

Adult bone-marrow stem cells and their potential in medicine

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An area of research that today generates great optimism is the use of stem cells for therapy of human diseases. Much of the excitement centres on embryonic stem cells, but this approach remains controversial for ethical reasons; moreover, routine clinical application of this strategy is many years away. By contrast, haematopoietic stem cells from adult bone marrow are well characterized and have long been used therapeutically.¹ An adult weighing 70 kg has a functional haematopoietic marrow volume of about 1.75 L and upon increased demands such as infection or haemorrhage it can increase sixfold.^{1,2} No moral controversy surrounds the use of these cells since they are either autologous or collected from a consenting donor. The potential applications of adult bone marrow cells have gained momentum with discoveries relating to the mesenchymal stem cell.

MESENCHYMAL STEM CELLS

Adult bone-marrow-derived mesenchymal stem cells (MSC) are capable of differentiation along several lineages (Box 1).³⁻¹⁵ They are positive for CD29, CD44, CD105 and CD166, have a doubling time of about two days, expand in culture up to sixfold and their biological functions are not altered by ageing.^{3,15} Box 2 lists some of the cytokine receptors expressed by these cells and the cytokines produced. Their features and properties are closely similar to those of counterpart cells isolated from fetal blood, liver and bone in the first and second trimesters, from amniotic fluid and umbilical cord blood, and from adult peripheral blood, compact bone and adipose tissue.²¹⁻²⁷ Moreover, a CD133-positive subpopulation of these cells, which can be expanded under defined conditions for more than one hundred population doublings without telomere shortening or karyotypic abnormality, has proved capable of differentiation not only into mesenchymal cell types (osteoblasts, chondrocytes, adipocytes, myocytes) but also into endothelium and cells with neuroectodermal phenotype and function.²⁸⁻³⁰ Previously, adult marrow-derived stem cells were believed to yield a limited number

Box 1 Differentiation potential of adult bone marrow mesenchymal stem cells (from refs 3-15)

Adipocyte	Myofibroblast
Osteoblast	Endothelial cell
Chondrocyte	Neural cell
Cardiac myocyte	Hepatic cell
Skeletal myocyte	Renal tubular cell
Tenocyte	

of cell types whereas embryonic cells were totipotent. The discovery of these multipotent adult stem cells has clearly narrowed the gap: they offer a very promising and much more abundant potential resource for therapy of inherited or degenerative diseases and for repair of tissues such as cartilage, bone and myocardium.

ADULT STEM CELL PLASTICITY

What is the mechanism of stem cell differentiation? When the phenomenon was first explored, the possibility of cell fusion was mooted—that is, hybridization with other cells rather than true plasticity. Indeed, embryonic stem cells were seen to hybridize with brain cells to form tetraploid cells with pluripotent character.³¹ However, *in-vitro* and *in-vivo* studies of adult bone marrow stem cells suggest a

Box 2 Cytokine expression and production of human adult bone marrow mesenchymal stem cells (Refs 3, 15-20)

Cytokine receptor expression	Cytokine production
c-kit (stem cell factor)	Stem cell factor
gp130	Interleukin-1, -6, -7, -8, -11, -12, -14, -15
Interleukin-1, -3, -4, -7	FLT-3 ligand
Leukaemia inhibitory factor	Leukaemia inhibitory factor
G-CSF	G-CSF, GM-CSF and M-CSF
TNF-alpha	
TGF-beta	
Basic fibroblast growth factor	
Platelet derived growth factor	
Epidermal growth factor	
Nerve growth factor (p75)	

G-CSF=granulocyte colony stimulating factor; TNF=tumour necrosis factor; TGF=transforming growth factor; GM-CSF=granulocyte macrophage CSF; FLT=tyrosine kinase receptor

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rate of cell fusion too low to account for the transdifferentiation.³² Moreover, single euploid bone marrow MSC, never co-cultured with tissue-specific cells or embryonic cells, have been seen to differentiate into cells of the three germ layers;³³ *in vivo*, the use of bone marrow cells selectively expressing the enhanced green fluorescent protein ruled out fusion as a mechanism for the generation of functional pancreatic islet beta cells;³⁴ and hepatocytes, cardiomyocytes, and pancreatic and endothelial cells have been described as physiologically either diploid or polyploid.^{35–37} Certain cytokines, including interleukins (IL) 1, 4, and 13, tumour necrosis factor alpha and interferon gamma, are involved in the generation of normal multinucleated cells such as osteoclasts and Langhans giant cells;^{38–40} thus, observations suggesting fusion of bone marrow cells with, for example, Purkinje neurons, cardiomyocytes and hepatocytes⁴¹ may instead simply reflect physiological polyploidy.

BIOLOGY OF ADULT MARROW MESENCHYMAL STEM CELLS

The direction in which bone marrow MSC differentiate is heavily influenced by cytokines (Table 1). For example, bone morphogenetic protein 6 (BMP-6) not only influences differentiation towards chondrogenesis or osteogenesis but may also serve to regulate the bone marrow environment via the effects of IL-6 on haematopoiesis and osteogenesis.⁵⁰ Two possible mechanisms have been proposed for a regulatory role of BMP-6 in the human bone marrow microenvironment: (i) it might enhance the osteoblastic differentiation of human MSC; or (ii) it might reduce the osteoclastic differentiation of haematopoietic marrow cells by decreasing interleukin-6 production in bone marrow stroma. MSC coexpressing CD133 and fetal liver kinase 1 generated endothelial cells in the presence of vascular endothelial growth factor, and functional hepatocytes in the presence of fibroblast growth factor-4 and hepatocyte

growth factor.^{29,30} Also, MSC coexpressing CD133, CD172 and nestin differentiated along a neural pathway in the presence of fibroblast growth factor or retinoic acid plus nerve growth factor.^{51,54} An MSC side-population with high efflux of DNA binding dye and expressing CD90 (Thy1) differentiated into mesangial renal cells.⁵⁵

MIGRATION/MOBILIZATION OF ADULT MARROW STEM CELLS

In animal models, transplanted bone marrow cells have been detected in skeletal and cardiac muscle,^{56–58} vascular endothelium,^{58,59} liver,^{60–62} lung, gut and skin epithelia,⁶² pancreatic beta cell islets,^{34,63} renal glomeruli,^{14,55} and neural tissue.^{33,64–69} When bone-marrow-derived MSC were injected intracerebrally in acid-sphingomyelinase-deficient mice, the onset of neurological abnormalities was delayed and the animals lifespan was extended.⁷⁰ Local transplantation of such cells is also reported to have regenerated bone^{71–73} and myocardium.^{74,75} It is noteworthy that no donor-derived tumours have been seen in these animal models—whereas with transplantation of undifferentiated embryonic stem cells teratoma development has been reported.⁷⁶ The results also differ from those of undifferentiated embryonic stem cell transplantation in that engraftment and tissue-specific differentiation are achieved without pretransplantation measures to induce differentiation down the lineage desired. The ability of marrow-derived cells to populate numerous body tissues—bone, liver, cardiac muscle, colon, skin—is well shown in patients who have received cells from gender-mismatched donors (Table 2).^{77–84} A post-mortem study revealed donor-derived neurons in the hippocampus and cerebral cortex of brain samples from women who had received bone marrow transplants from men.⁸⁵ Deductions from such findings must be qualified by the observation that women who have carried male fetuses may show long-term mosaicism with male cells;

Table 1 *In-vitro* differentiation conditions of human adult bone marrow mesenchymal stem cell

<i>Tissue-type generated</i>	<i>Cytokines, reagents and conditions</i>	<i>Ref.</i>
Chondrogenic cells	TGF-beta and dexamethasone	42
	Bone morphogenetic protein-6 (BMP-6)	43
	Prolactin	44
Osteogenic cells	Ascorbate, β -glycerophosphate and dexamethasone	45,46
	Parathyroid hormone vitamin D3 \pm BMP-6	3
Endothelial cells	Vascular endothelial growth factor	29
Functional hepatocytes	FGF-4 and hepatocyte growth factor on Matrigel	30
Neuronal cells	FGF and retinoic acid \pm NGF on fibronectin	51–54
Mesangial renal cells	Side population cell CD90+subset of MSC	13,55

FGF=fibroblast growth factor; NGF=nerve growth factor; TGF=transforming growth factor; MSC=mesenchymal stem cell

Table 2 Migration of human adult bone marrow stem cells in gender-mismatched bone marrow transplantation patients

Donor male cells in female recipient	Frequency	Ref.
Osteoblasts	2%	77
Hepatocytes	2%	78
Colon mucosal epithelial cells	13%	79
Hepatocytes, gut and skin epithelia	7%	80*
Buccal epithelial cells	–	81
Purkinje cells	–	82
Myocardial cells	10%	83,84
Cerebral (neural) cells	–	85

*Stem cells mobilized with granulocyte colony stimulating factor

nevertheless, the weight of the evidence is that donor bone-marrow-derived cells can migrate and give rise to tissues belonging to all three germ-cell layers.^{77–85} It is noteworthy that, in the transdifferentiation of these adult marrow stem cells, there was no evidence of cell fusion.^{77–85} Lately, work in mice indicated that such cells participate in skin regeneration and reconstitution and promote wound healing;^{86–88} and one research group reports a pilot study in three patients indicating that locally applied autologous bone marrow cells enhanced dermal building and closure of long-term non-healing wounds.⁸⁹

CLINICAL STUDIES

In animal models of myocardial infarction, stem cells were reported to participate in repair whether injected locally or stimulated in bone marrow by use of stem cell factor (SCF) and G-CSF.⁹⁰ In man, a randomized placebo-controlled study revealed increased coronary collateral flow in patients treated with intracoronary GM-CSF (molgramostim) followed by two weeks of subcutaneous administration.⁹¹

In the past decade the use of G-CSF (filgrastim) has transformed the treatment of cancer by facilitating marrow reconstitution after myeloablative therapy. We must hope for a similar breakthrough in the management of coronary heart disease.

In allogeneic transplantation, mesenchymal stem cells in bone marrow play a key part in immunomodulation and the induction of tolerance. MSC suppress the proliferation of T-lymphocytes induced by cellular or non-specific mitogenic stimuli⁹² and negatively influence B-cell lymphopoiesis.⁹³ Allogeneic/xenogeneic MSC transplants engraft in immunocompetent sheep and non-human primates.^{94–97} When a patient was treated, after myeloablation, with both haematopoietic stem cells and cultured MSC from a mismatched donor, only grade I graft-versus-host disease (GvHD) was observed.⁹⁸ That MSC can not only reduce GvHD but also facilitate haematopoietic engraftment is

evidenced by the rapid haematopoietic recovery of patients with breast cancer who received autologous blood stem cells together with culture-expanded MSC after high-dose chemotherapy.⁹⁹ In both clinical trials, MSC transplantation was well tolerated.

Osteogenesis imperfecta has been the focus of two studies in children. Allogeneic MSC transplantation, leading to successful osteoblast engraftment in 3 of 5 children with type III osteogenesis imperfecta, was associated with a 44–77% increase in bone mineral content, improved linear growth and reduced fracture frequency.^{77,100} In another cohort of 6 children with type III osteogenesis imperfecta who had received earlier bone marrow transplantation, MSC infusions from the original donor resulted in a 50% improvement in their growth velocity.¹⁰¹ Similar improvements were observed in children with metachromatic leukodystrophy and Hurler's syndrome after repeated allogeneic marrow MSC infusions.¹⁰²

Ten clinical studies have been reported on the effects of autologous bone marrow stem cell transplantation in patients with myocardial infarction or ischaemic heart failure (Table 3).^{103–112} In three pilot studies, two of them randomized controlled trials, bone marrow cells infused via a coronary catheter a few days after acute myocardial infarction led to significant improvement in coronary flow reserve and left ventricular ejection fraction.^{104,105,111} In the remaining seven, marrow cells injected directly into the myocardium of patients with chronic ischaemic heart disease yielded benefits in ejection fraction and also angina score.^{103,106–110,112}

Despite the impressive safety record of all these pilot clinical trials, the possibility of undesired differentiation into other tissues must be borne in mind in monitoring of future studies.

THE FUTURE

In the next decade, the approaches discussed above will clearly be developed and refined. Further avenues will open up. For example, bone-marrow-derived cells expressing stem cell factor have been shown to initiate endogenous pancreatic tissue regeneration in mice.¹¹³ If such cells could be used as pancreatic beta islet cell progenitors, there would be scope for autologous transplantation in patients with diabetes, avoiding the need for the immunosuppression necessary after allotransplantation and circumventing the scarcity of allogeneic material. Whereas the multipotent adult dermal stem cells from human scalp skin have shown mainly neural differentiation, suggesting a possible therapeutic role in neurodegenerative diseases,^{114,115} the bone marrow MSC show strong orientation towards bone, cartilage, endothelium and cardiac muscle.

In conclusion, the existing medical uses of bone marrow are likely to expand greatly with exploitation of the

Table 3 Clinical trials of adult bone marrow autotransplantation in ischaemic heart disease

No.	Bone marrow (BM) cells	Route of administration	Clinical outcome	Follow-up (months)	Ref.
<i>Acute myocardial infarction</i>					
10 (5–9 days after injury)	Mononuclear BM cell aspirate 2.1% CD34+, 0.6% CD133+ total 18 million CD34+ cells	Intracoronary, 6–7 injections of 2–3 mL containing 1.5–4 million CD34+ cells each	Increased contractility; reduced hypokinetic area	3	104
20 (4 days after injury) versus 11 control	Mononuclear BM cell aspirate (n=9) 1–7 million CD34+ or blood progenitor cells (n=11) 245 blood million cells	Intracoronary catheter 3 injections of 3 mL each	Increased global LVEF; reduced end-systolic; improved coronary flow reserve	4	105
30 (3–6 days after injury) versus 30 control	Mononuclear BM cell aspirate 4% CD34+, total 3–15 million CD34+ cells	Intracoronary, 4–5 injections of 5 mL each containing 0.6–3 million CD34+ cells	Increased global LVEF; increased systolic wall motion	6	111
<i>Chronic ischaemic heart disease</i>					
5	Mononuclear BM cell aspirate	With coronary artery bypass, 11 transendocardial injections of 0.1 mL	Improved coronary perfusion	12	103
6	Selected CD133+ BM cells; total 1.5 million CD133+ cells	With coronary artery bypass, 10 transendocardial injections of 0.2 mL	Improved coronary perfusion; improved global LVEF	6	109
14	Mononuclear BM cell aspirate	With coronary artery bypass	Improved global LVEF; increased cardiac function	10	110
12	Selected CD133+ BM cells; total 1.5 million CD133+ cells	With coronary artery bypass, 10 transendocardial injections of 0.2 mL	Improved local perfusion; improved global LVEF	12	112
10	Mononuclear BM cell aspirate	Endomyocardial catheter injection	Improved LVEF	3	106
14	Mononuclear BM cell aspirate	Endomyocardial catheter injection	Improved LVEF; reduced end systolic volume	2–4	107
8	Mononuclear BM cell aspirate	Endomyocardial catheter injection	Increased wall motion	3	106

LVEF=left ventricular ejection fraction

therapeutic potential of adult mesenchymal stem cells, with their capacity for many lines of differentiation. The next stage is to isolate the various subsets and investigate their mechanisms of differentiation and homing to tissues. This work has vast implications for human wellbeing, through cell and gene therapies, through tissue engineering and through immunotherapy.

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