Bone marrow stem cell transplantation for cardiac repair

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Haider, Kh Husnain, and Muhammad Ashraf. Bone marrow stem cell transplantation for cardiac repair. Am J Physiol Heart Circ Physiol 288: H2557–H2567, 2005; doi:10.1152/ajpheart.01215.2004.—Cardiomyocytes respond to physiological or pathological stress only by hypertrophy and not by an increase in the number of functioning cardiomyocytes. However, recent evidence suggests that adult cardiomyocytes have the ability, albeit limited, to divide to compensate for the cardiomyocyte loss in the event of myocardial injury. Similarly, the presence of stem cells in the myocardium is a good omen. Their activation to participate in the repair process is, however, hindered by some as-yet-undefined biological impediments. The rationale behind the use of adult stem cell transplantation is to supplement the inadequacies of the intrinsic repair mechanism of the heart and compensate for the cardiomyocyte loss in the event of injury. Various cell types including embryonic, fetal, and adult cardiomyocytes, smooth muscle cells, and stable cell lines have been used to augment the declining cardiomyocyte number and cardiac function. More recently, the focus has been shifted to the use of autologous skeletal myoblasts and bone marrow-derived stem cells. This review is a synopsis of some interesting aspects of the fast-emerging field of bone marrow-derived stem cell therapy for cardiac repair.

myocardial infarction; cardiomyocyte; plasticity; transdifferentiation

THE RESULTS OF STUDIES in both small and large animal models and phase 1 clinical studies in humans have shown the promise of bone marrow and bone marrow-derived stem cells in the treatment of diseased myocardium (111). The secret of this promise lies in the remarkable potential of bone marrow stem cells to undergo multilineage differentiation in general and cardiomyogenic potential in particular (66, 108).

Bone marrow consists of a nonhomogeneous population of cells composed of various lineages with differing abilities. The hematopoietic stem cell (HSC) fraction of the bone marrow is responsible for the production of >95% of the body’s blood cells. They have an extensive capacity of self-renewal. Recent reports (27, 65) have shown that HSC may also have the potential to differentiate into other lineages of cells, including their competence to attain the cardiac phenotype. The work of Matsuzaki and colleagues (81) suggests high-marrow seeding efficiency as a specific characteristic of primitive HSC in addition to their self-renewal and multipotent capacity. They have demonstrated a high “homing to niche” capacity of these cells. Over a long period of time, CD34, a cell surface molecule that is expressed on 1–3% of bone marrow cells, has been used as a convenient positive selection marker for HSC (94). However, several reports have raised serious questions about CD34 expression on hematopoietic cells in the mouse (36).

Sato and colleagues (112) have shown that CD34 expression in murine HSC reflects the activation state, and this may be so in humans. Moreover, the presence of human CD34− HSC has been reported as being more immature than CD34+ stem cells (15). Consequently, AC133 is being used as an alternative selection marker to CD34 antigen. The rationale to use purified AC133+ cells is to avoid injection of a large number of leukocytes and their progenitors, which have limited plasticity and the presence of which in large numbers may give rise to an unwanted inflammatory response at the site of the graft (152).

On the other hand, the non-HSC fraction is mainly composed of mesenchymal stem cells. They are clonogenic and possess the potential to develop into mature cells that produce fat, cartilage, bone, tendons, and muscle (108). They can be isolated from bone marrow and can easily be expanded in an in vitro cell culture without compromising their stem cell capabilities (86). Because of the ease of the method of their purification and multipotent nature, they have been extensively studied in both experimental and clinical settings. There is no universal surface marker for the identification of mesenchymal stem cells. They are negative for HSC markers CD31, CD34, and CD45 and express on their surface CD44, CD90, CD105, CD106, and CD166 (78). Besides, they have been reported to express T cell receptor components that enable them to remain in a state of readiness to enter various pathways of differentiation upon induction (158). Mesenchymal stem cells with similar potential have also been isolated from tissues other than bone marrow including peripheral blood and umbilical cord blood (61). Kawada et al. (59) have recently shown that CD34− nonhematopoietic bone marrow mesenchymal stem cells can differentiate into cardiomyocytes. The heterogeneous mononuclear fraction of the bone marrow (BMMNC) is the most promising and extensively studied stem cell population for cellular cardiomyoplasty (24, 47, 54, 56). Besides, CD117 and Sca-1+ cells have also been focused to assess their cardiomyogenic potential (72, 73, 76, 80, 152).

MALLEABLE NATURE OF BONE MARROW STEM CELLS

Intravenous administration of bone marrow-derived mesenchymal stem cells leads to their preferential migration to the...
Infracted myocardium (92), and tissue injury is considered as one important factor that influences the transdifferentiation of bone marrow cells to other lineages (1). BMMNCs taken from myocardial infarction patients have been found to show tropism for damaged cardiac tissue (10a). Similarly, there is a significant debate over the presence of stem cells from extra-cardiac origin in the myocardium (12, 110). Despite these interesting findings, the underlying mechanism is as to how the donor bone marrow stem cells home into the damaged myocardium and contribute toward improved cardiac function remains highly debatable (97, 118). However, it is considered to be the resultant effect of more than one mechanism. First, bone marrow cells undergo differentiation into cardiomycocytes, either due to preprogramming or under the influence of signals from myocardial microenvironment (64, 100, 121, 140). Second, they contribute toward improved wall thickness and a reduction in the infarct size. Bone marrow stem cells have been shown to express angiogenic growth factors in a paracrine way to stimulate neovascularization at the site of the cell graft and undergo transdifferentiation into endothelial cells to participate in angiogenesis, thus alleviating myocardial ischemia (42, 132, 139). The paracrine effect may not be the effect of individual cytokines themselves. Rather, this may be related with their synergistic interaction that may lead to the beneficial outcome in terms of neovascularization. Kinnaird and colleagues (62) have reported a full spectrum of arteriogenic cytokines released from bone marrow stromal cells. The expression of these growth factors gets accentuated under stressful hypoxic conditions (8). Mesenchymal stem cells grown under hypoxic conditions migrated and formed three-dimensional capillary-like structures on a Matrigel matrix. They also showed these effects when triggered by conditioned media taken from mesenchymal stem cells grown under hypoxic stress. These observations clearly depict the involvement of both paracrine as well as autocrine mechanisms. The role of paracrine signaling pathways as a mediator of bone marrow cell therapy has also been highlighted in vivo in the treatment of tissue ischemia, which clearly depicts that donor cell incorporation into vessels is not a prerequisite for their effects in angiogenesis (63).

An interesting study from Pak et al. (104) showed that bone marrow cell transplantation induces cardiac nerve sprouting and sympathetic hyperinnervation. Another proposed mechanism is the fusion of donor bone marrow cells with host cardiomycocytes to adopt their phenotype (123). The donor cell genes may supplement for the lost genetic material from the host nucleus during the ischemic injury. Out of all these mechanisms, the consensus is on transdifferentiation of bone marrow cells into cardiomycocytes and endothelial cell phenotypes and angiogenesis (Tables 1–3).

The ability of bone marrow-derived stem cells to break the barriers of lineage restriction and transdifferentiate to form different tissue cells are being extensively investigated (6, 37, 46, 53, 133, 135). A series of recent experiments have challenged the long-standing dogma of lineage restriction. The multipotential nature of bone marrow stem cells to transdifferentiate into various lineages has been shown in vitro (18, 21, 98, 137, 148, 151). Similar results have also been corroborated in vivo (9, 69, 95, 157). The underlying principle to these observations is that stem cells form the tissue in which they reside. Hence, it is thought that bone marrow stem cells under the influence of the new microenvironment after transplantation start to adopt the new phenotype and produce cells of the resident tissue (39, 52). However, these findings have been challenged by several research groups that have independently advocated the possibility of fusion between donor bone marrow cells with host cells to adopt the phenotype of the host tissue (134, 153). Recent publications from Alvarez and colleagues (5) have shown fusion between donor bone marrow cells and Purkinje fibers, cardiomycocytes, and hepatocytes. Thus there is a prevailing notion that fusion between donor stem cells and host tissue cells might be misleading the researchers to conclude that stem cells can cross the boundaries of lineage restriction.

Back-to-back recent reports from Balsam et al. (11) and Murry et al. (91) have clearly depicted a lack of ability of donor bone marrow cells to undergo differentiation into cardiomycocytes after transplantation. They have shown little evidence for transplanted bone marrow cells to form cardiomycocytes at the site of the graft posttransplantation. Similar concern has been shown by earlier researchers when isolated unfractonated bone marrow cells were found to be unable to differentiate into cardiomycocytes (13). These results are presenting a serious challenge to the findings that have been documented by Orlic and colleagues (101) and thus require an in-depth assessment of whether bone marrow stem cells have the required plasticity to transdifferentiate into cardiomycytes posttransplantation.

### Table 1. In vitro studies on BMC transdifferentiation into cardiovascular-related lineages

<table>
<thead>
<tr>
<th>References</th>
<th>Cells</th>
<th>Coculture</th>
<th>Pretreatment</th>
<th>Differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Makino et al. (77)</td>
<td>BMSC</td>
<td></td>
<td>5-Aza</td>
<td>Myocytes</td>
</tr>
<tr>
<td>Fukuda et al. (32)</td>
<td>BMSC</td>
<td></td>
<td>5-Aza</td>
<td>Myocytes</td>
</tr>
<tr>
<td>Fuchs et al. (30)</td>
<td>BMC</td>
<td>No</td>
<td>Yes</td>
<td>Myocytes</td>
</tr>
<tr>
<td>Toma et al. (137)</td>
<td>hBMMSC</td>
<td>ESC</td>
<td>IL-3 for 7 days</td>
<td>Myocytes</td>
</tr>
<tr>
<td>Terada et al. (134)</td>
<td>BMMNC</td>
<td></td>
<td></td>
<td>Myocytes</td>
</tr>
<tr>
<td>Eisenberg et al. (27)</td>
<td>HPC</td>
<td>Chick embryo heart tissue</td>
<td>RA and ECGS PGE$_2$ and IL-2</td>
<td>Myocytes</td>
</tr>
<tr>
<td>Fukuura et al. (33)</td>
<td>BMSC</td>
<td>Cardiomyocytes</td>
<td></td>
<td>Myocytes</td>
</tr>
<tr>
<td>Oswald et al. (103)</td>
<td>hBMMSC</td>
<td></td>
<td>VEGF</td>
<td>Endothelial cells</td>
</tr>
</tbody>
</table>

BMC, bone marrow cells; BMSC, bone marrow stromal cells; 5-Aza, 5-azacytidine; hBMMSC, human bone marrow mesenchymal stem cells; BMMNC, bone marrow mononuclear cells; ESC, embryonic stem cells; IL, interleukin; HPC, hematopoietic progenitor cells; RA retinoic acid; ECGS, endothelial cell growth supplement.
differentiation achieve the cardiomyocyte phenotype through the expression of specific genes encoding various transcription factors and structural and regulatory proteins (105). Among these are included GATA-4, Nkx 2.5, MEF2, and HAND. However, the exact role of these genes, the timing of their activation, and the signaling pathways involved in mediating cardiomyogenic differentiation remain unknown. Besides these, a recent report (41) has shown that they also express functional adrenergic and muscarinic receptors that modulate their function.

Various strategies have been adopted for directed differentiation of stem cells into cardiomyocytes (45). The induction of cardiomyogenic differentiation of stem cells has been achieved by culturing bone marrow cells in vitro using culture medium supplemented with retinoic acid, DMSO, and 5-azacytidine (5-Aza) (45). Cardiomyogenic differentiation of bone marrow in vitro has specifically been achieved using the demethylating agent 5-Aza (Table 1). Makino et al. (77) reported 5-Aza-induced differentiation of mice bone marrow stromal cells into cardiac-like cells. At a concentration of 3 μmol/l for 1 wk, 5-Aza induced bone marrow cells into cardiomyogenic cells. These cells stained positive for myosin, actin, and desmin and showed spontaneous beating at 3 wk after treatment. Electron microscopy revealed a cardiomyocyte-like ultrastructure, such as the presence of sarcomeres, centrally positioned nuclei, and atrial granules.

Coculture of bone marrow stem cells with cardiomyocytes has been shown to preprogram these cells to adopt a cardiac phenotype. Our research group (147) has recently shown that

<table>
<thead>
<tr>
<th>Model</th>
<th>Cells</th>
<th>Pretreatment</th>
<th>Angiogenesis</th>
<th>Differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomita et al. (138)</td>
<td>Cryoinjury</td>
<td>BMC</td>
<td>5-Aza</td>
<td>Yes</td>
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<tr>
<td>Wang et al. (143)</td>
<td>Normal heart</td>
<td>BMSC</td>
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<td>Yes</td>
</tr>
<tr>
<td>Wang et al. (144)</td>
<td>CAL</td>
<td>BMSC</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Davani et al. (24)</td>
<td>CAL</td>
<td>BMMPC</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Duan et al. (26)</td>
<td>CAL</td>
<td>BM-MSC</td>
<td>HGF transduction</td>
<td>Yes</td>
</tr>
<tr>
<td>Saito et al. (115)</td>
<td>CAL</td>
<td>BMSC</td>
<td>5-Aza</td>
<td>Yes</td>
</tr>
<tr>
<td>Sakaguchi et al. (116)</td>
<td>CAL</td>
<td>BM + myoblasts</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>Ishida et al. (54)</td>
<td>Doxorubicin</td>
<td>BMMNC</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Tang et al. (132)</td>
<td>CAL</td>
<td>BMMSC</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Zhang et al. (156)</td>
<td>CAL</td>
<td>BMMNC</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Orlic et al. (102)</td>
<td>CAL</td>
<td>Lin⁺ ckit⁺</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Jackson et al. (55)</td>
<td>CAL</td>
<td>SP</td>
<td>Yes</td>
<td>Myocytes and endothelial cells</td>
</tr>
<tr>
<td>Saito et al. (114)</td>
<td>CAL</td>
<td>BMSC</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Toma et al. (137)</td>
<td>Normal</td>
<td>hBMMSC</td>
<td>No</td>
<td>Myocytes</td>
</tr>
<tr>
<td>Agbulut et al. (3)</td>
<td>Doxorubicin</td>
<td>BMC</td>
<td>No</td>
<td>Myocytes</td>
</tr>
<tr>
<td>Kudo et al. (68)</td>
<td>CAL</td>
<td>Lin⁺ ckit⁺</td>
<td>No</td>
<td>Myocytes</td>
</tr>
<tr>
<td>Hiasa et al. (47)</td>
<td>CAL</td>
<td>BMMNC</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Murry et al. (91)</td>
<td>Normal/CAL</td>
<td>Lin⁺ ckit⁺</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Balsam et al. (11)</td>
<td>CAL</td>
<td>Lin⁺ ckit⁺ and Lin⁻ ckit⁻ Thy1.1⁺Sca⁺</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

CAL, coronary artery ligation; HGF, hepatocyte growth factor; SP, side population.

**Table 2. Small animal model studies used for BMC transplantation for cardiac repair**

**Table 3. Large animal studies using BMC for cardiac repair**

<table>
<thead>
<tr>
<th>Model</th>
<th>Cells</th>
<th>Pretreatment</th>
<th>Angiogenesis</th>
<th>Differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kamihata et al. (56)</td>
<td>LAD ligation</td>
<td>BMMNC</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Fuchs et al. (30)</td>
<td>Ameroid ring</td>
<td>BMC</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>Shade et al. (121)</td>
<td>60-min LAD occlusion</td>
<td>BMMSC</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Tomita et al. (139)</td>
<td>LAD occlusion</td>
<td>BMSC</td>
<td>5-Aza</td>
<td>Yes</td>
</tr>
<tr>
<td>Min et al. (84)</td>
<td>LAD branch ligation</td>
<td>hMSC+ hFC</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Pak et al. (104)</td>
<td>LAD microcoil embolization</td>
<td>BMSC</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Yau et al. (150)</td>
<td>Coil occlusion</td>
<td>BMSC</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Dog</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamano et al. (42)</td>
<td>LAD ligation</td>
<td>Fresh BMC</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Sheep</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bel et al. (13)</td>
<td>LCx ligation</td>
<td>Fresh BMC</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>Airey et al. (4)</td>
<td>In utero injection</td>
<td>hMSC</td>
<td>No</td>
<td>No</td>
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</tbody>
</table>

LAD, left anterior descending coronary artery; hMSC, human mesenchymal stem cells; hFC, human fetal cardiomyocytes; LCx, left circumflex coronary artery.
cell-to-cell contact during coculture of bone marrow cells with cardiomyocytes is imperative to trigger their cardiomyogenic transformation.

TRANSDIFFERENTIATION OF BONE MARROW STEM CELLS IN VIVO

Encouraged by the results from in vitro studies, both small and large animal studies have been carried out to show that the whole bone marrow and its subpopulations potentially repopulate the injured myocardium and undergo milieu-dependent differentiation to form endothelial cells and cells with a cardiomyocyte-like phenotype (Tables 2 and 3). It has been hypothesized that preprogramming donor bone marrow cells to adopt a cardiomyogenic differentiation pathway before their transplantation in animal models may have better chances of their transdifferentiation into cardiomyocytes than transplantation of uncommitted bone marrow cells (16, 138). The heart function of the 5-Aza-treated bone marrow cell transplanted group was better than that of other groups. Liecehty et al. (74) demonstrated in vivo site-specific differentiation of human bone marrow into cardiomyocytes. The cells were directly injected into the fetal sheep peritoneal cavity. Lin- ckit+ bone marrow cells developed into fetal and neonatal myocytes in infarcted mice hearts (101). They were positive for cardiac myosin, GATA-4, MEF2, and Csx/NKx 2.5. The cells were also positive for connexin43, indicating intercellular communication between the grafted cells and host tissue. A highly enriched hematopoietic CD34+ “side population” of bone marrow cells was transplanted into lethally irradiated C57BL/6-Ly-5.1 mice (55). The engrafted side population bone marrow cells differentiated into cardiomyocytes and endothelial cells. The derived cardiomyocytes were found primarily in the peri-infarct region. These studies suggested that signals originating from the cytokine-rich microenvironment of the injured myocardium and the direct cell-cell interaction between donor bone marrow cells and host cardiomyocytes triggered their myogenic differentiation. The injured heart is a more efficient inducer of bone marrow cells differentiation into cardiomyocytes (64). Shintani et al. (124) demonstrated that endothelial progenitor cells were mobilized into the peripheral blood and contributed to neovascularization in the heart during an acute infarction event in humans. These studies suggested that inflammatory cytokines and factors released by the infarcted heart into the peripheral blood may have the potential to trigger circulating bone marrow cells to differentiate into specific cell lineages. Condorelli et al. (23) observed that the majority of endothelial cells injected into infarcted mice differentiated into myosin-positive cells with a cardiomyocytes-like morphology, whereas only a very few of them differentiated into cardiomyocytes after injection into the normal mice heart. Our research group (68) has previously shown that transplantation of bone marrow-derived Lin- ckit+ cells significantly reduced infarction and fibrosis in an ischemic murine heart. The donor bone marrow cells differentiated into cardiomyocytes and endothelial cells in the ischemically damaged murine heart. Transplantation of bone marrow stromal cells has also been shown to improve the functioning of the healed infarcted rat heart through myogenesis and angiogenesis (99). The angiogenic potential of bone marrow cells posttransplantation may be enhanced by subjecting the cells to ischemic stress before transplantation (75). In recent study (117), granulocyte colony-stimulating factor (G-CSF)-mobilized human bone marrow CD34+ and CD34− cells were delivered intravenously into an infarcted nude rat heart model of myocardial infarction. The cells were found to migrate and repopulate the ischemic myocardium and induce neovascularization. There was a resultantly improved cardiac function and reduced remodeling.

To achieve controlled delivery of the transplanted bone marrow cells, Liu et al. (75) used a novel fibrin patch-based delivery of cells. A fibrin patch coated with autologous bone marrow cells was placed on the infarct site. The results revealed that the transplanted cells differentiated into cardiomyocytes and participated in the angiogenic process and became part of the capillary network.

Taken together, these data suggest that the injured heart might have a greater contribution to the milieu-dependent cardiomyocyte differentiation of bone marrow cells. However, the information on the signaling molecules and responding molecular mechanisms that recruit bone marrow cells to the injured heart and transdifferentiation into cardiomyocytes is still unclear. Myocardial infarction can mobilize bone marrow cells and recruit them to injured myocardium where they get stimulated for transformation into new cardiomyocytes.

One important consideration, however, is that the plastic and malleable nature of unselected multipotent bone marrow may be disadvantageous and results in unwarranted and unwanted outcomes. The presence of cells with osteogenic potential or a multipotent stem cell population together with local milieu of the cytokine-rich infarcted myocardium may favor osteogenic differentiation of bone marrow cells and enhance calcium deposition with an as-yet-undefined regulatory mechanism. It has been shown that transplantation of unselected bone marrow cells into the acutely infarcted myocardium may cause significant intramyocardial calcification, thus leading to contractile dysfunction or arrhythmias (155). The calcification in the bone marrow cell-transplanted hearts was identified in the peri-infarct area or normal myocardium, where the myocardial structure was relatively well preserved. Similarly, transplantation of undifferentiated mesenchymal stem cells has been shown to develop into fibroblastic scar tissue (144). These results underscore the importance of regulating cellular differentiation of adult stem cells in therapeutic applications.

CLINICAL STUDIES

Since the first human autologous skeletal myoblasts transplantation (82), more research groups have been involved in phase 1 safety and feasibility studies using adult stem cell transplantation for cardiac repair, including bone marrow cells. The underlying principle is to take advantage of the plastic nature of bone marrow stem cells, which enables them to cross the lineage restriction and participate in the reparative process by attaining the cardiac phenotype.

Whereas some of these studies have been carried out as the sole-therapy procedure using a bone marrow cell implantation approach (10a, 14, 31, 106, 141), others have been performed as an adjunct to either coronary artery bypass grafting (34, 109, 126, 127, 154) or percutaneous revascularization (10, 19, 22, 125, 129, 145). The small number of the patients in each study (without a blinded control group) and their performance as an adjunct procedure make it difficult to assess and attribute the

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real benefit and improvement in cardiac function to bone marrow cell transplantation. Invariably, all the studies have used autologous bone marrow cell transplantation. However, they differ in the processing, culture, and selection of the subpopulation before transplantation. For example, unlike Galinanes et al. (34), who used unmanipulated autologous bone marrow (34), and Tse et al. (141), who used unselected BM-MNC for transplantation, others have tried to purify a subpopulation of BM-MNC (106, 126, 129, 145). NOGA has been used for electromechanical mapping to discriminate between the ischemic and viable regions and for targeted delivery of cells (106, 141). The methods for the end-point measurement have been variable and include treadmill exercise duration, consumption of nitroglycerine, single photon emission computed tomography (SPECT) perfusion imaging, electromechanical mapping system (NOGA, EMM), echocardiography, [18F]fluorodeoxyglucose positron emission tomography (PET), and magnetic resonance imaging. In most cases, the cell transplantation procedure was uneventful, and the patients recovered well without any postprocedural complications. No arrhythmic changes have been reported on Holter monitoring during any study. Only two deaths, one each in the experimental and control groups, were reported by Perin et al. (106). However, both these deaths were unrelated to the cell transplantation procedure. Despite their observational status and design limitations, their outcome reveals the safety and feasibility of the approach. Taken together, the results of the phase 1 studies have been encouraging. Table 4 summarizes the bone marrow cell transplantation reports with the largest number of patients published in refereed journals.

The largest study, with 78 patients, was reported by Chen et al. (22). The patients were randomized to receive bone marrow cells (n = 34) and only percutaneous coronary intervention (n = 35) as the control group. After being cultured for 10 days in vitro, 8–10 × 10^6 cells/ml of autologous bone marrow cells were resuspended in 6 ml of heparinized saline solution and transfused into the infarct related target coronary artery through an inflated over-the-balloon catheter with high pressure (129). The control group patients received 6 ml of normal saline solution without cells using the same method. The patients underwent [18F]fluorodeoxyglucose PET scanning and echocardiography on the day of cell transplantation and were followed up at 3 and 6 mo. The follow-up results were encouraging and showed significant differences between the two groups of patients. The percentage of hypokinetic, akinetic, and dyskinetic areas in the left myocardium significantly decreased (from 32 ± 5% to 13 ± 11%), whereas wall motion velocity (from 2.17 ± 1.3 to 4.2 ± 2.5 cm/s) and left ventricular ejection fraction (LVEF; from 49 ± 9% to 67 ± 11%) registered significant increases in the cell transplanted patients together with significantly decreased perfusion defects as detected by PET. The most encouraging finding of the study was that the indicators of improvement in cardiac performance were maintained unaltered until 6 mo of observation.

The Bone Marrow Transfer to Enhance S-T Elevation Infarct Regeneration study, a recent randomized controlled clinical trial involving 60 patients, was reported by Wollert and colleagues (145). The patients were randomized to receive optimum medical treatment and PCI together with autologous bone marrow cell transplantation (n = 30) or without cell transplantation as the control group (n = 30). A total of 24.6 × 10^8 freshly isolated CD34^+ cells were transplanted by intracoronary infusion using a balloon catheter in the infarct related artery. Mean global LVEF showed a 7% increase in the stem cell transfer group compared with 0.7% in the medical therapy group (P = 0.0026). Study limitations included a lack of sham-operated control subjects and an inability to determine the mechanisms of action of bone marrow cells.

Zeiher’s group, in Germany, has documented preliminary results of the Transfer of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction study. The randomized study, which previously reported 20 patients, included 8 more patients to the cohort (10, 19). Interestingly, the study involves a comparison between BM-MNC and circulating progenitor cells (CPC) in blood for cardiac repair together with an internal reference group of 11 patients who were included as a control.

Table 4. Randomized clinical studies with control groups using BMC

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients</th>
<th>Cell type</th>
<th>Delivery Mode</th>
<th>Adjunct Procedure</th>
<th>Result Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assumus (10)</td>
<td>09/20</td>
<td>BMMNC</td>
<td>IC/CPC</td>
<td>PCI</td>
<td>Improved regional wall motion in the infarct area, enhanced LVEF, profoundly reduced LVESV, enhanced viability in the infarcted one by DS-echocardiography, and quantitative PET scan</td>
</tr>
<tr>
<td>Chen (22)</td>
<td>35/34</td>
<td>BMMNC</td>
<td>IC</td>
<td>PCI</td>
<td>Improved wall velocity in the infarcted segments, reduction in perfusion defects, improved LVEF, and significantly reduced LVESV by echocardiography, NOGA EMM, and PET scanning</td>
</tr>
<tr>
<td>Wollert (145)</td>
<td>30/30</td>
<td>CD34^+</td>
<td>IC</td>
<td>PCI</td>
<td>Improved LVEF 24-h Holter monitoring and functional MRI</td>
</tr>
</tbody>
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IC, intracoronary; CPC, circulating progenitor cells; PCI, percutaneous coronary intervention; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; DS, dobutamine stress; PET, positron emission tomography; NOGA, EMM, electromechanical mapping system.
control for comparison of results. BMMNC (CD34+/CD45+) were purified from autologous bone marrow aspirates of each patient undergoing BMMNC transplantation, and CPC were purified and expanded ex vivo for 3 days for each patient undergoing CPC transplantation from 250 ml venous blood. Except for one patient who suffered anterior wall infarction 3 days after cell transplantation, the whole exercise was without any problem. A 4 mo follow-up revealed improvement in global cardiac function; however, it did not differ between patients receiving BMMNC or CPC. The control group patients without cell therapy revealed no significant changes in the respective parameters of observation. The study results highlight the safety and efficacy of the procedure, like many of the previously reported studies. Moreover, it also showed that transplantation of CPC, the collection and availability of which is relatively easy and less invasive compared with BMMNC, is as effective as BMMNC in improving contractile function of the heart and coronary artery flow reserve.

**BONE MARROW STEM CELL MOBILIZATION**

BMMNC taken from myocardial infarction patients have been found to show tropism for damaged cardiac tissue in vitro (28). Moreover, there is evidence that repair of cardiac and vascular tissue by bone marrow stem cells are naturally occurring processes after injury (35, 124). Besides tissue-specific resident stem cells, a very low level of progenitor cells move from their bone marrow niche into peripheral blood under physiological conditions as a part of the homeostatic mechanism. However, in patients with acute myocardial infarction, circulating mononuclear cells are significantly increased (124). It is thought that tissue ischemia mobilizes mononuclear cells from the bone marrow to the peripheral blood, and the mobilized mononuclear cells home specifically to participate in neovascularization and differentiate into mature endothelial cells (130). It has been demonstrated that after myocardial infarction, mesenchymal stem cells are recruited to the injured heart, where they undergo milieu-dependent differentiation and may participate in the pathophysiological mechanisms of postinfarct remodeling, angiogenesis, and maturation of the scar (17). However, the process is far from being adequate to compensate for the massive loss of cardiomyocytes and needs to be supported for its inadequacy from an outside intervention. Attempts are being made to pharmacologically augment the process of mobilization of progenitor cells from the bone marrow niche for their homing into the infarcted myocardium (96).

Stem cell mobilization using various cytokines and growth factors is routinely practiced in hematological procedures. The use of cytokines for induction of endogenous bone marrow stem cells to home into the infarcted myocardium is gaining popularity. It is less invasive and obviates the use of cells from nonautologous sources and hence the risk of transmission of infectious agents and immunological rejection of the cell graft. These advantages of cytokine-induced mobilization of endogenous bone marrow stem cells make it a superior choice for regenerating infarcted myocardium. Various cytokines and growth factors either alone or in combination together can release HSC from the bone marrow into the peripheral blood (7, 85, 90, 100, 119, 149). G-CSF is currently the most commonly used agent for HSC mobilization (131). This is based on the fact that G-CSF is an effective chemoattractant for bone marrow stem cells and enhances the infiltration of the side population after myocardial infarction in mice (2). The outcome of the mobilization protocol is significantly influenced by the dose and route of G-CSF administration. Similarly, a comparative study (113) between continuous administration and bolus administration of G-CSF has shown that the former mode of administration reduced activation of polymorphonuclear cells. Treatment with G-CSF also induces an angiogenic response (120). Despite its effectiveness in translocation of bone marrow cells from their niche, the high dose of G-CSF that is required to achieve bone marrow cell mobilization is not without side effects (49, 58, 79). To overcome this problem, a combination of G-CSF with stem cell factor-1 (SCF-1) has been used (146). SCF is expressed constitutively in bone marrow stromal cells, fibroblasts, and endothelial cells and is essential to the survival, proliferation, adhesion/migration, and differentiation of HSC (20, 43). The synergistic interaction between G-CSF and other cytokines in mobilizing progenitor cells from the bone marrow has been demonstrated by multiple studies in animals and humans (38, 70, 89). Recent studies support the ability of HSC induced by cytokine(s) to transdifferentiate into cardiomyocytes; however, nothing is actually known regarding the process of transendothelial migration of HSC to the myocardium. In the hematopoietic microenvironment, bone marrow-derived endothelial cells may play an important role in the trafficking and maintenance of progenitor and stem cells due to adhesive interactions and paracrine secretion of hematopoietic growth factors.

It is hypothesized that bone marrow cells are integrated in their bone marrow niche with the bone marrow stroma. For the translocation of cells from their bone marrow niche under the influence of various cytokines, it is imperative to break these cytoadhesive interactions before they are blood borne (67). Besides others, CXCR-4 is a cell surface antigen expressed on hematopoietic progenitor cells including the primitive, pluripotent CD34+ subpopulation and is related to efficient SDF-1-induced migration in vitro (88, 107). Stromal cell derived factor-1 (SDF-1), through its receptor CXCR-4, is believed to be superior in stem cell mobilization, angiogenesis, and cell survival. Besides, G-CSF-induced HSC mobilization may also involve the SDF-1/CXCR-4 axis (107). Homing in of the mobilized stem cells to the injured myocardium is milieu dependent. Ischemia enhances the homing-in process. A multitude of local and systemic factors influence the homing-in of stem cells, including VEGF and its receptor-2, c-kit, and stem cell factors (93).

**FUTURE DIRECTIONS**

Stem cell therapy in general (44, 50, 51) and therapy based on bone marrow-derived stem cells in particular (31, 105, 127) is quickly emerging as an alternative strategy in cardiovascular therapeutics. Proof-of-concept animal studies and phase 1 clinical trials have already shown the safety and feasibility of this approach. However, there are some fundamental issues that need to be resolved to streamline research in this area. The ability of bone marrow cells to transdifferentiate into cardiomyocytes and integrate into the host tissue is being questioned. The proponents of fusion as the mechanism for the survival of donor cells posttransplantation are arguing for the development...
of hybrid cells as a result of fusion between donor cells and host cardiomyocytes, thus exhibiting a dual phenotype. Subsequently, the hybrid cells take on the function of the existing cells and proliferation results from the hybrid cells rather than stem cell transdifferentiation. However, they have failed to explain the extensive tissue regeneration that so many earlier studies have shown to occur after cell transplantation. These issues regarding the mechanistic basis of cell transplantation need extensive and in-depth studies. Resolving this issue is imperative because fused cells may have a different therapeutic potential and prognostic outcome compared with differentiated cells.

Combining bone marrow stem cell transplantation together with the delivery of therapeutic genes to the injured myocardium is an attractive alternative strategy. A previous study (40) has shown the effectiveness of overexpression of angiogenic genes together with stem cell transplantation in animal models. Genetic modulation of bone marrow stromal cells to overexpress the endothelial nitric oxide synthase gene did not interfere with their differentiation potential (25). Transplantation of mesenchymal stem cells overexpressing Akt has been shown to improve the survival of the donor cell and prevent ventricular remodeling together with improved cardiac function (78).

Age-related poor proliferative and differentiation potential of bone marrow stem cells is an important parameter that needs special consideration. Hence, in the case of old patients, the pros and cons of using autologous bone marrow cells for transplantation need to be carefully weighed. It has been shown that bone marrow undergoes age-related changes with respect to proliferative capacity and multilineage potential (87, 128). A clear relevance has been established between an intrinsic as-yet-undefined genetically controlled program of impaired stem cell functioning with aging. Interestingly, the stem cell pool in short-lived DBA/2 (D2) mice was reduced during aging, in contrast to long-lived C57BL/6 (B6) mice (57). Besides, growth of bone marrow stromal cells is better from bone marrow aspirates of younger patients (122). In the light of these observations, the age of the donor of bone marrow stem cells for transplantation has a strong influence on the outcome of the procedure.

Mobilization of bone marrow cells for cardiac repair is an interesting approach. Use of G-CSF has shown promise but has untoward effects that may limit its clinical application (49, 136). The combination of G-CSF with other cytokines and growth factors has been used for mobilization effects and to overcome this problem (93, 142). Studies have also shown that stem cells show a uniquely selective response to various cytokines and combinations of cytokines (146). The knowledge about a specific subpopulation of bone marrow cells that can differentiate into cardiomyocytes combined with the novel concept of lineage-specific mobilization may help to achieve better results with fewer side effects of the cytokine therapy.

Like numerous other tissues that house their resident stem cells, there is strong evidence for the presence of a myocardial stem cell-like population that retains the characteristics of other stem cells. However, with a decreased ability to change their genotype and gene expression profile, they are considered to have myocardium-restricted functions (48, 83). Hence, besides transplantation and mobilization strategies, activation of the resident stem cells for participation in the repair process may provide an alternative option with superior attributes in cell therapy for cardiac repair.

The results of the phase 1 clinical studies have been encouraging. However, in the absence of a blinded control group, and with a small number of patients involved in most of these studies, it is difficult to assert the beneficial effects of stem cell therapy. Furthermore, it is important to define the patient population that can benefit from cell therapy. For this purpose, a more concerted effort between the various research groups involved is needed.

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