI [60].

A number of researchers have looked for signs of histological and functional improvement after administering stem cells to rats or mice with transected or traumatized spinal cords. The studies lasted for different amounts of time, involved the use of a variety of cells (cultured vs noncultured; HSC vs MSC) and had a number of neurological end points. Based on what has been published, it appears that BMSCs have a positive effect on recovery from spinal cord injury [32,61–66], however, negative studies rarely see the light of day. Most groups used MSCs, although HSCs were also shown to differentiate into neural cells and aid in recovery [67]. In side-by-side assays, local administration of BMDSCs was found to be significantly more efficacious than intravenous administration in preventing functional deterioration following spinal cord injury [66]. Urdzikova and colleagues compared the effect of intravenous injection of MSCs, injection of the mononuclear fraction of BMCs or BMC mobilization induced by G-CSF in rats with a balloon-induced spinal cord compression lesion. While all three treatments had a beneficial effect, the MSC injection produced the best outcome [68]. The same group reported a possible positive effect of intravascular injection of MSCs in a small clinical trial [69]. The mechanisms responsible for the improvement observed following cord damage are the subject of speculation. As in stroke, the hypotheses include both cellular engraftment and secondary humoral (paracrine) effects.

Progressive neurological diseases

In MS, Parkinsonism, Alzheimer's disease, ALS and Huntington's disease, neurons slowly degenerate and die. The cause in each case is different. Owing to the progressive nature of these diseases, cell replacement and/or neuroprotection by enhanced release of growth factors have been recommended as options for stopping, or at least slowing, progression.

Multiple sclerosis is an autoimmune disorder in which myelinating cells (oligodendrocytes) are attacked by the immune system [74]. BM transplantation has been used in MS patients [75–77]. Historically, BM transplantation in MS (and other autoimmune diseases) was used in the hope of reprogramming the immune system, that is, deleting clones that are attacking the host. Recently, however, the possibility of repairing myelin by replacing the missing oligodendrocytes was entertained. Bonilla and colleagues have demonstrated that HSCs can differentiate into oligodendrocytes in the brains of neonatal mice [78]. Since Bonilla transplanted a sample enriched in HSCs, but not a pure cell population, it is possible that cells other than HSCs differentiated into oligodendrocytes. When cultured mouse BM cells were tagged with a retrovirus and injected into mice systemically, microglia were observed in the brain 8 weeks later. When the cells were injected directly into the striatum, however, they differentiated into neural cells, including oligodenedrocytes [79].

Models of Parkinsonism have been studied more extensively than those for any other degenerative disease. This is because the neurons that die due to the disease are well characterized and reside in one well-defined brain region, the substantia nigra. Most investigators have used neural stem cells or embryonic catecholaminergic cells to attempt to restore catecholamine levels in the striatum to normal [80,81]. In one of the first studies of human MSCs, Azizi demonstrated that the cells survive in the striatum when they are injected there, and that they downregulate fibronectin expression [82], that is, they become less mesenchymal in nature. In another series of experiments, rat and human MSCs were transduced using retroviruses that encode tyrosine hydroxylase (TH) and GTP cyclohydrolase I, the enzyme that is necessary for the production of the tetrahydrobiopterin co-factor for TH. When injected into the striatum of 2-methoxy-5-(2′,3′,4′-trimethoxyphenyl) tropone (MTPT)-lesioned rats, these cells survived and produced TH – although the production was very short lived [83]. MSCs transformed to express glial-derived neurotrophic factor (GDNF) were transplanted into mice in an effort to protect nigral neurons from degenerating following MPTP treatment. In the GDNF–BMT mice, 2 months after the neurotoxin exposure, more TH-immunoreactive nigral neurons and striatal terminals were found compared with the control–BMT group. A significant difference in motor activity was also detected between the two groups [84]. Intravenously injected congenic mononuclear cells and human cord blood cells were compared in mice with Parkinson's disease and were both significantly effective in extending survival. The

human cord blood cells appeared to have a greater effect [85]. Human MSCs were also shown to migrate and differentiate into neural cells and vascular endothelium in PD mice. The mice also showed functional improvement in rotational tests [86]. When Pavon-Fuentes implanted MSCs or ventral mesencephalic neurons into the striatum of hemi-Parkinsonian rats he found improvement in rotational tests, but no change in other motor skills in both cases [87]. In an elegant series of experiments, Dezawa and colleagues tranfected rat and human MSCs with the intracellular domain of Notch and treated the cells with FGF prior to transplanting them into the ipsilateral striatum of rats with PD. In culture, the transduced cells formed neural cells and many exhibited histological markers and electrophysiological properties of neurons [88]. In the animals that received the intrastriatal transplants, the apomorphin-induced rotational behavior significantly improved when compared with the control group. Lu and colleagues used genetically engineered MSCs using an adeno-associated virus to express TH, before transplantation into the striatum of PD rats. In these animals, increased dopamine production and functional improvement was demonstrated [89].

While there is no treatment to cure PD, there is therapy available to significantly slow down the progression of the disease. Conversely, ALS is one of the most devastating and quickly progressing neurological diseases with no known therapy. Thus, several groups tested the possible use of BMSCs in ALS. The transgenic mice with a mutation of the superoxide dismutase gene (*SOD1*) are used as a model since this gene has been associated with ALS in humans. Human cord blood mononuclear cells were intravenously administered to SOD1 mice that were previously irradiated. The lifespan of the treated mice increased by 30–40%, suggesting a beneficial effect of the treatment [90]. The study also demonstrated that pooled human cord blood (HCB) could be used without any complications. When wild-type BM was transplanted into SOD1 mice, the lifespan of the SOD1 mice was also significantly increased [91]. In a recent human clinical trial in seven patients, BM was taken from the iliac crest, the MSC were expanded in culture, added to cerebrospinal fluid and injected into the spinal cord using a pump. The patients had a significantly longer lifespan and better quality of life compared with untreated patients [92]. Human cord blood and BM were both shown to have beneficial effects in mouse models of Huntington's [93,94] and Alzheimer's disease [95].

Summary

Bone marrow transplants have been used in clinical work for almost 50 years [97]. Originally, they were employed to introduce or restore hematopoetic cell progenitors to genetically deficient patients or to people who had been given high levels of irradiation or chemotherapy to fight cancer. After data emerged in the literature suggesting that BM stem cells might have the potential to replace dying cells in a variety of tissues [75,98], including the brain [4,7,8,99], many studies were initiated to explore the possibility of using BM cells for tissue regeneration. Although the character of cells in the BM that are responsible for the beneficial effects observed is still debated, it appears that this mode of therapy should be further explored. HSCs in the BM demonstrated a much higher level of plasticity than was initially thought [100–102]. A subset of BMSCs is also thought to be pluripotent, and recently their immunomodulatory properties have excited considerable interest [75]. Furthermore, transduced BMSCs have been used successfully to deliver gene products to different tissues [31,36,89,103]. Although BM-derived (hematopoietic, mesenchymal and other) stem cells have shown promise in regenerative therapy, there are many questions to answer before they can routinely be used to help people. When they seem to be efficacious, we are not sure why they work; they may release cytokines or other protective factors, or 'trans'differentiate into other cell types that the organism needs. These two mechanisms are not mutually exclusive. In any given organ or disease state, and in subjects of different ages or genders, either mechanism or both could come into play.

Before we can take full advantage of BMSCs, we will have to learn the type(s) of cells that are responsible for the positive effects that have been seen. We need to discover how to expand the cells in culture without altering their beneficial properties, and how to administer them to patients without causing harm (e.g., histocompatibility problems, cancer growth or unexpected differentiation). The data cited in this review suggest that the answer to the question posed in the title is not simple. BMSCs are mostly 'masons' that secrete growth factors to help maintain the continuity of tissues that they

populate. They may, however, in times of extraordinary need, give rise to progeny that function as 'stones', replacing missing cells. In either case, we have to characterize the cytokines and growth factors that shape stem cell responses to specific environments and find ways to mimic their actions so that we can direct their migration and differentiation. Finally, we have to keep an open mind regarding the potential of stem cell therapy (and to remain sceptical about hyperbolic claims of success). It is time to reveal the secrets of these exciting cells.

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