This Review is part of a thematic series on Stem Cells, which includes the following articles:
Differentiation of Pluripotent Embryonic Stem Cells Into Cardiomyocytes
Derivation and Potential Applications of Human Embryonic Stem Cells
Stem Cells for Myocardial Regeneration
Neural Stem Cells: An Overview
Mesenchymal Stem Cells
Myocyte Death, Growth, and Regeneration in Cardiac Hypertrophy and Failure
Therapeutics and Use of Stem Cells

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Stem Cells for Myocardial Regeneration
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Abstract—Stem cells are being investigated for their potential use in regenerative medicine. A series of remarkable studies suggested that adult stem cells undergo novel patterns of development by a process referred to as transdifferentiation or plasticity. These observations fueled an exciting period of discovery and high expectations followed by controversy that emerged from data suggesting cell-cell fusion as an alternate interpretation for transdifferentiation. However, data supporting stem cell plasticity are extensive and cannot be easily dismissed. Myocardial regeneration is perhaps the most widely studied and debated example of stem cell plasticity. Early reports from animal and clinical investigations disagree on the extent of myocardial renewal in adults, but evidence indicates that cardiomyocytes are generated in what was previously considered a postmitotic organ. On the basis of postmortem microscopic analysis, it is proposed that renewal is achieved by stem cells that infiltrate normal and infarcted myocardium. To further understand the role of stem cells in regeneration, it is incumbent on us to develop instrumentation and technologies to monitor myocardial repair over time in large animal models. This may be achieved by tracking labeled stem cells as they migrate into myocardial infarctions. In addition, we must begin to identify the environmental cues that are needed for stem cell trafficking and we must define the genetic and cellular mechanisms that initiate transdifferentiation. Only then will we be able to regulate this process and begin to realize the full potential of stem cells in regenerative medicine. (Circ Res. 2002;91:1092-1102.)

Key Words: stem cells ■ plasticity ■ ischemia ■ infarction ■ myocardial regeneration

In this review, we address the concept of whether stem cells can repair injured tissues,1-4 with emphasis on ischemic heart disease. Ischemic heart disease accounts for 50% of all cardiovascular deaths and is the leading cause of congestive heart failure as well as premature permanent disability in workers.5 With ≈1.1 million myocardial infarctions and >400,000 new cases of congestive heart failure each year, cardiovascular disease severely impacts men and women as well as various ethnic groups. For patients diagnosed with congestive heart failure, a consequence of chronic heart disease, the 1-year mortality rate is 20%.

Myocardial Infarction and the Consequences of Ischemic Heart Disease
Myocardial infarction is, by nature, an irreversible injury.6 Regional systolic function and regional metabolism decrease within a few heartbeats of a sudden decrease in myocardial perfusion.7 In some patients, impaired diastolic relaxation...
may precede global systolic abnormalities. Irreversible cardiomyocyte injury begins after ≈15 to 20 minutes of coronary artery occlusion. The subendocardial myocardium has high metabolic needs and thus is most vulnerable to ischemia. The extent of the infarction depends on the duration and severity of the perfusion defect. However, the extent of infarction is also modulated by a number of factors including collateral blood supply, medications, and ischemic preconditioning. Beyond contraction and fibrosis of myocardial scar, progressive ventricular remodeling of nonischemic myocardium can further reduce cardiac function in the weeks to months after the initial event.

Many of the therapies available to clinicians today can significantly improve the prognosis of patients with acute myocardial infarction. Although angioplasty and thrombolytic agents can relieve the cause of the infarction, the time from onset of occlusion to reperfusion determines the degree of irreversible myocardial injury. No medication or procedure used clinically has shown efficacy in replacing myocardial scar with functioning contractile tissue. There is need for new therapeutics to regenerate normal cardiomyocytes.

Recent attempts to repair experimentally induced acute myocardial infarctions have provided encouraging but limited success in a number of animal models. The most promising results have been obtained after transplantation and mobilization of bone marrow cells to the area of infarction. We will review the emerging literature in the nascent field of stem cell plasticity and describe new instrumentation to deliver and monitor stem cell activity in myocardial therapy.

**Embryonic and Fetal Stem Cells**

The most primitive of all stem cell populations are the embryonic stem cells (ES cells) that develop as the inner cell mass at day 5 after fertilization in the human blastocyst. At this early stage, ES cells have vast developmental potential. They give rise to cells of the three embryonic germ layers. When isolated and transferred to appropriate culture media, mouse and human ES cells can undergo an undetermined number of cell doublings while retaining the capacity to differentiate into specific cell types, including cardiomyocytes.

Common teaching suggests that stem cells emerging during late embryonic and fetal development are restricted to the production of tissue-specific cell types. Specific gene expression patterns are imprinted and, although stem cells continue to self-renew in adult life, their ability to differentiate is limited to the tissue in which they reside. We will examine this dogma in light of recent remarkable data that indicate that adult stem cells retain a high degree of developmental plasticity. If this challenge to the traditional developmental paradigm of adult stem cell commitment is sustained, we are about to enter a revolutionary period in stem cell biology and regenerative medicine.

**Adult Mesenchymal Stem Cells (MSCs)**

MSCs can be derived from adult bone marrow and in vitro appear to have multilineage differentiation capacity. In culture, MSCs can maintain an undifferentiated, stable phenotype over many generations. However, controversy still exists regarding their precise phenotype, and there are no adequate markers to allow selection of purified cell populations. The DNA demethylating agent 5-azacytidine has been used to induce multiple new phenotypes, including cardiomyocytes. Additional unexpected differentiation pathways involving MSCs have been described for the formation of neural cells and the basis of neural cell–specific markers.

Preclinical models have shown the ability of undifferentiated human MSCs to undergo site-specific differentiation into a functional cardiac muscle phenotype after injection into sheep. Thus, they seem to avoid detection by the host immune system. Allogeneic bone marrow MSCs may therefore have potential clinical utility because of their lack of immunogenicity and relative ease of culture. As such they can be harvested and cryopreserved ready for infusion immediately after myocardial infarction.

Another subset of bone marrow stromal cells referred to as mesodermal progenitor cells or multipotent adult progenitor cells has been described. Multipotent adult progenitor cells copurify with MSCs. They proliferate extensively and differentiate in vitro into cells of all three germ layers. When injected in vivo they reconstitute bone marrow, liver, gut, lungs, and endothelium.

**Adult Hematopoietic Stem/Progenitor Cells**

Two categories of blood-forming stem cells exist in adult bone marrow. One population can provide permanent long-term reconstitution of the entire hematopoietic system. These cells, referred to as hematopoietic stem cells (HSCs), are rare, perhaps as few as 1:10 000 bone marrow cells. However, utilizing the specificity of monoclonal antibodies, HSCs can be enriched by flow cytometry to near purity on the basis of surface markers. As few as 20 to 100 highly purified mouse bone marrow HSCs can reconstitute the entire lymphohematopoietic system in myeloblasted adult mice. They can self-renew and can differentiate into the more mature progenitor cells in bone marrow.

The progenitor cells of bone marrow have a limited capacity for self-renewal and differentiation. They can only sustain hematopoiesis for 1 to 2 months and therefore are considered to be short-term repopulating stem cells. It is proposed that one subclass of mouse progenitor cells, the common lymphocytic progenitors (CLP), Lin c-kit Sca1 IL7Ra, is restricted to the generation of B and T lymphocytes, whereas another subclass, the common myelocytic progenitors (CMP), Lin c-kit Sca1 IL7Ra, is restricted to the generation of myelocytic cells. These CLP and CMP initiate hematopoietic activity by giving rise to precursor cells responsible for the formation of each blood cell lineage. Although in vivo assays for human CLP and CMP are not established, it is clear from in vitro studies that these progenitors exist in human bone marrow. In the treatment of blood disorders, it is now routine clinical practice to isolate and transplant CD34 stem cells. These include progenitor cells and HSCs that provide short-term and long-term hematopoietic reconstitution. Although several reports demonstrate the presence of HSCs in the Lin-CD34 cell population in mouse and human bone marrow, the role of CD34 HSCs in hematopoietic reconstitution is not well understood.
Hence, CD34⁺ HSCs are not used in bone marrow transplantation. We will use the term bone marrow stem cells (BMSCs) to signify the inclusion of both short- and long-term reconstituting stem cells in donor cell populations.

Short-term repopulating progenitor cells of bone marrow cannot self-renew indefinitely. Other tissue-specific stem/progenitor cells may also need to be continually replenished by a more primitive stem cell population. Emerging data suggest that BMSCs have the ability to differentiate into stem and progenitor cells that mature into functional cells in a variety of tissues including myocardium.

**Adult Stem Cells Engage in Normal Tissue Regeneration**

Stem cells are the ancestors of the specialized cells that impart function to tissues and organs. Throughout postnatal life, stem cells regenerate tissues that continually lose cells through maturation and senescence. These include the epithelial layers in skin; intestinal and pulmonary mucosal linings; and connective tissues such as bone, cartilage, muscle, blood, and bone marrow. Although previously considered to be postmitotic organs, recent evidence is persuading us to include brain, heart, and bone marrow on the list of adult tissues with regenerative capacity. The extremely low rate of neural and myocardial cell turnover may explain why renewing cells were not previously detected in these organs.

The origin of stem cells in regenerating adult tissues is now being called into question. It can no longer be assumed that tissue-specific stem cells are self-sustaining throughout life or that they are responsible for regenerating tissues damaged by radiation or chemotherapy treatments. As reported in a number of recent papers, bone marrow cells appear to have the capacity to repopulate many nonhematopoietic tissues. Thus, bone marrow may serve as a central repository for the primitive stem cells that can repopulate somatic tissues.

**Adult Mouse BMSC Plasticity**

In a series of reports, it has been suggested that adult BMSCs retain the capacity to produce cells of unrelated tissues. Based on evidence from several mouse models, tissues of all three germ layers can be derived from adult BMSCs (Figure 1). These include skeletal muscle, hepatocytes, neural cells, vascular endothelium, and epithelium of skin and several internal organs. Plasticity of transplanted BMSCs has been established by identifying specific cell surface markers or by fluorescence in situ hybridization identification of Y-positive nuclei in donor-derived cells that have acquired the capacity to synthesize specific protein in regenerating tissues.

In one study, a single male BMSC was transplanted by intravenous injection into lethally irradiated adult mice. At 11 months, Y-positive cells were identified in the liver, kidneys, skin, and epithelial lining of several internal organs including lung and small intestine. The highest percentage of Y-positive cells occurred in epithelial tissues. A common difficulty with this technique is the possibility that the percentage of Y-positive cells in any tissue will be underestimated. This inherent error relates to sampling, because a portion of the nucleus will often be excluded from the plane of the section. Hence, the Y chromosome in many male nuclei will not be recorded. Another difficulty involves the possibility that the Y-positive nucleus of a nearby blood cell may be recorded as belonging to the cell in question. This problem can be largely overcome by careful analysis using appropriate confocal microscopy techniques. Endogenous stem cells in these rapidly renewing tissues are likely to suffer severe depletion during the preconditioning total body radiation exposure leading to a critical need for recruitment of large numbers of exogenous stem cells. Thus, transplanted self-renewing Y-positive BMSCs may provide a new supply of stem/progenitor cells for epithelial and other somatic tissues in need of regeneration. Studies such as this mark an...
important beginning to our understanding of adult BMSC plasticity.

Can Stem Cell Plasticity Be Explained by Cell Fusion?

The enthusiasm generated by findings on stem cell plasticity has been countered by a degree of skepticism in many research centers. Several recent papers demonstrate in vitro cell-cell fusion of transgenic female-derived neural or bone marrow cells with male-derived ES cells. Cell fusion occurred at an estimated frequency of 1:10,000 or 1:100,000 cells, and the hybrid cells displayed a dual phenotype. They possessed a large nucleus that contained numerous nucleoli and a tetraploid number of chromosomes. The identification of XXXY-positive nuclei provided the strongest argument for the authors’ contention that cell fusion in animal studies may be a legitimate alternative to the concept of stem cell plasticity. In mouse studies that demonstrate 0.02% to 0.5% donor-derived cells, the cell fusion theory cannot be dismissed. In contrast to these reports of low-level reconstitution, wild-type BMSCs injected into genetically defective adult mice with a metabolic liver disorder resulted in the regeneration of significant liver mass. Also, in an experimental retinopathy study in mice, an extensive retinal capillary network was regenerated from BMSCs. Our own studies of myocardial regeneration demonstrated the formation of a new band of myocardium from BMSCs. It is unlikely that infrequent cell fusion events could explain the significant regeneration observed in liver, eye, and heart in these studies. Nevertheless, the possibility of cell fusion and tetraploidy must be ruled out in these studies as well.

Are Environmental Factors Involved in Stem Cell Migration?

The homing of stem cells to areas of tissue injury may potentially occur via two or more distinct scenarios. One hypothesis suggests that cell necrosis following an injury such as myocardial infarction may cause the release of signals that circulate and induce mobilization of stem cells from the bone marrow pool. The injured tissue may express appropriate receptors or ligands to facilitate trafficking and adhesion of stem cells to the site of injury where initiation of a differentiation cascade results in the generation of cells of the appropriate lineage. An alternative hypothesis suggests that stem cells are continually circulating with constant trafficking through all tissues, but only at the time of injury do they exit the blood and begin to infiltrate the site of injury. Both concepts support the view that there are circulating stem cells that could originate from a common pool in bone marrow. This is further supported by findings that show that stem cells isolated from skeletal muscle retain hematopoietic activity and are itinerant cells derived from bone marrow.

It is still unclear what environmental cues initiate mobilization and homing of adult BMSCs to normal and injured tissue. Likewise, we know little about the factors that induce these stem cells to differentiate along the appropriate organ-specific lineage. Below are described several receptor-ligand interactions that may regulate BMSC trafficking and that are the subject of intense investigation.

Stem Cell Factor (SCF) and c-kit

HSCs, neural crest cells, and germ line cells express c-kit, a tyrosine kinase receptor. The migration of these cells during embryonic development may be regulated by the c-kit ligand, SCF. SCF mRNA is expressed in fetal and neonatal hearts and by adult myocardial fibroblasts and macrophages. In theory, SCF may provide signals for stem cell migration in response to myocardial injury.

We hypothesize that there is an insufficient local stem cell pool to provide acute myocardial regeneration. Without augmentation of the number of circulating BMSCs through cytokine mobilization, the response to ischemia favors scar formation rather than cardiomyocyte regeneration. Recent exciting evidence has shown the central role of SCF, c-kit, and matrix metalloproteinase-9 in the mobilization of stem and progenitor cells from the bone marrow niche within hours of onset of myocardial necrosis.

The intense inflammatory reaction that initiates healing after a left ventricular (LV) myocardial infarction causes a local accumulation of mast cells and that are positive for CD117, the human equivalent of c-kit. They may migrate locally in response to macrophage secretion of SCF. This reinforces the idea that homing signals are released soon after myocardial injury.

CXCR4 and Stromal Cell–Derived Factor-1 (SDF-1)

CXCR4 is important for lymphocyte trafficking and recruitment at sites of inflammation, eg, after myocardial infarction. It appears that CXCR4 serves as a chemokine receptor and together with its ligand, SDF-1, plays an important role in vasculogenesis and hematopoiesis. These changes may occur in response to a disrupted interaction between SDF-1 and CXCR4. This hypothesis is supported in part by studies that show that SCF upregulates CXCR4 expression on human CD34+ stem/progenitor cells and enhances their migration in response to SDF-1. Migration of bone marrow CD34+ cells across endothelial barriers is modulated by a wide variety of chemokines, but the largest response is seen with α and β SDF-1.

Granulocyte Colony-Stimulating Factor (G-CSF)

The cytokine G-CSF is widely used to mobilize stem/progenitor cells that are harvested by leukapheresis, stored, and subsequently reinfused to support hematopoietic recovery in patients after chemotherapy or radiation treatment. How G-CSF mobilizes stem cells and progenitor cells from the bone marrow into the circulation is not clear because BMSCs do not generate G-CSF receptor. This suggests that an indirect mechanism may exist. For example, a single dose of G-CSF can induce a downregulation in SDF-1 levels within 24 hours and an upregulation of CXCR4 expression on hematopoietic cells. Accordingly, G-CSF stimulation potentiates the homing abilities of cytokine-stimulated BMSCs, an action that can be inhibited by pretreatment with anti-CXCR4 antibodies.

Vascular Endothelial Growth Factor (VEGF)/Flk-1

The roles of the VEGF isoforms and the tyrosine kinase VEGF receptors in endothelial cell proliferation and differ-
entiation are well described. However, evidence is emerging that VEGF receptor expression, most notably VEGF receptor-2 (KDR/flk-1), may define the point of divergence in the differentiation pathway of bone marrow–derived stem cells along a vascular progenitor lineage.

**BMSCs Regenerate Cells in Animal Models of Several Human Diseases**

**Hepatocyte Regeneration**
The capacity of highly enriched mouse long-term repopulating BMSCs to regenerate functional hepatocytes was tested in adult knockout female recipients deficient in fumarylacetate hydrolase (FAH−/−) synthesis. At 7 months after an intravenous injection of male BMSCs, donor Y-positive cells infiltrated the liver parenchyma and gave rise to nodules that comprised 30% to 50% of the liver mass. These wild-type donor-derived hepatocytes synthesized FAH, resulting in improved liver function and survival. This rescue of FAH−/− mutants demonstrated the efficacy of BMSC-induced repair of a genetic disease.

**Endothelial Regeneration**
GFP+ cells were isolated from transgenic mouse bone marrow on the basis of a Lin− c-kit− phenotype. A single transplanted GFP+ cell was injected into each of a large number of mice, and in several recipients the bone marrow was reconstituted within 6 months. The investigators then used an Argon Green laser system to induce photocoagulation in the retinal vasculature as a model for retinopathy. Damaged vessels were replaced within 3 weeks with a new, developing GFP+ capillary network. Because blood cells and endothelium were both derived from the same single GFP+ cell, the authors attributed this activity to adult bone marrow cells comparable to the embryonic hemangioblasts.

**Myocardial Regeneration**
Our data on regeneration in an adult mouse model of myocardial infarction demonstrate the ability of BMSCs to differentiate into cardiac myocytes, endothelial cells, and vascular smooth muscle cells. Ischemic injury to the myocardium of the left ventricle was produced by ligation of the descending branch of the left coronary artery (LCA) without reperfusion. The infarcts occupied as much as 70% of the free wall of the left ventricle with loss of myocytes and coronary vessels. When male BMSCs (phenotype, Lin− c-kit−) that carried the gene encoding enhanced green fluorescent protein (eGFP) were injected within 5 hours after coronary ligation (Figure 2), a band of regenerating myocardium was seen at 9 days after surgery (Figure 3A). This band consisted of Y-positive, eGFP+ cardiac myocytes and small coronary vessels. Regeneration was not observed (Figure 3B) in hearts that were transplanted with the subpopulation of bone marrow cells (phenotype, Lin− c-kit+) known to be devoid of stem cells. Cardiomyocytes (Figures 3C through 3E), smooth muscle cells, and endothelial cells were all eGFP positive. Early-acting cardiac-specific transcription factors GATA-4, Csx/Nkx2.5, and MEF-2 were expressed in the developing cardiomyocytes as well as cardiac myosin, sarcomeric α-actin, and connexin 43. The immature myocytes were arranged into what appeared to be an early form of an integrated syncytium with some connexin 43 at the lateral border of adjacent cells (Figures 3F and 3G). LV end-diastolic pressure and LV developed pressure improved 30% to 40% in hearts transplanted with BMSCs compared with negative control mice.

Myocardial regeneration was also examined in a mouse model in which BMSCs marked with the β-galactosidase gene were used to create chimeric bone marrow in adult mice. Subsequently, myocardial infarcts were induced, as in our study, by ligation of the LCA. β-Galactosidase–positive BMSCs were found to migrate to the site of injury and to give rise to new cardiomyocytes and endothelium. Although the level of β-galactosidase–positive cells was only 0.02% of the total myocardial cells counted, this study demonstrated a natural but very inefficient response by the BMSCs to repair the damaged myocardium.

We were able to demonstrate extensive regeneration in the mouse myocardial infarction model with cytokine-mobilized autologous BMSCs. After 5 daily injections of recombinant rat SCF and recombinant human G-CSF, the wave of circulating BMSCs reached a peak. Myocardial infarctions produced by ligation of the LCA showed a new band of myocardium. The new myocytes resembled fetal cardiac myocytes in size and gene expression. Their failure to mature and their continued proliferation remain unresolved issues. Numerous developing capillaries and arterioles were observed, and some contained red blood cells in their lumen. This suggested that anastomosis had occurred with the spared coronary vessels. Cytokine therapy improved hemodynamic functions, including ejection fraction, LV end-diastolic pressure, and LV end-systolic pressure, and resulted in markedly increased survival at 27 days.

These experiments demonstrated the capacity of adult BMSCs to give rise to new myocytes, endothelial cells, and smooth muscle cells in ischemic myocardium. However, they did not define whether one or more BMSC populations were responsible for the generation of these several myocardial cell types. Nor did they exclude the possibility that some stem...
cells with regenerative capability may have originated in other organs, including the heart.

Adult Human BMSC Plasticity

Y-Positive Cells Infiltrate Nonhematopoietic Organs

Therapeutic transplants of sex-mismatched bone marrow and orthotopic organ transplants in male recipients have been used to study human BMSC plasticity. Archival samples of liver and heart obtained from female recipients of a male bone marrow transplant and male patients who had received a liver or heart transplants from female donors were positive for Y-chromosomes.\textsuperscript{76,77} Although differing widely in percentage of Y-positive myocytes detected, several reports\textsuperscript{38,39} conclude that recipient cells repopulate the myocardium in transplanted hearts. Taken together, these data provide evidence that circulating human BMSCs traffic to nonhematopoietic organs, where they give rise to cells of a completely different origin and phenotype.

Clinical Trials to Regenerate Myocardium in Ischemic Heart Disease

In 10 patients with acute myocardial infarction, autologous mononuclear bone marrow cells were transplanted via the infarct-related artery after angioplasty. At 3 months after transplant, the infarct area had decreased significantly compared with 10 patients not given cell therapy. The treated patients also showed improved LV end-systolic volume and contractibility. This is the first report\textsuperscript{78} to demonstrate clinical feasibility of intracoronary infusion of bone marrow cells for myocardial repair.

Seiler et al\textsuperscript{79} recently reported the effects of intracoronary and systemic administration of granulocyte-macrophage colony-stimulating factor (GM-CSF) in patients with coronary artery disease. GM-CSF is a cytokine with effects on bone marrow similar to the more commonly utilized G-CSF, albeit with less potency for BMSC mobilization. Twenty-one patients who were not amenable to or refused coronary bypass surgery participated in this randomized, double blind, placebo-controlled study. Ten individuals received GM-CSF via intracoronary infusion into the vessel believed to subserve ischemic myocardium, followed by systemic administration of GM-CSF daily for 2 weeks. Analysis of mobilization of BMSCs was not performed in this study, but because the leukocyte counts were only twice the baseline values, it was suggested that only modest BMSC mobilization was achieved.

An invasive measure of collateral artery blood flow (estimated by coronary artery pressure distal to balloon occlusion) before and after administration of GM-CSF or placebo indicated improved collateral flow in the GM-CSF group at 2 weeks, but not in the placebo group, with reduced ECG signs of myocardial ischemia during coronary balloon occlusion. Because the quantity of BMSCs mobilized with GM-CSF was probably low, the coronary vascular benefit determined in this study may have resulted from direct effects of this cytokine on angiogenesis or on collateral vascular dilator tone with improved regional blood flow. No clinically relevant end points (eg, exercise-induced myocardial ischemia or LV contractile response to stress) were assessed in this study.

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**Figure 3.** BMSCs regenerate infarcted myocardium. Lin\textsuperscript{−} c-kit\textsuperscript{+} eGFP\textsuperscript{+} cells from transgenic mice were injected into myocardium near the site of an acute infarction. A, After 9 days, partial regeneration of structure and function were observed. Asterisk indicates necrotic myocytes; red, cardiac myosin; and green, nuclei labeled with propidium iodide. B, Lin\textsuperscript{−} c-kit\textsuperscript{−} bone marrow cells are devoid of stem cells and do not regenerate myocardium. Original magnification, \( \times \)50. C, Cardiac myosin (red), D, eGFP (green). E, Overlay of cardiac myosin (red) and eGFP (green). Propidium iodide–stained nuclei (blue). EN indicates endocardium; EP, epicardium; and arrows at subendocardium, area of myocardial infarction not regenerated. Original magnification, \( \times \)250. F, Adult control heart shows myocytes positive for connexin43 at intercalated disks (arrows) and at lateral margins of adjacent cells. G, Newly formed young myocytes in a Lin\textsuperscript{−} c-kit\textsuperscript{−}–treated heart show connexin43 in their cytoplasm with some distribution at lateral cell margins (arrows). Arrowheads indicate spared myocytes in the epicardium. First published in Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. Bone marrow cells regenerate infarcted myocardium. Nature. 2001;410:701–705.
These studies represent the first clinical attempts to regenerate myocardium after infarction. However, the empirical nature of these observations emphasizes the need for continued preclinical testing to determine the phenotype of the bone marrow cells involved in myocardial repair and to define the signaling required for their migration and differentiation into myocardial cells. When these questions are resolved, we can look to a time when transplanted or cytokine-mobilized stem cells may provide a new modality for the treatment of heart disease.

Role of Imaging in Assessing the Success of Stem Cell Therapy

Imaging will play an important role in assessing the myocardial response to stem cell therapy. In preclinical trials, analysis of tissue specimens allow detailed cellular characterization of genetic and cell surface markers. However, the need to euthanize the animal at fixed time intervals raises the complementary need for serial noninvasive imaging of myocardial function, perfusion, viability (Figure 4), and cell tracking.

Echocardiography assessment of myocardial function is well established. Doppler tissue imaging now provides real-time quantitative measurements of regional contractile function that have higher temporal resolution than any measurement scheme other than implanted ultrasonic crystals. Similarly, single photon emission computed tomography (SPECT) and positron emission tomography (PET) imaging can determine myocardial perfusion and viability. Developments in cardiovascular MRI warrant further discussion because this technology is well suited to serial studies.

MRI is generally accepted as a gold standard method for evaluating cardiac anatomy and volumes. Cine MRI provides excellent contrast between the myocardium and blood and performs with diagnostic accuracy comparable to that of dobutamine stress echocardiography. The programmable nature of the imaging planes allows reproducible and volumetric coverage of the heart. This leads to markedly smaller sample size requirements for clinical trials than can be achieved with echocardiography. The technology also scales well with subject size from mouse to human (Figure 5). There are several MRI-specific ways to quantitatively assess regional function including myocardial tagging, velocity-encoded imaging, and displacement-encoding methods.

Myocardial perfusion imaging using contrast-enhanced first-pass MRI can now obtain whole-heart coverage at a resolution double that of a PET scanner and 4 times the resolution of SPECT. Perfusion can be evaluated semiquantitatively in 250-μL samples of myocardium with high statistical certainty. This has translated to excellent diagnostic accuracy in patients with possible coronary artery stenosis when compared with gold standards of quantitative coronary angiography or PET scans. New MRI myocardial viability methods are now well validated. Gadolinium hyperenhancement correlates closely with infarcted myocardium defined by triphenyltetrazolium chloride. Gadolinium distributes in a pattern similar to that of sodium as demonstrated by electron probe x-ray microanalysis and by imaging. The gadolinium chelates used clinically equilibrate rapidly in the extracellular volume. Because of membrane rupture, the myocytes of acutely infarcted myocardium fail to exclude sodium and gadolinium from the intracellular space resulting in substantial contrast enhancement relative to normal myocardium. The volume of distribution remains high in chronic myocardial infarction. Furthermore, gadolinium enhancement differentiates stunned from infarcted myocardium with high specificity. The main advantage of this technique over nuclear methods is the high image resolution achievable. There is a relationship between the transmural extent of...
infarction and clinical definitions of viability\textsuperscript{107,108} (Figure 6). MRI assessment of viability correlates with PET except that MRI appears to detect subendocardial infarction missed by the lower-resolution nuclear techniques\textsuperscript{109}. Recent developments have taken MRI beyond traditional anatomic and functional imaging. Real-time visualization now allows identification of regions of myocardial infarction and precise MRI-guided delivery of therapeutic agents. Furthermore, the injection sites can be identified using contrast agents.\textsuperscript{110} It is also feasible to label cells with iron particles and detect their distribution in the body noninvasively.\textsuperscript{111} Gadolinium can be attached to antibodies for specific labeling of compounds in vivo.\textsuperscript{112} New contrast agents have allowed MRI visualization of gene expression at a cellular resolution.\textsuperscript{113} Apoptotic cells have been detected with targeted MRI contrast agents.\textsuperscript{114} Labeling and detection of stem cells is anticipated to enable MRI to trace their distribution in vivo.\textsuperscript{115}

Noninvasive imaging can monitor the response to stem cell therapy at approximately the level of gross pathology. There is still a need to develop techniques that can detect the cells introduced into the myocardium and to follow their division over time. There are important subtleties of cellular function, growth, and proliferation that cannot be imaged with the kinds of noninvasive methods described. Thus, there will remain a substantial need for detailed physiological and pathological methods to understand the myocardial response to stem cell therapy.

**Predicted Role for BMSCs in Regenerative Medicine**

It is now routine practice for patients about to undergo myeloablation to be treated with G-CSF to mobilize CD34\textsuperscript{+} BMSCs into the circulation for the purpose of collection and subsequent use for bone marrow reconstitution. If BMSC plasticity resides in the primitive CD34\textsuperscript{+} population as suggested in a recent study,\textsuperscript{48} this approach may have potential clinical utility in cardiac patients. In patients with refractory myocardial ischemia, safety feasibility studies have already begun.\textsuperscript{116} These studies are designed to determine whether BMSCs can traffic to the heart and develop into cardiomyocytes and coronary vessels.\textsuperscript{116}

Existing data indicate that BMSCs continually proliferate and enter the circulation. These circulating BMSCs appear to have an engraftment phenotype that fluctuates with the phase of cell cycle.\textsuperscript{117} Thus, BMSCs with different engraftment capabilities are continually passing through capillary networks in all tissues. Data showing production of a variety of bone marrow–derived cell types including hepatocytes and cardiomyocytes suggest that circulating BMSCs seed and differentiate into tissue-specific cells. It remains to be established whether this plasticity is attributed to a single subpopulation of BMSCs or to multiple subpopulations each being tissue restricted. At present, we can only acknowledge the ability of BMSCs to colonize skeletal muscle,\textsuperscript{55-57} skin,\textsuperscript{50} bone,\textsuperscript{117} liver,\textsuperscript{45} retina,\textsuperscript{49} and heart.\textsuperscript{54,53,54}

**Summary**

We propose, without much evidence, that the differentiation of BMSCs that seed peripheral organs is regulated by exposure to local environmental factors. Thus, a BMSC or its progeny that in bone marrow would give rise to granulocytes, erythrocytes, and platelets will give rise to lymphocytes in the thymus, hepatocytes in the liver, and cardiac myocytes in the heart. This has exciting clinical potential because BMSCs are self-renewing and can be easily harvested from bone marrow and peripheral blood. Furthermore, clinical experience shows that BMSC transplantation does not lead to neoplasia as may occur with other stem cell populations. The need to expand the scope of investigations using embryonic and fetal stem cells is of paramount importance and cannot be overstated, but adult BMSCs may offer the best near-term promise for tissue repair.

Although not conceived 3 to 4 years ago, tissue regeneration using adult BMSCs is now openly discussed among even the most conservative scientists and clinicians. If additional, encouraging preclinical data can be obtained, the next decade is likely to witness clinical trials aimed at testing the capacity of BMSCs to regenerate damaged tissues.
Appendix

Glossary of Terms

Stem cells: primitive cells that have the capacity for extensive self-renewal and the ability to differentiate into multiple cell types. Embryonic stem cells: pluripotent cells derived from the inner cell mass of the blastocyst; they give rise to cells of all three germ layers. Hemangioblasts: primitive embryonic cells that give rise to both HSCs and endothelial progenitor cells; they may also exist in adult bone marrow. Adult stem cells: present in all renewing tissues; these cells divide for self-renewal and differentiate into multiple progenitor cell types. Hematopoietic stem cells: rare adult stem cells present in blood and bone marrow; they give rise to several distinct populations of blood-forming progenitor cells. Progenitor cells: multipotential intermediate stem cells that serve as the direct precursors for tissue-specific mature cells. Endothelial progenitor cells: cells that are present in blood and bone marrow; they are involved in angiogenesis and postnatal neovasculogenesis. Mesenchymal stem cells: also referred to as marrow stromal cells; these cells differentiate in vitro along multiple pathways that include cardiac myogenesis. Plasticity or transdifferentiation: the capacity of adult stem cells that reside in one tissue to differentiate into mature cells of an unrelated tissue.

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