VEGF enhances functional improvement of postinfarcted hearts by transplantation of ESC-differentiated cells

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Abstract

After myocardial infarction (MI), dead myocardium is replaced by noncontractile fibrous scar tissue that leads to ventricular dysfunction. Although significant advances in diagnosis and treatment of cardiovascular diseases have been made in the last several decades, effective therapy for heart failure still remains a great challenge for medical professionals (8). The morbidity and mortality of heart failure are still high in developed countries (39). Limited proliferation of endogenous myocardial cells in infarcted myocardium has been reported (2). However, massive loss of mammalian myocardium is not sufficiently regenerated by the remaining myocytes. Therefore, the search for new and effective therapeutic methods for heart failure patients is warranted. In recent years, cell transplantation has emerged as a novel approach for generation of viable heart muscle cells (5, 19, 21, 37). Experimental data show that transplantation of bone marrow stem cells regenerated functional myocardium and improved ventricular function in animals with MI (15, 30, 40).

Cardiomyocytes derived from embryonic stem cells (ESCs) may be a viable source for donor cardiomyocytes (7). ESCs are pluripotent cells and retain the ability to differentiate in vitro into numerous cell types, including spontaneously contracting cardiomyocytes (18, 25, 36). Differentiation of ESCs to cardiomyogenic cells is accompanied by the expression of a number of cardiac and muscle-specific contractile proteins, including cardiac α- and β-myosin heavy chain (36), α-tropomyosin (29), phospholamban (10), and type B natriuretic factor (4). Klug et al. (17) have reported that transplantation of genetically selected cardiomyocytes from differentiating ESCs formed stable intracardiac grafts. Moreover, Etzion et al. (9) showed that transplantation of cultured cardiomyocytes dissected from embryos prevented the progression of heart failure in rat MI model. Our laboratory’s previous study showed that transplantation of ESCs to injured myocardium improved cardiac function (28).

One of the most important growth and survival factors for endothelium is vascular endothelial growth factor (VEGF). VEGF induces endothelial cell proliferation and angiogenesis. VEGF is a heparin-binding glycoprotein that is secreted as a 45-kDa homodimer (20). The development of new blood vessels, or angio-

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addition, Losordo et al. (24) showed that direct injection of plasmid VEGF DNA (pVEGF<sub>165</sub>) alone improved myocardial blood perfusion in patients with myocardial ischemia. Recently, in vitro, VEGF bone marrow-derived endothelial progenitor cell (EPC) gene transfer has been shown to enhance EPC proliferation, adhesion, and incorporation into endothelial cell monolayers. In vivo, gene-modified EPCs facilitate the strategy of cell transplantation by augmenting naturally impaired neovascularization in an animal model of experimentally induced limb ischemia (13).

Our present study was designed to evaluate whether transplantation of early-differentiated cells (EDCs) from ESCs could survive in injured myocardium and improve cardiac function in MI mice. In addition, we examined whether transplantation of EDCs with overexpression of VEGF produced a greater effect on improvement of cardiac function in MI mice. The effects of transplantation of EDCs alone or EDCs with overexpression of VEGF on neovascularization in ischemic myocardium were also evaluated and compared. This study would provide beneficial evidence of a novel approach to repair injured myocardium and improve impaired cardiac function by transplanting stem cells with overexpression of a vascular growth stimulator.

**METHODS**

Culture of embryonic stem cells. The mouse cell line ES-D3 was obtained from the American Type Culture Collection (Manassas, VA) and maintained in DMEM (GIBCO BRL, Grand Island, NY). The medium was supplemented with 15% fetal bovine serum, 0.1 mM β-mercaptoethanol, and 10<sup>4</sup> U/ml of leukemia inhibitory factor (LIF) (GIBCO BRL). To initiate differentiation, ESCs were dispersed with trypsin and resuspended in the medium without supplemental LIF and were cultured with the hanging-drops (~400 cells per 20 μl) method for 3 days (25, 42). The resulting embryoid bodies were transferred from the hanging drops into 100-mm dishes and cultured for another 5 days. Beating cardiomyogenic clusters were dissected by use of a sterile micropipette (25) and transferred into 100-mm culture dishes for 1–2 days.

Action potentials were recorded in cultured cardiac-like stem cells by the current-clamp technique (43), and cell shortening was measured by the edge-detection method (44). About 10 days after withdrawal of LIF from the conditioned culture medium and dissection of beating clusters, 40–50% of EDCs from mouse ESCs used for cell transplantation were spontaneously beating. The beating cells had spontaneous action potentials recorded by the zero current-clamp technique (Fig. IA). Some nonbeating cells had no spontaneous action potentials but demonstrated elicited action potentials after electrical stimulation (Fig. IB). Figure 1C shows that the increases in extracellular Ca<sup>2+</sup> concentrations enhanced the rate of spontaneous contraction and the amplitude of cell shortening in EDCs. In addition, after 11 days of culture by the hanging-drops method without micropipette dissection of beating clusters, flow cytometry revealed that 26 ± 1.2% (n = 5 runs) of EDCs were cardiac α-myosin heavy chain (α-MHC) positive. These results demonstrated that, after ~10 days in culture, ESCs in the absence of LIF are able, at least a portion of them, to differentiate into cardiac-like cells.

![Fig. 1. Action potentials recorded from embryonic stem cell (ESC)-derived cells. ESCs were cultured for ~10 days after withdrawal of leukemia inhibitory factor (LIF) from conditioned medium. A: spontaneous action potentials were observed with the zero-current clamp method in a representative beating cell. B: action potential was elicited in a nonbeating cell by intracellular injection of depolarizing current. C: changes in extracellular Ca<sup>2+</sup> concentrations altered the rate of spontaneous beats and the amplitude of cell shortening in cultured early-differentiated cells (EDCs) recorded by the edge-detection method.](image-url)

![Fig. 2. Schematic diagram of the ESC culture procedure used to obtain EDCs for cell transplantation in myocardial infarction (MI) animals.](image-url)
Transfection of green fluorescent protein gene. Before cell transplantation, EDCs were transfected with green fluorescent protein (GFP) cDNA to identify the survival of implanted cells. Plasmids with an hCMVIE promoter/enhancer driving GFP gene (5.7 kb) and the GenePORTER transfection reagent were obtained from Gene Therapy System (San Diego, CA). Briefly, EDCs were plated in 100-mm dishes and cultured to 60–90% confluence on the day of transfection. The GFP plasmid DNA (8 μg) was added to each dish with the calcium phosphate precipitation method (45). The GFP transfection efficiency was >90% as detected under fluorescent microscopy. After 2 days of GFP transfection, cultured EDCs were trypsinized and resuspended in Joklik modified medium (Sigma Chemical) with a density of 10^7 cells/ml. Figure 2 shows the entire procedure of cell culture to obtain EDCs for cell transplantation. Stable GFP expression was observed in cultured stem cells for 8 wk (data not shown), which is consistent with the results of a stable expression over 12 mo in cultured cells reported by others (11).

Transfection of phVEGF165. The plasmid containing VEGF cDNA (phVEGF165) was a generous gift from Dr. Kenneth Walsh (St. Elizabeth’s Medical Center, Tufts University School of Medicine, Boston, MA). It is a eukaryotic expression vector that uses the 736-bp cytomegalovirus promoter/enhancer driving VEGF expression (20). The 60–90% confluent EDCs were transfected with 8 μg phVEGF165 per 100-mm dish according to the manufacturer’s protocol (GIBCO BRL). Cells were trypsinized 48 h posttransfection and were resuspended in Joklik modified medium for transplantation. Overexpression of VEGF in cultured EDCs was observed by immunofluorescent assay. In brief, after 48 h of phVEGF165 transfection, EDCs were washed with PBS twice and then fixed in 4% paraformaldehyde. A rabbit anti-human VEGF antibody (Santa Cruz Biotechnology, Santa Cruz, CA) was incubated with the EDCs. A goat anti-rabbit IgG-conjugated fluorescent antibody (Fierce Chemical, Rockford, IL) was used as a second antibody to test for fluorescence. Western blot analysis of VEGF with the previous method (20) also showed a significant increase in VEGF-transfected EDCs.

Animal model of MI and EDC transplantation. The experiments were performed on 8- to 12-wk-old (20–30 g) male Friend leukemia virus, strain B mice (Charles River, Wilmington, MA). Myocardial infarction was induced by ligation of the left anterior coronary artery as described previously (28). Briefly, animals were anesthetized by intraperitoneal injection of pentobarbital sodium (40 μg/g body wt). A midline cervical skin incision was made, and an endotracheal tube was placed in the trachea. A lateral incision between the fourth and fifth ribs was made to open the chest. A rodent ventilator (Harvard Apparatus, Holliston, MA) was connected to the endotracheal tube to maintain animal respiration before opening of the chest. The heart was oriented to better expose the left main coronary artery system. Ligation proceeded with a 6-0 silk suture passed with a tapered needle underneath the left anterior coronary artery, ~2 mm posterior to the tip of the normally positioned left auricle. Experimental animals were randomized for each group. Fifteen minutes after MI induction, the EDC suspension (3 × 10^5 in 30 μl) was separately injected into three different sites (10 μl/per site) for each MI heart in the cell-transplanted group with a microliter syringe (Harvard Apparatus). Two injection sites were in the myocardium bordering the ischemic area and one within the ischemic area. Another MI group was transplanted with the same amount of cells with overexpression of VEGF in the same fashion as described above. Control MI animals received the same MI operation but were only injected with an equivalent volume of the cell-free medium. The sham group underwent the identical surgery with nei-
ther ligation of the coronary artery nor cell transplantation. The experimental protocol was approved by the Animal Care Committee of Beth Israel Deaconess Medical Center and was performed according to the Guide for the Care and Use of Laboratory Animals published by the U. S. National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Measurement of hemodynamics and isometric contraction of papillary muscles. Six weeks after MI operation and cell transplantation, hemodynamic measurements in vivo were performed with the methods described previously (28). After the measurements, the heart was rapidly removed from the killed mouse. Left ventricular papillary muscle strips were dissected and vertically connected to a strain-gauge tension transducer. Developed tension of muscle strips was recorded at their maximal length. The bath solution contained a modified Krebs-Henseleit solution and different concentrations of isoproterenol ($10^{-6}$, $10^{-5}$, and $10^{-4}$ M). The inotropic response of dissected ventricular papillary muscles from MI hearts to β-adrenergic stimulation was evaluated in MI control and cell-transplanted MI mice.

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Fig. 5. Hematoxylin and eosin staining of the cardiac sections obtained from a sham-operated mouse heart (A), a MI mouse heart injected with cell-free medium (B), and a MI mouse heart transplanted with EDCs (C). Panels for magnification ×40 and ×200 are from the corresponding square areas in the panels of ×10 and ×40, respectively. D: GFP-positive spots were observed under fluorescent microscopy (×100) in the myocardium sectioned from a MI heart with transplantation of GFP-transfected EDCs.
Histological and immunofluorescent analysis. The subsets of animals were killed 6 wk after MI induction. After quick removal of the hearts, the free wall of the left ventricle including the infarcted and peri-infarcted regions were embedded in tissue freezing medium (Fisher Scientific, Fair Lawn, NJ). Frozen tissue was sectioned to 10-μm slides and stained with hematoxylin and eosin. Survival of engrafted cells was confirmed by identification of GFP-positive spots under fluorescent microscopy.

To identify regenerated myocytes from engrafted EDC derived cardiomyocytes, we used an immunofluorescent technique to detect cardiac troponin-I (cTn-I) and α-MHC, two protein markers of myocardium. Frozen tissue sections were fixed in acetone for 10 min and then dried in air. Nonspecific binding was blocked by incubation in 1% bovine serum albumin in PBS. The samples were then reacted with an antitroponin-I antibody (goat polyclonal IgG, Santa Cruz Biotechnology) or a mouse anti-α-MHC monoclonal antibody (Berkeley Antibody, Richmond, CA) for 1 h. After washing with PBS, sections were incubated with a rabbit anti-goat conjugated rhodamine IgG (H + L) for cTn-I or a goat anti-mouse conjugated fluorescein IgG for α-MHC (Pierce Chemical). Fluorescent immunostaining for cTn-I and α-MHC was examined and photographed under fluorescent microscopy.

Double staining for GFP (Zymed Laboratories, San Francisco, CA) and connexin-43 (CX-43, Sigma Chemical) was carried out with mouse anti-GFP and rabbit anti-CX-43 antibodies in myocardial frozen sections to verify the formation of gap junctions in cell-transplanted myocardium. GFP labeling was detected with a goat anti-mouse antibody conjugated to FITC (Pierce Chemical). CX-43 labeling was detected with a goat anti-rabbit antibody conjugated to Texas Red (Vector Laboratories, Burlingame, CA). Fluorescent microscopy was applied to analyze immunofluorescent labeling of engrafted cells in injured myocardium with the antibodies recognizing GFP and CX-43.

Assessment of angiogenesis. Immunohistochemical staining for blood vessel endothelial cells was conducted by use of the anti-von Willebrand factor (vWF, DAKO LSAB Kit, DAKO, Carpinteria, CA) antibody to evaluate EDC-induced angiogenesis in infarcted myocardium. After tissue fixation in acetone, frozen sections were treated with 3% hydrogen peroxide for 5 min and then dried in air. Nonspecific binding was blocked by incubation in 1% bovine serum albumin in PBS. The samples were then reacted with an antitroponin-I antibody (goat polyclonal IgG, Santa Cruz Biotechnology) or a mouse anti-α-MHC monoclonal antibody (Berkeley Antibody, Richmond, CA) for 1 h. After washing with PBS, sections were incubated with a rabbit anti-goat conjugated rhodamine IgG (H + L) for cTn-I or a goat anti-mouse conjugated fluorescein IgG for α-MHC (Pierce Chemical). Fluorescent immunostaining for cTn-I and α-MHC was examined and photographed under fluorescent microscopy.

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Microscopy (×200 magnification). The number of capillaries in each section was presented as mean ± SE of blood vessels per unit area (mm²) for normal myocardium and infarcted areas with or without EDC transplantation.

**Data analysis.** The data are expressed as means ± SE. Statistical significance between two groups was determined by paired or unpaired Student’s t-test. Results for more than two experimental groups were evaluated by one-way ANOVA to specify differences between groups. A P value < 0.05 was considered significantly different.

**RESULTS**

*Improvement of myocardial contractility after EDC transplantation.* Six weeks after MI induction, hemodynamic measurements showed that the MI mice injected with the cell-free medium had a lower left ventricular (LV) systolic pressure (LVSP, P < 0.01), a higher LV end-diastolic pressure (LVEDP, P < 0.01), and a slower rate to reach peak LV systolic pressure (+dP/dt, P < 0.01) than those in sham-operated mice (Fig. 3 and Table 1). However, EDC implantation significantly improved LV function at 6 wk after MI induction and cell transplantation. Myocardial contractility reflected by the parameters of LVSP, LVEDP, and +dP/dt was significantly increased in cell-transplanted mice (P < 0.05 vs. MI control).

The developed tension of isolated papillary muscles was similar at baseline for the sham-operated mice and for the MI mice with or without cell transplantation (Fig. 4). β-Adrenergic stimulation with different concentrations of isoproterenol significantly increased the developed tension of papillary muscles isolated from the sham-operated mice (Fig. 4, Sham). In contrast, papillary muscles isolated from MI mice with intramyocardial injection of the cell-free medium did not respond well to β-adrenergic stimulation (Fig. 4, MI + Medium). However, papillary muscles isolated from MI mice transplanted with EDCs responded remarkably well to isoproterenol stimulation (Fig. 4, MI + EDCs), especially at 10⁻⁴ M concentration of β-adrenergic agonist (P < 0.05 vs. MI + Medium). These results indicate that intramyo-
cardiac transplantation of EDCs partially preserved the contractility of the LV papillary muscles.

**Histological analysis.** Hematoxylin and eosin staining shows the survival of engrafted cells in injured myocardium 6 wk after MI induction and cell transplantation (Fig. 5). Moreover, GFP-positive tissue was detected under fluorescent microscopy in frozen tissue sections prepared from MI hearts at 6 wk after MI induction and cell transplantation (Fig. 5D). These cells remained localized around the sites of injection because cross sections from other areas of the heart did not show any GFP-positive cells. Figure 6 shows that intensive immunostaining for α-MHC was observed in normal myocardium (Fig. 6A) and in infarcted myocardium with EDC transplantation (Fig. 6C). In contrast, the intensity of immunostaining for α-MHC was much lower in MI areas injected with the cell-free medium (Fig. 6B). The results of immunostaining for cTn-I were similar to those for α-MHC (data not shown). The results indicate that engrafted cells were able to regenerate myocardial tissue in injured hearts.

In addition, the amount of CX-43 was higher in normal myocardium (Fig. 7A) than in infarcted myocardium with medium injection (Fig. 7B), but GFP stained negatively in both the normal myocardium and MI hearts with medium injection (data not shown). However, double staining for GFP and CX-43 was positive in the injured myocardium with EDC transplantation (Fig. 7, C–E). The presence of connexin-43 points to cellular coupling and functional competence of the cells (37). Because GFP is a 238-amino acid polypeptide (~27 kDa) and gap-junction channels admit passage of small molecules (~1 kDa), it is impossible for GFP to cross connections with neighboring cells (32, 33). Also, GFP as an indicator to trace engrafted cells has successfully been used by other groups (30). These results demonstrate that engrafted cells not only survived in injured myocardium but also reached the functional and morphological competence of the cardiac muscle phenotype.

**Effects of transplantation of VEGF-overexpressed EDCs on heart function.** Application of VEGF improves myocardial blood perfusion by increasing collateral blood vessels in patients with myocardial ischemia (1, 24, 38). To test whether transplantation of EDCs overexpressing VEGF into injured myocardium would even further improve cardiac function, we transfected such cells with a VEGF_165 cDNA and implanted the VEGF-overexpressed EDCs into MI hearts. Figure 8 shows
that cultured EDCs expressed a certain level of VEGF detected by immunofluorescent staining (Fig. 8A). After the human VEGF165 gene was transfected into EDCs for 2 days, we observed high-intensity immunofluorescence of VEGF in transduced EDCs, indicating overexpression of the growth factor (Fig. 8B). Western blot analysis further confirmed that VEGF was increased threefold in cultured EDCs transfected with VEGF cDNA (Fig. 8C). In addition, experiments in vivo showed that improvement of LV function was significantly greater in MI mice transplanted with EDCs plus VEGF than in MI animals transplanted with EDCs alone. The differences of LVSP (P < 0.05) and LVEDP (P < 0.05) were statistically significant between the two groups (Fig. 9).

The effects of engrafted cells on neovascularization in the injured myocardium were evaluated by immunohistochemical analysis. An anti-vWF (a marker of endothelial cells) antibody was applied to confirm new blood vessels in the infarcted area. Compared with normal myocardium (Fig. 10A), the amount of vWF staining dramatically decreased in infarcted myocardium sectioned from the MI heart injected with the cell-free medium (Fig. 10B). However, transplantation of EDCs markedly increased the amount of vWF staining in infarcted myocardium. Figure 10, C (EDCs alone) and D (EDCs-VEGF), clearly shows neovascularization in the infarcted area with engrafted cells. In addition, we calculated capillary density and compared the differences among animals with various treatments. The number of capillaries was significantly greater (P < 0.001) in injured myocardium with EDC transplantation than in MI-medium hearts. Moreover, the capillary density in the EDCs-VEGF group was not only significantly greater (P < 0.001) than in the MI control mice but also the EDC group (Fig. 11). These results demonstrate that transplantation of EDCs with overexpression of VEGF produced a greater improvement of cardiac function in MI mice and that the better outcome may result from a stronger angiogenesis effect.

**DISCUSSION**

The data in the present study demonstrated that transplanted EDCs survived and differentiated, at least part of them, into cardiomyocytes and significantly improved cardiac function in MI mice. The improvement of cardiac function was even greater in the MI hearts transplanted with EDCs overexpressing VEGF. In addition, EDCs themselves expressed certain amounts of VEGF and were able to stimulate the growth of new blood vessels in injured myocardium. The angiogenesis effect was even stronger in infarcted myocardium when engrafted EDCs were transfected with VEGF. In our previous study, evidence shows that engrafted ESCs further proliferated and differentiated in vivo after cell transplantation (28). By calculation of single cardiomyocytes isolated from cell-transplanted MI hearts, the number of GFP-positive cells is at least fourfold greater (markedly underestimated) than the original cells implanted into the hearts. In this study, we speculate that further proliferation and differentiation might also occur in vivo, because the ventricular wall of the MI area with cell transplantation was significantly thicker compared with that in the MI control animals. However, although ~50% of cells used for cell transplantation in our experiments were cardiac-like cells, we did not find formation of teratomas in the EDC-transplanted hearts. This is consistent with the results reported by others with transplantation of cardiomyocytes differentiated from ESCs (17) or dissected from embryos (9). In view of significant improvement of cardiac function in the present study and our laboratory’s previous one (28), transplantation of mixed cells may demonstrate better effects in MI animals, because regeneration of cardiac tissue requires different types of cells. Therefore, EDCs may be an
important cell source for cell therapy in patients with MI-induced heart failure in the future.

Coronary bypass and heart transplantation are some alternatives to treat end-stage heart failure. Myocardial fibrosis and organ shortage, along with strict eligibility criteria, mandate the search for new approaches to treat the disease. Transplanted cardiomyocytes have been shown to survive, proliferate, and connect with the host myocardium (37). Engrafted cells may generate new cardiomyocytes to replace infarcted myocardium or serve as a source for therapeutic gene transfer to infarct areas (19). Li and co-workers (22, 23) demonstrated that transplanted fetal cardiomyocytes could form new cardiac tissue within the myocardial scar and significantly improve heart function. Bishop et al. (3) reported that embryonic myocardium dissected from pregnant rats could be implanted or cultured. In a recent review, Hescheler et al. (12) pointed out that pluripotent ESCs cultivated within embryonic

Fig. 10. Positive immunostaining for blood vessel endothelial cells by anti-von Willebrand factor (vWF) antibody in mouse myocardial sections. Left and right panels show ×200 and ×400 magnification, respectively. A: vWF staining (red) in a normal myocardial section demonstrates normal blood vessel distribution in a sham-operated mouse heart. B: vWF staining was significantly reduced in the infarcted myocardium injected with the cell-free medium. Sporadic vWF staining indicates few blood vessels in the MI region. C: transplantation of EDCs alone increased vWF staining in the infarcted myocardium. D: transplantation of EDCs with overexpression of VEGF increased more vWF staining in the infarcted myocardium.

Fig. 11. Effects of EDC transplantation on capillary density in infarcted myocardium. The average number of blood vessels is shown for sham (n = 8), MI + Medium (n = 7), MI + EDCs (n = 8), and MI + EDCs-VEGF (n = 7). **P < 0.001 vs. Sham; ##P < 0.001 vs. MI + Medium; ††P < 0.001 vs. MI + EDCs.
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 ESCs (16, 25). Transplanted bone marrow cells differentiate into new cardiomyocytes in cryoinjured myocardium (40) and in infarcted hearts (14, 30). In addition, transplantation of cardiomyocytes dissected from 15-day-old embryos attenuated LV dilation, infarct thinning, and myocardial dysfunction in an extensive MI rat model (9). Other studies also show that cell therapy could attenuate deleterious ventricular remodeling and improve cardiac performance in MI animals (15, 28). Moreover, gap junctions have been found between the engrafted fetal cardiomyocytes and the host myocardium (34, 35, 37), raising the possibility of electrical-contraction coupling between transplanted cells and the host tissue. Therefore, engrafted cells can restore damaged cardiac function.

We found that EDC transplantation significantly improved LV function and isometric contractility in post-MI mice. One possibility for the improvement of ventricular function is a reduction of infarct area by regeneration of myocardium from engrafted EDCs. Reduction of infarct size could prevent overstretching of the ventricle and preserve muscle contractile function (Frank-Starling Law). It has been reported that a reduction of chamber size improves cardiac performance (22). Our morphological data confirm that the engrafted cells survived in injured myocardium by identification of GFP-positive cells within implanted hearts at 6 wk after MI induction and cell transplantation. The intensive immunostaining for cTn-I and α-MHC in cell-transplanted MI hearts indicated differentiation and maturity of engrafted cells in injured myocardium. In contrast, both cTn-I and α-MHC were stained at a lower level in infarcted myocardium with medium injection. Previously we have observed the colocalization of GFP and cTn-I in injured myocardium with ESC transplantation (28). Furthermore, positive double staining for GFP and CX-43 in injured myocardium with EDC transplantation indicated possible formation of morphological and functional connections among engrafted and host heart cells. These results were consistent with the recent findings that transplantation of bone marrow cells generated large amounts (68%) of myocardium in infarcted mice (30).

Another beneficial effect of EDC transplantation is that these engrafted cells may induce angiogenesis in ischemically injured myocardium. In the present study, we found that transplantation of EDCs alone or EDCs plus VEGF significantly increased the density of capillary blood vessels in infarcted myocardium. In pig experiments, Van Meter and colleagues (41) showed that transplantation of human cardiomyocytes induced the growth of new blood vessels in the grafted area and host myocardium. The increase in microcirculation could provide the grafted cells with a blood supply and remove cellular debris after myocardial injury. More recently, Tomita et al. (40) counted the number of capillaries in the scar tissue. They found that the number of capillaries in the bone marrow cell transplanted group was significantly larger than that of the control group. Thus the improvement of LV function in postinfarcted failing hearts after cell transplantation might result from regeneration of cardiomyocytes and blood vessels. Subsequently, this regeneration attenuated infarct size and improved heart function.

Therapeutic angiogenesis is the controlled stimulation of collateral formation to reduce the unfavorable effects in ischemic tissue (6). Improvement of myocardial perfusion with administration of VEGF protein in porcine models has been demonstrated (31). VEGF gene therapy is particularly appealing because the VEGF gene encodes a signal sequence that permits the protein to be naturally secreted by intact cells (20). Previous studies demonstrated that both arterial and intramuscular gene transfer of naked DNA encoding VEGF resulted in significant improvement in neovascularization (1, 24, 38). We have gone another step by combining application of EDCs and VEGF in infarcted myocardium. Our data show that EDCs-VEGF transplantation provided an even more effective approach to improve cardiac function in postinfarcted failing hearts. After intramyocardial transplantation, these cells might communicate with their surrounding tissue, signaling the formation of blood vessels to nourish them. The capillary density was significantly higher in the EDC-treated MI animals than in the MI control mice. The difference between the EDCs and EDCs-VEGF groups was significant. This difference might enhance graft survival and account for the significantly greater improvement of ventricular function in the two groups. In addition, the increase in capillary density, if it occurs in a clinical setting, might be of significance for the quality of life in patients with heart failure.

Our present study shows that EDC transplantation was not only able to regenerate injured myocardium but also able to improve cardiac function in post-MI animals. Adding the VEGF gene to EDCs further enhanced the beneficial effects of cell transplantation in postinfarcted hearts. This synergistic approach resulted in a stronger neovascularization in ischemic area and greater improvement of damaged heart function. Our data may provide useful information for future clinical cell transplantation in patients suffering from heart failure after myocardial infarction.

REFERENCES


