Transplantation of embryonic stem cells improves cardiac function in postinfarcted rats

Jiang-Yong Min, Yinke Yang, Kimber L. Converso, Lixin Liu, Qin Huang, James P. Morgan, and Yong-Fu Xiao

Transplantation of embryonic stem cells improves cardiac function in postinfarcted rats. J Appl Physiol 92: 288–296, 2002.—Massive loss of cardiac myocytes after myocardial infarction (MI) is a common cause of heart failure. The present study was designed to investigate the improvement of cardiac function in MI rats after embryonic stem (ES) cell transplantation. MI in rats was induced by ligation of the left anterior descending coronary artery. Cultured ES cells used for cell transplantation were transfected with the marker green fluorescent protein (GFP). Animals in the treated group received intramyocardial injection of ES cells in injured myocardium. Compared with the MI control group injected with an equivalent volume of the cell-free medium, cardiac function in ES cell-implanted MI animals was significantly improved 6 wk after cell transplantation. The characteristic phenotype of engrafted ES cells was identified in implanted myocardium by strong positive staining to sarcomeric α-actin, cardiac α-myosin heavy chain, and troponin I. GFP-positive cells in myocardium sectioned from MI hearts confirmed the survival and differentiation of engrafted cells. In addition, single cells isolated from cell-transplanted MI hearts showed rod-shaped GFP-positive myocytes with typical striations. The present data demonstrate that ES cell transplantation is a feasible and novel approach to improve ventricular function in infarcted failing hearts.

MYOCARDIAL INFARCTION (MI) is a life-threatening event that may cause sudden cardiac death and heart failure. Despite considerable advances in the diagnosis and treatment of heart disease, cardiac dysfunction after MI is still the major worldwide cardiovascular disorder (5). After acute MI, the damaged myocardium is gradually replaced by fibrotic noncontractile cells. The developing ventricular dysfunction is primarily due to a massive loss of cardiomyocytes. A recent study showed that mild proliferating myocytes derived from resident cardiomyocytes or circulating stem cells may contribute to the increase in muscle mass of the infarcted human myocardium (1). However, hypertrophy and mild proliferation without effective therapy do not attenuate the onset and progress of cardiac dysfunction after MI. Therefore, finding effective new approaches to improve cardiac dysfunction after MI remains a major therapeutic challenge.

Cell transplantation has emerged as a potential new approach for repairing damaged myocardium in recent years. Transplanted cardiomyocytes have been shown to survive, proliferate, and connect with the host myocardium in murine models (28). Li and co-workers (15–17) demonstrated that transplanted fetal cardiomyocytes could form new cardiac tissue within the myocardial scar induced by cryoinjury and improve heart function. Bishop et al. (2) reported that embryonic myocardium of rats can be implanted and cultured in oculo and demonstrated that the engrafted embryonic cardiomyocytes proliferated and differentiated. In a recent review, Hescheler et al. (7) pointed out that pluripotent embryonic stem (ES) cells cultivated within embryonic bodies reproduced highly specialized phenotypes of cardiac tissue. Most of the biological and pharmacological properties of cardiac-specific ion currents were expressed in cardiomyocytes developed in vitro from pluripotent ES cells (7, 9). Recently, Etzion et al. (6) transplanted cardiomyocytes isolated from 15-day-old embryos into rat MI hearts and found that cell-transplanted MI animals had attenuated left ventricular (LV) dilatation, infarct thinning, and myocardial dysfunction. However, the significance of ES cell transplantation in postinfarcted failing hearts remains to be determined. This study was designed to determine whether transplanted ES cells could survive in injured myocardium and improve cardiac function in postinfarcted rats.

METHODS

Preparation of ES cells. The mouse ES cell line, ES-D3, was obtained from the American Type Culture Collection (Manassas, VA) and maintained with methods previously described (2).

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transplantation. Spontaneous action potentials (Fig. 1A) were recorded from a spontaneously beating ES cell (A), which was cultured 9 days after withdrawal of leukemia inhibitory factor from the medium. The whole cell zero-current clamp technique was applied to record spontaneous action potentials. The shape of the action potentials is very similar to that in mammalian neonatal cardiomyocytes (Fig. 1B). The spontaneous beating rate of this cell was 86 beats/min at room temperature.

described (27). Briefly, ES-D3 cells were cultured in DMEM on mitotically inactive mouse embryonic fibroblast feeder cells (American Type Culture Collection). The medium was supplemented with 15% fetal bovine serum, 0.1 mM β-mercaptoethanol (Sigma Chemical, St. Louis, MO), and 10^5 units/ml of leukemia inhibitory factor conditioned medium (BRL, Gaithersburg, MD) to suppress differentiation. To initiate differentiation, ES cells were dispersed with trypsin and resuspended in the medium without supplemental leukemia inhibitory factor and cultured with the hanging drops (approximate 400 cells per 20 μl) method for 5 days. They were then seeded into 100-mm cell culture dishes. Spontaneously beating clusters were dissected by use of a sterile micropipette and recultured for another 2–3 days at 37°C in a humidified atmosphere with 5% CO2.

Before cell transplantation, ES cells were transfected with the marker green fluorescent protein (GFP) to identify survival of engrafted ES cells. Plasmids with hCMV IE promoter/enhancer driving GFP gene (5.7 kb) and the Gene-PORTER transfection reagent were obtained from Gene Therapy Systems (San Diego, CA). ES cells were plated in 100-mm dishes to obtain 50–60% confluence on the day of transfection. Plasmid GFP DNA (8 μg) was added to each dish with a calcium phosphate precipitation method (32). After 2 days of GFP transfection, cultured ES cells were trypsinized and resuspended in Joklik modified medium (Sigma Chemical) with a density of 10^6 cells/ml for use in cell transplantation. Spontaneous action potentials (Fig. 1A) in cultured mouse ES cells (n = 4) were elicited by the patch-clamp technique (31). Electrophysiological properties of action potentials were similar to those recorded from cultured 1-day neonatal mouse cardiomyocytes (Fig. 1B).

**ES cell transplantation.** The experiments were performed in male Wistar rats (Charles River, Wilmington, MA) with an initial body weight of ~250 g. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and the protocol was approved by the Institutional Animal Care Committee. MI was induced by ligation of the left anterior descending coronary artery under anesthesia with pentobarbital sodium (60 mg/kg ip). The method to create a MI model in rats was described previously (20). Cell transplantation was performed within 30 min after induction of MI. ES cell suspension (30 μl) was injected into three sites, one within the infarct area and two in the myocardium bordering the ischemic area. Each injection was 10 μl of the medium containing beating ES cells (10^4 cells). Medium-treated animals received the same MI operation but were only injected with the equivalent volume of the cell-free medium. The sham group underwent an identical surgery with neither ligation of the coronary artery nor cell transplantation.

**Measurements of hemodynamics and isometric contraction.** Hemodynamics were measured before (baseline) and 6 wk after MI induction. Rats were anesthetized with pentobarbital sodium (60 mg/kg ip). A carotid artery was isolated and cannulated with a 3-Fr high-fidelity microtip catheter connected to a pressure transducer (Millar Instruments, Houston, TX). The Millar Micro-Tip catheter was advanced into the left ventricle to record ventricular pressure for a brief period of time. LV systolic and end-diastolic pressures, the maximum rate of LV systolic pressure rise (+dP/dt max), mean arterial pressure, and heart rate were monitored and recorded on a chart-strip recorder (Gould Series 2000). Analog signals were digitized by use of a data translation series (model DI-220) analog-digital converter (Data Instruments, Akron, OH) and then stored and analyzed on a Windaq data-acquisition system.

After final hemodynamic measurements, the posterior LV papillary muscle was dissected, and isometric contraction of the muscle was evaluated (20). Briefly, LV posterior papillary muscle was carefully dissected in a dissecting chamber containing a modified Krebs-Henseleit solution [in mM: 120 NaCl, 5.9 KCl, 5.5 dextrose, 25 NaHCO3, 1.2 NaH2PO4, 1.2 MgCl2, 1.0 CaCl2; pH 7.4; bubbled with carbogen (a mixture of 95% O2 and 5% CO2)] at room temperature and then fixed to a muscle holder with a spring clip. The tendinous end of the muscle was vertically connected to a strain-gauge tension transducer (model MBI 341, Sensotec, Columbus, OH) with a silk thread. The muscle was then mounted in a 50-ml tissue bath containing modified Krebs-Henseleit solution maintained at 30°C and continuously bubbled with carbogen. The isometric contraction of the papillary muscle was elicited by a punctate platinum electrode with square-wave pulses of 5-ms duration at 0.33 Hz. The voltage was set to 10% above

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**Table 1. General characteristics of sham-operated and MI rats**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI</th>
<th>MI + Medium</th>
<th>MI + ES Cells</th>
</tr>
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<tbody>
<tr>
<td>BW, g</td>
<td>445.7 ± 14.4</td>
<td>423.5 ± 24.7</td>
<td>422.5 ± 15.2</td>
<td>431.9 ± 19.9</td>
</tr>
<tr>
<td>LVW, mg</td>
<td>751.5 ± 19.4</td>
<td>954.5 ± 37.5*</td>
<td>986.5 ± 30.9*</td>
<td>814.0 ± 43.2†</td>
</tr>
<tr>
<td>LVW/BW, mg/g</td>
<td>1.69 ± 0.03</td>
<td>2.55 ± 0.2*</td>
<td>2.34 ± 0.1*</td>
<td>1.89 ± 0.1†</td>
</tr>
</tbody>
</table>

Values are means ± SD (n = 10 for each group). Sham, sham-operated rats without myocardial infarction (MI) induction; MI, postinfarcted rats without treatment; MI + Medium, postinfarcted rats injected with the cell-free medium; MI + ES Cells, postinfarcted rats with embryonic stem (ES) cell transplantation. BW, body weight; LVW, left ventricular weight; LVW/BW, ratio of left ventricular weight and body weight. Measurements were conducted 6 wk after operation. *P < 0.01 vs. Sham. †P < 0.05 vs. MI and MI + Medium.
threshold level. After a 30-min equilibration period, the muscle was carefully stretched to the length at which maximal tension developed. Developed tension (tension produced by the stimulated muscle) was recorded from each muscle at this maximal length. The response to a stepwise increase in the stimulated muscle was recorded from each muscle at the threshold level. After a 30-min equilibration period, the muscle was carefully stretched to the length at which maximal tension developed. Developed tension (tension produced by the stimulated muscle) was recorded from each muscle at this maximal length. The response to a stepwise increase in the stimulated muscle was recorded from each muscle at the threshold level. After a 30-min equilibration period, the muscle was carefully stretched to the length at which maximal tension developed. Developed tension (tension produced by the stimulated muscle) was recorded from each muscle at this maximal length. After a 30-min equilibration period, the muscle was carefully stretched to the length at which maximal tension developed. Developed tension (tension produced by the stimulated muscle) was recorded from each muscle at this maximal length. 

### Table 2. Improvement of left ventricular function after transplantation of ES cells

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI + Medium</th>
<th>MI + ES Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline (before operation)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>131.7 ± 5.9</td>
<td>134.3 ± 6.7</td>
<td>133.2 ± 8.5</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>5.5 ± 0.8</td>
<td>5.8 ± 1.1</td>
<td>5.7 ± 1.1</td>
</tr>
<tr>
<td>+dP/dt, mmHg/s × 10³</td>
<td>9.5 ± 0.6</td>
<td>9.8 ± 0.9</td>
<td>9.9 ± 1.0</td>
</tr>
<tr>
<td><strong>6 weeks after operation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>133.0 ± 7.2</td>
<td>82.3 ± 6.6*</td>
<td>97.4 ± 6.2††</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>5.7 ± 1.0</td>
<td>15.2 ± 2.2*</td>
<td>10.1 ± 1.4††</td>
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<tr>
<td>+dP/dt, mmHg/s × 10³</td>
<td>9.6 ± 0.7</td>
<td>6.3 ± 0.8†</td>
<td>7.9 ± 0.6*</td>
</tr>
</tbody>
</table>

Values are means ± SD (n = 10 for each group). LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; +dP/dt, rate of peak left ventricular systolic pressure rise. Measurements were carried out before and 6 wk after operation.

*P < 0.05, †P < 0.01 vs. Sham. ‡P < 0.05 vs. MI + Medium.

Fig. 2. ES cell transplantation improved left ventricular (LV) function [LV pressure (LVP) and peak LV systolic pressure (+dP/dt)] in post-myocardial infarction (MI) rats. Continuous chart strip recordings of hemodynamic measurement in anesthetized animals [sham-operated rats (A), postinfarcted rats injected with cell-free medium (B), and postinfarcted rats transplanted with ES cells (C)]. Measurement was conducted in rats 6 wk after the MI operation.
Table 3. Echocardiographic measurements of left ventricular function in vivo

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI + Medium</th>
<th>MI + ES Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>PW th, %</td>
<td>72.4 ± 21</td>
<td>41.6 ± 14*</td>
<td>61.3 ± 8§</td>
</tr>
<tr>
<td>AW th, %</td>
<td>65.2 ± 18</td>
<td>40.7 ± 10°</td>
<td>57.8 ± 9%</td>
</tr>
<tr>
<td>LVDd, mm</td>
<td>7.3 ± 0.4</td>
<td>10.0 ± 0.9*</td>
<td>8.2 ± 0.3§</td>
</tr>
<tr>
<td>LVDs, mm</td>
<td>4.6 ± 0.4</td>
<td>7.5 ± 1.1†</td>
<td>5.8 ± 0.7</td>
</tr>
<tr>
<td>En FS, %</td>
<td>36.8 ± 5.4</td>
<td>21.0 ± 2.6†</td>
<td>30.7 ± 4.3%</td>
</tr>
<tr>
<td>MW FS, %</td>
<td>20.6 ± 3.6</td>
<td>13.3 ± 1.8*</td>
<td>17.8 ± 2.6%</td>
</tr>
<tr>
<td>LV mass, g</td>
<td>0.65 ± 0.1</td>
<td>1.10 ± 0.2*</td>
<td>0.81 ± 0.2§</td>
</tr>
<tr>
<td>LVmass/BW, mg/g</td>
<td>1.4 ± 0.2</td>
<td>2.4 ± 0.2*</td>
<td>1.7 ± 0.4‡</td>
</tr>
</tbody>
</table>

Values are means ± SD. Each group has 5 rats. PW th, relative posterior wall thickness; AW th, relative anterior wall thickness; LVDd, left ventricular diastolic dimension; LVDs, left ventricular systolic dimension; En FS, endocardial fractional shortening; MW FS, midwall fractional shortening; LV mass/BW, the ratio of left mass and body weight. *P < 0.05, †P < 0.01 vs. Sham. §P < 0.05 vs. MI + Medium.

with a mouse monoclonal anti-GFP antibody (Zymed, San Francisco, CA), a goat polyclonal IgG anti-cTnI antibody (Santa Cruz Biotechnology, Santa Cruz, CA), or a mouse anti-α-MHC monoclonal antibody (Berkeley Antibody, Richmond, CA) for 60 min. After washing with PBS, sections were incubated with a rabbit anti-goat conjugated rhodamine IgG (for cTnI) or a goat anti-mouse conjugated fluorescein IgG (for α-MHC and GFP) antibody (Pierce Chemical, Rockford, IL). Finally, fluorescent staining for α-actin, α-MHC, and cTnI was detected and photographed under fluorescent microscopy.

**RESULTS**

Improvement of LV function after ES cell transplantation. Six weeks after infarction, the LV weight and ratio of LV weight to body weight were significantly increased in MI rats (P < 0.05 vs. the sham-operated group, Table 1). Intramyocardial transplantation of ES cells in MI rats significantly attenuated the severity of LV hypertrophy (Table 1). The area of infarcted myocardium was reduced from 40 ± 2% for MI rats injected with the cell-free medium to 35 ± 1% for MI rats transplanted with ES cells (P < 0.05).

The baseline values of hemodynamics were not significantly different among the three groups (10 rats per group). However, animals injected with the cell-free medium 6 wk after MI operation had a slower rate of reaching peak LV systolic pressure (dP/dt), a lower LV systolic pressure, and a higher LV end-diastolic pressure compared with those in sham-operated rats and self-baseline values (Table 2 and Fig. 2).
plantation of ES cells significantly improved ventricular contractility reflected by the increase in \( \frac{dP}{dt} \) and LV systolic pressure, as well as by the decrease in LV end-diastolic pressure.

Echocardiographic assessments in vivo were obtained in sham-operated and MI rats (Table 3) 6 wk after the operation. The LV geometry showed significant differences among the MI rats and sham-operated animals. The decrease in LV anterior and posterior relative wall thicknesses was comparable in sham-operated rats and MI rats with medium injection. During systole and diastole, infarcted hearts treated with the cell-free medium showed enlarged LV dimension. The prominent increase in cavity dimension of the infarcted hearts from the MI control group resulted in a significant decrease in anterior and posterior relative wall thicknesses. Both endocardial fractional shortening and midwall fractional shortening were depressed in MI control rats compared with age-matched sham animals. ES cell transplantation significantly blunted the development of LV remodeling with a lower ratio of LV weight to body weight than in MI rats injected with the cell-free medium. These results are similar to the data in Table 1. The LV anterior and posterior relative wall thicknesses were increased in MI rats with ES cell transplantation. Likewise, compared with MI rats injected with the cell-free medium, LV diastolic and systolic dimensions decreased in MI rats with ES cell transplantation. The parallel changes in relative wall thickness and cavity diameter resulted in the improvement of cardiac function.

Improvement of isometric contractility in papillary muscle after ES cell transplantation. At baseline, papillary muscles isolated from MI rats injected with the cell-free medium showed a significant decrease in developed tension (Fig. 3). In animals with intramyocardial injection of ES cells, developed tension appeared to be significantly preserved. Elevation of extracellular Ca\(^{2+}\) levels increased developed tension of papillary muscles isolated from all three groups of rats. The increase in developed tension was concentration dependent. However, the concentration-response curve of developed tension in MI rats injected with the cell-free medium was shifted downward significantly (Fig. 3). \( \beta \)-Adrenergic stimulation with cumulative concentrations of isoproterenol induced a pronounced increase in developed tension in papillary muscles isolated from sham-operated rats (Fig. 3). In contrast, papillary muscles isolated from MI rats injected with the cell-free medium had no positive inotropic response to isoproterenol stimulation. It is surprising that ES cell transplantation significantly partially restored the inotropic response to isoproterenol stimulation (Fig. 3).

Fig. 4. Presence of GFP-positive cells in injured myocardium and in isolated single cardiomyocytes. A: the frozen section was prepared from a MI heart 6 wk after ES cell transplantation. GFP-positive spots were detected under fluorescent microscopy (\( \times \)200). B: under fluorescent microscopy (\( \times \)200), single GFP-positive cells with rod shape and striations were detected in cells isolated from a post-MI heart 6 wk after ES cell transplantation. C: this panel corresponds to B. Isolated single cardiomyocytes from host myocardium were GFP negative with rod shaped and clear striations under light-contrast microscopy (\( \times \)200). Insets in B and C are further enlarged from the areas pointed to by the arrows.
Identification of transplanted ES cells. Frozen sections prepared from MI areas after 6 wk of cell transplantation showed GFP-positive spots under fluorescent microscopy (Fig. 4A). In contrast, sections from sham-operated hearts or MI control areas had no such GFP-positive tissue. In addition, single GFP-positive cells were detected under fluorescent microscopy in cells isolated from MI hearts 6 wk after transplantation (Fig. 4B). The rod-shaped GFP-positive cells had clear striations, which are characteristic of adult cardiomyocytes (Fig. 4, B and C). The isolated host cardiomyocytes were GFP negative (Fig. 4, B and C). In addition, cells isolated from the sham-operated or MI control hearts were unable to detect GFP-positive cells. In cell-transplanted animals, the average GFP-positive cells were $1,158,574 \pm 100,894 \ (n = 5)$ out of a total $15,949,206 \pm 406,249$ myocytes isolated from each MI left ventricle. The percentage of GFP-positive cells was 7.3%. According to our calculation in culture, the efficiency of transfection of GFP was 80–90% in cultured ES cells. Although we did not measure the exact size of GFP-positive cells, we found that the shape and size of mature GFP-positive myocytes did not significantly differ from those of host cardiomyocytes under a microscope (Fig. 4, B and C). However, the small round cell with GFP staining in Fig. 4B might be an immature cardiac cell, as reported by others (6). These results suggest that implanted ES cells not only survived in injured myocardium but also were able to differentiate into mature cardiomyocytes.

Histology and immunostaining of infarcted myocardium. Hematoxylin-eosin staining shows fibrosis in the infarction area in MI control hearts (Fig. 5, B and E) injected with the cell-free medium 6 wk after the MI operation. In ES cell-transplanted MI rats, characteristic phenotype of engrafted cells without evidence of immunorejection was detected in infarct areas (Fig. 5, C and F). Immunostaining confirmed that engrafted cells were distinct from host cardiomyocytes and infarct tissue. Implanted cells stained positively with the monoclonal antibody of antisarcomeric $\alpha$-actin (data not shown). However, the anti-$\alpha$-actin staining was negative in sham-operated and control MI hearts. In addition, engrafted cells stained positively to cardiac $\alpha$-MHC in cell-transplanted myocardium (Fig. 6C). Compared with MI hearts injected with cell-free medium (Fig. 6B), the sections from a MI cell-transplanted heart show much higher intensity of immunostaining for $\alpha$-MHC. To further confirm differentiation of engrafted cells into cardiomyocytes, we did double

![Fig. 5. Photomicrograms of transverse sections from rat left ventricles at the level of the posterior papillary muscle. Endocardium is on the left and epicardium is on the right for each image. Images were taken at low (A–C, \times40) or high magnification (D–F, \times200). A and D are from a rat with sham operation and show normal architecture and histology of myocardium. B and E are from a rat surviving acute MI 6 wk after ligation of the left anterior descending coronary artery and intramyocardial injection with the cell-free medium. The subepicardium was largely replaced by scar tissue, and there was granulation formation on the pericardium. The subepicardium was partially preserved. C and F are from a MI rat 6 wk after transplantation of ES cells. There was disruption of the myocardial architecture, and the subendocardial scar tissue was largely replaced by immature, hypertrophic cardiomyocytes, which might have differentiated from the implanted cells.](http://jap.physiology.org/)

J Appl Physiol • VOL 92 • JANUARY 2002 • www.jap.org
staining for GFP and cTnI in cell-transplanted myocardial sections. Figure 7 shows GFP- and cTnI-positive staining and their overlaps in numerous areas. These results confirm the survival and differentiation of implanted ES cells in injured myocardium.

DISCUSSION

The main findings of the present study are that 1) embryonic stem cells can be implanted and survive in injured rat myocardium and 2) transplantation of ES cells improves global cardiac function and myocardial contractility.

Several studies have demonstrated the feasibility of engrafting exogenous cells into host myocardium, including fetal cardiomyocytes (26, 28), atrial tumor (11), satellite cells (3), or bone marrow cells (29). These engrafted cells have been histologically identified in normal myocardium up to 4 mo after transplantation (3). Gap junctions have been found between the engrafted and host myocardium (8, 10, 28). Recently, myocyte transplantation has been extended into ischemically damaged myocardium with coronary artery occlusion in rats (29) or with cryoinjury in rats (15, 16) and dogs (3). ES cells are pluripotent cells derived from the early embryo and retain the ability to differentiate into all cell types, including cardiomyocytes (24, 25). One of the advantages of using ES cells is to reduce immunoreactivity, because ES cells express less immune-related cell-surface proteins (22). This study provides the evidence for survival of ES cells in injured myocardium after transplantation. Further, the isolated single GFP-positive cells were rod-shaped with clear striations. These characteristics of GFP-positive cells indicate that implanted ES cells, at least part of them, not only survived in injured myocardium, but also differentiated into mature cardiomyocytes after 6 wk of cell transplantation. Therefore, the improvement of ventricular function may result, at least partially, from cardiogenesis of implanted ES cells. This result is consistent with the recent findings of regeneration of infarcted myocardium in mice with transplantation of bone marrow cells (21, 29).

Large MI induced by permanent ligation of the left anterior descending coronary artery results in remark-
able impairment of cardiac function (23). We found that infarct size was reduced in MI rats implanted with ES cells. In addition, ES cell transplantation significantly improved LV function and isometric contractility in MI rats. One possibility for the improvement of cardiac function is a reduction of infarcted area by regeneration of myocardium after transplantation of ES cells. Reduction of the infarct size could prevent overstretching of the ventricle and preserve normal contractile function (Frank-Starling law). This is consistent with a previous report that reduction of chamber size improved heart performance (15, 16). In addition, myocardial regeneration by ES cells may improve global function of infarcted hearts, which provides beneficial effects on papillary muscle contractility. In contrast, papillary muscle contractility was decreased in untreated MI rat hearts, because enlarged failing hearts might overstretch and damage papillary muscles. Our morphological data confirm that engrafted ES cells survived in infarcted myocardium by identification of GFP-positive cells in cell-implanted hearts 6 wk after cell transplantation. GFP-positive cells isolated from post-MI hearts with ES cell transplantation were rod-shaped with clear striations that mimicked adult sarcomeric 

- MHC and double-stained to GFP and cTnI. In contrast, staining for 

- MHC was negative, and the intensity of MHC staining was lower in MI myocardium without ES cell transplantation. Calculation of the number of single GFP-positive cells from total isolated LV myocytes indicates that engrafted cells accounted for 7.3% of LV myocardium 6 wk after MI induction and cell transplantation. These data strongly suggest that cardiogenesis occurred in the infarcted myocardium after ES cell transplantation. Regeneration of myocardium and improvement of cardiac function also occurred in MI mice after intramyocardial implantation of bone marrow cells (21). Approximately 68% of the myocardium in the infarcted portion of the ventricle was newly formed in MI mice 9 days after cell transplantation (21). In addition, Beltrami et al. (1) found that there was myocyte proliferation in human heart after MI and that regeneration of myocytes may contribute to the increase in muscle mass of the myocardium. Therefore, myocardial regeneration by implanted stem cells may play a primary role in the improvement of ventricular function of infarcted failing hearts.

Another possible explanation of the beneficial effects of ES cell transplantation is that ES cells serve as platforms for the release of cardioprotective factors such as vascular endothelial growth factors. In normal porcine hearts, Van Meter et al. (30) showed that transplantation of either human atrial cardiomyocytes or fetal human ventricular cardiomyocytes can induce nascent blood vessel formation in grafted areas and the host ventricle. The increase in microcirculation provides the grafted cells with blood supply and is also an avenue for removal of cellular debris due to primary injury. Subsequently, attenuation of infarct size was also found in the present study. More recently, Tomita et al. (29) found that the number of capillaries in bone marrow cell-transplanted animals was significantly larger than that of the control. Therefore, the reduction of infarct size in MI rats transplanted with ES cells in our experiments may partially result from an improvement of blood supply. If the ischemic zone was reperfused, additional growth factors may reach other regions of the heart. The functional benefits of ES cell transplantation on papillary muscle contractility may relate to the release of growth factors whose positive effects on myocardial contractility have been reported by our laboratory (4) and others (14). Thus the improvement of ventricular function in postinfarcted hearts with ES cell transplantation may result from an increase in the pool of cardiomyocytes and from the paracrine effects of engrafted cells which facilitate the repair of injured cardiac tissue.

In conclusion, this study demonstrates the feasibility of transplanting ES cells into injured myocardium in rats. Transplanted ES cells were able to form stable intramyocardial grafts and to improve cardiac function in postinfarcted failing hearts. Our results raise the possibility that ES cell transplantation may provide a new and novel approach to improve cardiac function after a massive MI.

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