Chapter 12
Stem Cells in the Treatment of Myocardial Infarction and Cardiomyopathy

Robert J. Henning

Abstract Cardiovascular investigators are currently investigating adult bone marrow stem cells, cardiac stem cells, and adipose stem cells as potential new regenerative cell treatments for patients with acute myocardial infarctions and cardiomyopathies. The initial ten-year experience with autologous, unfractionated bone marrow aspirates, which contain hematopoietic and mesenchymal stem cells (MSCs), suggested that patients with myocardial infarctions who receive these cells demonstrate 2–3 % increases in the left ventricular (LV) ejection fraction of the heart, 4.8 ml decreases in the left ventricular end-systolic volume (LVESV), and approximately 5 % reductions in infarction size without experiencing significant side effects from these cells. The bone marrow stem cells are thought to act by releasing biologically active factors that limit myocardial inflammation, injury, and necrosis. The LateTIME, the TIME, and the Swiss Myocardial Infarction trials have recently addressed the questions of the optimal time for autologous, unfractionated bone marrow cell administration after acute myocardial infarction and coronary angioplasty and whether these cells limit myocardial damage in comparison with the patients treated with percutaneous coronary angioplasty and current medical care without cell transplantation. In these studies, the myocardial infarction sizes and the left ventricular ejection fractions (LVEFs) were not significantly different between the cell-treated patients with standard medical care and patients treated with standard medical care without bone marrow cells (BMCs). The lack of differences between treatments may have been due to the performance of coronary angioplasty within 4–5 h of the onset of patient's symptoms of myocardial infarction, but may also have been due to heterogeneous patient bone marrow cell populations, red blood cell contamination of stem cells, heparin inhibition of stem cell

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migration, and expulsion of the stem cells from the contracting heart shortly after injection. Current trials of patients with myocardial infarction are examining specific bone marrow stem cells including MSCs and $CD34^+$ endothelial stem cells. In addition, cardiac stem cells isolated from human hearts are being investigated in the treatment of patients with infarcted hearts. Cardiac stem cell treatments in patients with infarcted hearts have reportedly decreased left ventricular infarct scar sizes by 11.9–15.7 g and increased left ventricular viable mass by 17.9–22.6 g. Successful cell-based therapy for patients with heart disease requires the close cooperation and interaction of basic scientists and clinicians throughout the world. In this manner, the cell-based therapy in the twenty-first century will offer new hope to the millions of patients with heart disease throughout the world who would otherwise suffer from the inexorable downward progression of heart disease, heart failure, and death.

Keywords Stem cell · Myocardial infarction · Cardiomyopathy

12.1 Introduction

Each year in the USA, more than one million people experience a myocardial infarction and approximately 400,000 people die as a result of their myocardial infarction (Go et al. 2014). Single or recurrent myocardial infarction with loss of more than 25 % of the left ventricular myocardium is associated with low cardiac output and congestive heart failure in patients. Currently, five million people in the USA have congestive heart failure and more than one million people are hospitalized with heart failure each year (Go et al. 2014). In addition, approximately 58,000 patients with heart failure die annually.

Left ventricular scar due to myocardial infarction is largely acellular and causes left ventricular mechanical dysfunction, electrical uncoupling and cardiac arrhythmias, and ultimately cardiomyopathy. The cardiomyocyte deficit in patients with myocardial infarction and cardiomyopathy is approximately one billion cardiac myocytes (Beltrami et al. 1994). Although the heart has some ability to regenerate cardiac myocytes after myocardial infarction, endogenous myocardial muscle restoration is inadequate to compensate for the cardiac myocyte loss with myocardial infarction. Consequently, cardiovascular investigators are currently exploring the use of adult bone marrow stem cells, cardiac progenitor cells, and adipose stem cells as potential new regenerative cell treatments for patients with myocardial infarctions and cardiomyopathies.

12.2 Bone Marrow Stem Cells in Cardiac Repair: The First Ten Years

Due to the limited availability of human embryonic stem cells for cardiac repair, many cardiovascular investigators turned to adult bone marrow cells (BMCs) to potentially reduce in patients the size and the fibrosis of myocardial infarctions, limit

post-infarction left ventricular (LV) remodeling, and improve left ventricular wall thickening and compliance. Human bone marrow contains hematopoietic and mesenchymal stem cells (MSCs) which constitute less than 0.01 % of the BMCs. Bone marrow MSCs can generate in vitro myocytes as well as osteoblasts, chondrocytes, and adipose cells. Bone marrow hematopoietic stem cells can produce endothelial progenitor cells and also red blood cells, megakaryocytes, myeloid cells, and lymphocytes. Consequently, cardiovascular investigators theorized that bone marrow mesenchymal and hematopoietic stem cells could transdifferentiate into cardiomyocytes and vascular endothelial cells when implanted in infarcted myocardium. Subsequent investigations in research animals with myocardial infarctions demonstrated that BMCs could decrease infarct size and improve LV ejection fraction and paved the way for studies in patients with acute myocardial infarctions. Based on the studies in research animals, transdifferentiation of BMCs to cardiac myocytes and vascular endothelial cells in the infarcted LV does not appear to occur and does not explain the cardiac changes that can occur with bone marrow cell transplantation in the heart. Rather, bone marrow stem cells appear to act by paracrine mechanisms with the release of biologically active growth factors and anti-inflammatory cytokines that limit myocardial inflammation, injury, and infarct-associated decreases in heart contractility (Gnecchi et al. 2005; Kinnaird et al. 2004).

The first trials using autologous bone marrow stems cells for the treatment of patients with acute myocardial infarctions were reported in 2002. Since that time, many of the clinical trials have used autologous *unfractionated bone marrow mononuclear cells* that contain hematopoietic, MSCs, and other BMCs. The Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trial was a randomized study that reported in 2006 that bone marrow cell therapy significantly increased in patients with myocardial infarctions the left ventricular ejection fractions (LVEFs) and reduced the 1-year combined clinical endpoint of death, recurrence of myocardial infarction and revascularization especially in patients with myocardial infarctions and pulmonary congestion as determined by *n*-terminal brain natriuretic peptide (NT-BNP) values >733 pg/ml (Schaechinger et al. 2006a, b). In contrast to the REPAIR-AMI study, the Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration (BOOST) trial reported that adult bone marrow cell-treated patients with myocardial infarctions demonstrated an initial increase in LV ejection fraction at 6 months but no increase in LV ejection fraction after 18 months in comparison with patients treated with optimal medical treatment without bone marrow cells (Wollert et al. 2004; Meyer et al. 2006).

The discrepancies in these and other initial bone marrow stem cell studies in patients with myocardial infarctions are due to significant variations in bone marrow cell processing and characterization of cells, the timing of cell transplantation and the technique of injection, the number and volume of injected cells, the ability of autologous BMCs to migrate to ischemic and infarcted tissue and propagate in patients with chronic diseases, the presence of red blood cell contamination of BMCs, adjunctive medical therapy, and different observation periods after bone

marrow cell treatment (Kissel et al. 2007). In addition, the methodology of quantification of patient's cardiac performance after bone marrow cell transplantation is not standardized among cardiovascular investigators, which makes comparison of studies from different research investigators extremely difficult.

Several major meta-analyses of cardiac cell transplantation have been published during the first decade of stem cell transplantation into patient's hearts that permit general conclusions regarding the effects of BMCs in the treatment of patients with myocardial infarctions. An analysis by Lipinski et al. (2007) included 10 studies of 698 patients that were treated with percutaneous coronary angioplasty after acute myocardial infarction and then allocated to treatment with either intracoronary autologous adult bone marrow cell therapy or standard medical therapy. The patients were followed for a mean of 6 (range 3–18) months. Seven of the 10 studies randomized the patients to either cell treatment or placebo controls. In this meta-analysis, patients who received BMCs showed statistically significant 3 % (range 1.9–4.1 %) increases in LV ejection fraction, decreases in end-systolic volume of 7.4 ml (range −12.2 to −2.7 ml), and reductions in infarct size of 5.6 % (−8.7 to −2.5 %) (Lipinski et al. 2007). Patients who received intracoronary bone marrow cell infusions had a significant decrease in recurrent myocardial infarctions but no difference in rehospitalization for heart failure during the study. Table 12.1 summarizes the studies analyzed by Lipinski and coworkers (Schaechinger et al. 2006a, b; Meyer et al. 2006; Strauer et al. 2002; Bartunek et al. 2005; Li et al. 2007; Janssens et al. 2006; Wollert et al. 2004; Kang et al. 2006; Lunde et al. 2006, 2008; Ge et al. 2006; Meluzín et al. 2006, 2008; Dill et al. 2008).

Martin-Rendon et al. (2008) performed a Cochrane Systematic Review of 13 randomized controlled trials with 14 different comparisons involving 811 patients with acute myocardial infractions from 9 different countries that compared percutaneous coronary intervention plus autologous BMCs with percutaneous coronary angioplasty plus saline or heparinized plasma. All patients were followed for 3–6 months and 3 trials followed patients for greater than 12 months. Autologous bone marrow cell therapy was found to be safe and increased LV ejection fraction by 2.99 % (range 1.26–4.72 %), reduced LV end-systolic volume by 4.74 ml (range −7.84 to −1.64 ml), and decreased myocardial infarction size by 3.51 % (range −5.91 to −1.11 %) (Martin-Rendon et al. 2008). Subgroup analysis indicated that there was a statistically significant increase in LV ejection fraction when cells were infused within 7 days following the acute myocardial infarction and when the cell dose administered was greater than 10^8 cells. However, the trials in this Cochrane Review were too small to demonstrate whether bone marrow cell therapy reduced patient mortality after myocardial infarction.

Abdel-Latif examined 999 patients in a meta-analysis of 18 randomized and non-randomized trials of BMCs (Abdel-Latif et al. 2008). Twelve trials included patients with acute myocardial infarction, and 6 studies included patients with acute myocardial infarction and ischemic cardiomyopathy. The cells used for

Table 12.1 Adult bone marrow studies in patients with acute myocardial infarction

Study	Patient (*N*)	Randomized	Days post-MI	Cell dose	Baseline LVEF (%)	LVEF change (%)	Duration/ months	Other findings
Strauer et al. (2002)	20	Cohort	8	$2.8 \pm 2.2 \times 10^7$	57 ± 8	+5	3	Increased regional but not global LVEF; decreased LVESV and infarct size
Bartunek et al. (2005)	35	Cohort	10	$12.6 + 2.22 \times 10^6$	45 ± 2.5	+7	4	Increased LV regional function, perfusion, and restenosis
Li et al. (2007)	70	Cohort	6	$7.3 \pm 7.3 \times 10^7$	50 ± 8.2	+7	6	Decreased LVESV, LV wall motion score
Janssen et al. (2006)	67	Yes	1	172×10^6	48.5 ± 7.2	+3.3	4	Decreased infarct size
Wollert et al. (2004)	60	Yes	4.8	24.6×10^8	50.0 ± 10.0	No change	6–18	Increased LVEF at 6 but not at 18 months
Kang et al. (2006)	96	Yes	4	$1–2 \times 10^9$	52.0 ± 9.9	+5.1 AMI	6	Decreased LVESV and infarction in acute MI; no change ESV and no change OMI
Lunde et al. (2006, 2008)	100	Yes	6	68×10^6	41.3 ± 11.0	No change	6–12	Increased LVEF in treated and controls; no change EDV and infarct size
Ge et al. (2006)	20	Yes	1	4×10^7	53.8 ± 9.2	+4.8	6	Increased LV regional wall perfusion by SPECT
Meluzin et al. (2006, 2008)	66	Yes	5–9	10^7–10^8	42 ± 2	+3–5	3–12	LVEF increased 3 % with 10^7 LVEF increased 5–7 % with 10^8 at 3–12 months
Schachinger et al. (2006a, b), Dill et al. (2008)	204	Yes	3–8	2.4×10^8	48.3 ± 9.2	+6–7	4–12	Increased EF when Rx >4 days post-MI and when EF <49 %; increased LV perfusion

AMI Acute myocardial infarction; *BMC* Bone marrow cell; *CPC* Circulating progenitor cell; *EF* Ejection fraction; *ESV* End-systolic volume; *G-CSF* Granulocyte colony-stimulating factor; *IC* Intracoronary injection; *ICM* Ischemic cardiomyopathy; *IM* Intramyocardial injection; *LVEF* left ventricular ejection fraction; *LV* = Left Ventricle or Left Ventricular; *OMI* Old myocardial infarction; *SPECT* Single-photon emission computer tomography
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treatment included bone marrow mononuclear cells, MSCs, or bone marrow-derived circulating blood progenitor cells. The cells were administered into the coronary arteries of patients in 15 trials and directly into the myocardium in 3 studies. Compared with control patients, BMCs significantly increased LV ejection fraction by 3.66 % (range 1.93–5.4 %), decreased LV end-systolic volume by 4.8 ml (range −8.20 to −1.41 ml), and reduced infarct size by 5.49 % (range −9.10 to 1.88 %) (Abdel-Latif et al. 2008). There were no major local or systemic complications. In this meta-analysis, there was no significant difference between groups that received less or more than 80×10^6 cells. However, injection of BMCs 5–20 days after myocardial infarction resulted in a greater than threefold reduction in myocardial infarction size and better reduction in the LV end-systolic volume compared with injection of BMCs within the first 5 days after acute myocardial infarction and/or percutaneous coronary intervention (Abdel-Latif et al. 2008). Table 12.2 summarizes the 18 studies analyzed by Abdel-Latif and coworkers (Ge et al. 2006; Assmus et al. 2006; Bartunek et al. 2006; Chen et al. 2004; Erbs et al. 2005; Ge et al. 2006; Hendrikx et al. 2006; Katritsis et al. 2005; Mocini et al. 2006; Perin et al. 2004; Ruan et al. 2005; Strauer et al. 2005).

None of the meta-analyses of bone marrow trials reported an increased incidence of cardiac arrhythmias with bone marrow cell transplantation into the heart muscle. The meta-analyses suggest that in patients with myocardial infarctions, modest decreases in infarct size and increases in left ventricular ejection fraction can be achieved with bone marrow cell therapy and that the therapy is safe.

Although the increases in cardiac LV ejection fraction with bone marrow stem cell therapy are modest, they are comparable to what has been reported with pharmacology therapy and with angioplasty in patients with myocardial infarctions and ischemic cardiomyopathies. In the Valsartan in Acute Myocardial Infarction (VALIANT) Study, treatment of patients with myocardial infarctions with the drug valsartan increased the LV ejection fraction 1.3 ± 6.7 %, while treatment with captopril increased the LV ejection fraction by 2.7 ± 7.2 %, and combined valsartan and captopril treatment increased the LV ejection fraction by 1.9 ± 7.3 % (Solomon et al. 2005). In the intravenous streptokinase in acute myocardial infarction (ISAM) trial, the LV ejection fractions in patients treated with thrombolytic streptokinase therapy averaged 56.8 ± 0.7 % versus 53.9 ± 0.7 % in control patients (The I.S.A.M. Study Group 1986). In a comparison of thrombolytic therapy with streptokinase versus acute coronary angioplasty in the treatment of acute myocardial infarction, the LV ejection fraction was 45 ± 12 % in patients treated with streptokinase and 51 ± 11 % in patients with acute coronary angioplasty (Zijlstra et al. 1993). Consequently, the meta-analyses suggest that the increases in cardiac function with stem cell therapy are comparable to pharmacologic therapy and angioplasty for the treatment of acute myocardial infarction.

Table 12.2 Bone marrow and circulating progenitor cells in coronary artery disease patients

Study	Patient (*N*)	Randomized	Time-post PCI and/or MI (days)	Cells dose	Injection route	Baseline LVEF (%)	LVEF change (%)	Duration (months)	Other findings
Assmus et al. (2006)	92	Yes	2348–2470	22 ± 10^6 CPC $205 \pm 110 \times 10^6$ BMC	IC	CPC 39 ± 10; BMC 41 ± 11	CPC: -0.4 BMC: +2.9	3	Patients with previous MI; increased LVEF in BMC but not CPC treatment
Bartunek et al. (2005, 2006)	35	Cohort	10	$12.6 \pm 2.2 \times 10^6$	IC	45 ± 2.5	+7	4	Increased LV regional function, increased perfusion, and restenosis
Chen et al. (2004)	69	Yes	18.4 ± 0.5	$8–10 \times 10^9$	IC	49 ± 9	+18	6	Increased LVEF by ventriculogram; increased perfusion; decreased ESV
Erbs et al. (2005)	26	Yes	225 ± 87	$69 \pm 14 \times 10^6$	IC	51.7 ± 3.7	+7.2	3	Patients with chronic CAD occlusion Rx with CPC; EF by MRI; infarct size decreased 16 %
Ge et al. (2006a, b)	20	Yes	1 day	$39 \pm 22 \times 10^6$	IC	53.8 ± 9.2	+4.8	6	Increased perfusion by SPECT
Hendrikx et al. (2006)	20	Yes	217 ± 162	$60 \pm 31 \times 10^6$	IM	42.9 ± 10.3	+5	4	CABG in patients with previous CAD; increased regional but not global LV function; 6/9 with induced VTach

(continued)

Table 12.2 (continued)

Study	Patient (*N*)	Randomized	Time-post PCI and/or MI (days)	Cells dose	Injection route	Baseline LVEF (%)	LVEF change (%)	Duration (months)	Other findings
Janssen et al. (2006)	67	Yes	1	172×10^6	IC	48.5 ± 7.2	+3.3	4	Decreased infarct size
Kang et al. (2006)	96	Yes	<14 AMI >14 OMI	$1–2 \times 10^9$	IC	52.0 ± 9.9	+5.1 AMI	6	G-CSF for 3 days; decreased ESV and infarct size in AMI; no change EF, ESV, and infarct size in OMI
Katritsis et al. (2005)	22	Cohort	224 ± 470	$2–4 \times 10^6$	IC	39.7 ± 9.3	+1.6	4	Increased regional but not global LV function
Lunde et al. (2006, 2008)	100	Yes	6 ± 1.3	68×10^6 (median) $54–130 \times 10^6$	IC	41.3 ± 11.0	No change	6–12	Increased LVEF in treated and controls; no change EDV and infarct size
Meyer et al. (2006)	60	Yes	4.8 ± 1.3	$24.6 \pm 9.4 \times 10^8$	IC	50 ± 10	+5.9	18 ± 6	Increased LVEF by MRI significantly at 6 but not at 18 months
Mocini et al. (2006)	36	Cohort	AMI < 6 months	$292 \pm 232 \times 10^6$	IM	46 ± 6	+5	3–12	CABG in all; troponin increased
Perin et al. (2004)	20	Cohort	ICM	$25.5 \pm 6.3 \times 10^6$	IM transendocardial	30 ± 6	+5.1	12	LVEF no change controls; increased LV perfusion and exercise

(continued)

Table 12.2 (continued)

Study	Patient (*N*)	Randomized	Time-post PCI and/or MI (days)	Cells dose	Injection route	Baseline LVEF (%)	LVEF change (%)	Duration (months)	Other findings
Ruan et al. (2005)	20	Yes	~1	NR	IC	53.5 ± 5.8	+5.8	6	Increased LV segmental contraction
Schachinger et al. (2006a), Dill et al. (2008)	204	Yes	3–8	2.4×10^8	IC	48.3 ± 9.2	+6–7	4–12	Increased EF when Rx >4 days post-MI and when EF ≤49 %; increased LV perfusion
Strauer et al. (2002)	20	Cohort	5–9	$2.8 \pm 2.2 \times 10^7$	IC	57 ± 8	+5	3	Increased regional but not global LVEF; decreased ESV and infarct size
Strauer et al. (2005)	36	Cohort	823 ± 945	9.0×10^7	IC	52 ± 9	+8	3	Decreased infarct size 30 %; increased VO_2 max
Li et al. (2007)	70	Yes	7 ± 5	$7.3 \pm 7.3 \times 10^7$	IC	50 ± 8.2	+7	6	G-CSF for 5 days; decreased LV ESV; decreased LV wall motion score

AMI Acute myocardial infarction; *BMC* Bone marrow cell; *CABG* Coronary artery bypass surgery; *CPC* Circulating progenitor cell; *EF* Ejection fraction; *ESV* End-systolic volume; *G-CSF* Granulocyte colony-stimulating factor; *IC* Intracoronary injection; *ICM* Ischemic cardiomyopathy; *IM*: Intramyocardial injection; *LVEF* left ventricular ejection fraction; *NR* Not recorded; *OMI* Old myocardial infarction; *PCI* Percutaneous coronary intervention; *Rx* Treatment; *SPECT* Single-photon emission computer tomography; *VTach* Ventricular tachycardia

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12.3 Bone Marrow Stem Cells in Ischemic Cardiomyopathy

In contrast to the large number of bone marrow cell trials in patients with acute myocardial infarctions, few clinical trials have investigated the use of stem cells in the treatment of patients with chronic ischemic cardiomyopathies. Moreover, the cardiomyopathy trials frequently often involved small numbers of patients and often did not randomize patients to bone marrow cell treatment or no cell treatment. Nevertheless, the trials demonstrated that BMCs could be safely administered to patients with ischemic cardiomyopathies.

In a randomized study of patients with ischemic cardiomyopathies who were at least 3 months of post-myocardial infarction, Assmus injected into the coronary arteries autologous BMCs or a placebo into the patients' coronary arteries (Assmus et al. 2006). At 3 months post-injection, LV ejection fraction, determined by LV ventriculograms with contrast material, increased significantly by 2.9 ± 3.6 % among patients receiving BMCs but declined by 1.2 ± 3.0 % among patients who did not receive cell infusions. Magnetic resonance imaging (MRI) of LV regional function in a subgroup of patients treated with BMCs demonstrated that the hypocontractile segments decreased from 10.1 ± 3.6 segments to 8.7 ± 3.6 segments and the normal contractile segments increased significantly from 3.8 ± 4.5 to 5.4 ± 4.6 segments.

Strauer reported in a non-randomized study the five-year follow-up of the intracoronary administration of BMCs in 191 patients with ischemic cardiomyopathies with LVEF <35 % and chronic heart failure (Strauer et al. 2010). All patients received dobutamine intravenously to augment contractility for 24 h after the coronary injection of BMCs. In 181 of these patients at 60 months, BMCs increased the LVEF by 6.2 ± 8.4 %, improved the New York Heart Association Classification from 3.2 ± 0.5 to 1.5 ± 0.5, and increased the survival in patients with heart failure compared to the group not treated with BMCs (Strauer et al. 2010).

In contrast to the studies by Assmus and Strauer, several trials have failed to confirm the beneficial effects of intracoronary delivery of BMCs in patients with ischemic cardiomyopathies. In a study of 20 patients with chronic ischemic heart disease, Ang et al. (2008) injected autologous BMCs either directly into myocardial scars or into a coronary artery graft supplying scarred myocardium in 10 patients. In this study, BMCs did not significantly increase myocardial regional contractile thickening and LV ejection fraction or decrease infarct scar volume when measured by dobutamine stress echocardiography and magnetic resonance imaging (MRI) 6 months after cell transplantation in comparison with patients treated with myocardial revascularization without cell transplantation.

Hendrikx et al. (2006) reported that autologous BMCs directly injected into the infarct scar borders in 10 patients with ischemic cardiomyopathy at the time of coronary artery bypass surgery improved regional myocardial wall function

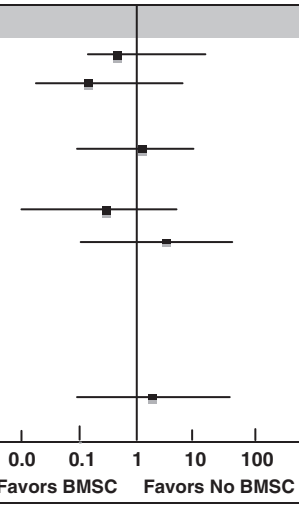
but not global contractile function when measured by MRI at 4 months after cell transplantation in comparison with 10 patients who received coronary artery bypass grafts without bone marrow stem cells. Monomorphic ventricular tachycardia was induced during electrophysiological studies in 5 patients, and polymorphic ventricular tachycardia was induced in 1 patient in in a total of ten patients who received BMCs (Hendrikx et al. 2006). Automatic implantable defibrillators were inserted in 3 patients, and 3 patients were treated with amiodarone.

The studies by Ang and Hendrikx suggest that stem cells when injected into myocardial scars in patients with cardiomyopathies are associated with little or no left ventricular hemodynamic improvement. In contrast, stem cell injection into *viable myocardium* in patients with ischemic cardiomyopathies, either at the time of cardiac surgery or with the use of the NOGA catheter navigation system during LV catheterization, appears to be associated with modest hemodynamic improvement.

The Cochrane Collaboration has published an evaluation of 23 randomized control trials that investigated the use of bone marrow stem cells in the treatment of patients with chronic ischemic heart disease and congestive heart failure (Fisher et al. 2014). Co-interventions, such as primary angioplasty, coronary bypass surgery, or administration of stem cell mobilizing agents, were included in the meta-analysis when the interventions were equally administered to stem cell-treated and control patients. The 23 studies included 659 patients treated with bone marrow-derived stem cells and 478 control patients. The quality of the evidence in the 23 studies was stated by the Cochrane Collaboration to be *low to moderate quality* (Fisher et al. 2014). Nevertheless based on the data available, the collaboration concluded that bone marrow stem cell treatment in patients with ischemic heart disease was associated with a reduction in left ventricular end-systolic volume (LVESV) (mean difference −14.64 ml, 95 % confidence intervals (CI) −20.88 to −8.39 ml in 153 patients), an increase in LV stroke volume index (mean difference 6.52, 95 % CI 1.51–11.54 in 62 patients), an improvement in LVEF (mean difference 2.62 %, 95 % CI 0.50–4.73 % in 6 studies), a reduced incidence of mortality (risk ratio 0.28, 95 % CI 0.14–0.53), and rehospitalization due to heart failure (relative risk 0.26, 95 % CI 0.07–0.94) over ≥12 months (Fisher et al. 2014) (see Table 12.3). Patients with LVEF less than 30 % or patients with symptomatic congestive heart failure benefited more from BMC treatment than patients with LVEF >30 % and/or patients without symptoms of heart failure. Of 19 trials in which adverse events were reported, adverse events due to either BMC treatment or related procedures occurred in only four individuals. No long-term adverse events were reported. The Cochrane Collaboration emphasized the low to moderate quality of the evidence and concluded that there is a need for large-scale, adequately powered studies with well-defined participant cohorts and long-term follow-up to confirm the modest beneficial effects of BMCs for patients with chronic ischemic heart disease and congestive heart failure (Fisher et al. 2014).

Table 12.3 Comparison of stem cell versus no stem cell treatment on all mortality

Study or subgroup	BMSC n/N	no BMSC n/N	Risk Ratio M-H, Fixed, 95% CI
Short-term follow-up (<12 months)			
Ang 2008	1/42	1/19	
Assmus 2006	0/52	1/23	
Erbs 2005	0/12	0/11	
Hendrikx 2006	1/11	1/12	
Honold 2012	0/22	0/10	
Hu 2011	0/31	1/28	
Kang 2006	1/16	0/16	
Losordo 2007	0/18	0/28	
Losordo 2011	0/111	0/55	
Patel 2005	0/10	0/10	
Perin 2011	0/20	0/10	
Perin 2012a	1/61	0/31	
Perin 2012b	0/10	0/10	



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12.4 Bone Marrow Cells in Non-ischemic Cardiomyopathies

Patients with non-ischemic cardiomyopathies have dilated cardiac ventricles, often with non-homogenous LV tissue perfusion, which makes targeted administration of stem cells through coronary arteries challenging. However, these patients often have greater numbers of progenitor cells in the systemic circulation with better functional capacity than patients with ischemic cardiomyopathies, which suggests that bone marrow cell therapy might be beneficial in patients with non-ischemic cardiomyopathies (Vrtovec et al. 2011). See Table 12.4.

Fifty-five patients with non-ischemic cardiomyopathies in an open-labeled study received intracoronary bone marrow $CD34^+$ endothelial progenitor cells or placebo. At 5 years after treatment, stem cell therapy was associated with an increase in left ventricular ejection fraction (from 24.3 ± 6.5 to 30.0 ± 5.1 %), an increase in 6-min walk distance (from 344 ± 90 to 477 ± 130 m), and a decrease in pulmonary congestion as determined by N-terminal B-type natriuretic peptide (from 2322 ± 1234 to 1011 ± 893 pg/ml) (Vrtovec et al. 2011). The improvement in the LVEF was most significant in patients with the greatest attraction of injected $CD34^+$ cells to the myocardium. In this study, patient mortality at five years was 14 % in the stem cell treatment group and 35 % in the non-stem cell-treated patients (Vrtovec et al. 2011).

In the Autologous Bone Marrow Cells in Dilated Cardiomyopathy (ABCD) trial of 24 patients with non-ischemic cardiomyopathy who received intracoronary BMCs, the LVEF increased by an average of 5.9 % (22.5 ± 8.3 % to 28.4 ± 11.8)

Table 12.4 Prospective randomized trials of stem cell therapy in non-ischemic heart failure

Study	Patient (*N*)	Randomized	Cell dose	Injection type	Baseline LVEF	LVEF increase	Duration (months)
Bocchi et al. (2010)	22	No	GSF stimulation BMC	IC	21	8.8	15
Fischer-Rasokat et al. (2009)	33	No	$259 \pm 135 \times 10^6$ BMC	IC	30	3.4	3
Seth et al. (2010)	85	Yes	$168 \pm 96 \times 10^6$ BMC	IC	23	5.9	36
Vrtovec et al. (2011)	55	Yes	$123 \pm 23 \times 10^6$ BMC	IC	26	4.6	12
Vrtovec et al. (2013)	55	Yes	$113 \pm 26 \times 10^6$ BMC	IC	24	5.6	60

LVEF Left ventricular ejection fraction; *BMC* Bone marrow cells; *IC* Intracoronary injection
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which was associated with an improvement in New York Heart Association functional class III and IV patients (Seth et al. 2010). The three-year follow-up showed persistent improvement in LVEF due to decreases in LVESV. However, this hemodynamic improvement was not associated with significant improvement in patient survival because 12 (24.4 %) patients died in the treated group and 14 (30 %) patients died in the control group (Seth et al. 2010).

In a third study of non-ischemic cardiomyopathy patients, Fischer-Roasokat infused BMCs into the coronary arteries of 33 patients and analyzed patient hemodynamics at 3 months (Fischer-Roasokat et al. 2009). In this study, the LVEF increased by 3.2 % from 30.2 ± 10.9 to 33.4 ± 11.5 %, and the NT-proBNP decreased at one year from 1610 ± 993 to 1473 ± 1147 pg/ml. The increase of regional contractile function and LVEF was directly related to the functionality of the infused cells as measured by their colony-forming capacity (Fischer-Rasokat et al. 2009). Based on this study and the previous two studies, the number of BMCs retained in the myocardium and the functionality of the BMCs appear to be important factors in the response of patients with cardiomyopathy to bone marrow cell therapy.

The studies of bone marrow stem cells in patients with ischemic and non-ischemic cardiomyopathies are promising. However, the effects of BMCs in patients with cardiomyopathies require much larger numbers of patients in clinical trials for longer periods of time in order to permit definitive conclusions about the effects of stem cells in these patients. The amount of hibernating myocardium in patients with ischemic cardiomyopathies varies greatly, and patients with non-ischemic cardiomyopathies frequently have myocardial fibrosis and non-homogenous myocardial perfusion. These confounding factors contribute to the heterogeneity of the treatment responses in the different studies. In addition, the type of cells injected (adult bone marrow mononuclear cells, $CD34^+$ cells, or

mesenchymal cells), the location of injection (viable LV myocardial tissue or scar), the methods of measurement of LV function, and the duration of patient follow-up should be standardized in order to permit comparisons of different studies and ultimately permit reasonable conclusions regarding the amount of benefit of bone marrow cell therapy in patients with ischemic and non-ischemic cardiomyopathies.

12.5 The Second Decade: TIME Trials of Bone Marrow Stem Cells in Cardiac Repair

The initial ten-year experience with stem cells, primarily from bone marrow aspirates, suggested that patients with myocardial infarctions who received autologous unfractionated bone marrow mononuclear cells showed significant 2–3 % (range 1.9–5.4 %) increases in LVEF, decreases in LVESV of 4.8 ml (range −1.4 to −8.2 ml), and reductions in infarct size of approximately 5 % (−1.9 to −9.1 %) without experiencing significant side effects (Henning 2011, 2012, 2013). However, many of the initial bone marrow cell studies consisted of small numbers of patients and not all the studies randomized patients to treatment with bone marrow mononuclear cells or placebo.

Despite promising but modest results from the initial studies, major questions have persisted in the treatment of patients with acute myocardial infarctions with bone marrow mononuclear cells or other cells for heart repair. What is the optimal cell for treatment of patients with myocardial infarction? When is the optimal time to inject cells in patients with myocardial infarctions? What is the viability of the stem cells prior to injection into patients? What is the best technique to monitor cardiac patients after stem cell treatment? The LateTIME, the TIME, and the Swiss Myocardial Infarction trials were multicenter trials that addressed the questions whether unfractionated bone marrow mononuclear cells and standard of care limit myocardial damage in comparison with patients treated with standard of care and what is the optimal time for cell administration after acute myocardial infarctions.

12.6 LateTIME Trial: Transplantation in Myocardial Infarction Evaluation 2–3 Weeks Following Acute Myocardial Infarction (AMI)

The LateTIME trial was a randomized, double-blind, placebo-controlled trial designed to determine whether unfractionated bone marrow mononuclear cells administered to patients 2–3 weeks after AMI would be safe and effective in limiting infarct size and improving LV function (Traverse et al. 2011). All patients were successfully treated initially with primary percutaneous coronary angioplasty (PTCA) within a median time of 4 h after the onset of chest pain. More than 90 % of the patients had anterior wall myocardial infarctions.

All patients underwent bone marrow aspirations. The bone marrow aspirate was processed at each site with a closed, automated cell processing system (Sepax, Biosafe SA) to ensure a uniform cellular product for administration. The BMCs contained 2.6 % $CD34^+$ and 1.2 % $CD133^+$ hematopoietic cells, and the viability of the cells was >70 %. Fifty-eight patients were given 150×10^6 autologous bone marrow mononuclear cells into the infarct-related coronary artery, and 20 patients were given 5 % human serum albumin plus 100 μl of autologous blood as a placebo, 2–3 weeks after acute myocardial infarction (Traverse et al. 2011). All patients received heparin during the procedure as well as aspirin, clopidogrel, and American Heart Association guideline recommended post-AMI medications.

Infarct volume and global and regional LV function were measured by MRI with gadolinium prior to each intracoronary injection and at 6 months after injection. The LVEFs prior to infusion of cells or placebo averaged 48.7 % in the BMC group and 45.3 % in the placebo group. The changes between baseline and 6 months in BMC group for infarct volume, LVEF, wall motion in the infarct zone, and wall motion in the border zone of the infarction were not statistically different from the placebo group (Traverse et al. 2011). No significant improvement was observed in the recovery of LV function in the group of AMI patients with the most depressed LVEF at baseline. However, the BMC treatment group had fewer clinical adverse events than the placebo group, and the bone marrow cell infusions were felt to be safe.

The LateTIME trial investigators concluded that among patients with AMI and LV dysfunction following reperfusion with PCI, intracoronary infusion of autologous unfractionated BMCs 2–3 weeks after PCI did not improve global or regional LV function at 6 months (Traverse et al. 2011).

12.7 TIME Trial: Transplantation in Myocardial Infarction Evaluation

In 2006, the investigators in the REPAIR-AMI trial reported that delivery of BMCs to patients 5–7 days after AMI resulted in a 5.1 % absolute increase in LV ejection fraction (Schaechinger et al. 2006b). This increase in LV ejection fraction contrasted with patients treated 3–4 days after AMI in which there was no significant increase in LVEF in comparison with placebo-treated patients (Schaechinger et al. 2006b). Based on the REPAIR-AMI trial, additional studies were recommended. The TIME trial was a double-blind, placebo-controlled trial that investigated the intracoronary administration of autologous bone marrow mononuclear cells or placebo in patients 3 or 7 days after an acute myocardial infarction (Traverse et al. 2012). All patients had successful coronary reperfusion with coronary angioplasty within a median time of 3–4 h after the onset of ischemic symptoms. More than 81 % of the patients had anterior wall infarctions.

All patients had bone marrow aspirations. The mean time from PCI to bone marrow aspiration and cell processing was 3.3 days in the 3 day and 7.4 days in the 7 day group. Bone marrow mononuclear cells were isolated in each center with the Sepax system (Biosafe), and the cells or placebo was infused within 12 h of bone marrow aspiration and cell processing. The BMCs contained 2.3 % $CD34^+$ and 1.1 % $CD34^+$ plus $CD131^+$ hematopoietic cells. All patients had baseline cardiac MRIs with gadolinium at day 3 or at day 7 after AMI and at 6 months after the AMI (Traverse et al. 2012).

Forty-three patients received unfractionated BMCs on day 3, and 36 patients received unfractionated BMCs on day 7 after AMI. Each patient received approximately 147×10^6 bone marrow mononuclear cells within 12 h of aspiration and cell processing. Forty-one patients received a placebo. All patients received heparin during the procedure as well as aspirin and clopidogrel.

The differences between the BMC treatment and the placebo treatments in the 3 day group and in the 7 day group were not significant (Traverse et al. 2012). When both BMC groups were combined ($n = 75$) to include patients with MRI measurements at baseline and at 6 months and compared with the combined placebo group ($n = 37$), there was no significant increase in the LVEF for the BMC group in comparison with the placebo group. Moreover, there was no significant difference between the changes in regional wall motion in the infarct zone and the border zone between BMC and placebo groups. Infarct volumes uniformly decreased in both groups, but the differences were not statistically significant. Major coronary adverse events were rare among all treatment groups.

The TIME trial investigators concluded that among patients with ST segment elevation myocardial infarction treated with primary PCI, the administration of intracoronary autologous unfractionated BMCs at either day 3 or day 7 after AMI had no significant effect on recovery of global or regional LV function compared with placebo (Traverse et al. 2012).

12.8 Swiss Multicenter Intracoronary Stem Cell Study in Acute Myocardial Infarction Trial (Swiss AMI Trial)

The Swiss Multicenter Intracoronary Stem Cell Study in Acute Myocardial Infarction trial randomized patients with AMIs with LVEF <45 % by ventriculography or echocardiography, who had been successfully treated with PCI of the infarct-related artery within a median of 5 h of onset of chest pain, to either the intracoronary administration of 140–160 million autologous bone marrow mononuclear cells at a median of 6 days after AMI (early group $n = 58$) or at a median of 24 days after AMI (late group, $n = 49$) or to a placebo group ($n = 60$) (Sürder et al. 2013). Ninety-two percent of the patients had anterior wall infarctions. Bone marrow aspirates were performed only in patients assigned to the BMC treatment. Each 10 ml aspirate was treated with 1000 IU heparin to prevent clot formation. The bone marrow mononuclear cell fraction was isolated by density gradient

centrifugation at a centralized processing facility and contained 1–1.3 % $CD34^+$ hematopoietic cells. The median percentage of mononuclear cells that exhibit migration capacity was only 29 % (Sürder et al. 2013).

Cardiac MRI with gadolinium was performed on patients at baseline prior to infusion and at 4 months after the injection of BMCs into the infarct-related coronary artery and was compared with MRIs of control patients treated with best medical care at the same times.

At 4 months after coronary infusion, there were no significant differences in infarct scar size or LV myocardial wall thickening in patients treated with BMCs at either 5–7 days or 3–4 weeks after AMI in comparison with control patients. Moreover, LV function did not significantly improve at 4 months after the intracoronary infusion of autologous BMCs in either the early or late treated groups in comparison with the placebo group. In all cell and placebo treatment groups, LV scar, determined by late gadolinium enhancement on MRI, decreased by more than 10 g with a 4–5 % decrease in the ratio of myocardial scar to myocardial mass. There were no significant differences in adverse events between BMC-treated and control patients (Sürder et al. 2013).

12.9 Critique of LateTIME, TIME, and Swiss Bone Marrow Cell Trials

The primary endpoints of the LateTIME, TIME, and Swiss Bone Marrow Cell trials were not met, and the functional benefit of autologous unfractionated bone marrow mononuclear cells remains in doubt. Nevertheless, these trials provide insights into stem cell trial designs and stem cell functions in patients with AMIs.

Patients with AMIs in the LateTIME, TIME, and Swiss Multicenter trials were treated with percutaneous coronary angioplasty within a median of approximately 4–5 h of the onset of chest pain. Thereafter, the patients were treated with American and European Heart Association guided best medical therapy. Consequently, myocardial infarction sizes and the extent of LV remodeling in the trial patients were significantly limited, and the differences between BMC-treated patients and placebo-treated patients were small. Although the initial qualifying LVEFs by echocardiography after PCI in the LateTIME and TIME trials patients were <45 %, the LVEFs by MRI at the time of BMC injection were larger than 45 %. BMCs are much less effective in patients with small myocardial infarctions with near normal LVEFs. Moreover, placebo-treated patients continue to improve with best medical therapy after myocardial infarctions as exemplified by the control patients in the BOOST trial in which the LVEFs continued to improve and equaled or exceeded the increases in the LVEFs in the BMC-treated patients at 18 months after AMI (Meyer et al. 2006). In addition, the Valsartan in Acute Myocardial infarction trial and trials of neurohormonal blockade of patients with acute myocardial infarctions have demonstrated that optimal medical therapy of patients with AMIs can increase LVEF by a mean of 2.7 % points at 20 months

(Solomon et al. 2005; Henning 2011). Consequently, much larger numbers of patients will be required in clinical trials to demonstrate statistically significant differences between BMC-treated patients and placebo-treated patients who receive PCI early after the onset of AMI and guideline directed optimal medical therapy. The BAMI trial (the effect of intracoronary reinfusion of bone marrow-derived mononuclear cells on all-cause mortality in acute myocardial infarction) is recruiting 3000 patients with LVEFs <45 % within 7 days of AMIs, who have undergone successful coronary reperfusion therapy, for randomization into treatment with either intracoronary autologous unfractionated bone marrow mononuclear cells or placebo (Mathur 2013). Perhaps, the BAMI trial will provide a definitive answer to the question not only whether autologous unfractionated BMCs can significantly decrease patient mortality due to myocardial infarction but also substantially reduce infarct size and improve LVEF in comparison with patients treated with best medical therapy over three years.

The lack of differences between bone marrow cell-treated patients and placebo-treated patients with AMIs in the LateTIME, the TIME, and the Swiss Multicenter trials may be due to important factors other than prompt coronary angioplasty after AMI and optimal medical therapy in these trials. Several important factors are discussed in the following sections.

12.9.1 Heterogeneous Bone Marrow Cell Populations

Unfractionated bone marrow mononuclear cells are a heterogeneous group of cells that contain less than 3 % $CD34^+$ and 1 % $CD34^+/CD133^+$ hematopoietic progenitor cells and <1 % $CD105^+$ MSCs when marrow cells are separated by Ficoll density gradient-based separation. However, the bone marrow aspirates in the LateTIME and TIME trials were separated by an automated cell process system (Sepax, Biosafe), which recovered only 23.6 % of the total nucleated cells (Richman et al. 2012). Consequently, the bone marrow mononuclear cells delivered in the LateTIME and TIME trials may have contained smaller numbers of $CD34^+$ and $CD105^+$ cells. In addition, 150–160 $\times$ 10^6 unfractionated BMCs may not be the most optimal dose of BMCs for stem cell treatment of patients with AMI. In addition, bone marrow mononuclear cells from patients with advanced age and patients with chronic diseases, such as ischemic heart disease or diabetes mellitus, are often functionally impaired, propagate poorly, and have a shortened life span (Kissel et al. 2007; Fadini et al. 2010; Orlandi et al. 2010). In meta-analyses of stem cell trials of patients with myocardial infractions or ischemic cardiomyopathies, bone marrow mononuclear cells produce only a modest increase in the LVEF of approximately 2–3 % (Henning 2011, 2012). Consequently, despite well-conducted clinical trials, autologous unfractionated BMCs have a small therapeutic effect and may not be the most optimal cells for the treatment of patients with AMIs or ischemic cardiomyopathies.

12.9.2 Red Blood Cell Contamination of Stem Cells

Red blood cell contamination of bone marrow mononuclear cells can significantly decrease the migration ability and the efficacy of BMCs. Large numbers of red blood cells in the cell preparations cause reduced BMC viability and decreased colony-forming unit capacity and are associated with reduced recovery of LVEF in patients with myocardial infarctions (Assmus et al. 2010). In patients in the REPAIR-AMI trial, univariate and multivariate analysis demonstrated that red blood cell contamination of the BMCs prior to infusion into patients with myocardial infarctions independently predicted reduced recovery of LVEF (Schaechinger et al. 2006b). Moreover, the addition of red blood cells to BMCs dose-dependently decreased neovascularization in ischemic hind-limbs of research animals compared to treatment with BMCs without red blood cells (Assmus et al. 2010). The mechanism by which red blood cells interfere with bone marrow cell propagation, migration, and neovascularization involves a dose-dependent reduction of BMC mitochondrial membrane potential and a decrease in BMC mitochondrial adenosine triphosphate (ATP) production (Assmus et al. 2010). As a consequence, mitochondrial metabolism and function, stem cell self-renewal, and differentiation are decreased.

12.9.3 Heparin Decreases Stem Cell Migration

Heparin is another factor that can impact on the efficacy of BMCs in patients with AMIs. Heparin in a dose-dependent manner can inhibit stromal cell-derived factor 1 (SDF-1) induced BMC migration (Seeger et al. 2012; Heeschen et al. 2004; Murphy et al. 2007). In this regard, homing of BMCs to areas of myocardial ischemia is primarily guided by SDF-1 and its receptor termed chemokine receptor 4 (CXCR4). Heparin can bind to SDF-1 and CXCR4 and thereby block CXCR4 signaling (Seeger et al. 2012). Incubation of BMCs with 20 U/ml of heparin for 30 min abrogates SDF-1 BMC migration by 84 % in vitro and significantly reduces the homing of injected BMCs to injured and infarcted myocardium by 50 % in research animals (Seeger et al. 2012). Decreased migratory capacity of BMCs also correlates with reduced neovascularization and functional capacity in research animals with limb ischemia (Heeschen et al. 2004). The minimal dose of heparin that inhibits SDF-1-induced migration of BMC is 0.05 U/ml which is significantly less than the heparin dose used in several large trials of BMCs in AMI (Seeger et al. 2012). Heparin also interferes with activation of the cell survival factor Akt (Protein Kinase B) by SDF-1 and CXCR4 and in this manner interferes with cell survival and growth. In addition, heparin decreases the levels of vascular endothelial growth factor and in this manner limits neovascularization (Seeger et al. 2012). In contrast, the thrombin inhibitor bivalirudin does not appear to interfere with BMC homing or SDF-1/CXCR4 signaling and does not decrease vascular endothelial growth factor.

Consequently, not only the BMC isolation protocol but also the use of anticoagulants such as heparin can have major impact on the functional activity of BMCs (Seeger et al. 2007, 2012). The assessment of bone marrow cell number and viability by Trypan Blue staining does not accurately reflect the functional capacity of BMCs or other stem cells when injected into patients with AMIs. Colony-forming unit capacity is a better measure of stem cell viability than Trypan Blue staining.

12.9.4 Stem Cell Expulsion from Myocardium

An important factor that impacts on the efficacy of stem cells in patients with myocardial infarctions and ischemic cardiomyopathies is the time the stem cells actually reside in the myocardium. The majority (90–97 %) of unfractionated BMCs injected directly into the myocardium or into the coronary arteries leave the myocardium in less than 2 h (Hofmann et al. 2005; Hou et al. 2005). Most of the cells are ejected out of the myocardium through the injection sites or through the coronary veins and lymphatics into the right heart due to the massaging action of the contracting myocardium. The cells are ultimately lodged in the lungs, liver, spleen, and kidneys. In addition, approximately 12 % of cells are retained in the catheter delivery system after injection (Hou et al. 2005). With the intravenous injection of bone marrow or other cells for cardiac repair, the majority of the cells become entrapped in the lungs. Consequently, fourfold or greater numbers of stem cells are required above that required for intramyocardial or intracoronary injection for repair of myocardial infarctions (Henning 2011).

12.10 Unfractionated Bone Marrow Stem Cells: Quo Vadas? (“Whither Goest Thou?”)

A meta-analysis published in 2014 concluded after reviewing 22 randomized control trials between 2002 and 2013 of unfractionated bone marrow-derived mononuclear cell therapy in patients with acute myocardial infarction that BMC therapy is safe but does not significantly enhance cardiac function based on MRI-derived parameters and does not improve 6-month patient outcome (de Jong et al. 2014). The results of the BAMI trial, which examines the effects of BMC on patient mortality over 3 years, will be important in determining the future of autologous, unfractionated bone marrow mononuclear cells in the treatment of patients with AMIs (Mathur 2013). In the interim, MSCs, adipose-derived stem cells (ADSCs) (which include MSCs), and cardiac stem cells are being investigated for cardiac repair in patients with myocardial infarctions and cardiomyopathies.

12.11 Mesenchymal Stem Cells in Cardiac Repair

In 1970, Friedenstein demonstrated that bone marrow contains not only hematopoietic stem cells but also a small population of MSCs which are also known as stromal cells (Friedenstein et al. 1968). These MSCs support the hematopoietic stem cells and the development of hematopoietic lineages but also can differentiate in vitro into osteoblasts, chondrocytes, adipocytes, and a myocyte phenotype.

The International Society for Cell Therapy has published specific criteria for identifying MSCs that include (1) the expression of cell surface proteins CD73, CD90, and CD105 in the absence of surface proteins such as CD34, CD45, HLA-DR, CD14, CD11b, CD79a, or CD19 when cells are analyzed by fluorescence-activated cell sorting; (2) cell adherence to plastic culture dishes during standard cell culture conditions; and (3) a cell capacity for differentiation in vitro into osteoblasts, adipocytes, and chondroblasts (Dominici et al. 2006).

Human MSCs express modest levels of major histocompatibility complex class I human leukocyte antigens (HLA), lack major histocompatibility complex class II expression, and do not express co-stimulatory molecules B7 and CD40 ligand (Williams and Hare 2011; Majumdar et al. 2003). Consequently, MSCs do not cause T-cell proliferation in mixed lymphocyte cultures.

Autologous MSCs have been examined in patients with acute myocardial infarctions and ischemic cardiomyopathies and do not cause cardiac arrhythmias or significant patient side effects. In this regard, 69 patients who underwent primary percutaneous coronary angioplasty within 8 ± 3.7 h after onset of acute myocardial infarction were randomized to autologous MSCs or saline 3 weeks after angioplasty. In the 34 MSC-treated patients, LVEF increased from 49 ± 9 to 67 ± 3 % at 6 months in comparison with the 35 patients in the control group in which the LVEF increased from 48 ± 10 to 54 ± 5 % (Chen et al. 2004).

In a separate study, 20 patients were treated with intravenous allogeneic MSCs from a single healthy donor 1–10 days post-myocardial infarction and were compared with 14 patients treated with placebo (Hare et al. 2009). Although the baseline LVEF was similar in both groups (47.3 ± 3.3 % vs. 45.2 ± 3.4 %), the MSC-treated patient showed a 5.2 ± 1.9 % increase in MRI-determined LVEF relative to baseline after 12 months, whereas the control patients showed an increase of only 1.8 ± 1 %.

To date, three studies have examined MSCs in patients with ischemic cardiomyopathies. In 2007, eight patients with chronic heart failure were reported that received MSCs and endothelial progenitor cells at the time of revascularization with either coronary artery bypass grafting or percutaneous coronary angioplasty (Mohyeddin-Bonab et al. 2007). The LVEF increased significantly from 38.7 ± 13 % at baseline to 48.8 ± 6.4 % at 18 months in the cell treated patients in comparison with a control group in which the LVEF only slightly increased from 41.9 ± 8.4 to 42.5 ± 8.9 %. Infarct scar size, measured by Thalium scan, decreased from 11 segments to 7.75 segments in the cell treatment group and also decreased slightly but significantly from 10.88 to 9.75 segments in the control group (Mohyeddin-Bonab et al. 2007).

In the POSEIDON trial, either autologous or allogeneic MSCs were administered to 30 patients with ischemic cardiomyopathies (Hare et al. 2012). This was an open-label study without a control group. In this study, the LVEF did not increase, but allogeneic and autologous MSCs reduced LV scar by 31.6 % (C.I. −49.24 to −13.99 %) and 34.9 % (C.I. 48.18–21.68 %), respectively, and decreased LV remodeling. The 6-min walk test increased significantly by 65.8 m at 12 months in the autologous MSC group but also increased slightly but not significantly in the allogeneic MSC group by 19.7 m (Hare et al. 2012).

In the C-CURE trial, 32 patients with ischemic cardiomyopathies were treated with MSCs that were injected with the aid of the NOGA endocardial catheter mapping system into viable LV myocardium (Bartunek et al. 2013). Before injection, the MSCs were treated with a cardiogenic cocktail (transforming growth factor, bone morphogenetic protein, activin, fibroblast growth factor, cardiotrophin, thrombin, diaminopyrimidine) that triggered expression of cardiac transcription factors. In the treated patients, LVEF, measured by echocardiography, improved with MSC therapy from 27.5 ± 1.0 to 34.5 ± 1.1 % versus patients treated with standard care in which the LVEF did not significantly change at 6 months after treatment. Cell therapy also improved the 6-min walk test in MSC-treated patients by 62 ± 18 m versus a decrease in the 6-min walk test of 15 ± 20 m in the standard care group (Bartunek et al. 2013).

The studies of MSCs in patients with myocardial infarctions and ischemic cardiomyopathies are promising, but clinical conclusions are limited due to the small numbers of studies, the small numbers of patients in each of the studies, and the relatively short-term follow-up of patients that received MSCs. Nevertheless, the fact that these cells can improve quality of life as evidence by an increase in 6-min walk test in patients with ischemic cardiomyopathy is encouraging and suggests that the otherwise dire prognosis of these patients with cardiomyopathies can be improved at least for the short term. The Safety Study of Allogeneic Mesenchymal Precursor Cell Infusion in MyoCardial Infarction (AMICI) in www.ClinicalTrials.gov and the Safety and Efficacy of Adipose-Derived Regenerative Cells (ADRCs) Delivered Via the Intracoronary Route in the Treatment of Patients With ST-elevation Acute Myocardial Infarction (ADVANCE) in www.ClinicalTrials.gov are investigating the effects of mesenchymal and mesenchymal-like cells on cardiac repair in more than 200 patients with myocardial infarctions.

12.12 Adipose-Derived Stem Cells (ADSCs)

Adipose tissue is mesodermally derived tissue that consists of adipocytes of various sizes. The adipocytes are interspersed in stroma composed of endothelial cells, preadipocytes, smooth muscle cells, fibroblasts, leukocytes, and macrophages, which are collectively termed the adipose stromal cell fraction (Lin et al. 2010). The frequency of adipose stem cells in adult human adipose tissue ranges from 1:30 to 1:100 cells per total nucleated cells and is dependent on the location of

the adipose tissue and the age of the patient. ADSCs contain mesenchymal surface markers such as CD 105, CD90, CD73, CD51, CD49e, and CD29 and also express surface markers CD166, CD13, and CD44 but do not express hematopoietic cell markers. Microarray analysis and real-time polymerase chain reaction (PCR) determinations of ADSCs and BM-MSCs demonstrate that these cells exhibit virtually identical transcriptional profiles for the stem-related genes OCT4, Nanog, and Sox2 (Witkowska-Zimny and Walenko 2011).

Less than 1 % of ADSCs expresses HLA-DR protein, which makes these cells suitable for allogeneic transplantation without significant risk of rejection (Russo et al. 2014). ADSCs can suppress peripheral blood mononuclear cell proliferation in vitro and shift lymphocyte Th1 cytotoxic responses to lymphocyte Th2 anti-inflammatory responses. This anti-inflammatory ADSC effect exceeds that of bone marrow MSCs (Witkowska-Zimny and Walenko 2011; Russo et al. 2014).

Adipose cells can be readily obtained by liposuction of a patient and immediately prepared for autologous transplantation without the need for adipose cell culture and expansion. Human ADSC has significantly reduced myocardial infarction size in 8/9 animal studies, substantially improved LVEF, determined by echocardiography, in 9/10 animal studies, and significantly increased the number of blood vessels in the infarcted area in 10/10 animal studies (Naaijkens et al. 2014). The predominant mechanism of action of ADSC in AMI is through the paracrine release of antiapoptotic, immunomodulatory, and proangiogenic factors (Yang et al. 2013). These factors are thought to salvage injured cardiomyocytes and stimulate neoangiogenesis in the infarct border zone, thereby limiting scar size and left ventricular remodeling after myocardial infarction. Consequently, ADSCs currently represent an alternative to bone marrow MSCs for the treatment of patients with myocardial infarctions.

Based on the promising results of ADSCs in animal studies, several studies of adipose stem cells have been performed in patients with acute myocardial infarction and ischemic cardiomyopathy.

The APOLLO trial was a randomized, double-blind, placebo-controlled clinical trial of ADSCs, in concentrations of 17.4 ± 4.1 million cells, administered into the coronary arteries of 10 patients with acute ST-elevation myocardial infarction (Houtgraaf et al. 2012). In the ADSC-treated patients, the percentage of infarcted left ventricle was significantly reduced from 31.6 ± 5.3 to 15.3 ± 2.6 % at six months after infarction in contrast to no change in infarct size in the placebo-treated AMI patients (24.7 ± 9.2 % vs. 24.7 ± 4.1). This decrease in infarct size in ADSC-treated patients was associated with a significant decrease of the LV perfusion defect in ADSC-treated patients from 16.9 ± 2.1 to 10.9 ± 2.4 % at six-month follow-up. However, the LVEF in the ADSC-treated patients, measured by single-photon emission computerized tomography (SPECT), did not significantly differ from the placebo group. This study suggests that autologous ADSCs can be safely obtained by liposuction and administered via intracoronary infusion to patients with AMI and can produce some reduction of cardiac damage (Houtgraaf et al. 2012).

In the PRECISE trial, patients with ischemic cardiomyopathies with Canadian Cardiovascular society Class II to IV angina and/or New York Heart Association Class II to II heart failure, not amendable to coronary revascularization, were randomized to receive either the stromal vascular fraction (SVF) of autologous adipose tissue or placebo (Perin et al. 2014). Twenty-one patients received the SVF and 6 patients received placebo. The SVF or placebo was injected transendocardially into ischemic areas, which was determined by NOGA unipolar catheter LV voltages > 6.9 mV. In the SVF-treated patients, the total LV mass, determined by cardiac MRI, increased from baseline to 6 months from 128.1 ± 26 to 149.5 ± 32 g but did not significantly change in the control group (from 144.6 ± 52.7 to 152.6 ± 59.6 g). The absolute mass of the LV infarctions did not significantly change (35.1 + 20.4 to 34 + 16.5 g) in the treated patients but increased in the control group over 6 months from 29.6 ± 15.9 to 39.0 ± 15.4 g. The global LV wall motion score index improved slightly but significantly in the SVF-treated patients but did not significantly change in the control patients (Perin et al. 2014). The exercise tolerance, measured by metabolic equivalent (MET) values, was preserved over time in the ADSC-treated group (4.9 ± 0.8 to 4.9 ± 1.4) but decreased significantly (5.3 ± 2.5 to 4.2 ± 2.1) in the control group at 18 months (Perin et al. 2014).

Other trials that are examining ADSC include the ATHENA trial (Taylor and Dabkowski 2012) and the MyStromalCell trial (Qayyum et al. 2012), which are two prospective, randomized, double-blind, placebo-controlled Phase II studies that are investigating the effects of ADSCs in patients with chronic ischemic heart disease.

Although cellular therapy using adipose tissue in patients with acute myocardial infarctions and ischemic cardiomyopathies is promising, we currently do not know the most optimal adipose cell type (mesenchymal cell vs. SVF), the most optimal cell number to inject, the most optimal time to inject these cells in patients with myocardial infarction, or the most optimal technique (intracoronary or transendocardial) with which to inject these cells. Large patient randomized studies of ADSCs and SVF are necessary to answer these important questions. In addition, MSCs from adipose tissue should be compared with MSCs from bone marrow MSCs for the treatment of patients with acute myocardial infarction and ischemic cardiomyopathies.

12.13 Cardiac Stem/Progenitor Cells

Cardiovascular investigators have sought alternative stem cells to unfractionated bone marrow stem cells and adipose stem cells for cardiac repair in patients with ischemic heart disease. Cardiac stem cells are multipotent stem cells that are present in niches in the heart. These cells contribute to the physiological turnover of myocytes and vascular endothelial cells in the heart. The number of cardiac stem cells in the heart is small with an estimated one cardiac stem cell per 10,000

cardiac myocytes (Beltrami et al. 2003). Consequently, endogenous cardiac stem cells are not normally able to reverse heart damage due to myocardial infarctions. The physiologic turnover of myocytes by cardiac stem cells in the heart occurs at rates of approximately 1 % per year and is dependent on the age, sex, and the health of the individual (Kikuchi and Poss 2012).

Autologous cardiac stem cells have been isolated, cultured, propagated, and delivered to patients with injured and infarcted myocardium. Two major cardiac stem cell types have been investigated in the SCIPIO and CADUCEUS clinical trials in patients with ischemic cardiomyopathies: c-kit$^+$ lineage negative cardiac stem cells isolated from right atrial appendages and cardiosphere-derived cells (CDCs) from cardiospheres grown from right ventricular muscle biopsies.

12.14 C-Kit$^+$ Stem Cells

C-kit$^+$ cardiac stem cells have the capacity for self-renewal, clonogenicity, and multi-potency (Bearzi et al. 2007; Anversa et al. 2013). These stem cells can express the cardiac transcription factors GATA-4, Nkx2.5, and MEF2 and are reported to differentiate into myogenic, vascular endothelial, and smooth muscles cells (Bearzi et al. 2007; Kajstura et al. 2010). C-kit is a cell surface receptor for stem cell factor, and stem cell factor can chemoattract these stem cells to ischemic and injured myocardium. In research animals with myocardial infarctions, c-kit$^+$ cardiac stem cells are reported to form new myocytes in the heart (Bearzi et al. 2007). Consequently, these cells represent an important area of investigation for cardiac repair.

Autologous c-kit cardiac stem cells from right atrial appendages have recently been investigated for the treatment of patients with ischemic cardiomyopathies in the open-labeled Cardiac Stem Cell Infusion in Patients with Ischemic Cardiomyopathy (SCIPIO) trial (Bolli et al. 2011; Chugh et al. 2012; Bolli 2012). In this trial, the right atrial appendage was removed from patients during cardiopulmonary bypass for coronary artery surgery. C-kit positive stem cells were then isolated from each patient's atrial appendage and propagated in cell culture. Four months later, approximately one million autologous cardiac stem cells were injected back into each patient's saphenous vein grafts and coronary arteries supplying the infarcted myocardium.

In the SCIPIO trial, the LVEF, which was measured by three-dimensional echocardiography and also by MRI with gadolinium in patients who received cardiac stem cells, increased by 11.9 ± 2.7 % absolute units in 12 patients at 2 years after treatment (Bolli 2012). Left ventricular infarct scar in 6 patients, determined by cardiac MRI, decreased by 15.7 ± 4.7 g at 2 years. This decrease in myocardial scar was associated with an increase in viable muscle of 17.9 ± 12.1 g ($N = 6$) at 2 years (Bolli 2012). New York Heart Association Functional Class score improved in these patients by 0.9 ± 0.2 at 2 years ($N = 13$). In this study, c-kit cardiac stem cells were postulated to chemoattract the patients' native stem cells

to areas of myocardial injury and also to transdifferentiate to myocytes for cardiac repair. A Phase 2 trial of safety and efficacy of c-kit cardiac stem cells in a larger group of patients with cardiomyopathy is currently being planned.

12.15 Cardiosphere-Derived Cells (CDCs)

Percutaneous endomyocardial biopsy specimens of the right ventricular septal wall in patients, when grown in culture, can yield spherical multicellular clusters termed cardiospheres. Cardiospheres are a mixture of stromal, mesenchymal, and hematopoietic progenitor cells that contain cells that express CD 105 (a transforming growth factor beta-receptor subunit commonly associated with MSCs) and partially express c-kit (Smith et al. 2007; Li et al. 2012). CDCs, when injected into the border zone of myocardial infarctions in mice, engrafted and increased the viable myocardium (Li et al. 2012). The functional benefit of CDCs is thought to be predominantly due to the secretion of growth factors and the recruitment of endogenous stem cells to injured and infarcted myocardium for myocyte generation (Li et al. 2012; Chimenti et al. 2010). In this regard, cardiospheres and CDCs can secrete the growth factors angiopoietin-2, basic fibroblastic growth factor, hepatocyte growth factor, insulin-like growth factor-1, stromal-derived factor-1, and vascular endothelial growth factor (Li et al. 2012; Chimenti et al. 2010).

Autologous CDCs have been investigated in the treatment of patients with ischemic cardiomyopathies in the open-labeled Cardiosphere-derived Autologous Stem Cells to Reverse Ventricular Dysfunction (CADUCEUS) trial (Makkar et al. 2012). In this trial, 17 patients, post-myocardial infarction with LVEFs of 25–45 %, underwent endomyocardial biopsies of the right ventricular septum. CDCs were obtained from cultures of the endomyocardial biopsies from each patient, and the cells were propagated in cell culture. A total of 12.5 and 25 million autologous CDCs were then given directly into the infarct-related coronary artery of each of the 17 patients 1.5–3 months after their myocardial infarctions in the CADUCEUS trial. The one-year follow-up of 12 of the 17 patients treated with autologous CDCs and 8 control patients have been presented (Makkar et al. 2012; Malliaras et al. 2014). Cardiac MRI with gadolinium was used for the determination of most endpoints. Left ventricular scar mass significantly decreased by a mean of 11.9 ± 6.8 g in CDC-treated patients and by 1.7 ± 7.8 g in patient controls. Left ventricular viable mass increased substantially by a mean of 22.6 ± 9.4 g in treated patients in comparison with 1.8 ± 8.7 g in patient controls. LVEFs did not significantly increase but the regional wall function of infarcted segments did increase and correlated with the decrease in LV myocardial scar size (Makkar et al. 2012; Malliaras et al. 2014). Although adverse events were slightly greater in the treated patients than in the control patients, the events were not significantly different between the two groups. The ALLSTAR trial is a Phase 2 study of CDCs currently in progress that involves *allogeneic* CDCs for the treatment of patients after myocardial infarction (www.ClinicalTrials.gov).

12.16 Critique of the Scipio and Caduceus Trials

The SCIPIO and CADUCEUS trials utilized unique cardiac stem cell populations in a highly selected patient population with myocardial infarctions. In the SCIPIO trial, 1545 patients were evaluated. Two hundred and thirteen patients had LVEFs <40 %, and 20 patients were treated with CSCs. All patients had echocardiographic determinations of LVEF, and 12 of 20 patients had MRI determinations of left ventricular function. Control patients did not have MRI determinations of left ventricular function. In the CADUCEUS trial, approximately 436 patients were evaluated and 17 patients received CDCs. Consequently, these trials report a highly selected patient population, and the results of these trials cannot be applied to all patients with myocardial infarctions and ischemic cardiomyopathies. Much larger trials of each of these cell types in patients with myocardial infarctions are necessary.

In each of these studies, LV infarction was defined by MRI of delayed enhancement of myocardium in the region of coronary artery occlusion/reperfusion due to gadolinium that leaked from myocardial capillaries and pooled in the myocardial interstitial space and intracellular spaces of infarcted myocytes. In these patients, the gadolinium volume of distribution was increased and washout from the myocardium was reduced. However, c-kit$^+$ cardiac stem cells and CDCs can incorporate into damaged blood vessels in infarcted myocardium. In addition, these stem cells can chemoattract endogenous stem cells that can form entirely new blood vessels (vasculogenesis) and can also secrete angiogenic growth factors that stimulate new blood vessels from preexisting vessels (angiogenesis). Consequently, the blood vessels in the damaged myocardium of patients treated with these stem cells were possibly less permeable to gadolinium (Murry 2012). Infarct scars can potentially appear smaller on MRI due to less gadolinium leak as well as myocardial infarction contracture, and therefore, "viable" myocardium can actually appear larger with MRI. MRI also cannot distinguish hypertrophic cardiac myocytes from myocyte hyperplasia (Murry 2012). Moreover, inter-scan variability and intra- and inter-observer variability in infarct measurements and interpreting MRI scans can account for some myocardial changes between pre- and post-stem cell infusion (Kwong and Farzaneh-Far 2011). Rebuttals to these arguments against the use of contrast-enhanced MRI in estimating infarct size and myocardial regeneration after stem cell treatment have been published by the Caduceus Investigators (Malliaras et al. 2013). The rebuttal is based on a porcine myocardial infarction study in which allogeneic CDCs decreased infarct scar size and lead to cardiomyocyte hyperplasia on MRI and also on histological examination (Malliaras et al. 2013). Nevertheless, anatomical and histological examinations of myocardial biopsies or myocardial autopsy examinations of patients treated with these stem cells are necessary to determine whether the infarct fibrosis is significantly decreased and whether the substantial generation of new myocytes occurs in patients treated with c-kit$^+$ cardiac stem cells and CDCs.

The fact that there is long-term improvement in the LVEFs by echocardiography and MRI in the SCIPIO trial and improvement in LV regional wall motion and LV thickening by MRI in the CADEUCUS trial suggests that the cardiac stem cells can reduce myocardial inflammation and scar formation, preserve injured myocytes, and chemoattract endogenous stem cells for myocardial repair. Trials of larger numbers of patients treated with CDCs and cardiac stem cells, such as the ALLSTAR trial (Marban NCT0 1458) and the proposed Phase II SCIPIO trial (Loughran et al. 2012), are warranted to determine the precise mechanisms of cardiac stem cell action and their benefit in patients over long periods.

12.17 Other Cell Types: Human Umbilical Cord Blood Stem Cells (hUCBC)

Four million births occur each year in the USA and approximately one hundred and thirty-four million births occur each year throughout the world. Human umbilical cord blood mononuclear cells (hUCBCs) are a source of hematopoietic, endothelial, and MSCs (Broxmeyer 1998; Broxmeyer et al. 1992; Bieback et al. 2004). The total content of hematopoietic progenitor cells in umbilical cord blood equals or exceeds that of bone marrow, but the highly proliferative hematopoietic stem cells are eightfold higher in hUCBC than in bone marrow and can be enriched by as much as 77–95 % (Broxmeyer et al. 1989; Piacibello et al. 1997). Human umbilical cord mesenchymal stem cells, which are present in cord blood and also umbilical cord tissue, are in the G0/G1 stage of the cell cycle but are capable of proliferating with a population-doubling time of 48 h (Bieback et al. 2004; Erices et al. 2000).

Human cord blood mononuclear cells are currently used for repopulating BMCs in patients treated for acute leukemia, chronic myeloid leukemia, myelodysplastic syndrome, neuroblastoma, and non-malignant diseases such as Fanconi's anemia and aplastic anemia (Broxmeyer 1998; Gluckman 2009). These cord blood cells contain less $CD3^+$, $CD4^+$, and $CD8^+$ immune cells than human adult blood cells and rarely express HLA class II antigens. In addition, cord blood T cells express CD45RA antigen which indicates that they are immunologically naïve (Broxmeyer 1998). This significantly reduces the risk of rejection by the host (Broxmeyer 1998; Henning et al. 2004). Moreover, hUCBC can be cryopreserved for periods of 20 or more years with the recovery of 60–100 % viable cells (Broxmeyer 1998). Consequently, hUCBC can be readily available for the treatment of damaged hearts.

Human umbilical cord mononuclear cells have been given to research animals with acute myocardial infarctions. In animal studies, hUCBCs have significantly limited the size of myocardial infarctions by $\geq$50 % and reduced LV remodeling thereby preserving LV ejection fraction and the rate of rise and fall of LV pressure (dP/dt) without requirements for host immune suppression (Henning et al. 2004, 2006, 2007, 2008, 2010). The optimal number of hUCBC for infarct size reduction

in rodents with myocardial infarctions is four million cord cells when administered directly into the peri-infarct area or into the coronary arteries and 16 million cord blood cells when administered intravenously (Henning et al. 2007). The optimal time for injection of these cells in order to minimize infarct size is 2–24 h after the onset of acute myocardial infarction (Henning et al. 2006).

Human umbilical cord blood cells significantly limit the expression of inflammatory cytokines in acutely inflamed and infarcted myocardium. Within 12 h of acute infarctions in untreated research animals, the myocardial concentration of tumor necrosis factor alpha, monocyte chemoattraction protein, fractalkine, IL6 ciliary neurotrophic protein, macrophage inflammatory protein, and interferon-gamma increases two to as much as eightfold in comparison with cytokine concentrations in non-infarcted myocardium (Henning et al. 2008). In contrast, these inflammatory cytokines do not significantly change between 2 and 72 h after coronary occlusion in myocardial infarctions treated with hUCBC (Henning et al. 2008). Moreover, hUCBCs also significantly limit the myocardial infiltration of inflammatory neutrophils and lymphocytes into acute infarctions. For example, the percentage of neutrophils in untreated myocardial infarctions within 12 h of coronary occlusion significantly increases more than 130-fold from 0.04 ± 0.2 to 5.3 ± 1.2 %/50,000 ventricular myocytes in research animals (Henning et al. 2008). In contrast in the hUCBC-treated myocardial infarctions, the percentage of neutrophils is significantly less and averages only 1.3 ± 0.7 %/50,000 heart myocytes. Moreover, the percentages of neutrophils/50,000 cardiac myocytes at 24 and 72 h in hUCBC-treated infarcted hearts are not significantly different from normal controls (Henning et al. 2008). Similarly, at 24 and 72 h after coronary occlusion, the percentage of CD3 and CD4 lymphocytes in infarcted myocardium are twofold greater in untreated infarcted hearts in comparison with hUCBC-treated infarcted hearts (Henning et al. 2008). The hUCBC-induced reduction in inflammatory cells and inflammatory cytokines in these investigations is associated with left ventricular infarct sizes that are more than 40–50 % smaller in hUCBC-treated infarctions and LV ejection fractions that are more than 10 % greater at 1 and 2 months post-infarction than untreated infarctions (Henning et al. 2008).

An additional mechanism whereby hUCBC may be beneficial in ischemic/infarcted myocardium is by stimulating new blood vessel formation (Henning et al. 2004; Ma et al. 2005; Murohara et al. 2000). Endothelial progenitor cells are normal components of umbilical cord blood that can release pro-angiogenic molecules such as vascular endothelial growth factor (Ma et al. 2005; Murohara et al. 2000). These cells can also express KDR, Tie2/Tek, and VE-cadherin, which are expressed by endothelial cells during new blood vessel formation (Murohara et al. 2000; Nieda et al. 1997). In addition, $CD34^+$ hUCBCs integrate into the walls of blood vessels in the periphery of injured tissue and can increase capillary density in ischemic/infarcted muscles (Murohara et al. 2000; Pesce et al. 2003; Hirata et al. 2005).

When subjected to 1 % oxygen or hydrogen peroxide (H_2O_2)-induced free oxygen radical stress, hUCBC significantly increase the secretion of hepatocyte growth factor, insulin-like growth factor vascular endothelial cell growth factor,

placental growth factor, IL-10, and angiogenin (Pesce et al. 2003; Henning et al. 2012; Jin et al. 2013; Henning et al. 2014). These hUCBC paracrine factors can significantly increase in cardiac myocytes the activation of the cell survival protein Akt (Protein kinase B) which can decrease activation of the myocyte death proteins JNK and p38 and thereby preserve myocyte viability by limiting or preventing myocyte apoptosis and necrosis (Henning et al. 2012; Jin et al. 2013; Henning et al. 2014).

Currently, hUCBCs are being processed for the development of vascular grafts and heart valves for the treatment of newborns with congenital heart defects. In addition, Phase 1 studies are being performed with the use of hUCBC in the treatment of patients with angina pectoris that is refractory to medical therapy and who are not candidates for surgical or angioplasty coronary revascularization.

12.18 A Stem Cell Perspective

Although different stem cells are available for cardiac repair, the optimal stem cell for the treatment of all patients with infarcted myocardium remains to be determined. The optimal stem cell should permit transplantation into different patients without requirements for patient immune suppression therapy. Current cell candidates that are undergoing investigations in patients include allogeneic bone marrow MSCs and CDCs. New techniques must be developed to enhance the survival and propagation of these stem cells without increasing the risks of neoplastic differentiation.

Cell banks should be established that provide readily available, undifferentiated, but accurately characterized allogeneic stem cells that have significant capacity for in vitro and in vivo propagation for the treatment of patients with heart disease. The optimal number of stem cells and the optimal timing of stem cell transplantation into patients' hearts after myocardial infarction must be systematically investigated to maximize the chemoattraction of stem cells to ischemic and infarcted myocardium and facilitate myocardial healing. In this regard, the repeated administration of stem cells to patients will probably be necessary via intracoronary or intravenous injections and should be investigated.

Investigations must determine whether intramyocardial, intracoronary, or intravenous injection is most optimal for cardiac repair. With intracoronary or intravenous injections, large numbers of MSCs can cause cell clumping and microinfarctions in the heart or lungs. Multiple intramyocardial injections can be associated with high rates of stem cell leakage from the myocardium, disruption of the extracellular matrix of the myocardium, and scar formation, thereby potentiating the formation of arrhythmogenic foci. Although some stem cells injected intravenously do reach the heart, many stem cells become lodged in the lungs. Consequently with intravenous injections, the number of stem cells required for cardiac repair can be fourfold greater than the numbers required for

intramyocardial or intracoronary injection for cardiac repair (Henning et al. 2007). Pulmonary function studies and oxygen saturation levels should be monitored. Moreover, strategies must be developed to facilitate the homing to the heart of stem cells that are injected intravenously.

Enhancement of stem cell engraftment in the heart is mandatory for optimizing the therapeutic benefits of these cells. Currently, less than 10 % of the stem cells remains in the heart 1–2 h after injection into the beating heart (Hofmann et al. 2005; Hou et al. 2005). Ninety percent or more of the cells are expelled from the intramyocardial injection site or are extruded from the myocardium through the coronary veins and lymphatics due to the massaging action of the heart. The stem cells then migrate to the lungs, liver, spleen, and kidneys (Hofmann et al. 2005). Potential treatment strategies to ensure that stem cells remain in the myocardium include co-delivery of stem cells with extracellular matrix molecules, nanofibers, fibrin glues, or other drugs, or applying stem cell patches to the epicardium (Henning 2011, 2012).

Significant discrepancies exist between the paucity of stem cells that actually engraft in the heart and the improvement in heart function that can occur with stem cell therapy. This suggests that the beneficial effects of stem cells are due to the release of biologically active growth factors and anti-inflammatory cytokines that protect cardiomyocytes and vascular endothelial cells in the injured myocardium. These biologically active factors can potentially limit myocyte apoptosis, necrosis, and extracellular matrix remodeling, stimulate angiogenesis, and recruit endogenous stem cells to the damaged myocardium. Consequently, growth factors and anti-inflammatory cytokines secreted by stem cells must be isolated, identified, purified, expanded, and investigated as new pharmacologic therapies for cardiac repair.

Imaging and hemodynamic measurement endpoints must be uniformly employed to demonstrate benefit, permit comparisons of different stem cell investigations, and provide insights into stem cell mechanisms of action. In this regard, MRI should be uniformly employed to measure changes in cardiac regional wall motion, ejection fraction, ventricular end-systolic and end-diastolic volumes, and left ventricular mass.

Additional basic science, preclinical, and clinical studies are required in order to address and answer the unresolved issues discussed in this chapter regarding stem cells in cardiac repair. These studies will require the close cooperation, the interaction, and the financial support of basic scientists and clinicians throughout the world. In this way, cell-based therapy in the twenty-first century will offer new hope to the millions of patients with heart disease who would otherwise suffer from the inexorable downward progression of the heart disease and heart failure.

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