Multimodality Noninvasive Imaging Demonstrates In Vivo Cardiac Regeneration After Mesenchymal Stem Cell Therapy

Luciano C. Amado, MD,*† Karl H. Schuleri, MD,*† Anastasios P. Saliaris, MD,*† Andrew J. Boyle, MBBS, PhD,*† Robert Helm, MD,* Behzad Oskouei, MD,*† Marco Centola, MD,*† Virginia Eneboe, VT,*† Randell Young, MVD,‡ Joao A. C. Lima, MD,* Albert C. Lardo, PhD,* Alan W. Heldman, MD,*‡ Joshua M. Hare, MD*†

Baltimore, Maryland

OBJECTIVES The purpose of this study was to test the hypothesis, with noninvasive multimodality imaging, that allogeneic mesenchymal stem cells (MSCs) produce and/or stimulate active cardiac regeneration in vivo after myocardial infarction (MI).

BACKGROUND Although intramyocardial injection of allogeneic MSCs improves global cardiac function after MI, the mechanism(s) underlying this phenomenon are incompletely understood.

METHODS We employed magnetic resonance imaging (MRI) and multi-detector computed tomography (MDCT) imaging in MSC-treated pigs (n = 10) and control subjects (n = 12) serially for a 2-month period after anterior MI. A sub-endocardial rim of tissue, demonstrated with MDCT, was assessed for regional contraction with MRI tagging. Rim thickness was also measured on gross pathological specimens, to confirm the findings of the MDCT imaging, and the size of cardiomyocytes was measured in the sub-endocardial rim and the non-infarct zone.

RESULTS Multi-detector computed tomography demonstrated increasing thickness of sub-endocardial viable myocardium in the infarct zone in MSC-treated animals (1.0 ± 0.2 mm to 2.0 ± 0.3 mm, 1 and 8 weeks after MI, respectively, p = 0.028, n = 4) and a corresponding reduction in infarct scar (5.1 ± 0.5 mm to 3.6 ± 0.2 mm, p = 0.044). No changes occurred in control subjects (n = 4). Tagging MRI demonstrated time-dependent recovery of active contractility paralleling new tissue appearance. This rim was composed of morphologically normal cardiomyocytes, which were smaller in MSC-treated versus control subjects (11.6 ± 0.2 μm vs. 12.6 ± 0.2 μm, p < 0.05).

CONCLUSIONS With serially obtained MRI and MDCT, we demonstrate in vivo reappearance of myocardial tissue in the MI zone accompanied by time-dependent restoration of contractile function. These data are consistent with a regenerative process, highlight the value of noninvasive multimodality imaging to assess the structural and functional basis for myocardial regenerative strategies, and have potential clinical applications. (J Am Coll Cardiol 2006;48:2116–24) © 2006 by the American College of Cardiology Foundation

Ischemic cardiomyopathy due to myocardial infarction (MI) is the major cause of congestive heart failure and death in the Western world (1). There is accumulating experimental support for the application of cellular transplantation as a strategy to improve myocardial function (2–8) and reduce scar burden due to MI (2–4,8); however, controversy exists regarding the underlying mechanism(s) for this effect. In particular, the question of whether transplanted cells regenerate new myocardium (either through differentiation or cell–cell signaling to endogenous precursor cells) to replace those lost to MI (9,10) remains unanswered. Additionally, the use of different cell populations and varying clinical protocols and their application in diverse patient groups has hampered any effort to compare outcomes using the various cell types with one another (11).

Critical in assessing the efficacy of any novel regenerative therapy is the ability to noninvasively image tissues within the heart at various time points. Moreover, it is essential to determine whether regenerated tissue is functional (i.e., does the new tissue contract with the rest of the heart or is it non-contractile and mechanically uncoupled from the rest of the heart), in which case it might actually be detrimental to cardiac function.

Implantation of cells such as bone marrow-derived mesenchymal stem cells (MSC) restores the function of the damaged heart and decrease necrotic tissue (8,12). Whether they do so by generating new, electromechanically coupled...
myocardium (13,14) or by altering the composition of the evolving scar, thereby preventing remodeling (15,16), remains controversial. Some groups advocate that implanted cells are not contractile but rather that new viable tissue serves to decrease myocardial wall tension at the infarcted region by strengthening the infarct scar and preventing ventricular dilatation and thus improving diastolic function and enhancing global cardiac function (16,17). Others suggest there is actual regeneration of functional myocardium that replaces the area occupied by scar (3). Whether this occurs by (trans)differentiation of the implanted cells into new cardiomyocytes, whether there is fusion of the donor cells with the surviving cells inducing cellular turnover, or whether the injected cells stimulate endogenous myocytes or myocyte precursors to divide via a paracrine or cell–cell signaling action is incompletely understood.

To address this important issue we used multimodality imaging, with both magnetic resonance imaging (MRI) and contrast-enhanced multi-detector computerized tomography (MDCT) to serially monitor the regional and global structure and function of the porcine heart after MI in response to allogeneic MSC therapy. We have previously demonstrated that MSC therapy leads to the development of a sub-endocardial rim of muscle and improvement in overall cardiac function (12); however, whether this rim of myocardium is functionally coupled to the rest of the heart remains unknown. We therefore tested the hypothesis that MSC cellular cardiomyoplasty stimulates the regeneration of viable, contractile myocardium adjacent to evolving MI scar thereby enhancing regional systolic function.

METHODS

Experimental protocol. All animal studies were approved by the Johns Hopkins University School of Medicine Institutional Animal Care and Use Committee and comply with the “Guide for the Care and Use of Laboratory Animals” (National Institutes of Health [NIH] Publication no. 80–23, revised 1985). Twenty-three female Yorkshire pigs (25 to 30 kg) underwent left anterior descending coronary artery occlusion followed by reperfusion and then were randomized to receive MSCs (n = 10) or not receive MSCs (n = 12, non-treated group) 3 days later. Mortality was not different between groups. In the control group, 2 animals died within 72 hours of MI and 3 animals died during the follow-up period, resulting in 7 animals being available for the imaging and histology protocols. In the MSC-treated group, 1 animal died after cell injection and 2 animals died during the follow-up period, resulting in 7 animals that were available for imaging and pathology examinations. Cine and tagging MRI assessment was performed at 4 time points after injection: 2 days and 1, 4, and 8 weeks. Also, MDCT was performed at 1 week and 8 weeks after MI. Detailed protocols are in the following sections. Partial data—ejection fraction and infarct size obtained from MRI, and histologically measured rim thickness—from a subset (n = 6 MSC and n = 6 control subjects) of these animals have previously been reported (12).

MI creation and intramyocardial MSC injection. The protocol used here has been previously described (12). In brief, intravascular sheaths were placed in the right carotid artery (8-F), and a coronary angioplasty balloon (2.5 × 20 mm) was advanced into the proximal left anterior descending coronary artery. Myocardial infarction was induced by inflating the angioplasty balloon for 60 min, followed by artery reperfusion. After reperfusion, the catheter sheath in the carotid artery was removed, and the carotid artery was permanently closed. All animals were adequately heparinized during the surgical procedure.

Three days after MI, animals randomized for MSC treatment received intramyocardial injections of allogeneic porcine MSCs (2.0 × 10^8 cells, Osiris Therapeutics, Baltimore, Maryland) under fluoroscopy, with a pistol-needle tip injection catheter advanced to the LV through a steerable guide catheter (Stiletto, Boston Scientific, Natick, Massachusetts).

Hypokinetic, akinetic, and dyskinetic areas were identified during contrast ventriculography, and MSC injections were performed within and at the borders of the dysfunctional area, as defined by bi-plane ventriculography. A total of 15 injections were performed in each animal, each injection containing 1 cc, thereby delivering a total of 200 million cells/animal. Each injection was fluoroscopically guided to distribute cells evenly throughout the entire infarct and border zones.

MRI protocol. The MRI images were acquired with a 1.5-T MR scanner (CV/i, GE Medical Systems, Waukesha, Wisconsin) at 4 time points after injection: 2 days and 1, 4, and 8 weeks. Global LV function was assessed with a steady-state free precession pulse sequence (18). Eight to 10 contiguous short-axis slices were prescribed to cover the entire heart from base to apex. Image parameters were the following: repetition time (TR) = 4.2 ms and echo time (TE) = 1.9 ms; flip angle = 45°; 256 × 160 matrix; 8-mm slice thickness/no gap; 125 kHz; 28-cm field of view (FOV); and 1 number of signal average (NSA). To assess regional cardiac function, tagging MRI images were acquired with an electrocardiography-gated, segmented K-space, fast gradient recalled echo pulse sequence with spatial modulation of magnetization to generate a grid tag pattern (19,20). Images were obtained at the same
location as the cine-MRI images, and image parameters were as follows: TR = 6.7 ms and TE = 3.2 ms; flip angle = 12°; 256 × 160 matrix; views/s: 4; 8-mm slice thickness/no gap; 31.25 kHz; 28-cm FOV; 1 NSA; and 6 pixels tagging space.

**MRI analysis.** Cine-MRI images were analyzed with a custom research software package (Cine Tool, GE Medical Systems). Endocardial and epicardial contours were done manually at end-diastolic and end-systolic phases of the cardiac cycle, and global cardiac function was assessed on the basis of Simpson’s rule method.

The high resolution of current generation MRI scanners allows differential assessment of contractile function at various levels within the myocardium at any given circumferential point. We assessed contraction in 24 circumferential areas of the LV, and in each of these areas we assessed contractile function from 3 different layers of the myocardial wall: endocardial, mid-myocardial, and epicardial layers. Tagged images were quantitatively analyzed with a custom software package (Diagnosoft HARP, Diagnosoft Inc., Palo Alto, California) (21). Regional strain magnitude was determined from the 24 radially displaced segments for each short-axis section covering the entire LV and averaged among slices for each region and each time point, as previously described (22), generating a strain map for each point over the cardiac cycle. The peak systolic circumferential strain (Ecc) was determined from the strain map for each point. This approach is most similar to many commonly used indexes based on tissue Doppler functional imaging. Negative Ecc values represent myocardial contraction, whereas values of increasing strain (toward positive values) reflect worsening contractile function in that region. Less negative Ecc values represent hypokinetic myocardium, and a positive value represents dyskinetic myocardium. A value of 0 represents akinetic non-contractile myocardium, and a positive value represents dyskinetic myocardial segments. Peak systolic circumferential strain (peak Ecc) was calculated for each layer as previously described (21). Also, peak Ecc was separated from infarcted and non-infarcted (remote) areas for each animal.

**MDCT imaging protocol.** The MDCT images were obtained in the first week after MI (baseline) and 8 weeks after MSC injection in a subset of animals (n = 4 in each group) with a 32-detector scanner (Aquilion 32 Toshiba Medical Systems Corporation, Otawara, Japan). The specific protocol used has been previously described and validated by Lardo et al. (23). In brief, a stack of axial slices (0.5-mm thickness) was prescribed to cover the entire heart. Images were acquired 5 min after contrast injection (iodixanol 150 ml, 5 cc/s), the ideal time for infarct visualization. Imaging acquisition parameters were as follows: gantry rotation time = 400 ms; detector collimation = 0.5 mm × 32; pitch = 7.2; tube voltage = 135 kV; tube current = 420 mAs; and scanning FOV = 13.2 mm.

**MDCT imaging analysis.** Axial images were reconstructed at 75% of the cardiac cycle and at 2-mm slice thickness. Image analysis was performed with a custom research software package (Cine Tool, GE Medical Systems), and areas of necrosis were identified as hyper-enhanced areas. The multi-detector slice CT technique was chosen owing to its high spatial resolution and ability to avoid the partial volume effect, allowing for better characterization of scar region (24). In the infarct zone, thickness measurements of the entire infarct zone, the scar, and the endocardial rim were made at 4 points in each slice and 4 slices for each animal, and the average was taken.

**Stem cell harvest and isolation.** Male swine MSCs (Osiris Therapeutics) were obtained, isolated, and expanded as previously described (12,25). All used cells were harvested when they reached 80% to 90% confluence at passage 3, and then cells were placed in cryo bags at a concentration of 10 to 15 million MSCs/ml and frozen in a control rate freezer to −180°C until the day of implantation. Trypan blue staining was performed to attest viability of thawed MSC lots before injection. Only MSC lots containing 85% or more of viable cells were used in the study.

**Postmortem analysis.** Quantification of viable myocardium in the subendocardial border of the MI was confirmed by gross pathology. For this purpose, at the end of the 8-week period, animals were humanely killed and hearts were excised and sectioned into 8-mm-thick short-axis slices. Each slice was digitally photographed. For each slice, non-viable infarcted areas and viable subendocardial border thickness were manually measured with a custom research software package (Image J 1.34s; NIH, Bethesda, Maryland), and an average of 6 measurements were taken for each specimen that was used. The amount of viable subendocardial tissue is expressed in absolute terms and as a ratio of viable tissue (mm width) normalized by infarct transmurality (mm width) of each slice. The subendocardial rim thickness data from a subset of these animals have previously been reported (12). The LV circumference was measured at the endocardium and epicardium, as was the circumference of the infarct zone, and the infarct zone was expressed as the percentage of the LV circumference average of the endocardial and epicardial measurements.

After photographs were taken, myocardial tissue samples were obtained from 2 regions: infarcted and remote (normal) areas. Hematoxylin and eosin (H&E) stain was performed, and myocyte length was measured from the remote and infarcted areas (viable tissue at subendocardial border), with a custom research software package (Scion Image Analysis program; Beta version 4.0.2; Scion Corporation, Frederick, Maryland). One hundred myocytes were measured at each site from 2 control and 2 MSC animals with similar initial infarct size and morphology.

**Statistical analysis.** All values are expressed as mean ± SEM. Indexes of cardiac function as well as amount of viable tissue at day 2 and week 8 were compared between groups with non-paired independent samples Student t test. The change in sub-endocardial rim thickness and scar thickness within groups from baseline to 8 weeks were
analyzed with a paired Student t test. Differences in ejection fraction and infarct size during the 8-week period were compared within the groups with repeated measurements analysis of variance (ANOVA) and between groups with 2-way ANOVA with an interaction term. Simple linear regression analysis was performed to compare peak Ecc between groups at different time points, and the Huber/White Sandwich estimator of variance was used to take correlation between the animals into account. A level of p < 0.05 was considered statistically significant.

RESULTS

Serial imaging demonstrates reappearance of myocardial tissue. There were no baseline differences between groups (Table 1). We examined the tissue characteristics of the infarcted regions in vivo with high definition MDCT imaging at several time points and compared this with histological analysis. The CT images offered important details regarding changing scar morphology and tissue characteristics due to MSC treatment. The MDCT images in the first week after MI (Fig. 1) demonstrated the presence of a rim of tissue at the endocardial layer of the MI scar, which had a tissue density similar to remote myocardium (130.9 ± 31.6 Hounsfield units). This rim increased in thickness during an 8-week interval, consistent with the development of potentially viable myocardial tissue. Additionally, there was a distinct difference in tissue density between viable myocardium and the infarct scar (235.9 ± 48.6 Hounsfield units) and this viable sub-endocardial tissue. Image-based quantification of this sub-endocardial region over 8 weeks revealed no change in the overall thickness of the infarct zone but an increase in thickness of the viable sub-endocardial tissue in the MSC-treated group, from 1.02 ± 0.16 mm to 2.02 ± 0.28 mm (p = 0.028, n = 4). There was a corresponding reduction in the thickness of the scar in this region from 5.1 ± 0.5 mm to 3.6 ± 0.2 mm (p = 0.044, n = 4) (Fig. 2). However, in the untreated group, the total infarct zone thickness, the scar thickness, and the rim thickness remained unchanged from week 1 to week 8 (p = NS, n = 4).

The generation of a sub-endocardial rim of myocardium was confirmed by pathologic analysis. The MSC-treated animals had a 2.9 ± 0.4-mm sub-endocardial rim of tissue, representing 35 ± 3% of the wall thickness in the infarct zone (n = 6). Untreated animals (n = 7), however, had a thinner sub-endocardial rim, 1.4 ± 0.1 mm (p = 0.02 vs. MSC group), accounting for 21 ± 2% of the wall thickness (p = 0.004 vs. MSC-treated) (Fig. 3). Additionally, volumetric analysis of the infarct scar in 2 animals from each group demonstrated that MSC injection significantly reduced the volume of myocardial infarct scar tissue over the course of the study period (49.5 ± 9.9% decrease in infarct size at 8 weeks after MI; p < 0.05 vs. baseline), whereas no change in infarct size was observed in non-treated animals (p < 0.05 vs. MSC-treated animals).

The circumferential extent of the infarcted zone was not different between groups (22 ± 3% vs. 24 ± 4% of the LV circumference in MSC-treated and untreated animals, respectively, p = 0.56). Thus, regeneration of the infarct zone

Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MSC-Treated</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI size (% of LV volume)*</td>
<td>18.3 ± 3.4</td>
<td>20.2 ± 2.3</td>
<td>NS</td>
</tr>
<tr>
<td>LVED mass*</td>
<td>48.2 ± 2.9</td>
<td>56.7 ± 4.7</td>
<td>NS</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>29.8 ± 1.9</td>
<td>29.1 ± 2.6</td>
<td>NS</td>
</tr>
<tr>
<td>MI zone thickness (mm)†</td>
<td>6.2 ± 0.3</td>
<td>6.3 ± 0.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Baseline characteristics were not different between groups. *Measured by cine magnetic resonance imaging. †Measured by Multi-slice detector computed tomography.

LVED = left ventricular end-diastolic; LVEF = left ventricular ejection fraction; MI = myocardial infarction; MSC = mesenchymal stem cell.

Figure 1. Multi-detector computed tomography scan of a pig 1 week after myocardial infarction. The different tissue densities of the infarct scar and the sub-endocardial rim of viable tissue are clearly apparent. The sub-endocardial rim has a density similar to the myocardium outside the infarct zone. The relative densities in Hounsfield units (HU) are also represented. MSC = mesenchymal stem cell.
was not due to regeneration from the border zone inward into the scar, but due to regeneration of the sub-endocardial rim. Histological analysis confirmed that this rim at the endocardial region of the infarct zone contained properly aligned cardiac myocytes (Fig. 4A). These myocytes were smaller than normal, with myocyte width of 11.6 ± 0.2 μm vs. 12.6 ± 0.2 μm (p < 0.05) (Fig. 4B) for cardiomyocytes in the region remote from the infarct. This finding excludes myocyte hypertrophy as a cause for the increase in sub-endocardial rim thickness and is consistent with myocardial regeneration in this region (3,4,8,12).

Regenerated myocardium has coordinated contraction. Next we examined whether the reduction in infarct size and its replacement with new myocardium resulted in recovery of contractile function in this region. For this purpose, we used MRI tagging to calculate peak Ecc. Representative tagged imaging with color mapping to demonstrate normal and abnormal contraction is shown in Figure 5.

As depicted in Figure 6, both groups of animals had hypokinetic wall motion in the endocardial layers of the infarct zone at baseline, −5.5 ± 0.3% and −5.5 ± 0.5%, respectively, for MSC-treated and non-treated groups (p = NS). However, whereas this area remained hypokinetic in control animals for the 8-week duration of the study, the sub-endocardial area of MSC-treated pigs where regeneration of myocardium occurred began to increase peak negative strain by 4 weeks, followed by near-full peak Ecc recovery, comparable to that of remote or non-infarcted myocardium, by 8 weeks after injection (peak Ecc at week 8: −11.8 ± 0.9% vs. −5.1 ± 0.5 in MSC-treated vs. control subjects, p < 0.05).

The epicardial layer of myocardium exhibited a somewhat different course over the 8-week study period. As shown in Figure 6, a depressed regional function was observed in both groups with peak Ecc −4.2 ± 0.3% and −5.5 ± 0.5% for MSC-treated and control groups, respectively (p = NS). However, improvement in cardiac function was observed in both groups by the end of the 8-week study period (−9.4 ± 0.3 and −6.8 ± 0.9 in MSC-treated and control groups, respectively; p < 0.05 vs. baseline for both and p = NS between groups). This improvement in regional function is expected, because the region most likely contains viable myocardium, as confirmed previously by histological analysis (26).

To complete the analysis of regional function of the whole wall, we examined the temporal course of peak Ecc in the mid-wall region. Interestingly, despite the improvement observed at the epicardial layer in the non-treated animals, no improvement or movement gathering occurred in the mid-wall layer. Initial peak Ecc was depressed at −4.1 ± 0.4% at baseline and did not improve in the course of the 8 weeks in the non-treated animals, with a peak Ecc of −4.7 ± 0.6% (p = NS vs. baseline). However, MSC-treated animals demonstrated an important improvement in regional peak Ecc shortening at the mid-wall region, from −4.6 ± 0.3% to −10.8 ± 0.6%, leading to near normal levels at 8 weeks after injection (p < 0.05 vs. non-treated).

These data are consistent with restoration of contractile function in the endocardial and mid-wall areas of the infarcted myocardium in animals treated with MSC injection, not evident in untreated animals. It is important to note that the entire infarct zone of both groups remained hypokinetic at the time of the week-1 MRI, demonstrating that the development of contractility in the MSC-treated group developed over several weeks. The timescale of improvement coupled with the CT findings of scar replacement with myocardium in this area strongly suggests that regeneration of functional myocardium rather than just
recovery of stunned myocardium is the mechanism of recovery.

Regenerated myocardium improves global cardiac function. Enhancement of regional cardiac function in the infarct zone leads to improvement in global cardiac function (Fig. 7). As previously reported, the LV ejection fraction improved in a time-dependent manner in the MSC-treated group but not in the control group (12). Importantly, this improvement followed a time course similar to that of the observed myocardial regeneration and the improvement in regional contraction.

**DISCUSSION**

Here we show that multimodality imaging with MRI and MDCT allows precise characterization of tissue morphology and regional and sub-regional cardiac function that demonstrates the structural and functional response to cell-based therapy in a large animal model. Together, our results demonstrate that transplantation of allogeneic MSCs in pigs 3 days after MI stimulates cardiac repair by leading to the reappearance and/or growth of new tissue along the sub-endocardial rim of the MI that partially
replaces the infarct scar. This tissue is contractile and mechanically coupled with the existing myocardium, demonstrating that cellular cardiomyoplasty with MSCs likely stimulates regeneration of myocytes as the basis for the improvement in regional and global function and the reduction in infarct size. Importantly, these insights were gleaned from imaging modalities that did not require labeling of injected cells.

Figure 4. Hematoxylin and eosin (H&E) stain of tissue obtained from the subendocardial rim (A) and the remote zone (B) of mesenchymal stem cell (MSC)-treated animals. As shown, the rim of tissue is made up of cardiomyocytes with normal ultrastructural architecture. (C) There is no difference in the size of myocytes taken from the remote myocardium of both control and MSC hearts. However, the myocytes from the infarct rim are significantly smaller in MSC-treated animals versus control animals (n = 2 each, >100 cells/heart), suggesting that the enhanced rim size in MSC animals might be due to new cardiomyocyte regeneration. *p < 0.001 MSC rim versus control rim.

Figure 5. Color map depicting non-contractile and dysfunctional areas of myocardium detected by tagging magnetic resonance imaging. Bar color demonstrates the spectrum in change of systolic cardiac function: blue and green colors identify normal contractile areas and red colors represent dysfunctional areas. Non-treated animals exhibited worsening regional systolic function during the 8-week period, whereas a visual improvement was noticed in the mesenchymal stem cell (MSC)-treated animals.

Figure 6. Plot of peak circumferential strain (peak Ecc) over time comparing mesenchymal stem cell (MSC)-treated and non-treated animals for 3 myocardial layers (endocardial, epicardial, mid-wall layers). Peak negative Ecc values represent myocardial shortening, whereas increasingly positive values reflect relative myocardial dysfunction. As can be appreciated, peak negative Ecc values at the endocardial and mid-wall layers for MSC-treated infarct regions decrease (i.e., improve) over the 8-week follow-up period compared with the control group, which remained dysfunctional. †p < 0.05 difference within the group; ‡p < 0.05 difference between groups; n = 6 in each group. MI = myocardial infarction.
is not due to hypertrophy of the cardiomyocytes but rather suggests growth of new tissue. It has previously been shown that the cardiomyocytes in this region are ki67 positive, also suggesting growth of new tissue (12). However, whether this occurs due to MSC differentiation into cardiomyocytes, to proliferation of endogenous cardiomyocytes or their precursors, or to other currently unappreciated mechanisms remains to be determined. Studies aimed at elucidating the relative contributions of these mechanisms are currently ongoing.

There are other postulated potential mechanisms of action. First, MSCs likely stimulate homing of endogenous precursor cells (33,34). Systemically injected MSCs home to the site of tissue damage after MI (35,36), demonstrating their participation in cellular trafficking. Additionally, MSCs themselves secrete stromal derived factor 1 (SDF-1), a stem cell chemoattractant cytokine (37) and might therefore promote trafficking and/or differentiation of other stem cells, such as endothelial progenitor cells (EPCs) or endogenous cardiac stem cells. It is attractive to speculate that MSCs might act as an amplifier to enhance the function of endogenous cardiac stem cells in effecting myocardial regeneration. Indeed, it has been shown that exogenous signals introduced after MI can cause proliferation and differentiation of endogenous cardiac stem cells, resulting in myocardial regeneration and improved LV function (38). Mesenchymal stem cell transplantation might act in a paracrine or cell–cell signaling fashion to effect cardiac repair via cytokine-induced enhancement of endogenous cardiac stem cell function.

Another proposed mechanisms for new tissue regeneration is cell fusion (39) of the implanted MSCs with host cells. However, fusion seems increasingly unlikely given the accumulation of abundant data revealing diploidy of regenerated cells (40–42). Our study has not specifically addressed the issue of how myocyte regeneration occurs, but we have confirmed that it occurs, that it happens in a time-dependent manner, that it develops along the subendocardial surface of the scar, and that it results in functional contractile improvement. Further studies are underway to more fully elucidate the mechanism by which MSC therapy induces myocardial regeneration.

In summary, we have used multimodality state-of-the-art imaging to serially follow the response of a large-animal model of MI to MSC cell therapy and demonstrate the presence of myocardial regeneration over several time points. In addition, we demonstrate that this new tissue enhances regional systolic function, which in turn leads to an improvement in global chamber performance. These data demonstrate the efficacy of intramyocardial MSC injection after MI and highlights the value of noninvasive multimodality imaging not only in elucidating mechanisms underlying new tissue regenerative therapies but also in serially assessing these tissue changes and their functional consequences. These results have implications for the design of clinical trials of stem cell therapy.
REFERENCES


26. Reimer KA, Jennings RB. The “wavefront phenomenon” of myocardial ischemic cell death. II. Transmural progression of necrosis within the framework of ischemic bed size (myocardium at risk) and collateral flow. Lab Invest 1979;40:633–44.


