2 Hematopoietic Stem Cells for Myocardial Regeneration

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SUMMARY

Adult bone marrow consists of several populations of stem cells that are the focus of investigations into their potential to regenerate nonhematopoietic tissues. According to this hypothesis, bone marrow stem cells display a plasticity not previously recognized. Although data supporting bone marrow stem cell plasticity is extensive, many researchers dispute this concept. One of the most controversial aspects of stem cell plasticity relates to regeneration of heart muscle following acute myocardial infarction (MI). When experimentally induced MIs in rodents were analyzed for regeneration of the heart tissues, it was reported that cardiomyocyte renewal was achieved as a result of bone marrow stem cell infiltration of the damaged tissue. Evidence continues to accumulate in support of and against the potential for myocardial regeneration, indicating the need for a better scientific basis for the possible involvement of bone marrow-derived stem cells in myocardial regeneration.

In order to achieve a higher level of acceptance for myocardial regeneration, researchers must develop more exacting methodologies to monitor repair over time at the cellular level. A major effort must be undertaken to identify the signals required for stem cell mobilization and trafficking to infarcted cardiac tissue and to define the genetic mechanisms involved in stem cell plasticity. To answer these questions it will be necessary to establish the exact identity of the stem cell population involved. Controversies regarding myocardial regeneration in rodent models will require additional experiments using large animal models, with an emphasis on tracking of labeled donor cells. These preclinical experiments will also enable testing cell-delivery devices and noninvasive modalities for detecting improvement in regional and global myocardial function. If key questions relating to transdifferentiation potential, cell survival, and function can be resolved, we may one day be able to fully exploit the potential of stem cells for myocardial repair.

Key Words: Myocardial regeneration; stem cell plasticity; ischemia; myocardial infarction.

STEM CELLS FOR TISSUE REGENERATION

Investigators in the nascent field of stem cell therapy propose the lofty goal that one day it may be within our capacity to regulate the regeneration of tissues and organs. There is a biological basis for some of the enthusiasm for regenerative medicine: in

From: *Contemporary Cardiology: Stem Cells and Myocardial Regeneration*
Edited by: M. S. Penn © Humana Press Inc., Totowa, NJ

normal growth and especially following injury, tissues undergo regeneration, and in many instances the stem cells involved in regeneration have been identified.

The most thoroughly investigated developmental pathway from a stem cell to a mature functional cell is the renewal of blood cells through hematopoiesis. From early fetal life and throughout adult life, the bone marrow consists of hematopoietic stem cells (HSCs) that give rise to multiple populations of descendants referred to as progenitor cells. The progeny of these progenitor cells acquire specific molecular patterns that characterize unique blood cell lineages. Each blast cell transits through several levels of cell maturation accompanied by a series of cell divisions, leading to the formation of a cluster of 8–32 erythrocytes or leukocytes.

HSCs in adult bone marrow are a rare population of cells. The best estimates of their frequency, obtained largely from mouse studies, suggest that they occur at a ratio of approx 1:10,000 or 1:100,000 cells. Their enrichment, utilizing lineage-specific markers and several HSC-specific markers, by flow cytometry has enabled basic researchers and clinicians to better define the molecular and cellular events that occur in bone marrow and achieve a degree of control over hematopoietic tissue regeneration. Advances such as these, over many years, have provided insight into normally occurring processes in tissue regeneration.

Currently, several stem cell populations, most prominently HSCs and neural stem cells (NSCs), are being investigated for a newly proposed attribute referred to as stem cell plasticity or transdifferentiation. According to this hypothesis, stem cells from one specific tissue may differentiate into cells of a different tissue, even one whose origin is from another embryonic germ layer. The concept of stem cell plasticity has provoked enormous interest from biologists and clinicians. The excitement in some quarters, however, is matched by skepticism in others. In this chapter we will attempt to define what has been achieved thus far and what future studies may be needed to establish bone marrow stem cell plasticity as a basic component of today's science as well as its potential in treating human diseases.

STEM CELLS IN ADULT BONE MARROW

Initially, hematopoietic stem cells arise in the yolk sac *(1)* and aorta–gonad–mesonephros *(2,3)* region of the developing embryo. During fetal development, these stem cells are believed to colonize the liver, spleen, and, at mid-gestation, the bone marrow. After birth, hematopoietic activity in bone marrow is under the control of resident HSCs. However, in addition to HSCs, the cells that infiltrate the cavities of fetal bone marrow also give rise to mesenchymal stem cells (MSCs), which survive the lifetime of the individual, and perhaps a third class of stem cells referred to as multipotent adult progenitor cells (MAPCs). With this hierarchy in mind, it is clear that HSCs and bone marrow stem cells (BMSCs) are not synonymous. Thus, HSCs are one of several stem cell populations in adult bone marrow (Fig. 1). Unfortunately, the literature is replete with examples in which authors use the term HSCs to describe transdifferentiation events when working with a combination of BMSCs.

Hemangioblasts are believed to represent a population of bone marrow cells more primitive than HSCs. They are present initially in yolk sac blood islands, where they appear to give rise to the primitive red blood cells of the embryo and the endothelial cells that form channels, the vitelline veins, through which newly formed red cells circulate to the embryo proper. This developmental pattern may simply be an attribute

of a cell population with dual differentiation pathways. Alternatively, because endothelial cells and red blood cells do not share a common morphology or function, hemangioblast activity may represent an example of stem cell plasticity in adult bone marrow. Although their phenotype is not well characterized, hemangioblasts appear to co-purify with HSCs. A recent investigation showed that a single donor lineage-negative (Lin^-) Sca-1^+ $CD45^+$ green fluorescent protein-positive (GFP^+) bone marrow-derived stem cell reconstituted ablated recipient bone marrow within 30 days of transplantation. Subsequently, following laser beam-induced damage to the retinal vasculature, the progeny of this single GFP^+ bone marrow reconstituting cell trafficked to the site of retinal ischemia and engaged in neovasculogenesis *(4)*. From this observation and many others *(5–12)*, there is growing support for the concept of a rare population of adult bone marrow cells that is endowed with hematopoietic and vasculogenic potential.

HSCs are capable of unlimited cell proliferation in bone marrow. They have a relatively well-defined surface phenotype (Fig. 1) by which they can be enriched using fluorescence-activated flow cytometry. Mouse HSCs are classified as Lin^- Sca-1^+ *(13)* and c-kit^+ *(14,15)*. However, they cannot be purified because their phenotype is shared in part with their immediate progeny, the committed progenitor cells that give rise to the myeloid and lymphoid lineages, and also, to a more limited extent, with MSCs, MAPCs, and the bone marrow-derived endothelial progenitor cells (EPCs), which have the potential to generate endothelium. By comparison, based on difficulties involved in following HSC activity in human bone marrow, the phenotype of human HSCs is less well defined. There is general agreement, however, that human HSCs are Lin^- $CD34^+$ $CD38^-$ cells, but several cell types share the Lin^- $CD34^+$ phenotype, and CD38 is not a well-defined marker. Finally, HSCs, the ultimate ancestor of the blood cell hierarchy, share with all developing and mature blood cells the CD45 common leukocyte marker.

Are HSCs the bone marrow component that some believe exhibit plasticity, and, if so, should they be considered prime candidates for initiatives in cellular therapy? This concept is fraught with difficulties. For decades scientists and hematologists have struggled with the difficulty that HSCs cannot be purified based on phenotypical characteristics and, perhaps more importantly, cannot be expanded and cloned ex vivo. Recent evidence has emerged suggesting that HSCs can be expanded ex vivo *(16)* and that this occurs by forced expression of the Polycomb group gene *Bmi-1 (17)*, but there is still no evidence to support the idea of clonality. For these reasons HSCs are not ideally suited for in vitro experiments designed to test plasticity. In this regard HSCs differ dramatically from MSCs in bone marrow and NSCs in the central nervous system, both of which can be clonally derived and tested for multiple differentiation pathways. Some of the best evidence to date regarding HSC plasticity is from mouse experiments that involved the injection of a single bone marrow-derived stem cell that initially reconstitutes the bone marrow and subsequently gives rise to cells with a capacity to form endothelium *(4)* and epithelium in multiple organs *(18)*.

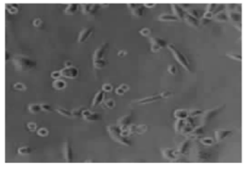
Although HSCs reside primarily in bone marrow, small numbers can be isolated from the circulation. However, the relatively few HSCs in blood under normal conditions can be greatly enhanced in response to a series of daily injections of granulocyte–colony-stimulating factor (G-CSF) and stem cell factor (SCF) *(19,20)*. The cytokine G-CSF induces neutrophils in bone marrow to release their granular content of proteolytic enzymes, including matrix metalloproteinase-9 and elastase *(21–24)*. This change in the bone marrow microenvironment alters the steady-state conditions, and following proteolytic cleavage of stromal-derived factor-1 (SDF-1) from its receptor

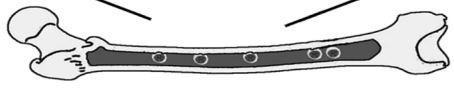
Hematopoietic Stem Cells

	Mouse	Human
plastic adherent:	no	no
expandable ex vivo:	no	no
phenotype:	lineage-	lineage-
	c-kit+	c-kit+ (CD117)
	Sca-1+	Sca-1-
	CD34 (?)	CD34+
	CD45+	CD45+
plasticity:	yes	yes

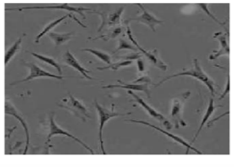
Multipotent Adult Progenitor Cells

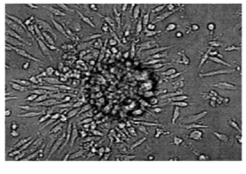
	Mouse	Human
fibronectin adherent:	yes	yes
expandable ex vivo:	yes	yes
cell doublings:	120	180
phenotype:	CD34-	CD34-
	CD45-	CD45-
	CD44-	CD44-
	c-kit-	c-kit- (CD117)
	class I HLA-	class I HLA-
plasticity:	yes	yes





Bone Marrow Stem Cells





Mesenchymal Stem Cells

	Mouse	Human
plastic adherent:	yes	yes
expandable ex vivo:	yes	yes
cell doublings:	14-21 days	14-21 days
	CD34-	CD34+
	CD45-	CD45-
	CD31-	CD31-
	Sca-1+	CD90+
	Class II HLA-	CD106+
plasticity:	yes	yes

Endothelial Progenitor Cells

	Mouse	Human
fibronectin adherent:	yes	yes
expandable ex vivo:	yes	yes
phenotype:	CD133+	CD133+
	KDR+	KDR+
	CD31-	CD31-
	???	VEGFR2+
plasticity:	yes (?)	yes (?)

CXCR4, the previously tethered HSCs are released into the circulation. HSCs obtained from bone marrow and blood can reconstitute bone marrow, but there is evidence that some physiological features of HSCs residing in bone marrow differ from those within the circulation. Gene expression analysis, using cDNA microarray technology, has identified nine genes associated with cell cycling expressed at two- to fivefold higher levels in $CD34^+$ cells residing in bone marrow compared with circulating $CD34^+$ cells *(25)*. This raises the question: Is one or the other population more suited to respond to chemokine signals that emanate from injured myocardium by homing to the zone of infarction? This and many other questions remain unanswered; however, cytokine mobilization of stem cells retains its appealing and innovative promise for the initiation of clinical trials involving cell therapy in patients with cardiac disease.

Mesenchymal or stromal cells are a second population of stem cells in bone marrow (Fig. 1). They are a Lin^- CD34 low/- c-kit^+ Sca-1^+ $CD45^-$ nonhematopoietic cell population in bone marrow *(26,27)* and are generally considered to be a structural component of bone marrow with little or no ability to enter the circulation. Recent experiments demonstrate their capacity to produce soluble factors important for establishing the bone marrow microenvironment *(28)* needed for HSC homing and tethering during steady-state conditions *(29–31)*. It is also becoming clear that mesenchymal/stromal stem cells are capable of multilineage differentiation *(32)*. This finding has generated excitement because MSCs appear to avoid detection by the immune system of recipients following transplantation *(33)*. Thus, they are prime candidates for regenerative cell therapy.

MAPCs isolated from mouse bone marrow are a less well-defined bone marrow stem cell subpopulation *(34)* (Fig. 1). They co-purify with MSCs in the bone marrow mononuclear cell fraction, are $CD45^-$, are $TER119^-$, and display adherence to the surface of culture dishes. When injected into the tail vein of nonirradiated nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice, MAPCs colonized several but not all rapidly renewing epithelial structures. Importantly, they were not detected in the heart and brain. These reported regenerative findings have yet to be confirmed in parallel studies, but it is hoped that the study of MAPCs will contribute substantially to the study of stem cell plasticity.

Endothelial progenitor cells, or angioblasts, derived from bone marrow enter the blood in small numbers and are the immediate precursors for endothelial cells during neovasculogenesis (for review, *see* ref. *35*) (Fig. 1). Their phenotype includes the markers CD34, CD133, and one of the receptors for vascular endothelial growth factor (VEGFR-2) *(36,37)*. Within a few days to a week in culture, EPCs differentiate into

Fig. 1. *(Opposite page)* Adult bone marrow is the source of several populations of stem cells. These are rare cells. Estimates indicate that hematopoietic stem cell incidence ranges from 1:10,000 to 1:100,000 and mesenchymal stem cells may be as rare as 1:200,000 bone marrow mononuclear cells. The number of hematopoietic stem cells remains relatively constant in vivo, with little or no capacity to proliferate ex vivo. In contrast, mesenchymal stem cells, multipotent adult progenitor cells, and endothelial progenitor cells proliferate extensively ex vivo. Some of the similarities and differences between mouse and human stem cells within each stem cell population are indicated. Each stem cell population is characterized by its specific surface phenotype and its ability to differentiate into multiple cell types. We thank the publishers for permission to reproduce the photographs of mesenchymal stem cells from Cardiovasc Res 2005;65:334–344 and of multipotent adult progenitor cells from J Clin Invest 2005;25(5):535–537.

mature $CD31^+$ $CD144^+$ endothelial cells that bind acetylated low-density lipoprotein and synthesize nitric oxide. The level of circulating EPCs measured by colony formation in vitro proved to be a strong indicator of endothelial function and, by extension, potential cardiovascular risk among men of average age 50 years *(38)*. Mouse *(39)* and human *(40)* bone marrow-derived EPCs are capable of restoring vasculogenesis in aging and immunodeficient murine recipients, respectively. In addition to the evidence for EPC origin of endothelial cells, several reports suggest that cells positive for the monocytic surface marker CD14 show outgrowth of endothelial cells from clusters grown on fibronectin-coated dishes *(41,42)*.

In summary, HSCs, MSCs, MAPCs, and EPCs are distinct stem/progenitor cell populations in bone marrow. These cell types differ in size, surface markers, and ability to proliferate and differentiate. For these reasons the term "bone marrow stem cell" may be more suitable when referring to findings based on transplants consisting of a mononuclear fraction of bone marrow cells. Reference to HSCs, which may be uniquely committed to hematopoiesis, rather than BMSCs has created considerable confusion among researchers in the nascent field of cellular plasticity.

THE CONTROVERSY: STEM CELL PLASTICITY OR CELL FUSION?

Many studies that report BMSC generation of multiple cell types (Fig. 2), including skeletal myocytes *(43–46)*, hepatocytes *(18)*, epithelium *(47,48)*, neurons *(49,50)*, and cardiomyocytes *(55,56)*, have been criticized recently. Some of the criticism derives from utilizing the Y chromosome as the primary indicator of transdifferentiation. More exacting studies, based on karyotyping and DNA content, have provided in vitro evidence of fusion of male bone marrow mononuclear cells with female-derived embryonic stem cells *(57)*. Individual cells within the clones produced by these fused cells displayed tetraploidy—three X chromosomes and one Y chromosome—and contained a 4 N nuclear content of DNA. Additional studies have demonstrated in vivo cell–cell fusion following transplantation of *Cre* recombinase engineered bone marrow cells into transgenic R26R, β-galactosidase-positive (β-gal^+) recipients *(58)*. Cell–cell fusion was observed following *Cre* recombinase excision of the *loxP*-flanked stop cassette in recipient nuclei resulting in expression of the *LacZ* reporter.

Fusion occurred in hepatocytes, neurons, and cardiomyocytes at a frequency of approx 1:1000 cells.

HSCs cannot be cloned, unlike mouse NSCs (mNSCs), and thus cannot be analyzed in vitro for plasticity. When enhanced GFP^+ ($EGFP^+$) mNSCs were co-cultured with fresh or paraformaldehyde-fixed human endothelial cells (hECs), the mNSCs were coaxed into adopting a mEC phenotype *(59)*. Cell surface contact was proposed as the mechanism driving transdifferentiation. These findings challenge the theory that no cellular crossover of the embryonic germ layer boundaries occurs in adult tissues and establish the experimental standard needed to achieve acceptance for BMSC plasticity.

DO STEM CELL NICHES EXIST IN MYOCARDIUM?

The existence of cellular niches in the microenvironment of tissues has been extensively studied in bone marrow. Although without compelling evidence, local niches are nevertheless considered the basis for HSC homing to specific sites within bone marrow following their exit from the circulation *(60–63)*. These presumed but poorly

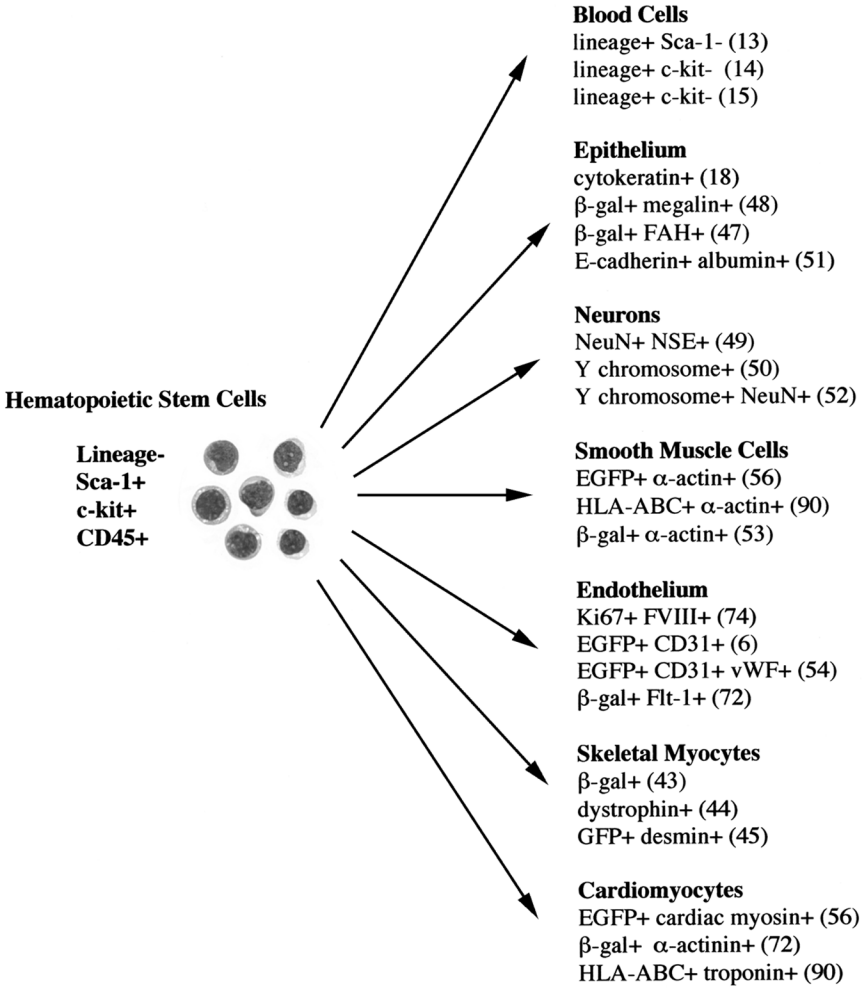


Fig. 2. The most widely studied stem cells in bone marrow are the hematopoietic stem cells. Their role in blood cell formation is well characterized. Of great interest is the recent flurry of papers describing their possible role in the generation of cells outside the hematopoietic system. The claims of hematopoietic stem cell plasticity are based largely on molecular features that the cells acquire during their transdifferentiation into cells of nonmesodermal origin. However, many of the molecular findings that support the concept of hematopoietic stem cell plasticity are being called into question. EGFP, enhanced green fluorescent protein; HLA, human leukocyte antigen; GFP, green fluorescent protein.

defined niches in bone marrow may be the product of secretions from osteoblasts and/or the endosteal cells that form the boundary between bone and bone marrow *(62,63)*. The molecular components of bone marrow niches are believed to provide the appropriate signals needed to anchor stem cells in a milieu conducive for self-renewal and differentiation.

Recent attempts to define microenvironmental niches have been spurred by studies suggesting that BMSCs home to ischemic tissue when they engage in tissue regeneration. Although niches are not well defined within infarcted myocardium, infiltrating or local inflammatory cells, as well as damaged fibroblasts, endothelial cells, mast cells, and

even cardiomyocytes may help to establish the myocardial niche by secreting cytokines, chemokines, and angiogenic factors. This hypothesis suggests that neovasculogenesis and myogenesis may occur in the ischemic zone of infarcted myocardium when BMSCs respond to locally secreted β-fibroblast growth factor, VEGF, angiopoietin 1 and 2, interleukin (IL)-1β and -6, and SDF-1 *(64–69)*. One example of early changes in the myocardial microenvironment involves the accumulation of SDF-1 within the zone of infarction induced by ligation of the left anterior descending coronary artery in a rat model. The level of SDF-1 increased within hours and remained high for a week before dropping to preinfarction levels *(70)*. Expression of SDF-1 in ischemic myocardium was found to induce circulating $CXCR4^+$ c-kit^+ stem cells to home to the infarction, resulting in improved cardiac function *(70)*. A major difficulty in proposing that vascular endothelial cells, fibroblasts, and especially cardiomyoctes within the zone of infarction participate in niche formation derives from indications that apoptosis is initiated within 30 minutes of the onset of ischemia *(71)*. Exploration of the manner by which constituents of the microenvironment are generated is at a rudimentary stage, but it is likely that real progress in cardiovascular repair will be achieved only when our understanding of the molecular signaling pathways is more complete.

Regardless of our inability to define the composition of stem cell niches in mouse myocardium, it was reported that nonmobilized $EGFP^+$ bone marrow stem cells traffic to the zone of infarction, albeit in small numbers, where they infiltrate the tissue *(72)*. Their numbers increased substantially following daily cytokine therapy with G-CSF and SCF *(73),* resulting in improved cardiac function. In a nonhuman primate model, cytokine mobilized stem cells homed to the site of myocardial infarctions and provided evidence of neovascularization accompanied by a 26% increase in blood flow in the zone of infarction *(74)*. It is unclear whether the cytokine therapy utilized in these experiments also induced mobilization of stem cells in other organs. G-CSF and/or SCF therapy may trigger mobilization of endogenous cardiac stem cells residing in atrial and/or ventricular myocardium *(75)* or bone marrow-derived stem cells present in skeletal muscle *(76–78)*.

DOES MYOCARDIUM REGENERATE?

The dogma that defines myocardium as a tissue incapable of self-renewal may no longer be tenable. Several reports now suggest that cardiomyocytes are produced throughout the lifetime of the adult *(79,80)*, but at a low frequency compared with rapidly renewing tissues such as epithelium and bone marrow. However, even a low rate of cardiomyocyte proliferation coupled with an extended cellular half-life may account for a significant level of myocardial renewal during the lifetime of an individual. Although medication is effective in prolonging life in patients with heart disease, there is a strong interest among basic researchers and clinicians to develop a means for repair of injured myocardium. This research is focused on attempts to identify a population of stem cells that would expand the naturally occurring low level of regenerative potential that exists in myocardium. As indicated previously, many believe that myocardial renewing cells can be derived from bone marrow. In addition, skeletal muscle *(81)* and cardiac tissue *(82–87)* appear to contain stem cells with a capacity for myocardial regeneration. To date, none of these three sources has emerged as the leading candidate for repair, but BMSCs have been most frequently studied (Table 1).

Table 1
Animal Studies of Myocardial Cell Therapy Using Bone Marrow-Derived Stem Cells[a]

Reference	*Cell source*	*Donor species*	*Cardiac injury*	*Follow-up*	*Outcome*
Jackson et al., 2001 *(72)*	Nonmobilized BM cells	Mouse	LAD ligation	2 and 4 weeks	Cardiomyocyte and endothelial cell regeneration
Orlic et al., 2001 *(56)*	Lin- c-kit+ BM cells	Mouse	LAD ligation	11 days	Myocardial regeneration, improved LVEDP and LVDP
Orlic et al., 2001 *(73)*	G-CSF/SCF mobilized BM cells	Mouse	LAD ligation	28 days	Myocardial regeneration, improved LVEF
Kamihata et al., 2001 *(88)*	BM-derived mononuclear cells	Rat	LAD ligation	3 weeks	Neovascularization, cytokine secretion
Kocher et al., 2001 *(89)*	Mobilized CD34+ BM cells	Human	LAD ligation	2 and 15 weeks	Vasculogenesis, improved systolic function
Yeh et al., 2003 *(90)*	Nonmobilized PB CD34+ cells	Human	LAD ligation	2 months	Myocardial regeneration
Norol et al., 2003 *(74)*	G-CSF/SCF mobilized BM cells	Baboon	CCA ligation	2 months	Angiogenesis, no improvement in LV systolic function
Balsum et al., 2004 *(91)*	Lin- c-kit+ BM cells	Mouse	LAD ligation	2 and 6 weeks	No myocardial regeneration, improved LVEDD, LVESD, FS
Murry et al., 2004 *(92)*	Lin- c-kit+ BM cells	Mouse	LAD ligation	2–10 weeks	No myocardial regeneration
Henning et al., 2004 *(93)*	CB-derived mononuclear cells	Human	LAD ligation	1–4 months	Reduced infarct size, improved LVEF and LV dP/dt
Soukiasian et al., 2004 *(94)*	B2-microglobulin- BM cells	Rat	Cryoinjury	6 and 8 weeks	Cardiomyocyte regeneration
Zhang et al., 2004 *(95)*	Nonmobilized PB CD34+ cells	Human	LAD ligation	60 days	Cardiomyocyte and endothelial cell regeneration

(Continued)

Table 1 *(Continued)*

Reference	*Cell source*	*Donor species*	*Cardiac injury*	*Follow-up*	*Outcome*
Thompson et al., 2005 *(96)*	Mixed BM-derived Progenitor cells	Rabbit	Cryoinjury	4 weeks	Cardiomyocyte regeneration, improved LVEDP and LV dP/dt
Fukuhara et al., 2005 *(97)*	G-CSF mobilized BM cells	Mice	LAD ligation	4 weeks	Myocytes derived mostly from non-BM sources
Yoshioka et al., 2005 *(98)*	BM CD34+ cells	Monkeys	LAD ligation	2 weeks	No cardiomyocyte regeneration, donor cell secretion of VEGF
Deten et al., 2005 *(99)*	G-CSF/SCF mobilized and iv injected BM cells	Mice	LAD ligation	6 weeks	No cardiac regeneration, LVSP decreased, LVEDP increased
Yoon et al., 2005 *(100)*	Multipotent BM-derived stem cells	Human	LAD ligation	4 weeks	Myocardial regeneration, improved LVEDD, LVESD, FS

[a]Mesenchymal/stromal stem cells, multipotent adult progenitor cells and endothelial progenitor cells are present in adult bone marrow but are not included in this table. BM, bone marrow; LAD, left anterior descending (coronary artery); LVEDP, left ventricle end diastolic pressure; LVDP, left ventricle-developed pressure; G-CSF, granulocyte-colony stimulating factor; SCF, stem cell factor; LVEF, left ventricle ejection fraction; PB, peripheral blood; CCA, circumflex coronary artery; LVEDD, left ventricle end diastolic diameter; LVESD, left ventricle end systolic diameter; FC, fractional shortening; CB, cord blood; LV dP/dt, left ventricle rate of pressure change; VEGF, vascular endothelial growth factor; LVSP, left ventricle systolic pressure.

CAN TRANSPLANTED STEM CELLS REGENERATE MYOCARDIUM?

At 600 beats per minute, the anterior wall of the left ventricle in adult mice is a difficult target for cell transplantation, especially if aiming for the border zone of an infarction. In our experience, of 30 mice injected with a 2.5-μL bolus of $EGFP^+$ Lin^- $c\text{-}kit^+$ BMSCs, only 12 displayed tissue regeneration *(56)*. Upon examination by confocal microscopy and immunochemistry, the regenerating $EGFP^+$ cardiomyocytes resembled fetal cardiomyocytes. They expressed Nkx2.5, MEF-2, and GATA-4, transcriptional factors associated with early cardiomyocyte maturation. $EGFP^+$ endothelial cells expressed Ki67, suggesting proliferation and a role in neovascularization. Following stem cell therapy, left ventricular function was improved. In contrast, when the stem cell-depleted Lin^- $c\text{-}kit^-$ fraction of bone marrow *(15)* was injected, there was no improvement in cardiac function. Because cardiomyocytes undergo apoptosis soon after exposure to ischemia, and because developing $EGFP^+$ cardiomyocytes averaged 500–2500 μm^3, whereas mature mouse cardiomyocytes average 25,000 μm^3, our findings are consistent with the concept of cardiomyocyte renewal rather than cell fusion.

In subsequent investigations, human $CD34^+$ cells isolated from peripheral blood *(69,90)* or cord blood *(11)* were injected into ischemic myocardium of NOD/SCID mice or nude rats. These studies and those involving a swine model of myocardial infarction *(68)* demonstrated an increase in the number of capillaries lined with human $CD34^+$ endothelial cells and improved regional blood flow. In contrast, several studies failed to achieve cardiomyogenesis or neovasculogenesis in infarcted mouse hearts *(91,92,101)*. In addition, they reported either no evidence of donor cell–cardiomyocyte fusion or cardiomyocyte–donor cell fusion in less than 1:1000 cardiomyocytes counted *(101)*. Of interest, in one experiment *(91)*, although microscopic analysis did not reveal any myocardial regeneration at an early time interval, mice examined at 6 weeks postinfarction demonstrated significantly improved cardiac function. Unfortunately, the basis for the partial recovery was not determined. Since scar tissue is expected to be well formed at 6 weeks post infarction, it is unfortunate that the contractile basis for this improvement was not established.

The controversy arising from these contrasting animal findings may serve to motivate researchers in this field to be more rigorous in experimental design and data reporting. It is clear that to advance research on BMSCs in regenerative medicine, it is incumbent on us to resolve these many issues. This lack of agreement regarding myocardial regeneration, however, has not halted ongoing clinical trials that continue to provide a modest degree of encouragement. Indeed, early data suggested that intracardiac injection of host-derived bone marrow mononuclear cells may provide benefit for patients with heart disease.

CLINICAL TRIALS IN ISCHEMIC HEART DISEASE

Heart disease is a leading cause of death worldwide, with nearly 50% of deaths resulting from ischemic heart disease. Nearly 1.1 million myocardial infarctions occur in the United States alone each year. Myocardial infarction is an irreversible injury that severely affects both men and women. When a coronary artery is occluded, regional systolic function and metabolism decrease suddenly and the affected cardiomyocytes undergo changes, leading to apoptosis within 30 minutes of the onset of ischemia *(71)*. Angioplasty and thrombolytic agents can significantly limit the extent of the infarction by reducing the duration and severity of the perfusion defect and thus improve the prognosis of patients suffering an infarction, but there is no treatment to replace a

myocardial scar with healthy contractile tissue. Thus, there is a need to investigate possible regenerative therapies.

Several clinical trials are currently underway in Europe, the Far East, Brazil, and the United States to test the regenerative capacity of autologous bone marrow-derived cells following an acute myocardial infarction. With one exception in which $CD133^+$ cells were injected *(102)*, most trials to date have utilized density gradient separated bone marrow mononuclear cells *(103–113)* consisting of a mixture of primitive hematopoietic, endothelial, and mesenchymal stem cell populations as well as mature monocytes and lymphocytes. The cells were delivered percutaneously to the zone of infarction by a series of transendocardial injections or were infused in a series of pulses into the infarct-related coronary artery using a balloon catheter. These trials were based on results from animal experiments that were not designed to determine the appropriate cell type for transplantation or the optimum number of cells needed to achieve a positive outcome. Likewise, the most efficacious route for cell delivery is still an open question. Several reports indicated a modest degree of short-term (2–4 month) efficacy in regard to reperfusion of the infarcted zone with improved survival of cardiomyocytes distal to the occluded artery. However, critics point to major shortcomings of these trials, including the small number of patients enrolled and the fact that the studies, with a single exception thus far, were not randomized or double-blinded. Even stronger criticism is directed at the concept of initiating clinical protocols prior to establishing positive outcomes in nonhuman primate studies. However, clinicians conducting trials argue, with considerable justification, that patients with severely damaged heart muscle are in need of novel attempts to improve symptoms and prognosis. They also argue that no adverse effects have been observed to date among the more than 200 patients treated with stem cell therapy.

One clinical trial *(108)* that received considerable attention was designed to satisfy some of the objections raised against the earlier trials. Sixty patients were enrolled, with 30 randomly assigned to the control group and 30 to the cell therapy group. All patients received percutaneous coronary intervention with stent implantation prior to entry in the trial and were maintained on medication. Magnetic resonance imaging of global left ventricular ejection fraction was designated the primary end point. Autologous bone marrow cells were obtained from the posterior iliac crest within 4–8 days after percutaneous coronary intervention but prior to the onset of fibrous tissue formation. A total of approx $2.5–3.0 \times 10^9$ mononuclear cells, including $1.0–1.2 \times 10^7$ $CD34^+$ cells, were infused during four or five occlusions of the infarct-related coronary artery via an over-the-wire inflated balloon catheter to prevent retrograde cell migration. Each occlusion lasted 2.5–4 minutes, after which the balloon was deflated and the tissue reperfused for several minutes to prevent mini-infarctions. Six months after treatment, all patients were assayed by scintillation angiography using fluorodeoxyglucose–positron emission tomography. The cell therapy group demonstrated enhanced global and regional contraction and a modest but significant 6% increase in left ventricular ejection fraction from a baseline value of 51 to 57% ($p = 0.0026$). The 30 patients enrolled as control subjects showed a nonsignificant 0.7% increase in left ventricle ejection fraction. No attempt was made to uncover the molecular or cellular mechanisms responsible for this improvement, but it has been suggested that the transplanted cells may have secreted cytokines or chemokines that favored cardiomyocyte recovery from the ischemic episode. Of concern, the heart sizes in diastole were greater in cell therapy-treated patients

compared with untreated patients, which is contrary to expectation of a favorable effect on healing of the infarct.

In a follow-up to the early 6-month report on the BOOST trial in which 60 patients were randomized to receive placebo or BM transfer, cardiac MRI was repeated at 18 months after treatment. The significant improvement in mean global LV ejection fraction seen early in the study was no longer apparent at 18 months (3.1% increase in controls vs 5.7% in BM recipients, $p = 0.27$). The authors concluded that a single dose of BM cells infused via the infarct-related coronary artery did not provide long-term improvement in LV systolic function *(109)*. This observation differed substantially from the data obtained in the TOPCARE-AMI trial that demonstrated a continuous rise in LV ejection fraction in a cohort of similarly treated patients assessed at 4, 12, and 24 months after BM cell transfer. Global LV ejection fraction in the BM treated group increased progressively from 47 + 10% to 63 + 10% for a mean increase of 15.8% at 24-month follow-up *(110)*.

A randomized, double blind, placebo-controlled study was recently reported that included 67 patients with ST-elevation acute myocardial infarction *(111)*. Patients received autologous BM cells or placebo by intracoronary transfer within 24 hours following reperfusion therapy. Global LV ejection fraction assessed by MRI at 4 months did not show improvement following BM cell transfer. The increase from 48.5 to 51.8% was comparable to the increase from 46.9 to 49.1% observed in control patients ($p = 0.36$). In contrast, infarct size decreased significantly from 21 to 10 g in recipients of BM cells compared with a reduction from 22 to 15 g in the placebo controls. This 28% treatment effect ($p = 0.036$) may represent a favorable effect on myocardial remodeling. Similarly, findings were obtained when intracoronary transplantation was performed on a small cohort of 18 patients with chronic coronary artery disease *(112)*. Infarct size was reduced by 30% at 3 months following transplantation along with a 15% improvement in global LV ejection fraction and a 57% increase in infarction wall movement velocity.

The infarct-related coronary artery is the most common route for infusing BM cells acutely following an infarction. However, in treating patients with chronic ischemic heart failure an attempt has been made to utilize the transendocardial route for cell delivery *(113)*. This clinical trial followed a study in adult pigs *(114)*, in which transendocardial injections of autologous BM cells resulted in enhanced collateral perfusion. In the clinical study, a cohort of 18 nonrandomized patients received transendocardial transplantation of autologous BM cells using a NOGA catheter. At 4 months, the authors reported an improvement in LV ejection fraction from 20 to 27% ($p = 0.003$) and a significant reduction in end systolic volume ($p = 0.03$).

In addition to clinical trials involving coronary artery or transendocardial infusion of BM stem cells, several papers report the use of subcutaneous injections of G-CSF in order to mobilize BM stem cells. Regardless of whether cytokine treatment was initiated on day 1 or day 5 after acute myocardial infarction there was no detectable influence on LV ejection fraction between the G-CSF group and the placebo group (0.5 vs 2.0%, $p = 0.14$) *(115)*, no reduction in infarct size (6.2 vs 4.9%, $p = 0.56$) *(115)* and no systolic wall thickening in the infarct area (17 vs 17%, $p = 1.0$) *(116)* at 4–6 months compared with randomized, double-blind, placebo-controlled patients. It was concluded that G-CSF treatment is safe but fails to produce positive effects in acute myocardial infarction patients. The 4- to 5-day delay in achieving large numbers of mobilized BM CD34+ cells following the onset of G-CSF therapy may in part be responsible for the lack of a positive outcome.

SUMMARY

Since the mid-1990s when reports began to appear suggesting the possibility of regenerating damaged myocardial tissue following coronary artery occlusion or cryoinjury, numerous studies have explored the potential regenerative capacity of embryonic stem cells, fetal stem cells, cardiac stem cells, and adult BMSCs. These early experiments in rodents, dogs, pigs, and nonhuman primates have provided some insight into myocardial regeneration, but much remains obscure. Perhaps the highest priority at this time is the need to precisely identify the cells with the best prospect for tissue regeneration. This has not been accomplished in any of the animal models to date, but once identified it will be possible to study the genetic and cellular regulatory mechanisms involved. There is an urgent need to expand the use of large animal models in regenerative studies. This will enable researchers to determine the optimum time and route of stem cell delivery and establish the number of stem cells required to regenerate a unit volume of infarcted myocardium.

Reports indicating some success in regenerating myocardium in small animal studies have stimulated a desire among clinicians to initiate trials in patients with acute myocardial infarction and ischemic heart failure. Most of these clinical efforts have utilized a mixture of adult bone marrow cells that included several populations of stem cells. These trials have provided modest but encouraging achievements, and, not withstanding all the controversy, it is clear that we are entering an exciting period in cardiovascular medicine. We eagerly await the outcome from long-term randomized trials conducted at multiple clinical centers. If stem cell therapy for regenerative medicine can be widely validated, perhaps one day it will become standard therapy.

ACKNOWLEDGMENT

This research was supported by the Intramural Research Program of the National Heart Lung and Blood Institute, NIH, Bethesda, MD.

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http://www.springer.com/978-1-58829-664-1

Stem Cells and Myocardial Regeneration
Penn, M.S. (Ed.)
2007, 400 p. 67 illus., 10 illus. in color., Hardcover
ISBN: 978-1-58829-664-1
A product of Humana Press