MINIREVIEW

Mesenchymal Stem Cells and the Treatment of Cardiac Disease

JOSÉ J. MINGUELL,*,†,[1] AND ALEJANDRO ERICES*,‡

*Laboratorio de Trasplante de Médula Osea, Clínica Las Condes, Santiago, Chile; †TCA Research, Covington, Louisiana 70433; and ‡Laboratorio de Biología Celular y Farmacología, Departamento de Ciencias Biológicas, Facultad de Ciencias de la Salud, Universidad Andrés Bello, Santiago, Chile

The ischemia-induced death of cardiomyocytes results in scar formation and reduced contractility of the ventricle. Several preclinical and clinical studies have supported the notion that cell therapy may be used for cardiac regeneration. Most attempts for cardiomyoplasty have considered the bone marrow as the source of the "repair stem cell(s)," assuming that the hematopoietic stem cell can do the work. However, bone marrow is also the residence of other progenitor cells, including mesenchymal stem cells (MSCs). Since 1995 it has been known that under *in vitro* conditions, MSCs differentiate into cells exhibiting features of cardiomyocytes. This pioneer work was followed by many preclinical studies that revealed that *ex vivo* expanded, bone marrow–derived MSCs may represent another option for cardiac regeneration. In this work, we review evidence and new prospects that support the use of MSCs in cardiomyoplasty. Exp Biol Med 231:39–49, 2006

Key words: mesenchymal stem cells; cardiomyoplasty; infarcted myocardium; cell therapy

Myocardial dysfunction resulting from atherosclerosis-related myocardial infarction (MI) is a widespread and important cause of morbidity and mortality among adults. Due to scar- and ischemia-related postinfarction events, clinical manifestations are enormous and heterogeneous. The damaged left ventricle undergoes progressive "remodeling" and chamber dilation, with myocyte slippage and fibroblast proliferation. These events reflect an apparent lack of effective intrinsic mechanisms for myocardial repair and regeneration. Unless deep (and still unknown) modifications are introduced in the area proximate to the damage to force the proliferation of resident cardiac progenitor cells (1–3), all restorative therapies must consider the use of exogenous multipotent stem cells capable to differentiate, at least, into cardiomyocytes (4–6). From this point of view, bone marrow–located stem cells have been considered to display the required biologic properties for a cell therapy approach to treat patients with MI (7, 8).

With the use of animal models, a near normalization of ventricular function after acute MI was observed after injection of bone marrow–derived precursor cells (9). However, it was not made clear whether the beneficial effect produced by the graft was elicited by hematopoietic stem cells, precursors for cardiomyocytes, and/or endothelial cells, or was simply due to contamination with other unidentified cells. On the other hand, the use of unfractionated sheep bone marrow did not result in any beneficial effect in the chronically infarcted myocardium (10).

In addition to the use of bone marrow–derived hematopoietic precursor cells, cellular, molecular, and preclinical data have shown that bone marrow–derived mesenchymal stem cells represent a suitable cell archetype for regenerative purposes after MI. In this review, we will analyze the experimental evidence that warrants the utilization of mesenchymal stem cells (MSCs) in the treatment of MI.

This work was supported by grants 1030304 and 1040881 from Fondo Nacional de Investigación Científica y Tecnológica (FONDECYT-Chile) and grant 2003/03 from Dirección Académica, Clínica Las Condes (Chile).

[1] To whom correspondence should be addressed at Laboratorio de Trasplante de Médula Osea, Clínica Las Condes, Lo Fontecilla 441, Las Condes, Santiago, Chile. E-mail: jminguel@inta.cl

1535-3702/06/2311-0039$15.00

Biologic Features of MSCs

Under proper stimulation, MSCs can be induced to differentiate into adipocytes, osteoblasts, chondrocytes, tenocytes, myocytes, and hematopoietic-supporting stroma (11–14). Furthermore, MSCs may also give rise to other lineages such as endothelial, kidney, and neural, revealing a high degree of plasticity (15–17). MSCs that are isolated from several human sources, including bone marrow and peripheral and umbilical cord blood, exhibit a high *ex vivo* expansion capacity. This property has been used to assess the biologic properties of MSCs (11, 12, 18–20) to perform transfection with viral vectors (21, 22) and initiate studies toward the use of MSCs in clinical strategies (23, 24).

The promising therapeutic effect(s) of MSCs relies on their capacity to engraft and survive long term in distinctive target tissue. Using animal models, it has been demonstrated that after the syngeneic and/or xenogeneic transplantation of MSCs, donor cells engraft into the various mesenchymal tissue of the recipient animal (25–28).

Differentiation of MSCs to Cells of the Cardiovascular Tissue

Data from a number of laboratories have shown that MSCs, once exposed to a variety of physiologic or nonphysiologic stimuli, differentiate into cells displaying several features of cardiomyocytes-like cells (Table 1). Under these conditions, *ex vivo* differentiated MSCs exhibit a myotube-like structure and a time-dependent competence to synchronously beat. In turn, electron microscopic analysis revealed a cardiomyocytes-like ultrastructure including typical sarcomeres, a centrally positioned nucleus, and atrial granules. These cells show several functional features of a developing cardiomyocyte including the production of peptides and the expression of multiple structural and contractile proteins. They also display, at least, sinus node–like and ventricular cell–like action potentials (29–31, 36, 38, 43, 45–47).

Both well-defined mediators and direct cell-to-cell contacts induce the differentiation of MSCs into cardiomyocytes. Thus, by co-culturing human MSCs with human cardiomyocytes, it was demonstrated that the stem cell acquired a cardiomyocytes-like phenotype characterized by the expression of myosin heavy chain, beta-actin, and troponin T. However, when MSCs were incubated with a cardiomyocyte-conditioned medium, only beta-actin expression was observed. Thus, it seems that direct cell-to-cell contact is obligatory in relaying "cardiac environmental or microenvironmental" signals for MSC differentiation into a cardiomyogenic lineage (37, 39). Furthermore, human MSCs exhibit cell-to-cell coupling to each other and to ventricular myocytes *via* specific gap junctions (42, 48). The observation that atrial and ventricular cardiac myocytes in culture (49) exhibit remarkable cellular and molecular similarities with the cardiomyocytes-like cell differentiated from the MSC represents an important step for a better comprehension of the intercellular cross-talk between primary adult and MSC-derived cardiomyocytes. Similarly, the understanding of mechanisms involved in endothelin peptide and angiogenic growth factor production by myofibroblasts (50, 51) could be helpful for developing new therapeutic interventions in cardiac tissue repair/remodeling.

Reports have shown that MSCs differentiate not only into cardiomyocytes, but also into vascular smooth muscle cells/pericytes (vSMC/PC) progenitors and endothelial cells. These cell types are involved in the development of vascular systems, including angiogenic sprouting and vessel enlargement. Previous data have shown that *de novo* formation of vSMC/PC occurs after the differentiation of perivascular mesenchymal cells, in a platelet-derived growth factor B (PDGF-B)–dependent process (52). In turn, after the intramyocardial injection of MSCs, histopathologic and immunohistochemical analyses revealed the differentiation of infused cells into cardiomyocytes, vSMC/PC, and endothelial cells (53, 54).

Concomitantly, an increase in vessel density was observed (54). Similarly, in dogs with chronic ischemia the intramyocardial injection of MSCs resulted in increased vascularity and improved cardiac function. An immunofluorescence analysis revealed a co-localization of MSCs with endothelial and smooth muscle cells, but not with myocytes (55).

Thus, these data strongly suggests that MSCs supply an ideal donor source of a vast repertoire of cardiovascular cells for patients after MI. The mechanisms underlying MSC differentiation to cardiovascular cells, and subsequent improvement in neovascularization and cardiac function, involve the paracrine secretion of growth factors by MSCs (56–59). Such relationships between specific growth factors and MSC development are not without precedent. We have demonstrated that FGF2, a mostly mitogenic protein, is produced by MSCs and stimulates sustained quiescence and proliferation in uncommitted and committed MSCs, respectively (60). Uncommitted MSCs represent the small pool of quiescent precursors which, on commitment and maturation, give raise to the vast range of terminally differentiated mesenchymal lineages (20, 61).

The statement that MSCs have the competence to differentiate into several cardiac phenotypes is based, in most cases, on immunohistologic analysis of frozen tissue samples showing the co-localization of lineage-specific markers in fixed cells. Until now, it has not been conclusively demonstrated that the claimed immunophenotype(s) detected in the recipient heart after MSC infusion represent the *bona fide* revelation for myogenic and/or vascular lineage differentiation and not artifacts. Similar issues have been extensively discussed after methodologic development in the field of bone marrow stem-cell plasticity (8). Thus, evolving studies on the differentiation of MSCs into cardiac cells must be performed by a suitably methodologic analysis that ensures accurate, reproducible,

Table 1. Differentiation of MSC into Cardiomyocyte-Like Cells[a]

MSC source[b]/type of study	Stimuli	Differentiation features	References
Rat/*in vitro*	5-Azacytidine, amphotericin b	Multinucleated myotubes, spontaneous beating	29
Murine/*in vitro*	5-Azacytidine	Myotube-like, spontaneous beating, desmin, myosin, actinin, natriuretic peptides, others	30
Rat/*in vivo*	Intramyocardial Tx 5-azacytidine–treated MSC	Myotube-like, troponin I-C	31
Human/*in vivo* (mice)	Intramyocardial Tx with fetal cardiomyocytes	Cardiac alpha-myosin heavy chain, troponin I	32
Human/*in vivo* (mice)	Intramyocardial Tx	Desmin, beta-MHC, alpha-actinin, troponin T, phospholamban, sarcomeric organization	33
Human, cord blood/*in vitro*	5-Azacytidine	Cardiomyocyte-like, troponin T	34
Human, cord blood/*in vivo* (mice)	Intravenous Tx	Human DNA (PCR human beta-globin) in cardiac muscle	35
Murine/*in vitro*	5-Azacytidine	Cardiomyocyte-like, isoproterenol-dependent beating, action potentials, alpha1A, 1B, 1D; beta1 and beta2 adrenergic and M1 and M2 muscarinic receptors	36
Murine/*in vitro*	Co-culture with neonatal cardiomyocytes	Beta-MHC, troponin I, atrial natriuretic peptide, connexin 43	37
Rat/*in vivo*	Intramyocardial Tx, MSC encoding prosurvival protein	Stop remodeling, reduced intramyocardial inflammation, collagen deposition and hypertrophy; normalized myocardial volume and cardiac function	38
Human/*in vitro*	Co-culture with human cardiomyocytes	Beta MHC, beta-actin, troponin	39
Swine/*in vivo* (pig)	Intramyocardial Tx MSC inside fibrin matrix patches	X-gal$^+$–myocyte-like, vWF$^+$ angioblasts/capillaries	40
Rat/*in vivo*	Intravenous Tx	Desmin, troponin T, connexin 43; cells in vascular structures are vWF$^+$; increase in capillary density, cardiac function; decrease infarct size	41
Human/*in vivo* (dog)	Subepicardial Tx MSC transfected with pacemaker gene	Spontaneous rhythms left-sided origin; MSCs form gap junctions with adjacent myocytes	42
Human/*in vitro*	Insulin, dexamethasone, ascorbic acid	Troponin I, sarcomeric tropomyosin, titin, alpha actinin-positive Z bands	43
Human, cord blood/*in vitro*	Fibronectin, laminin, ECM-like peptides	Titin, myosin H/L chains, transcriptional activators (Nkx 2.5, GATA-4, E-HAND, D-HAND, MEF-2A)	44
Human/*in vitro*	5-Azacytidine	Beta-MHC, desmin, alpha cardiac actin, myofilament-like, troponin T	45

[a] Data show a selective, but not exhaustive, list of findings ordered by year of publication. Tx, transplantation; PCR, polymerase chain reaction for human beta-globin.
[b] Tissue source is bone marrow unless indicated.

and sustained data. This will help avoid redundant controversy such as the transdifferentiation of marrow hematopoietic stem cells into cardiac phenotypes and the subsequent effect on myocardium regeneration (62, 63).

Preclinical Studies

Experimental studies performed in rodent, sheep, dog, swine, or monkey infarct models have shown that cardiac transplantation of a number of cell types is feasible and contributes to the improvement of the contractile performance of the infarcted myocardium (6, 10, 64, 65). Cell types included a source of autologous, unpurified bone marrow, bone marrow mononuclear cells, purified bone marrow–derived cells ($CD34^+$ and/or $CD113^+$), cardiomyocytes, fibroblasts, and myoblasts. Despite variations in the infusion procedure (intramyocardial, intracoronary, or intravenous), the number of injected cells, and the cardiac condition of the receptor, an immune or toxic response was not detected after transplantation.

Bone marrow–derived MSCs have also been considered as potential candidates for cellular therapy for heart diseases. The promising effects of MSC infusion rely on their proven capacity to lodge and populate recipient tissue in a time-dependent and tissue-specific manner (27, 66). Because cardiac tissue was a preferred destination site (28, 67), these results put forward the concept that MSC infusion may play a significant role in the pathophysiology of postinfarct remodeling, angiogenesis, and maturation of the scar (68). In this regard, preclinical data have shown that after intracoronary or intramyocardial infusion, engrafted MSCs persisted in the myocardium and underwent a milieu-dependent (microenvironment) cardiomyogenic differentiation. Engrafted cells displayed *de novo* expression of cardiomyocyte markers, like beta-myosin heavy chain, alpha-actinin, cardiac troponin T, and phospholamban. Furthermore, engrafted cells develop into myofibers containing striated sarcomeric myosin heavy chain and cell-to-cell junctions (5, 33, 41, 69–71).

Because the pig heart is anatomically similar to the human heart, it has been selected as a model for studies related to MI and general cardiovascular studies (72). By using this model, it has been possible to gain valuable information on the tracking of injected MSCs into normal and infarcted myocardium and the resulting cardiac effects after immediate and long-term engraftment. With the help of magnetic resonance fluoroscopy, investigators have identified target sites like the border between infarcted and normal tissue, to guide intramyocardial MSC injections. In addition, iron fluorophore–particle labeling of infused MSCs has permitted their detection in the beating heart after transplantation, both in the normal and the infarcted pig myocardium (73–75). Using the swine model, it was established that 2 weeks after intramyocardial implantation, robust engraftment of labeled MSCs had occurred and was associated with the coexpression of several muscle-specific proteins. This observation suggests that MSC differentiation into cardiomyocytes-like cells was followed, 2 weeks later, by a significant attenuation of contractile dysfunction. Concurrently, wall thinning was remarkably reduced (76).

During cardiomyoplasty, an important issue to consider is related to the optimization of safety and feasible procedures for cell delivery. Using large animal models (i.e., sheep, dogs, swine), most authors have demonstrated that the intramyocardial infusion of progenitor cells across the infarcted area is safe and feasible. In the case of MSCs and using the pig model, the intramyocardial injection of cells (range: 10^4–10^8) proved to be safe and produce neither detectable immune nor other toxicity responses (32, 73–76). Further evidence for the procedural safety of the intramyocardial injection of MSCs was established in a canine chronic ischemia model (54). It was demonstrated that dogs undergoing intramyocardial injections of MSCs (1×10^8 total cells) survived the procedure without complications and without showing signs of arrhythmias, the heart's ST-T wave changes, or onset of Q waves. Moreover, myocardial damage was excluded because creatine kinase (CK)-MB and troponin I levels, after an initial mild increase, decreased to baseline levels. In turn, histopathologic analysis revealed no MI. However, the safety of the intramyocardial injection of MSCs has been challenged. A recent study revealed that acute myocardial ischemia and subacute myocardial microinfarction occurred after the intracoronary arterial administration of canine MSCs ($\sim 10 \times 10^6$) to normal dogs (77). According to this study, the primary insult for the development of these profound cardiac changes was the onset of an ischemic condition due to vascular occlusion brought out by the large size (18μ–20μ) of the MSCs. Because a control group of catheterized dogs not receiving cells was not included in this influential study, it is difficult to establish whether cell diameter, the catheterization procedure *per se*, or other factors (78) was instrumental in the onset of myocardial ischemia and microinfarction. Vulliet's results run contrary to a growing body of evidence, including studies in humans, on the safety and effectiveness of the intracoronary infusion of stem cells (79, 80).

The impact that these preclinical studies might have in MI patients is difficult to assess. In terms of procedural safety, one may assume that the dependable security data unveiled by the swine studies may be relevant to humans. However, there is no doubt that more studies are required (81). In terms of effectiveness, results unveiling a time-dependent retention, engraftment, migration, and differentiation strengthen the concept that MSC transplantation is an alternative therapy for ischemic heart failure.

Clinical Studies

In the last 3 years, several clinical trials have been initiated to assess the effect of transplantation of autologous cells in myocardial regeneration after acute MI. In most of

these studies, the source of "repairing" cells has been the heterogeneous fraction of bone marrow cells, named bone marrow–derived mononuclear cells (BM-MNCs). BM-MNCs contain at least several subpopulations of lymphocytes, early myeloid cells, endothelial progenitors, and an extremely low number of hematopoietic and/or MSCs. In addition to this source of "repairing" cells, more purified fractions of marrow cells, like those enriched in $CD34^+$ or $CD133^+$ progenitors as well as skeletal myoblasts, have also been used for severe postinfarction left ventricular dysfunction. In all cases, the "autologous repair cell" has been administered by intracoronary, intramyocardial, or transepicardial procedures. Results have shown that the implantation procedure is safe, feasible, and effective in terms of improving the perfusion rate of the infarcted myocardium (79, 80, 82–87). The latter has been, in most cases, attributed to angiogenic events elicited either by the endothelial progenitors present in the BM-MNCs (51, 88–90) and/or by secreted angiogenic cytokines (91–94). Despite the accumulated information regarding MSC differentiation and utilization in animal models, there are few clinical trials assessing their "cardiac functional effectiveness" in patients with myocardium infarct.

Recently, Chen *et al.* (95) conducted a randomized study to investigate the effectiveness of intracoronary injection of MSCs in patients with acute MI. After occlusion of the infarct-related coronary artery, a suspension of autologous MSCs was directly injected into the target coronary artery through an inflated, over-the-wire balloon catheter. Cardiographic evaluation demonstrated significant variation in the group of patients that received MSCs in comparison to controls. The percentage of hypokinetic, akinetic, and dyskinetic segments decreased in treated patients, while wall movement velocity over the infarcted region and left-ventricular ejection fraction increased significantly in the MSC group. The report by Chen *et al.* (95) demonstrates a significant and sustained improvement in global left-ventricular ejection fraction, even larger than that detected after infusion of hematopoietic cells (80), suggesting that MSC infusion triggers in the human heart the formation of new cardiomyocytes and neoangiogenesis (41). In addition, it provided evidence that the intracoronary infusion of MSCs does not produce any cell size–related adverse effect, as previously reported (77, 96).

In a prospective, nonrandomized, clinical, phase 1 trial initiated at our institution, we assessed the feasibility, safety, and effectiveness of the intramyocardial injection of a mixture of autologous MSCs and MNCs to patients during coronary artery bypass grafting (CABG) surgery. Patients with MI and candidates for CABG for persistent ischemia were enrolled in this study. Bone marrow was aspirated from the patients (study group) and processed for isolation and expansion of MSCs (12). A second bone marrow aspirate was taken (study group) on the day of surgery and used to prepare the MNCs. Once all bypass-to-coronary-artery anastomoses had been completed, a mixture of MSCs and MNCs was injected along the circumference of the infarct border. All patients survived the procedure and did not manifest operative complications. Unexpected increases in serum markers after cell infusion were not detected. Four months after surgery, a cardiac magnetic resonance imaging analysis revealed that global left-ventricular ejection fraction was increased in patients who received the cell infusion as compared with baseline values and with control patients. Furthermore, cell-treated patients displayed a significant reduction in MI volume. Despite the limited number of patients and the complexity in separating the effects of surgery from those produced by the cell infusion, these results suggest that infusion of a mixture of autologous MSCs and BM-MNCs is feasible, safe, and most likely favorable (Florenzano and Minguell, unpublished data).

The rationale for using a mixture of "repair cells" instead of a single cell type lies in the following foundations. First, BM-derived MNCs represent an important source of endothelial progenitors (51, 90). Clinical data have shown that the implantation of MNCs to MI patients (79), as well as to patients with ischemic limbs (97), was effective in promoting therapeutic angiogenesis. Second, as discussed earlier, MSCs have the capability to differentiate into cardiomyocyte-like cells. Third, MSCs produce angiogenic growth factors like basic fibroblast growth factor, vascular endothelial growth factor, and stem-cell homing factor (57, 60, 98–100). Therefore, the co-transplantation of MSCs and MNCs may result in the enhancement of both cardiomiogenesis and angiogenesis. Myocardial co-transplantation of MSCs with other cells is not without precedent; results have shown that intramyocardial transplantation of MSCs either with fetal cardiomyocytes or bone marrow cells resulted in a marked increase in myocardial regeneration. The latter was probably due to triggering cellular and molecular events associated with neocardiomyogenesis, neoangiogenesis, and/or nerve sprouting and atrial sympathetic hyperinnervation (32, 101).

Additional Prospects for MSC Therapy in Myocardial Diseases

Human Cord Blood Mesenchymal Stem Cells.

Embrionary development requires specific proliferation and differentiation genetic programs supported by a broad spectrum of fetal stem cells (19, 102–104). Fetal stem cells have migratory properties and use fetal circulation as a vehicle to target new tissue in formation (105, 106). In addition, this migratory capacity includes the circulation of fetal stem cells in maternal blood where they can be detected long after pregnancy (107, 108).

Hematopoietic activity is supported by hematopoietic stem cells (HSCs) which, in succession, home fetal liver, spleen, and bone marrow. By using early circulation, HSCs migrate from one hemopoietic tissue to the next, finally reaching the bone marrow where HSCs settle and persist during adult life (106, 109–111). Marrow stromal cells also

Table 2. MSCs and the Allogeneic Immune Cell Response[a]

Type of reaction	Comments for MSC involvement	References
MSC auto/allo transplant security issues	Cells home and survive in recipient, no adverse reactions	28, 35, 122–124
Lymphocyte alloreactivity	Suppress proliferation of allogenic lymphocytes, alloreactive T cells and B cell proliferation (by MSC-soluble factors), suppression *via* release of indoleamine 2,3-dioxygenase and activation of apoptosis	118–120, 125–133
APC function	Inhibit: maturation APC/DCs; differentiation/function of monocyte-derived DCs; alloantigen-induced differentiation DC	133–136
	Decrease: cytokine secretion by DC, naive and effector T and NK cells; allostimulatory ability of MSC-treated DC	
	Induce: formation $CD4^+$ T regulatory/suppressive cells	
Lysis by cytotoxic T and NK cells	Inhibit formation of cytotoxic lymphocytes; escape lysis but not interfere with lytic capacity of cytotoxic T lymphocytes and NK cells	137
Induction tolerogenic response to allogeneic tissue	Induce prolonged skin graft survival	125
GVHD after BM-allotransplants	Decrease severity acute GVHD after co-transplantation with identical sibling/unrelated HSC	123–138
Tumor growth	Potentiate proliferation melanoma cells	139

[a]APC, antigen-presenting cell; DCs, dendritic cells; GVHD, graft-versus-host disease; BM, bone marrow.

mobilize through fetal blood, thus allowing the transit of hemopoiesis from an immature into a more mature hemopoietic site (112–113). MSCs have been identified in fetal tissue and, as circulating cells, in human cord blood (19, 47, 102, 103, 114–116).

Cord blood MSCs (CB-MSCs) exhibit a morphology and immunophenotype, which is similar to that of adult MSCs. However, *in vitro* studies have established that the frequency and number of mesenchymal colonies, generated by a fixed number of nucleated cells, is much higher in fetal than in adult tissue (102). In terms of differentiation potential, and despite reports claiming a broad differentiation capacity (117), CB-MSCs, as well as adult MSCs, give rise to essentially the same mesenchymal lineages.

As indicated in Table 1, CB-MSCs can be induced *in vitro* to express a cardiac phenotype and, once transplanted, migrate, survive in the myocardium, and improve cardiac function after MI (34, 35, 44, 46, 47). In terms of immunogenicity, CB-MSCs express class I human leucocyte antigen (HLA) antigens, whereas class II HLA antigens are expressed only after prolonged exposure to interferon-γ (INF-γ). Immunologic responses elicited by cord blood and adult MSCs are comparable (118–120).

Therefore, in terms of differentiation and immunosuppressive properties, no main differences can be found among fetal and adult MSCs. However, in terms of practical issues related to their use as "repair cells," CB-MSCs seem to present more advantages than their adult counterpart in four ways. First, due to their high proliferation rate, less culture time is required to get a fixed number of *ex vivo* expanded CB-MSCs. This will result in less subcultivated cells and, thus, fewer chances of expressing apoptotic features (12). Second, fetal cells and probably CB-MSCs have an increased transendothelial migration capacity (121), which should be important throughout intracoronary cell delivery. Third, CB-MSCs are obtained from a source that, still in many countries, is usually wasted. Finally, due to their immunosuppressive properties (as discussed next), CB-MSCs may be used for allogeneic transplantation.

MSCs and the Allogeneic Immune Cell Response

In addition to the capacity of MSCs to differentiate into mesenchymal and nonmesenchymal tissue, they exhibit an interesting (and probably unique) feature in playing roles as modulators in the allogeneic immune cell response. A summary of immunomodulatory processes mediated by MSCs is shown in Table 2.

Studies to assess the expression of HLA antigens by bone marrow–derived and cord blood–derived MSCs have shown the expression of surfaces associated with HLA class I, but not HLA class II, antigens. However, bone marrow–derived MSCs contain intracellular HLA class II molecules, which are translocated to the cell surface after exposure to INF-γ (118). This pattern of expression of HLA antigens is stable and not significantly modified by MSC differentiation (118). Typical immunologic antigens, like B7–1, B7–2, CD40, CD40L, CD80, and CD86, are not expressed by MSCs (11, 13, 122–140). However, MSCs share with thymic epithelium the expression of antigens involved in T-cell interactions (e.g., VCAM-1, ICAM-1, LFA-3; Refs. 11, 13, 26, 141). Because the expression of these adhesion molecules is modulated by IL1-α, it has been proposed that the immune reactivity of MSCs is regulated by microenvironmental clues (142).

The molecular mechanisms involved in the immunosuppressive properties of MSCs are still not completely

understood. Up to now, the following studies have provided suggestions for candidate molecules and mechanisms involved. First, the immune effect elicited by MSCs is mediated by interactions with lymphocytes and results in the inhibition of splenocytes and T- and B-lymphocyte proliferation (132) and the expression of activation markers by phytohemagglutinin-activated lymphocytes (123). Interaction also involves $CD4^+$ T-cell differentiation to a regulatory phenotype (136) and cytokine secretion by effector T and NK cells (135). These effects are also mediated by soluble immunomodulatory factors like IL-10, TGF-β, HGF, and prostaglandin E2 (126, 128, 139, 140). Second, on induction of monocytes with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4, MSCs strongly inhibit early steps in monocyte differentiation to dendritic cells (DCs; Ref. 133). In addition, MSCs induce DCs to attain a more anti-inflammatory or tolerant phenotype (135). Third, MSCs seem to induce general and antigen-specific immunosuppression. This is consistent with the inhibition of alloantigen-induced DC differentiation and the preferential activation of T-cell subsets with a regulatory/suppressive phenotype (136). In the same vein, MSCs inhibit alloreactive T cells (134). Finally, by producing indoleamine 2,3-dioxygenase and the concomitant formation of a tryptophan-depleted milieu, MSCs also promote immunosuppression (131). It is without doubt that a better understanding of candidate molecules and mechanisms involved will contribute to optimize and open new alternatives for the preferential utilization of MSCs in (allogeneic) cellular therapy.

The differentiation, immunologic, and other attributes exhibited by adult-derived and cord blood–derived MSCs give additional support to the affirmation that mesenchymal progenitors are "no longer second class marrow citizens" (143). We anticipate that, very soon if not already, the distinctive attributes of MSCs will be used for the development of new clinical protocols for the treatment of myocardial infarct and other diseases.

1. Beltrami AP, Urbanek K, Kajstura J, Yan SM, Finato N, Bussani R, Nadal-Ginard B, Silvestri F, Leri A, Beltrami CA, Anversa P. Evidence that human cardiac myocytes divide after myocardial infarction. N Engl J Med 344:1750–1757, 2001.
2. Muller P, Beltrami AP, Cesselli D, Pfeiffer P, Kazakov A, Bohm M. Myocardial regeneration by endogenous adult progenitor cells. J Mol Cell Cardiol 39:377–387, 2005.
3. Urbanek K, Torella D, Sheikh F, De Angelis A, Nurzynska D, Silvestri F, Beltrami CA, Bussani R, Beltrami AP, Quaini F, Bolli R, Leri A, Kajstura J, Anversa P. Myocardial regeneration by activation of multipotent cardiac stem cells in ischemic heart failure. Proc Natl Acad Sci U S A 102:8692–8697, 2005.
4. Warejcka DJ, Harvey R, Taylor BJ, Young HE, Lucas PA. A population of cells isolated from rat heart capable of differentiating into several mesodermal phenotypes. J Surg Res 62:233–242, 1996.
5. Wang JS, Shum-Tim D, Galipeau J, Chedrawy E, Eliopoulos N, Chiu RC. Marrow stromal cells for cellular cardiomyoplasty: feasibility and potential clinical advantages. J Thorac Cardiovasc Surg 120:999-1005, 2000.
6. Siminiak T, Kurpisz M. Myocardial replacement therapy. Circulation 108:1167–1171, 2003.
7. Wulf GG, Jackson KA, Goodell MA. Somatic stem cell plasticity: current evidence and emerging concepts. Exp Hematol 29:1361–1370, 2001.
8. Herzog EL, Chai L, Krause DS. Plasticity of marrow-derived stem cells. Blood 102:3483–3493, 2003.
9. 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 410:701–705, 2001.
10. Bel A, Messas E, Agbulut O, Richard P, Samuel JL, Bruneval P, Hagege AA, Menasche P. Transplantation of autologous fresh bone marrow into infarcted myocardium: a word of caution. Circulation 108:247–252, 2003.
11. Pittenger M, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147, 1999.
12. Conget PA, Minguell JJ. Phenotypical and functional properties of human bone marrow mesenchymal progenitor cells. J Cell Physiol 81: 67–73, 1999.
13. Deans RJ, Moseley AB. Mesenchymal stem cells: biology and potential clinical uses. Exp Hematol 28:875–884, 2000.
14. Minguell JJ, Erices A, Conget P. Mesenchymal stem cells. Exp Biol Med 226:507–520, 2001.
15. Ito T, Suziki A, Okabe M, Imai E, Hori M. Application of bone marrow-derived stem cells in experimental nephrology. Exp Nephrol 9:444–450, 2001.
16. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 418:41–49, 2002.
17. Neuhuber B, Gallo G, Howard L, Kostura L, Mackay A, Fischer I. Reevaluation of in vitro differentiation protocols for bone marrow stromal cells: disruption of actin cytoskeleton induces rapid morphological changes and mimics neuronal phenotype. J Neurosci Res 77:192–204, 2004.
18. Fernandez M, Simon V, Herrera G, Cao C, Del Favero H, Minguell JJ. Detection of stromal cells in peripheral blood progenitor cell collections from breast cancer patients. Bone Marrow Transplant 20: 265–271, 1997.
19. Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. Br J Haematol 109:235–242, 2000.
20. Conget PA, Allers C, Minguell JJ. Identification of a discrete population of human bone-marrow derived mesenchymal cells exhibiting properties of uncommitted progenitors. J Hematother Stem Cell Res 10:749–758, 2001.
21. Conget PA, Minguell JJ. Adenoviral-mediated gene transfer into ex vivo expanded human bone marrow mesenchymal progenitor cells. Exp Hematol 28:382–390, 2000.
22. Partridge K, Yang X, Clarke NM, Okubo Y, Bessho K, Sebald W, Howdel SM, Shakesheff KM, Oreffo RO. Adenoviral BMP-2 gene transfer in mesenchymal stem cells: in vitro and in vivo bone formation on biodegradable polymer scaffolds. Biochem Biophys Res Commun 292:144–152, 2002.
23. Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, Sussman M, Orchard P, Marx JC, Pyeritz RE, Brenner MK. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med 5:309–313, 1999.

24. Barry FP, Murphy JM. Mesenchymal stem cells: clinical application and biological properties. Int J Biochem Cell Biol 36:568–584, 2004.
25. Piersma AH, Ploemacher RE, Brockbank KG. Transplantation of bone marrow fibroblastoid stromal cells in mice via the intravenous route. Br J Haematol 54:285–290, 1983.
26. Pereira RF, Halford KW, O'Hara MD, Leeper DB, Sokolov BP, Pollard MD, Bagasra O, Prockop DJ. Cultured adherent cells from marrow can serve as long-lasting precursor cells for bone, cartilage, and lung in irradiated mice. Proc Natl Acad Sci U S A 92:4857–4861, 1995.
27. Devine SM, Cobbs C, Jennings M, Bartholomew A, Hoffman R. Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into non human primates. Blood 101:2999–3001, 2003.
28. Allers C, Sierralta WD, Neubauer S, Rivera F, Minguell JJ, Conget PA. Dynamic of distribution of human bone marrow-derived mesenchymal stem cells after transplantation into adult unconditioned mice. Transplantation 78:503–508, 2004.
29. Wakitani S, Saito T, Caplan A. Myogeneic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. Muscle Nerve 18:1417–1426, 1995.
30. Makino S, Fukuda K, Miyoshi S, Konishi F, Kodama H, Pan J, Sano M, Takahashi T, Hori S, Abe H, Hata J, Umezawa A, Ogawa S. Cardiomyocytes can be generated from marrow stromal cells in vitro. J Clin Invest 103:697–705, 1999.
31. Bittira B, Kuang JQ, Al-Khaldi A, Shum-Tim D, Chiu RC. In vitro preprogramming of marrow stromal cells for myocardial regeneration. Ann Thorac Surg 74:1154–1159, 2002.
32. Min JY, Sullivan MF, Yang Y, Zhang JP, Converso KL, Morgan JP, Xiao YF. Significant improvement of heart function by cotransplantation of human mesenchymal stem cells and fetal cardiomyocytes in postinfarcted pigs. Ann Thorac Surg 74:1568–1575, 2002.
33. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. Circulation 105:93–98, 2002.
34. Cheng F, Zou P, Yang H, Yu Z, Zhong Z. Induced differentiation of human cord blood mesenchymal stem/progenitor cells into cardiomyocyte-like cells in vitro. J Huazhong Univ Sci Technolog Med Sci 23:154–157, 2003.
35. Erices AA, Allers CI, Conget PA, Rojas CV, Minguell JJ. Human cord blood-derived mesenchymal stem cells home and survive in the marrow of immunodeficient mice after systemic infusion. Cell Transplant 12:555–561, 2003.
36. Fukuda K. Application of mesenchymal stem cells for the regeneration of cardiomyocyte and its use for cell transplantation therapy. Hum Cell 16:83–94, 2003.
37. Fukuhara S, Tomita S, Yamashiro S, Morisaki T, Yutani C, Kitamura S, Nakatani T. Direct cell-cell interaction of cardiomyocytes is key for bone marrow stromal cells to go into cardiac lineage in vitro. J Thorac Cardiovasc Surg 125:1470–1480, 2003.
38. Mangi AA, Noiseux N, Kong D, He H, Rezvani M, Ingwall JS, Dzau VJ. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. Nat Med 9:1195–1201, 2003.
39. Rangappa S, Entwistle JW, Wechsler AS, Kresh JY. Cardiomyocyte-mediated contact programs human mesenchymal stem cells to express cardiogenic phenotype. J Thorac Cardiovasc Surg 126:124–132, 2003.
40. Liu J, Hu Q, Wang Z, Xu C, Wang X, Gong G, Mansoor A, Lee J, Hou M, Zeng L, Zhang JR, Jerosch-Herold M, Guo T, Bache RJ, Zhang J. Autologous stem cell transplantation for myocardial repair. Am J Physiol Heart Circ Physiol 287:501–511, 2004.
41. Nagaya N, Fujii T, Iwase T, Ohgushi H, Itoh T, Uematsu M, Yamagishi M, Mori H, Kangawa K, Kitamura S. Intravenous administration of mesenchymal stem cells improves cardiac function in rats with acute myocardial infarction through angiogenesis and myogenesis. Am J Physiol Heart Circ Physiol 287:H2670–H2676, 2004.
42. Potapova I, Plotnikov A, Lu Z, Danilo P Jr, Valiunas V, Qu J, Doronin S, Zuckerman J, Shlapakova IN, Gao J, Pan Z, Herron AJ, Robinson RB, Brink PR, Rosen MR, Cohen IS. Human mesenchymal stem cells as a gene delivery system to create cardiac pacemakers. Circ Res 94:952–959, 2004.
43. Shim WS, Jiang S, Wong P, Tan J, Chua YL, Tan YS, Sin YK, Lim CH, Chua T, Teh M, Liu TC, Sim E. Ex vivo differentiation of human adult bone marrow stem cells into cardiomyocyte-like cells. Biochem Biophys Res Commun 324:481–488, 2004.
44. Vanelli P, Beltrami S, Cesana E, Cicero D, Zaza A, Rossi E, Cicirata F, Antona C, Clivio A. Cardiac precursors in human bone marrow and cord blood: in vitro cell cardiogenesis. Ital Heart J 5:384–388, 2004.
45. Xu W, Zhang X, Qian H, Zhu W, Sun X, Hu J, Zhou H, Chen Y. Mesenchymal stem cells from adult human bone marrow differentiate into a cardiomyocyte phenotype, *in vitro*. Exp Biol Med 229:623–631, 2004.
46. Hirata Y, Sata M, Motomura N, Takanashi M, Suematsu Y, Ono M, Takamoto S. Human umbilical cord blood cells improve cardiac function after myocardial infarction. Biochem Biophys Res Commun 327:609–614, 2005.
47. Zhao P, Ise H, Hongo M, Ota M, Konishi I, Nikaido T. Human amniotic mesenchymal cells have some characteristics of cardiomyocytes. Transplantation 79:528–535, 2005.
48. Valiunas V, Doronin S, Valiuniene L, Potapova I, Zuckerman J, Walcott B, Robinson RB, Rosen MR, Brink PR, Cohen IS. Human mesenchymal stem cells make cardiac connexins and form functional gap junctions. J Physiol 555:617–626, 2004.
49. Bird SD, Doevendans PA, van Rooijen MA, Brutel de la Riviere A, Hassink RJ, Passier R, Mummery CL. The human adult cardiomyocyte phenotype. Cardiovasc Res 58:423–434, 2003.
50. Katwa LC. Cardiac myofibroblasts isolated from the site of myocardial infarction express endothelin de novo. Am J Physiol Heart Circ Physiol 285:H1132–H1139, 2003.
51. Rafii S, Avecilla S, Shmelkov S, Shido K, Tejada R, Moore MA, Heissig B, Hattori K. Angiogenic factors reconstitute hematopoiesis by recruiting stem cells from bone marrow microenvironment. Ann N Y Acad Sci 996:49–60, 2003.
52. Hellstrom M, Kalen M, Lindahl P, Abramsson A, Betsholtz C. Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. Development 126:3047–3055, 1999.
53. Gojo S, Gojo N, Takeda Y, Mori T, Abe H, Kyo S, Hata J, Umezawa A. In vivo cardiovasculogenesis by direct injection of isolated adult mesenchymal stem cells. Exp Cell Res 288:51–59, 2003.
54. Davani S, Marandin A, Mersin N, Royer B, Kantelip B, Herve P, Etievent JP, Kantelip JP. Mesenchymal progenitor cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a rat cellular cardiomyoplasty model. Circulation 108:253–258, 2003.
55. Silva GV, Litovsky S, Assad JA, Sousa AL, Martin BJ, Vela D, Coulter SC, Lin J, Ober J, Vaughn WK, Branco RV, Oliveira EM, He R, Geng YJ, Willerson JT, Perin EC. Mesenchymal stem cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a canine chronic ischemia model. Circulation 111:150–156, 2005.
56. Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchs S, Epstein SE. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. Circ Res 94:678–685, 2004.
57. Tang YL, Zhao Q, Zhang YC, Cheng L, Liu M, Shi J, Yang YZ, Pan C, Ge J, Phillips MI. Autologous mesenchymal stem cell trans-

plantation induce VEGF and neovascularization in ischemic myocardium. Regul Pept 117:3–10, 2004.

58. Vandervelde S, van Luyn MJ, Tio RA, Harmsen MC. Signaling factors in stem cell-mediated repair of infarcted myocardium. J Mol Cell Cardiol 39:363–376, 2005.
59. Harada M, Qin Y, Takano H, Minamino T, Zou Y, Toko H, Ohtsuka M, Matsuura K, Sano M, Nishi J, Iwanaga K, Akazawa H, Kunieda T, Zhu W, Hasegawa H, Kunisada K, Nagai T, Nakaya H, Yamauchi-Takihara K, Komuro I. G-CSF prevents cardiac remodeling after myocardial infarction by activating the Jak-Stat pathway in cardiomyocytes. Nat Med 11:305–311, 2005.
60. Benavente CA, Sierralta WD, Conget PA, Minguell JJ. Subcellular distribution and mitogenic effect of basic fibroblast growth factor in mesenchymal uncommitted stem cells. Growth Factors 21:87–94, 2003.
61. Minguell JJ, Fierro FF, Epuñan MJ, Erices AA, Sierralta WD. Nonstimulated human uncommitted mesenchymal stem cells express cell markers of mesenchymal and neural lineages. Stem Cells Dev 14: 408–414, 2005.
62. Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, Pasumarthi KB, Virag JI, Bartelmez SH, Poppa V, Bradford G, Dowell JD, Williams DA, Field LJ. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature 428:607–608, 2004.
63. Kajstura J, Rota M, Whang B, Cascapera S, Hosoda T, Bearzi C, Nurzynska D, Kasahara H, Zias E, Bonafe M, Nadal-Ginard B, Torella D, Nascimbene A, Quaini F, Urbanek K, Leri A, Anversa P. Bone marrow cells differentiate in cardiac cell lineages after infarction independently of cell fusion. Circ Res 96:127–137, 2005.
64. Agbulut O, Menot ML, Li Z, Marotte F, Paulin D, Hagege AA, Chomienne C, Samuel JL, Menasche P. Temporal patterns of bone marrow cell differentiation following transplantation in doxorubicin-induced cardiomyopathy. Cardiovasc Res 58:451–459, 2003.
65. Haider HK, Ashraf M. Bone marrow stem cell transplantation for cardiac repair. Am J Physiol Heart Circ Physiol 288:H2557–H2567, 2005.
66. Gao J, Dennis JE, Muzic RF, Lundberg M, Caplan AI. The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. Cells Tissues Organs 169:12–20, 2001.
67. Liechty KW, MacKenzie TC, Shaaban AF, Radu A, Moseley AM, Deans R, Marshak DR, Flake AW. Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep. Nat Med 6:1282–1286, 2000.
68. Bittira B, Shum-Tim D, Al-Khaldi A, Chiu RC. Mobilization and homing of bone marrow stromal cells in myocardial infarction. Eur J Cardiothorac Surg 24:393–398, 2003.
69. Barbash IM, Chouraqui P, Baron J, Feinberg MS, Etzion S, Tessone A, Miller L, Guetta E, Zipori D, Kedes LH, Kloner RA, Leor J. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infracted myocardium: feasibility, cell migration, and body distribution. Circulation 108:863–868, 2003.
70. Saito T, Kuang JQ, Lin CC, Chiu RC. Transcoronary implantation of bone marrow stromal cells ameliorates cardiac function after myocardial infarction. J Thorac Cardiovasc Surg 126:114–123, 2003.
71. Hattan N, Kawaguchi H, Ando K, Kuwabara E, Fujita J, Murata M, Suematsu M, Mori H, Fukuda K. Purified cardiomyocytes from bone marrow mesenchymal stem cells produce stable intracardiac grafts in mice. Cardiovasc Res 65:334–344, 2005.
72. Bloor CM, White FC, Roth DM. The pig as a model of myocardial ischemia and gradual coronary artery occlusion. In: Swindle MM, Ed. Swine as Models in Biomedical Research. Ames, IA: Iowa State University Press, pp163–175, 1992.
73. Dick AJ, Guttman MA, Raman VK, Peters DC, Pessanha BS, Hill JM, Smith S, Scott G, McVeigh ER, Lederman RJ. Magnetic resonance fluoroscopy allows targeted delivery of mesenchymal stem cells to infarct borders in swine. Circulation 108:2899–2904, 2003.
74. Hill JM, Dick AJ, Raman VK, Thompson RB, Yu ZX, Hinds KA, Pessanha BS, Guttman MA, Varney TR, Martin BJ, Dunbar CE, McVeigh ER, Lederman RJ. Serial cardiac magnetic resonance imaging of injected mesenchymal stem cells. Circulation 108:1009–1014, 2003.
75. Kraitchman DL, Heldman AW, Atalar E, Amado LC, Martin BJ, Pittenger MF, Hare JM, Bulte JW. In vivo magnetic resonance imaging of mesenchymal stem cells in myocardial infarction. Circulation 107:2290–2293, 2003.
76. Shake JG, Gruber PJ, Baumgartner WA, Senechal G, Meyers J, Redmond JM, Pittenger MF, Martin BJ. Mesenchymal stem cell implantation in a swine myocardial infarct model: engraftment and functional effects. Ann Thorac Surg 73:1919–1925, 2002.
77. Vulliet PR, Greeley M, Halloran SM, MacDonald KA, Kittleson MD. Intra-coronary arterial injection of mesenchymal stromal cells and microinfarction in dogs. Lancet 363:783–784, 2004.
78. Kovacic JC, Graham RM. Stem-cell therapy for myocardial diseases. Lancet 363:1735–1736, 2004.
79. Assmus B, Schachinger V, Teupe C, Britten M, Lehmann R, Dobert N, Grunwald F, Aicher A, Urbich C, Martin H, Hoelzer D, Dimmeler S, Zeiher AM. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). Circulation 106:3009–3017, 2002.
80. Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P, Breidenbach C, Fichtner S, Korte T, Hornig B, Messinger D, Arseniev L, Hertenstein B, Ganser A, Drexler H. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. Lancet 364:141–148, 2004.
81. Jaquet K, Krause KT, Denschel J, Faessler P, Nauerz M, Geidel S, Boczor S, Lange C, Stute N, Zander A, Kuck KH. Reduction of myocardial scar size after implantation of mesenchymal stem cells in rats: what is the mechanism? Stem Cells Dev 14:299–309, 2005.
82. Strauer BE, Brehm M, Zeus T, Kostering M, Hernandez A, Sorg RV, Kogler G, Wernet P. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation 106:1913–1918, 2002.
83. Menasche P, Hagege AA, Vilquin JT, Desnos M, Abergel E, Pouzet B, Bel A, Sarateanu S, Scorsin M, Schwartz K, Bruneval P, Benbunan M, Marolleau JP, Duboc D. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. J Am Coll Cardiol 41:1078–1083, 2003.
84. Pagani FD, DerSimonian H, Zawadzka A, Wetzel K, Edge AS, Jacoby DB, Dinsmore JH, Wright S, Aretz TH, Eisen HJ, Aaronson KD. Autologous skeletal myoblasts transplanted to ischemia-damaged myocardium in humans: histological analysis of cell survival and differentiation. J Am Coll Cardiol 41:879–888, 2003.
85. Perin EC, Dohmann HF, Borojevic R, Silva SA, Sousa AL, Mesquita CT, Rossi MI, Carvalho AC, Dutra HS, Dohmann HJ, Silva GV, Belem L, Vivacqua R, Rangel FO, Esporcatte R, Geng YJ, Vaughn WK, Assad JA, Mesquita ET, Willerson JT. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. Circulation 107:2294–2302, 2003.
86. Stamm C, Westphal B, Kleine HD, Petzsch M, Kittner C, Klinge H, Schumichen C, Nienaber CA, Freund M, Steinhoff G. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. Lancet 361:45–46, 2003.
87. Tse HF, Kwong YL, Chan JK, Lo G, Ho CL, Lau CP. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. Lancet 361:47–49, 2003.
88. Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, Kearne M, Magner M, Isner JM. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physio-

logical and pathological neovascularization. Circ Res 85:221–228, 1999.

89. Kawamoto A, Asahara T, Losordo DW. Transplantation of endothelial progenitor cells for therapeutic neovascularization. Cardiovasc Radiat Med 3:221–225, 2002.
90. Tepper OM, Capla JM, Galiano RD, Ceradini DJ, Callaghan MJ, Kleinman ME, Gurtner GC. Adult vasculogenesis occurs through in situ recruitment, proliferation, and tubulization of circulating bone marrow-derived cells. Blood 105:1068–1077, 2005.
91. Yoshida S, Ono M, Shono T, Izumi H, Ishibashi T, Suzuki H, Kuwano M. Involvement of interleukin-8, vascular endothelial growth factor, and basic fibroblast growth factor in tumor necrosis factor alpha-dependent angiogenesis. Mol Cell Biol 17:4015–4023, 1997.
92. Presta M, Dell'Era P, Mitola S, Moroni E, Ronca R, Rusnati M. Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. Cytokine Growth Factor Rev 16:159–178, 2005.
93. Gupta K, Zhang J. Angiogenesis: a curse or cure? Postgrad Med J 81: 236–242, 2005.
94. Yoshioka T, Ageyama N, Shibata H, Yasu T, Misawa Y, Takeuchi K, Matsui K, Yamamoto K, Terao K, Shimada K, Ikeda U, Ozawa K, Hanazono Y. Repair of infarcted myocardium mediated by transplanted bone marrow-derived CD34+ stem cells in a nonhuman primate model. Stem Cells 23:355–364, 2005.
95. Chen SL, Fang WW, Ye F, Liu YH, Qian J, Shan SJ, Zhang JJ, Chunhua RZ, Liao LM, Lin S, Sun JP. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. Am J Cardiol 94:92–95, 2004.
96. Florenzano F, Minguell JJ. Autologous mesenchymal stem cell transplantation after acute myocardial infarction. Am J Cardiol 95: 435, 2005.
97. Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, Amano K, Kishimoto Y, Yoshimoto K, Akashi H, Shimada K, Iwasaka T, Imaizumi T. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. Lancet 360:427–435, 2002.
98. Haynesworth SE, Baber MA, Caplan AI. Cytokine expression by human marrow-derived mesenchymal progenitor cells in vitro: effects of dexamethasone and IL-1 alpha. J Cell Physiol 166:585–592, 1996.
99. Erices A, Conget P, Rojas C, Minguell JJ. Gp130 activation by soluble interleukin-6 receptor/interleukin-6 enhances osteoblastic differentiation of human bone marrow-derived mesenchymal stem cells. Exp Cell Res 280:24–32, 2002.
100. Tang YL, Zhao Q, Qin X, Shen L, Cheng L, Ge J, Phillips MI. Paracrine action enhances the effects of autologous mesenchymal stem cell transplantation on vascular regeneration in rat model of myocardial infarction. Ann Thorac Surg 80:229–236, 2005.
101. Pak HN, Qayyum M, Kim DT, Hamabe A, Miyauchi Y, Lill MC, Frantzen M, Takizawa K, Chen LS, Fishbein MC, Sharifi BG, Chen PS, Makkar R. Mesenchymal stem cell injection induces cardiac nerve sprouting and increased tenascin expression in a swine model of myocardial infarction. J Cardiovasc Electrophysiol 14:841–848, 2003.
102. Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood 98:2396–2402, 2001.
103. Romanov YA, Svintsitskaya VA, Smirnov VN. Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. Stem Cells 21:105–110, 2003.
104. Igura K, Zhang X, Takahashi K, Mitsuru A, Yamaguchi S, Takashi TA. Isolation and characterization of mesenchymal progenitor cells from chorionic villi of human placenta. Cytotherapy 6:543–553, 2004.
105. Hann IM, Bodger MP, Hoffbrand AV. Development of pluripotent hematopoietic progenitor cells in the human fetus. Blood 62:118–123, 1983.
106. Tavassoli M. Embryonic and fetal hemopoiesis: an overview. Blood Cells 17:269–281, 1991.
107. Bianchi DW, Zickwolf GK, Weil GJ, Sylvester S, DeMaria MA. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. Proc Natl Acad Sci U S A 93:705–708, 1996.
108. O'Donoghue K, Choolani M, Chan J, de la Fuente J, Kumar S, Campagnoli C, Bennett PR, Roberts IA, Fisk NM. Identification of fetal mesenchymal stem cells in maternal blood: implications for non-invasive prenatal diagnosis. Mol Hum Reprod 9:497–502, 2003.
109. Dennis JE, Charbord P. Origin and differentiation of human and murine stroma. Stem Cells 20:205–214, 2002.
110. Oberlin E, Tavian M, Blazsek I, Peault B. Blood-forming potential of vascular endothelium in the human embryo. Development 129:4147–4157, 2002.
111. Peault B, Tavian M. Hematopoietic stem cell emergence in the human embryo and fetus. Ann N Y Acad Sci 996:132–140, 2003.
112. Nieda M, Nicol A, Denning-Kendall P, Sweetenham J, Bradley B, Hows J. Endothelial cell precursors are normal components of human umbilical cord blood. Br J Haematol 98:775–777, 1997.
113. Mayani H, Gutierrez-Rodriguez M, Espinoza L, Lopez-Chalini E, Huerta-Zepeda A, Flores E, Sanchez-Valle E, Luna-Bautista F, Valencia I, Ramirez OT. Kinetics of hematopoiesis in Dexter-type long-term cultures established from human umbilical cord blood cells. Stem Cells 16:127–135, 1998.
114. in 't Anker PS, Noort WA, Scherjon SA, Kleijburg-van der Keur C, Kruisselbrink AB, van Bezooijen RL, Beekhuizen W, Willemze R, Kanhai HH, Fibbe WE. Mesenchymal stem cells in human second-trimester bone marrow, liver, lung, and spleen exhibit a similar immunophenotype but a heterogeneous multilineage differentiation potential. Haematologica 88:845–852, 2003.
115. Bieback K, Kern S, Kluter H, Eichler H. Critical parameters for the isolation of mesenchymal stem cells from umbilical cord blood. Stem Cells 22:625–634, 2004.
116. Fan CG, Tang FW, Zhang QJ, Lu SH, Liu HY, Zhao ZM, Liu B, Han ZB, Han ZC. Characterization and neural differentiation of fetal lung mesenchymal stem cells. Cell Transplant 14:311–321, 2005.
117. Kogler G, Sensken S, Airey JA, Trapp T, Muschen M, Feldhahn N, Liedtke S, Sorg RV, Fischer J, Rosenbaum C, Greschat S, Knipper A, Bender J, Degistirici O, Gao J, Caplan AI, Colletti EJ, Almeida-Porada G, Muller HW, Zanjani E, Wernet P. A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential. J Exp Med 200:123–135, 2004.
118. Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringden O. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. Exp Hematol 31:890–896, 2003.
119. Gotherstrom C, Ringden O, Westgren M, Tammik C, Le Blanc K. Immunomodulatory effects of human foetal liver-derived mesenchymal stem cells. Bone Marrow Transplant 32:265–272, 2003.
120. Gotherstrom C, Ringden O, Tammik C, Zetterberg E, Westgren M, Le Blanc K. Immunologic properties of human fetal mesenchymal stem cells. Am J Obstet Gynecol 190:239–245, 2004.
121. Yong KL, Fahey A, Pahal G, Linch DC, Pizzey A, Thomas NS, Jauniaux E, Kinnon C, Thrasher AJ. Fetal haemopoietic cells display enhanced migration across endothelium. Br J Haematol 116:392–400, 2002.
122. Koc ON, Gerson SL, Cooper BW, Dyhouse SM, Haynesworth SE, Caplan AI, Lazarus HM. Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. J Clin Oncol 18:307–316, 2000.
123. Le Blanc K, Rasmusson I, Gotherstrom C, Seidel C, Sundberg B, Sundin M, Rosendahl K, Tammik C, Ringden O. Mesenchymal stem cells inhibit the expression of CD25 (interleukin-2 receptor) and CD38

on phytohaemagglutinin-activated lymphocytes. Scand J Immunol 60: 307–315, 2004.

124. Le Blanc K, Gotherstrom C, Ringden O, Hassan M, McMahon R, Horwitz E, Anneren G, Axelsson O, Nunn J, Ewald U, Norden-Lindeberg S, Jansson M, Dalton A, Astrom E, Westgren M. Fetal mesenchymal stem-cell engraftment in bone after in utero transplantation in a patient with severe osteogenesis imperfecta. Transplantation 79:1607–1614, 2005.
125. Bartholomew A, Sturgeon C, Siatskas M, Ferrer K, McIntosh K, Patil S, Hardy W, Devine S, Ucker D, Deans R, Moseley A, Hoffman R. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. Exp Hematol 30:42–48, 2002.
126. Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, Grisanti S, Gianni AM. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood 99:3838–3843, 2002.
127. Le Blanc K, Tammik L, Sundberg B, Haynesworth SE, Ringden O. Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. Scand J Immunol 57:11–20, 2003.
128. Krampera M, Glennie S, Dyson J, Scott D, Laylor R, Simpson E, Dazzi F. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. Blood 101:3722–3729, 2003.
129. Maitra B, Szekely E, Gjini K, Laughlin MJ, Dennis J, Haynesworth SE, Koc ON. Human mesenchymal stem cells support unrelated donor hematopoietic stem cells and suppress T-cell activation. Bone Marrow Transplant 33:597–604, 2004.
130. Le Blanc K, Rasmusson I, Sundberg B, Gotherstrom C, Hassan M, Uzunel M, Ringden O. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 363:1439–1441, 2004.
131. Meisel R, Zibert A, Laryea M, Gobel U, Daubener W, Dilloo D. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. Blood 103:4619–4621, 2004.
132. Augello A, Tasso R, Negrini SM, Amateis A, Indiveri F, Cancedda R, Pennesi G. Bone marrow mesenchymal progenitor cells inhibit lymphocyte proliferation by activation of the programmed death 1 pathway. Eur J Immunol 35:1482–1490, 2005.
133. Jiang XX, Zhang Y, Liu B, Zhang SX, Wu Y, Yu XD, Mao N. Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. Blood 105:4120–4126, 2005.
134. Beyth S, Borovsky Z, Mevorach D, Liebergall M, Gazit Z, Aslan H, Galun E, Rachmilewitz J. Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T-cell unresponsiveness. Blood 105:2214–2219, 2005.
135. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 105:1815–1822, 2005.
136. Maccario R, Podesta M, Moretta A, Cometa A, Comoli P, Montagna D, Daudt L, Ibatici A, Piaggio G, Pozzi S, Frassoni F, Locatelli F. Interaction of human mesenchymal stem cells with cells involved in alloantigen-specific immune response favors the differentiation of CD4+ T-cell subsets expressing a regulatory/suppressive phenotype. Haematologica 90:516–525, 2005.
137. Rasmusson I, Ringden O, Sundberg B, Le Blanc K. Mesenchymal stem cells inhibit the formation of cytotoxic T lymphocytes, but not activated cytotoxic T lymphocytes or natural killer cells. Transplantation 76:1208–1213, 2003.
138. Lazarus HM, Koc ON, Devine SM, Curtin P, Maziarz RT, Holland HK, Shpall EJ, McCarthy P, Atkinson K, Cooper BW, Gerson SL, Laughlin MJ, Loberiza FR Jr, Moseley AB, Bacigalupo A. Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. Biol Blood Marrow Transplant 11:389–398, 2005.
139. Djouad F, Plence P, Bony C, Tropel P, Apparailly F, Sany J, Noel D, Jorgensen C. Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals. Blood 102:3837–3844, 2003.
140. Tse WT, Pendleton JD, Beyer WM, Egalka MC, Guinan EC. Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. Transplantation 75:389–397, 2003.
141. Barda-Saad M, Rozenszajn LA, Ashush H, Shav-Tal Y, Ben Nun A, Zipori D. Adhesion molecules involved in the interactions between early T cells and mesenchymal bone marrow stromal cells. Exp Hematol 27:834–844, 1999.
142. Majumdar MK, Thiede MA, Mosca JD, Moorman M, Gerson SL. Phenotypic and functional comparison of cultures of marrow-derived mesenchymal stem cells (MSCs) and stromal cells. J Cell Physiol 176: 57–66, 1998.
143. Gerson SL. Mesenchymal stem cells: no longer second class marrow citizens. Nat Med 5:262–264, 1999.