Homing of intravenously infused embryonic stem cell-derived cells to injured hearts after myocardial infarction

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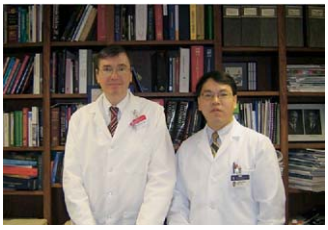

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Objective: The present study was designed to test whether intravenously infused embryonic stem cell-derived cells could translocate to injured myocardium after myocardial infarction and improve cardiac function.

Methods: Cultured embryonic stem cell-derived cells were transfected with green fluorescent protein. Embryonic stem cell-derived cells were administered through the tail vein (approximately 10^7 cells in 1 mL of medium for each rat) every other day for 6 days in 45 rats after myocardial infarction. Six weeks after myocardial infarction and cell infusion, cardiac function, blood flow, and the numeric density of arterioles were measured to test the benefits of cell therapy. An in vitro Transwell assay was performed to evaluate the embryonic stem cell migration.

Results: Ventricular function, regional blood flow, and arteriole density were significantly increased in rats receiving intravenously infused embryonic stem cell-derived cells compared with control rats after myocardial infarction. Histologic analysis demonstrated that infused embryonic stem cell-derived cells formed green fluorescent protein-positive grafts in infarcted myocardium. Additionally, positive immunostaining for cardiac troponin I was found in hearts after myocardial infarction receiving embryonic stem cell-derived cell infusion that corresponded to the green fluorescent protein-positive staining. The Transwell migration assay indicated that cultured neonatal rat cardiomyocytes with overexpression of tumor necrosis factor α induced greater migration of embryonic stem cells compared with cardiomyocytes without tumor necrosis factor α expression.

Conclusions: Our data demonstrate that intravenously infused embryonic stem cell-derived cells homed to the infarcted heart, improved cardiac function, and enhanced regional blood flow at 6 weeks after myocardial infarction. The in vitro migration assay suggested that such a homing mechanism could be associated with locally released cytokines, such as tumor necrosis factor α, that are upregulated in the setting of acute myocardial infarction and heart failure.

In recent years, cell transplantation has emerged as a potential therapy for heart failure caused by myocardial infarction (MI).[1-6] Initial efforts at cellular cardiomyoplasty have transplanted satellite cells,[1] skeletal myoblasts,[2] bone marrow-derived cells,[5] and fetal cardiomyocytes.[6] Embryonic stem cells (ESCs), which have better plasticity and cardiomyogenic capacity than the cell types listed above, have also been successfully transplanted into infarcted

Abbreviations and Acronyms

cTnI	= cardiac troponin I
DAPI	= 4′-6-diamidino-2-phenylindole
+dP/dtmax	= maximum rate of LV systolic pressure increase
−dP/dtmax	= maximum rate of LV systolic pressure decrease
EDC	= embryonic stem cell-derived cell
ESC	= embryonic stem cell
GFP	= green fluorescent protein
LV	= left ventricular
LVSP	= left ventricular systolic pressure
MI	= myocardial infarction
TNF	= tumor necrosis factor

rodent hearts[3,4] and the left ventricular (LV) wall of dystrophic mice.[7] Most of the previous studies delivered donor cells through intramyocardial injection after cardiac surgery. The advantage of this approach is that it traps implanted cells in selected injured areas of the heart. However, the procedure of intramyocardial injection is invasive and might not be suitable for patients with acute MI or severe congestive heart failure. Recently, Chiu and colleagues[8,9] demonstrated the feasibility of delivering bone marrow stromal cells with coronary infusion and indicated that marrow stromal cells could traffic through the coronary system into injured myocardium and form cardiomyocytes. Mobilized bone marrow cells, stimulated by stem cell factor or granulocyte colony-stimulating factor, have been shown to repair the infarcted mouse heart and improve ventricular function.[10] It appears that somatic stem cells can migrate to heart tissue and further differentiate into cardiomyocytes.[11]

MI is associated with inflammatory responses that include upregulation of mast cells, macrophages, and associated inflammatory cytokines. Experimental MI is also associated with activation of a series of cytokines.[12] Mast cell-derived tumor necrosis factor α (TNF-α) released after myocardial ischemia represents an "upstream" cytokine responsible for initiating the inflammatory cascade.[12] Released cytokines from injured myocardium might act as chemoattractants for circulating donor cell migration. Our previous study indicated that mouse ESC-derived cells (EDCs) infused intravenously were able to migrate into injured myocardium caused by encephalomyocarditis virus[13] and increased the survival rate of recipient mice.

The present study was designed to investigate whether intravenously infused EDCs could translocate to injured myocardium in response to locally released cytokines after MI and improve cardiac function. An in vitro culture system was used for testing whether TNF-α, an inflammatory cytokine that is upregulated in the setting of acute MI and heart failure, could facilitate EDC migration responding to cytokine stimulation.

Materials and Methods

EDC Preparation and Transplantation

The mouse ESC line ES-D3 was purchased from the American Type Culture Collection (Manassas, Va) and cultured with the handing drops method, as previously described.[3,4] Before transplantation, cells dissected from beating clusters were transfected with green fluorescent protein (GFP), a marker for identification of infused cells from host myocardium. Plasmids with an hCMV IE promoter/enhancer-driving GFP gene (5.7 kb) and Gene PORTER transfection reagent were obtained from Gene Therapy Systems Inc (San Diego, Calif). GFP-labeled EDCs could be detected under fluorescent microscopy at the second day of transfection, and the transfection efficiency was greater than 90% (Figure 1). Our previous study demonstrated that the action potentials recorded from spontaneously beating EDCs are very similar to those recorded in neonatal mouse cardiomyocytes.[3] Two days after GFP transfection, cultured EDCs were trypsinized and resuspended in Joklik modified medium (Sigma, St Louis, Mo), with a density of approximately 10^7 cells/mL for cell infusion.

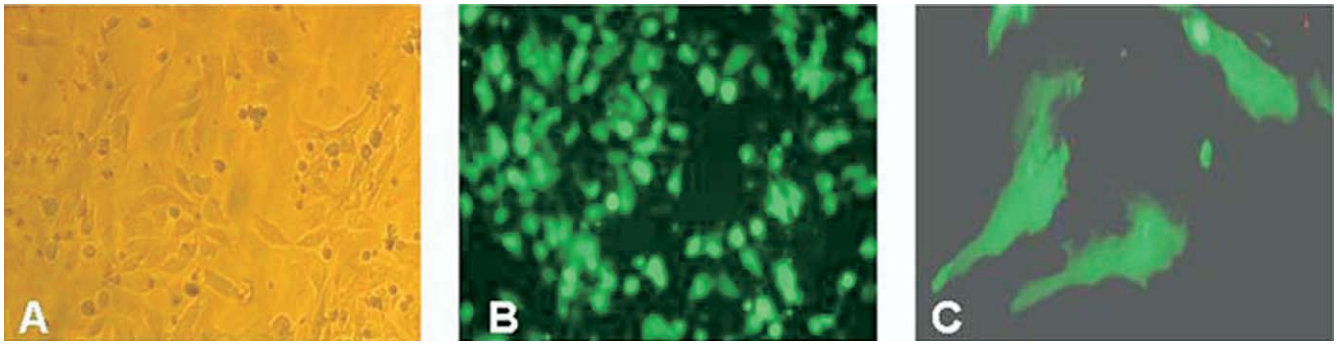

Figure 1. Confluent culture of EDCs under phase-contrast (A, original magnification 40×) and fluorescent (B, original magnification 40×; C, original magnification 400×) microscopy at 3 days after GFP transfection.

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Experiments were performed in 45 Wistar male rats (Charles River Laboratories, Wilmington, Mass) aged 3 months. The investigation conformed to the "Guide for the care and use of laboratory animals" published by the US National Institutes of Health (publication no. 85-23, revised 1996), and the protocol was approved by our Institutional Animal Care Committee. MI was created by means of ligation of the left coronary artery during anesthesia with pentobarbital (60 mg/kg administered intraperitoneally), as previously described.[3,4] Approximately 20 minutes after ligation of the coronary artery, EDCs were administered through the tail vein (approximately 10^7 cells in 1 mL of medium for each rat) every other day for 6 days in experimental rats after MI. Control rats after MI received the same operation but were only infused with the equivalent volume of the cell-free medium. The sham group underwent an identical operation with neither ligation of the coronary artery nor cell transplantation.

Measurements of Cardiac Function and Infarct Size

Hemodynamic measurements (8 per group) in vivo were performed with a modified method, as described previously,[3,4] at 6 weeks after MI and cell infusion. The Millar catheter was carefully advanced into the left ventricle through the carotid artery. The LV systolic pressure (LVSP), the LV end-diastolic pressure, the maximum rate of LV systolic pressure increase (+dP/dtmax), and the maximum rate of LV systolic pressure decrease (−dP/dtmax) were recorded on a computer and analyzed by using a PowerLab data-acquisition system (model ML820; ADInstruments, Colorado Springs, Colo). The rat heart was harvested after hemodynamic measurement, weighed, and normalized by body weight. Subsequently, the hearts were transversely sectioned into 4 pieces from the apex to the base and prepared for infarct size measurement and histologic study.[3,4]

Measurements of Regional Blood Flow and Numeric Density of Arterioles

In another set of animals (7 per group), stable isotope-labeled microspheres (15 μm; BioPAL Inc, Worcester, Mass) were used to determine the regional blood flow in anesthetized rats. The method was modified from a previous publication.[14] In brief, a set of microspheres (1.25×10^6 in 0.5 mL) was diluted in 0.5 mL of sanSaline saline (BioPAL Inc) and injected into the left atrium over 10 seconds. Reference blood samples were withdrawn by using a standard syringe pump (model PHD 2000; Harvard-Apparatus, Holliston, Mass) at a constant rate of 2-minute intervals through the femoral artery, resulting in a 2-mL sample used to calculate absolute myocardial blood flow. The rat heart was then harvested after achievement of anesthesia. The left ventricle was surgically isolated and cut into transmural slices, which were further subdivided into transmural segments. Each segment contained approximately equal concentrations of the endocardium and epicardium. The average myocardial sample weighed approximately 0.15 g. Finally, the tissues and blood samples were shipped to BioPAL Inc for measurement of isotope microspheres and determination of myocardial blood flow. In addition, the numeric density of arterioles larger than 20 μm in diameter was counted in each area observed (5 rats for each group and 5 random high-power fields in each rat) on hematoxylin and eosin-stained slides under light microscopy at 400× magnification. The number of arterioles in each section was averaged and expressed as the number of arterioles per square millimeter.

Histologic Study to Identify Donor Cells

The survival of engrafted EDCs was identified by using GFP-positive tissues in paraffin-embedded sections made from rat hearts after MI and cell infusion. Transverse sections were also made across the major axis of the liver, lung, kidney, thymus, and spleen in each rat. The hearts were sectioned at 5-μm thickness and stained with hematoxylin and eosin. Survived infused cells were confirmed by means of identification of GFP expression under fluorescent microscopy and immunostaining for GFP antibody (Abcam, Cambridge, Mass). The secondary antibody was anti-rabbit IgG horseradish peroxidase, and the color was produced with diaminobenzidine (DAKO, Carpinteria, Calif). Endogenous peroxides were blocked with DAKO peroxide-blocking reagent (DAKO). Rabbit IgG was used as an isotype control. New differentiated cardiac tissues from infused EDCs were verified by means of positive immunostaining of goat anti-cardiac troponin I (cTnI) polyclonal antibodies (Santa Cruz Biotechnology Inc, Santa Cruz, Calif). Positive stains were recognized by adding secondary antibodies of chicken anti-goat IgG 594 (Molecular Probes, Eugene, Ore). Nuclei were identified by means of 4′-6-diamidino-2-phenylindole (DAPI) staining. Nonspecific binding was blocked through incubation with protein-blocking solution (DAKO). We then estimated the area of GFP-positive spots in each sample of myocardial sections (5 rat hearts received EDC infusion) and counted the GFP-positive spots in eight 200× fields that covered the whole heart section. The percentage of surviving cells was calculated by dividing the estimated total area of the left ventricle by the area of GFP-positive spots by using a National Institutes of Health imaging system. The resulting percentage was assumed to be the percentage of surviving cells in the heart that derived from donor stem cells previously infused.

ESC Migration Assay In Vitro

Migration of ESCs was assessed in Transwell plates of 6.5-mm diameter with 5-μm pore filters (Figure 2, *A*). Mouse embryonic fibroblast feeder cells (STO, American Type Culture Collection) were plated at 3×10^4 cells/well on fibronectin-coated filters. The adherent cells were cultured for 2 days to obtain confluent STO monolayers. Each lower compartment at the bottom was plated with neonatal rat myocytes. Myocytes in the treated group were transfected with TNF-α cDNA. Cultured neonatal rat myocytes (80% confluence) plated in TI-75 flasks were transfected with TNF-α cDNA (5 μL, 9.9×10^{11} particles/mL). The content of TNF-α released by cardiomyocytes into the culture medium was measured with a commercial enzyme-linked immunosorbent assay kit specifically designed for detection of mouse TNF-α (R&D Systems, Inc, Minneapolis, Minn; n = 7). Before adding ESCs to the upper compartments, STO monolayers were treated with mitomycin-C (10 g/mL) for 90 minutes and washed 3 times with culture medium. GFP-tagged ESCs (6×10^4 cells) were added to each upper

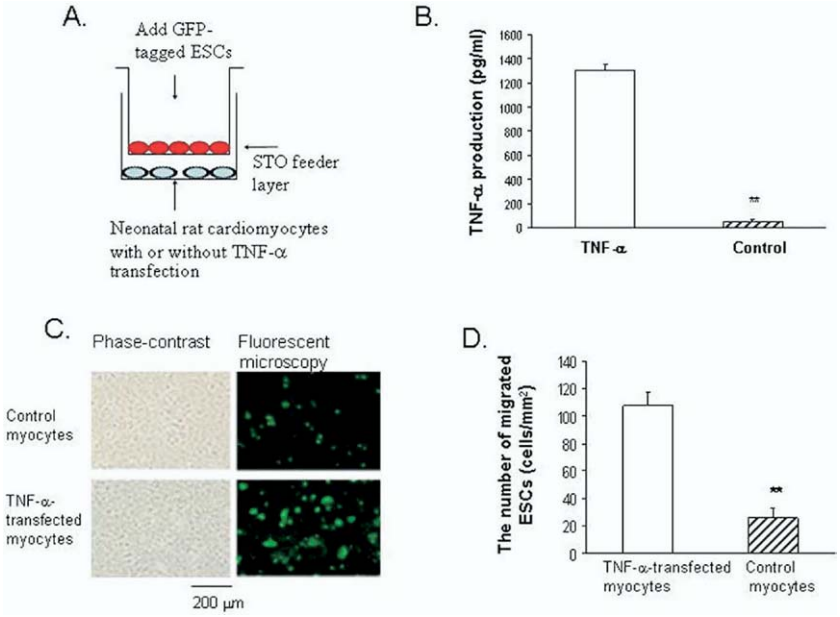

Figure 2. Diagram of embryonic stem cell *(ESC)* migration assay with Transwell plates (A). Each lower compartment of Transwell plates contained one 3-mm glass cover slip plated with neonatal rat myocytes. Green fluorescent protein *(GFP)*-tagged ESCs were added to the upper compartments of the Transwell dishes and cocultured with myocytes for 24 hours. Migrated GFP-positive cells on each cover slip were counted during fluorescent microscopy for control and tumor necrosis factor α (TNF-α)–transfected myocytes. Efficient production of TNF-α in control and TNF-α transfected myocytes from the lower compartments of the Transwell is shown in panel B. Panel C demonstrates that more GFP-positive ESCs were detected on the cover slips seeded with TNF-α-transfected myocytes than on those with control myocytes, which indicated that greater ESC migration responded to TNF-α overexpression. The average GFP-positive cells in the lower compartments that were migrated from the upper compartments at 24 hours after culture are shown in panel D. *TNF-α*, The group of TNF-α-transfected neonatal rat myocytes; *Control*, the group of neonatal rat myocytes without TNF-α transfection. **$P < .01$, Control group versus TNF-α group.

compartment. Twenty-four hours after incubation, the number of migrated GFP-positive cells in the lower compartments (n = 5 runs) was counted during fluorescent microscopy.

Data Analysis

All values are presented as means ± standard deviation. Data derived from 3 groups in the animal study with repeated measurements were evaluated by means of 1-way analysis of variance (ANOVA). If ANOVA showed a significant difference, an unpaired Student *t* test with the Bonferroni correction was used to compare 2 individual groups. The results of migration assay between 2 individual groups were compared by using the unpaired Student *t* test.

Results

Improvement of Cardiac Function and Blood Perfusion After Intravenous Infusion of EDCs

Six weeks after intravenous infusion of EDCs, the ratio of heart weight to body weight was significantly increased in the MI control and MI-EDC infusion groups compared with that seen in the sham-operated group. Cell therapy in rats after MI partially attenuated not only the severity of cardiac hypertrophy but also the infarcted area versus that seen in control rats after MI (Table 1). Ad-

TABLE 1. General characteristics of sham-operated rats and rats after MI after EDC infusion

	Sham	MI + control	MI + IV EDCs
BW (g)	432 ± 12	411 ± 15	421 ± 18
HW (g)	1.0 ± 0.1	1.3 ± 0.4	1.2 ± 0.3
HW/BW*100	0.23 ± 0.03	0.32 ± 0.04*	0.28 ± 0.03*†
HR (beats/min)	390 ± 26	402 ± 27	411 ± 30
Infarct size (%)		41 ± 6	32 ± 4†

Values are presented as means ± standard deviation. Measurements were conducted at 6 weeks after embryonic stem cell-derived cell or medium infusion in 8 rats for each group. *Sham*, Sham-operated rats; *MI+control*, myocardial infarction rats with cell-free medium infusion; *MI+IV EDCs*, myocardial infarction rats with embryonic stem cell-derived cell infusion; *BW*, body weight; *HW*, heart weight; *HW/BW*, ratio of heart weight/body weight; *HR*, heart rate. *$P < .01$ versus sham group; †$P < .05$, MI + IV EDC group versus MI + control group.

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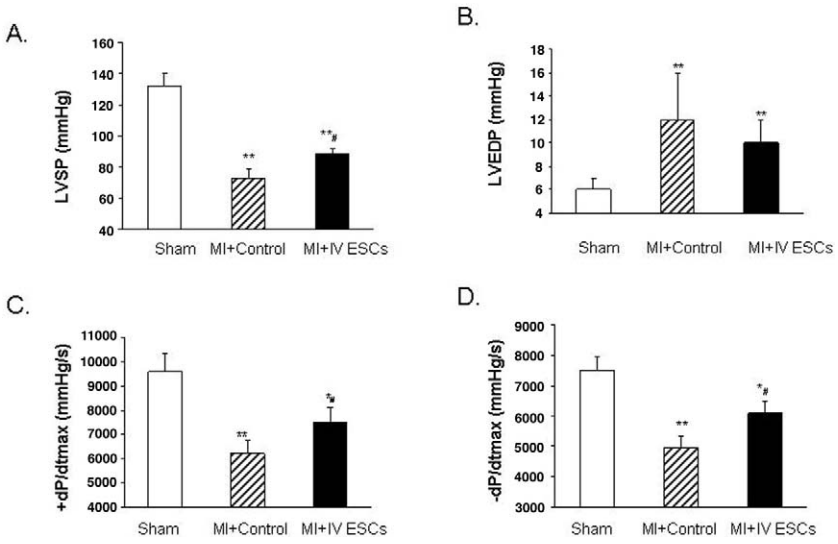

Figure 3. Cardiac function assessed with a Millar catheter in experimental rats at 6 weeks after myocardial infarction *(MI)* and embryonic stem cell-derived cell *(EDC)* infusion through tail vein injection. A, Left ventricular systolic pressure *(LVSP)*; B, left ventricular end-diastolic pressure *(LVEDP)*; C, maximum rate of peak left ventricular systolic pressure increase *(+dP/dtmax)*; D, maximum rate of peak left ventricular systolic pressure decrease *(−dP/dtmax)*. *Sham*, Sham-operated rats; *MI+Control*, rats after with cell-free medium infusion; *MI+IV EDCs*, rats after MI with EDC infusion (n = 8 in each group). **P* < .05 and ***P* < .01 versus sham group; #*P* < .05 versus MI+Control group.

ditionally, the control rats after MI had a lower LVSP, lower +dP/dtmax, lower −dP/dtmax, and higher LV end-diastolic pressure compared with values in the sham and MI cell-treated groups (Figure 3). EDC infusion at 6 weeks after MI significantly improved cardiac function, reflected by an increase in LVSP, +dP/dtmax, and −dP/dtmax (Figure 3). Additionally, the regional blood flow assessed by isotope microspheres and the arteriole density were significantly decreased in the rats after MI (Figure 4). EDC infusion significantly increased the LV myocardial blood perfusion and the numeric density of arterioles compared with that seen in the MI control group.

Histologic Study and Identification of Infused EDCs

Hematoxylin and eosin staining of heart sections from rats after MI receiving cell therapy showed less necrosis in infarcted and surrounding areas at 6 weeks after cell infusion (Figure 5). Significant fibrosis was found in postinfarcted myocardium in the control hearts after MI without cell infusion. Paraffin-embedded sections from rat hearts after MI at 6 weeks after cell treatment showed GFP-positive spots under fluorescent microscopy that were further stained positively with GFP antibody (Figure 6). In contrast, sections from sham-operated hearts and control hearts after MI had no such GFP-positive tissue stained with GFP antibody. Positive immunostaining for cTnI was found in EDC-infused hearts after MI that corresponded to GFP-positive spots (Figure 7). These data suggest that infused EDCs not only survived in injured myocardium but also differentiated into cardiac tissue. In addition, GFP-positive cells were not identified outside the infarcted hearts, except in the

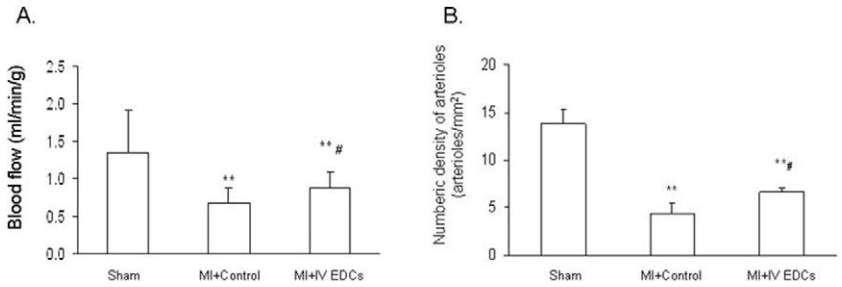

Figure 4. Left ventricular blood flow measured with isotope microspheres is shown in panel A (7 for each group). Numeric densities of arterioles are shown in panel B (8 for each group). The results indicated that intravenously infused embryonic stem cell–derived cells *(EDCs)* significantly improved regional blood perfusion and enhanced angiogenesis at 6 weeks after myocardial infarction *(MI)* and EDC treatment. *Sham*, Sham-operated rats; *MI+Control*, rats after MI with cell-free medium infusion; *MI+IV EDCs*, rats after MI with EDC infusion. ***P* < .01 versus sham group; #*P* < .05 versus MI+Control group.

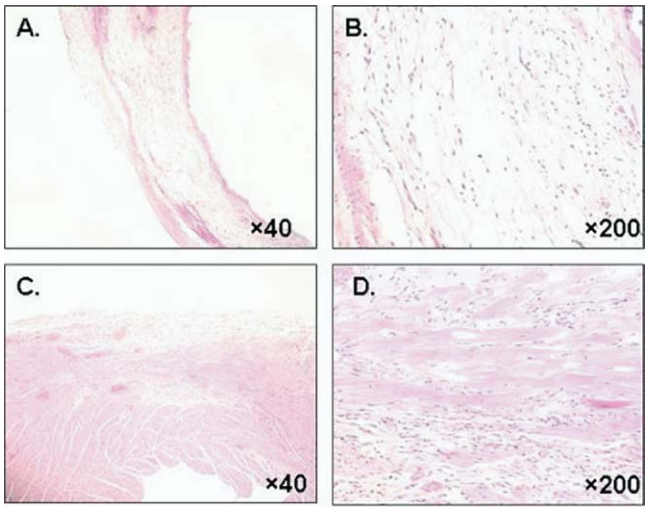

Figure 5. Representative rat myocardium stained with hematoxylin and eosin were from a rat at 6 weeks after myocardial infarction *(MI)* with cell-free medium infusion (A and B) and a rat at 6 weeks after MI and embryonic stem cell-derived cell *(EDC)* tail vein infusion (C and D). Cell grafts were clearly found within the infarcted zone in infracted myocardium after EDC infusion. In contrast, the tissue in the infarcted regions of the control myocardium appeared fibrotic and relatively acellular.

spleen, which contained some GFP-positive areas but stained negative to troponin I (data not shown). The surviving cells examined in a cross-section of the heart were identified as expressing GFP in the group of rats after MI receiving cell infusion. The average percentage of GFP-positive cells was 5.3% of the total left ventricle.

In Vitro Assay of TNF-α-Induced ESC Migration

The lower compartments of the Transwell plates were cultured with neonatal rat cardiomyocytes with or without transfection of TNF-α cDNA. The amount of TNF-α production was significantly increased in myocytes transfected with TNF-α compared with that from the control myocytes in culture medium of the lower compartments (Figure 2, *B*; n = 7 runs for each). Figure 2, *C* and *D* (n = 5 runs for each), demonstrate significantly greater ESC migration in response to TNF-α incubated for 24 hours after adding GFP-tagged ESCs to the upper compartments. These data suggest that cultured neonatal rat cardiomyocytes with overexpression of TNF-α attract more ESCs migrating from the upper compartment to the lower compartment.

Discussion

Cell transplantation has emerged as a potential therapy to treat cardiac dysfunction resulting from MI. Various cell types appear to be promising candidates because of their ability to integrate into the host heart tissue[1-6,15,16] and improve cardiac function.[1-6,17] Differentiation efficiency of bone marrow-derived stem cells into adult cardiomyocytes appears limited.[18] ESCs, on the other hand, are pluripotent cells derived from the early embryo and retain the ability to differentiate into all cell types, including cardiomyocytes.[19,20] The availability of human ESCs and the technique of development for enrichment of cardiomyocytes derived from human ESCs has paved a possible way to use ESCs for a therapeutic approach for animal study and clinical application. Our previous studies[3,4] demonstrated that ESCs could be implanted into rat myocardium with intramyocardial injection after MI. The viability and regeneration capacity of engrafted ESCs were demonstrated by their positive immunostaining for α-myosin heavy chain and cTnI. Studies have also shown the feasibility of transcoronary infusion of donor cells into the intact rat heart,[21] doxorubicin-induced failing rat heart,[22] and infarcted rat heart.[9] However, the clinical situation during the early stage of MI might be suitable neither for invasive cardiothoracic surgery with intramyocardial injection of cells nor for relatively less invasive interventional catheterization. Such surgical or invasive procedures might be associated with high mortality in critically ill patients. Moreover, intracoronary

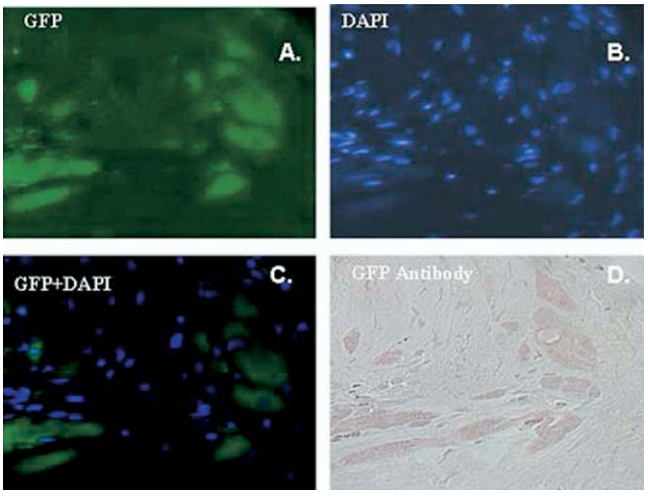

Figure 6. Infused embryonic stem cell-derived cells (EDCs) in postinfarcted rat myocardium were identified at 6 weeks after myocardial infarction (MI) and tail vein injection. Infused cells were clearly seen in the infarcted area with green fluorescent protein (GFP) fluorescence (A) and further verified positive to GFP antibodies (D), which demonstrated that circulating infused EDCs could home to injured myocardium. Panel B shows the nuclear staining with DAPI, and panel C shows the merge of GFP and DAPI staining. (Original magnification 200×.)

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cell transplantation has an apparent disadvantage because of the risk of coronary embolism.[21]

Recent studies demonstrated that intravenously infused donor cells can migrate to an injury site[23,24] and induce angiogenesis[24] in a rat model of stroke. It has been shown that an animal model of MI[25] is associated with inflammatory infiltration, which is abundant on days 2 to 3 but gradually decreases and disappears on day 7 after MI. Our present study indicates that intravenously administrated EDCs can traffic through the circulation to postinfarcted myocardium and differentiate into cardiac tissue. The mechanism might be associated with the inflammatory response after MI, and locally released cytokines might facilitate infused EDC migration toward injured myocardium.

Orlic and associates[10] reported that subcutaneous injection of stem cell factor or granulocyte colony-stimulating factor facilitated translocation of native bone marrow cells into infarcted myocardium, resulting in a significant degree of tissue differentiation 27 days later. However, recruitment of endogenous adult stem cells after ischemic injury might not be enough to achieve functional improvement. Our data demonstrated that intravenously infused EDCs can translocate to injured myocardium through the circulation. No significant numbers of GFP-positive donor cells were found in noninjured organs, including the lung, kidney, and liver, except for the spleen, in which a few GFP-positive spots were found (data not shown). Trapped EDCs might infiltrate through vessels into injured myocardium. Within a suitable surrounding niche, infused EDCs differentiated into new cardiac-like tissue to replace dead or damaged myocardium, which was supported by positive staining to cTnI in the grafts. Moreover, cardiac protective factors (eg, vascular growth factor) might be released from infused EDCs, which not only rescue damaged cardiac tissue but also promote angiogenesis, as reflected by the enhancement of regional blood perfusion and increased numbers of arterioles observed in the present study. Therefore the beneficial effects on improvement of cardiac function and the reduction of infarct size after EDC infusion might result from the synergistic effects of myogenesis and angiogenesis. Other factors, such as the antiapoptotic role of engrafted stem cells, might also partially contribute to the functional benefits. More experimental studies are needed to address the mechanisms of cell transplantation therapy.

The homing response of intravenously infused EDCs might be associated with the response to cytokines that are released locally in injured myocardium. TNF-α, a member of the proinflammatory cytokines, is considered to participate in the interactive signal-transduction cascade that regulates inflammatory and immunologic responses and triggers leukocyte infiltration through its chemotactic properties.[26] After MI, there is an immediate and rapid upregulation of TNF-α in the myocardium as part of the immune system response in the rat MI model.[27] Furthermore, detectable expression of TNF-α persisted to day 35 after MI. Levels of TNF-α protein in the infarct and peri-infarct zones increased early to 8- to 10-fold above normal levels and increased to 4- to 5-fold in the contralateral zone.[27] Thus it is reasonable to use TNF-α as a representative factor in the cytokine family to test whether cytokines released from local injured myocardium after MI facilitate migration of infused EDCs. During fluorescent microscopy, we detected more GFP-positive cells on the cover slips seeded with TNF-α-transfected cardiomyocytes than on cover slips plated with control cardiomyocytes. Cardiomyocytes with overexpression of TNF-α attracted more ESCs, which migrated from one compartment to another in response to TNF-α stimulation. This finding suggests that cytokines produced by injured myocardium after acute MI perform a major role as chemoattractants for intravenously infused EDCs and result in infused cells migrating into sites of injury. Using a Dunn chamber (a direct viewing chemotaxis chamber with concentric wells), we recently showed that ESCs are highly motile and respond to different concentrations of TNF-α in a dose-related manner.[28] In addition, our previous study[29] indicated that the molecular pathway of stem cell migration stimulated by excessive TNF-α is through the type II TNF-α receptor. Activation of p38 and JNK is required for TNF-α-enhanced stem cell migration.

There are several limitations to the present study. Interpreting the functional benefits of EDC infusion in rats after MI should be done cautiously because cardiac function was measured in unloaded experimental condition and different loading conditions might affect functional results measured with a Millar catheter. A previous study[30] demonstrated that intravenously infused bone marrow-derived mesenchymal stem cells at 1 week after infusion were trapped either in the infarcted or border zone and also lodged in the lung, liver, spleen, and bone marrow. However, the present study indicated that infused donor cells were identified only in the infarcted heart and the spleen at 6 weeks after MI and cell infusion. These differential results might be related to the different time points chosen to track the fate of infused donor cells among various studies.

In conclusion, the present study indicates that intravenously infused EDCs can traffic through the circulation into injured myocardium. The migration of infused EDCs might be associated with local cytokine release after acute MI. EDCs homing to injured myocardium because of chemotactic properties of inflammatory cytokines re-

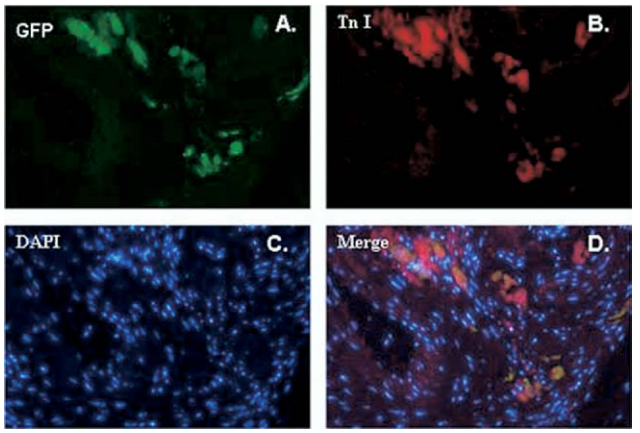

Figure 7. Myocardial sections from postinfarcted myocardium 6 weeks after embryonic stem cell–derived cell *(EDC)* infusion. Infused green fluorescent protein *(GFP)*–positive EDCs (A) homing into the infarcted area were stained positive to cardiac troponin I (*TnI*; B). DAPI showed nuclear staining (C). The merge (D) of GFP, cardiac TnI, and nucleus of cells demonstrated that infused GFP-labeled EDCs could not only home to the infarcted zone but also differentiate into cardiac tissue. (Original magnification 200×.)

leased locally could differentiate into cardiac tissue and improve ventricular function. The functional benefits are also related to enhancement of regional blood perfusion in infarcted myocardium after EDC infusion. The present strategy simplifies the pathway for cell delivery by using intravenous infusion. This approach could potentially be used in either severe heart failure or during the acute phase of MI in a critically ill patient who might not tolerate cell transplantation through surgical operation or invasive catheterization.

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