Beneficial Effect of Autologous Cell Transplantation on Infarcted Heart Function: Comparison Between Bone Marrow Stromal Cells and Heart Cells

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Background. **Cell transplantation may restore function after myocardial infarction, but the optimal cell type remains controversial. We compared autologous bone marrow stromal cells (BMCs) with autologous heart cells (HCs) in a porcine myocardial infarction model.**

Methods. **Yorkshire pigs underwent coil occlusion of the left anterior descending artery. Bone marrow stromal cells were obtained from sternal marrow and HCs were obtained by left ventricular biopsy, then cultured for 4 weeks. Four weeks after infarction, a ^{99m}Tc-sestamibi single-photon emission tomography (^{99m}Tc-MIBI SPECT) scan was performed and the pigs were then transplanted with BMCs (n = 7), HCs (n = 7), or culture medium (n = 14). Four weeks after transplantation, ^{99m}Tc-MIBI SPECT scanning was repeated to evaluate regional perfusion. Pressure-volume loops were constructed from micromanometer and conductance catheter data to evaluate left ventricular function. Hearts were evaluated histologically.**

Results. **Bone marrow stromal cells and HCs engrafted within the infarct and assumed a myocyte morphology. SPECT MIBI scans showed increased perfusion in the infarct in cell-transplanted pigs, while perfusion decreased in the control pigs. Heart cell transplantation improved preload-recruitable stroke work and HC and BMC transplantation both shifted the end-systolic pressure–volume relation to the left. Both BMCs and HCs prevented thinning and expansion of the infarct region, and some BMCs differentiated into endothelial cells in newly formed blood vessels perfusing the infarct.**

Conclusions. **Both BMCs and HCs engrafted in the infarct region and improved let ventricular function by preventing infarct thinning. Bone marrow stromal cells demonstrated greater plasticity in vivo, and may offer a practical alternative to HC transplantation to restore function and perfusion after a myocardial infarction.**

(Ann Thorac Surg 2003;75:169–77)

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Cell transplantation has undergone intense scrutiny in the last decade as a potential novel therapy for patients with postinfarction ventricular dysfunction and congestive heart failure [1]. Cardiomyocytes [2], skeletal myoblasts [3, 4], smooth muscle cells [5], endothelial cells [6], and fibroblasts have all been transplanted into infarcted rat [7], rabbit [4], and porcine hearts [8]. In most studies, the transplanted cells limited postinfarction ventricular dilatation, improved left ventricular (LV) dysfunction, and induced angiogenesis. Transplanted cells may also serve as vehicles for therapeutic gene delivery [9].

The first clinical application of skeletal myoblast transplantation was reported in November 2000, and ongoing follow-up of those patients has suggested both evidence of viability of the transplanted cells and concern about potential arrhythmogenesis (P. Menasche, presented at American Heart Association Scientific Sessions, November 2001) [10]. Other cell types are being considered for clinical evaluation.

The optimal type of cell to be transplanted remains controversial, however, and selection of a particular cell type may be influenced by a number of factors including the ease with which cells may be obtained from a patient; the time required to culture and expand the cell number; and the ability of those cells to alter ventricular scar remodeling or to form a functional syncytium with the surviving native myocardium, to improve regional contractility and to exert a long-lasting benefit without oncogenic, arrhythmogenic, or other undesirable effects.

Several studies have suggested that bone marrow mesenchymal stem cells may offer an attractive alternative to transplantation of myocytes. These cells may be induced to express cardiomyocyte-like characteristics by incubation with 5-azacytidine [11], and when transplanted into the scarred myocardium, limit ventricular

Presented at the Thirty-eighth Annual Meeting of The Society of Thoracic Surgeons, Fort Lauderdale, FL, Jan 28–30, 2002.

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Drs Weisel, Mickle, and Li disclose that they have a financial relationship with Genzyme Corp.

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Published by Elsevier Science Inc

0003-4975/03/$30.00
PII S0003-4975(02)04290-X

Ann Thorac Surg
2003;75:169–77

dilatation and improve function [12]. Some evidence suggests that these cells may be mobilized from the bone marrow and migrate to the heart in the presence of injury [13]. Bone marrow cells (BMCs) from a male recipient can be observed in a transplanted female donor heart [14]. These cells migrate through the blood and differentiate into cardiomyocytes within the donor myocardium. The study has also suggested that adult heart cells (HCs) can replicate and may contribute to cardiac regeneration in pathological conditions [14].

In the present study, we sought to directly compare the effects of BMC and HC transplantation on regional perfusion, left ventricular (LV) function, and infarct zone morphometry and histology in a clinically relevant porcine model of left anterior descending coronary artery (LAD) infarction. Autologous cells were used to eliminate the effect of rejection on these outcomes.

Material and Methods

Experimental Animals

Adult female domestic Yorkshire swine (31 ± 4 kg) (Charles River Canada Inc, Quebec, Canada) were used. All procedures were approved by the Animal Care Committee of the University Health Network and performed according to the "Canadian Council on Animal Care and Use of Laboratory Animals," published by the National Institutes of Health.

Creation of the Myocardial Infarction

All surgical procedures on swine were performed under general anesthesia and continuous electrocardiographic monitoring. Animals were premedicated with ketamine (20 to 30 mg/kg, intramuscular) before anesthetic induction with 4% isoflurane. Anesthesia was maintained with isoflurane 1% to 2.5%. Pigs underwent myocardial infarct generation by coil and Gelfoam embolization of the distal LAD (N = 28) under fluoroscopic guidance as previously described [8]. Occlusion of the distal one third of the LAD was confirmed angiographically.

Preparation and Identification of Heart Cells

A flexible bioptome was introduced retrogradely into the left ventricle from the carotid artery under fluoroscopic guidance, and five biopsies were obtained from varying sites on the interventricular septum. Tissue samples were placed immediately into ice-cold sterile cell-culture dishes containing culture medium for subsequent cell isolation and culture. Under sterile conditions, porcine HCs were isolated by enzymatic digestion as we have described previously [8]. Cells were cultured for 4 weeks before transplantation.

The HCs in the culture dish were identified using monoclonal antibodies against troponin I and myosin heavy chain for cardiomyocytes, smooth muscle actin for smooth muscle cells, and factor VIII for vascular endothelial cells as we have described previously [5, 8]. Briefly, cultured cells were fixed with 100% methanol at −20°C for 20 minutes. Endogenous peroxidase was then blocked using 3% hydrogen peroxide for 10 minutes at 42°C and 2 N HCl for 30 minutes at room temperature. After rinsing with phosphate-buffered saline (PBS) three times, the cells were incubated with antibodies against troponin I, myosin heavy chain, smooth muscle actin, or factor VIII in a moist chamber for 16 hours at room temperature. Negative control samples were incubated in PBS without the primary antibodies under the same conditions. After being rinsed with PBS three times (15 minutes each) the cells were then incubated with goat anti-rabbit immunoglobulin G conjugated to peroxidase, at 37°C for 45 minutes. The samples were washed three times (15 minutes each) with PBS and then immersed in diaminobenzidine-H_2O_2 (2 mg/mL diaminobenzidine, 0.03% H_2O_2 in 0.02 mL/L phosphate buffer) solution for 15 minutes. After washing with PBS, the samples were covered with a crystal mount and photographed.

To identify the transplanted cells in the recipient myocardium, 10% of the HCs (3 dishes out of a total of 30 dishes for each animal) were labeled with bromodeoxyuridine (BrdU) (0.1 mg BrdU/mL culture medium) for 2 days before transplantation.

Preparation and Identification of Bone Marrow Cells

First, 10 mL of sternal bone marrow was aspirated using a bone marrow aspiration needle and then cultured in Iscove's modified Dulbecco's medium with 20% fetal bovine serum, penicillin G (100 U/mL), streptomycin (100 μg/mL), and amphotericin B (0.2 μg/mL). The cells were incubated with 95% air and 5% CO_2 at 37°C. After 48 hours, 5-azacytidine was added into the culture medium at a final concentration of 10 μmol/L. The medium was changed 24 hours later to remove the 5-azacytidine, and the cells maintained in culture for 4 weeks. The cultures were depleted of erythroid progenitor cells by removing cells that were not adherent to the culture dish at each change of medium, every 2 to 3 days.

Thirty percent of the BMCs were labeled with BrdU (0.1 mg BrdU/mL culture medium) for 2 days before transplantation to facilitate subsequent identification. The cells were again incubated with 5-azacytidine for the 24-hour period immediately before transplantation.

Cell Transplantation

Four weeks after infarct creation, the animals were anesthetized and the heart was exposed through a left thoracotomy as previously described. The cultured BMCs and HCs were dissociated from the culture dishes with 0.05% trypsin in phosphate-buffered saline and centrifuged at 570g to remove the trypsin. The cells were resuspended in culture medium. The suspension of BMCs (2 mL, 1×10^8 cells, n = 7) or HCs (2 mL, 2×10^7 cells, n = 7) was then injected into the infarct region with a tuberculin syringe in a star pattern. One half of the suspension was delivered into the center of the infarct, and the remaining half was divided between four or five injections in the periphery of the infarct zone, to facilitate any potential connection between the transplanted cells and the nor-

mal myocardium. In the control pigs (n = 14), only culture medium, without cells, was injected into the infarct region.

^{99m}Tc-Sestamibi Single-Photon Emission Tomography

Four weeks after infarct creation, and again 4 weeks after transplantation with BMCs, HCs, or culture medium, myocardial perfusion was assessed by injecting 10 mCi ^{99m}Tc-sestamibi single-photon emission tomography (^{99m}Tc-MIBI SPECT) (Dupont) 30 minutes before electrocardiogram-gated SPECT acquisition as per our standard clinical protocol. Attenuation was calculated by determining the distance from the detector to the center of the myocardium and counting the activity in venous blood samples. Quantitative perfusion was measured by ^{99m}Tc-MIBI SPECT in each of 20 segments in cross-sectional views at the apical and midventricular levels, and a vertical long-axis view, as per our usual clinical protocol, by a cardiologist blinded to the treatment protocol. Perfusion scores were normal in all animals in the basal regions. All infarct-related values were obtained in eight apical, periapical, and midventricular segments. These eight regions contained all of the infarct areas as assessed by pretransplant ^{99m}Tc-MIBI SPECT scans, were the areas in which the cells were injected, and were therefore used to assess any changes occurring with cell transplantation. Differences in pre- and posttransplantation values were normalized to baseline values in each heart.

Evaluation of Ventricular Function by Pressure-Volume Loops

Four weeks after transplantation, ventricular function in the control and transplanted animals was evaluated. Through a sternotomy, a conductance catheter and a micromanometer (Millar Instruments, Austin, TX) were inserted into the LV apex. Pressure and volume data were acquired, and real-time pressure-volume loops constructed, at baseline and during caval occlusion, in triplicate. Data points from the pigs in each group were pooled for analysis. Analysis of covariance was performed on these points using end-diastolic volume (EDV) as a covariate, group as a predictor variable, and LV stroke work (LVSW) as the outcome variable, to evaluate preload-recruitable stroke work (the slope of the LVSW–EDV relation) as a measure of myocardial performance. A similar analysis of covariance was performed using LV end-systolic volume as a covariate, group as a predictor, and LV end-systolic pressure as the outcome, to evaluate end-systolic elastance and systolic function.

Morphological and Histological Studies

After the invasive studies of ventricular function were complete, the heart was arrested with potassium chloride and rapidly excised. The atria were removed and the heart was weighed. The coronary arteries were then perfused with 100 mL 10% formaldehyde, and the heart was fixed in diastole with an intraventricular pressure of 30 mm Hg in formaldehyde solution for 7 days before sectioning for histology.

After fixation, the hearts were sliced into 5-mm thick slices and each section photographed. The mean scar length in each section was calculated as the mean of the epicardial scar length and the endocardial scar length. The scar area was then calculated as the mean scar length for that section multiplied by 0.5 cm. Total scar area was calculated as the sum of scar areas for all sections. The thickness of the scar was calculated in each section, and scar volume was calculated as total scar area multiplied by the mean scar thickness [4]. A cube of tissue from the center of the infarct zone measuring 5 mm on each side was embedded in paraffin and cut into 10-μm sections for staining with hematoxylin and eosin, as well as for BrdU, to localize the transplanted cells.

For immunohistochemical studies, tissue slices were serially rehydrated in 100%, 95%, and 70% ethanol after deparaffinization with toluene. Endogenous peroxidase in the sample was blocked and the samples were stained with antibodies against BrdU for identification and troponin I for contractile protein as described above.

Statistical Analysis

All data are expressed as the mean ± standard error. ^{99m}Tc-MIBI SPECT-derived perfusion values and LV volumes were subjected to analysis of variance. Left ventricular pressure and volume data were subjected to analysis of covariance, evaluating group, time, and group × time interactive effects over a range of ventricular volumes. Other variables were assessed by two-way analysis of variance or, when appropriate, by *t* test.

Results

Bone Marrow Stromal Cell Characterization Before Transplantation

From the 10 mL of bone marrow aspirate, 1.5 to 4 × 10^8 BMCs were obtained after 4 weeks in culture (Fig 1A). The plates of BMCs were generally split at 60% confluence, but in a subset of plates allowed to reach full confluence, 5% to 15% of the cells were noted to have formed myotube-like structures.

Heart Cell Characterization Before Transplantation

A mixed cell type (Fig 1B) was identified by immunohistochemistry. Positive staining for troponin I was noted in 74% ± 16% of the cultured HCs. Staining for factor VIII was noted in 6% ± 4% of the cells, and these cells were assumed to be endothelial cells. The remainder of the cells (20% ± 18%) were spindle-shaped, and were presumed to be fibroblasts and smooth muscle cells.

Body Weight

Body weight increased slightly in all pigs between the time of infarct generation (30.7 ± 0.9 kg) and transplantation (37.5 ± 1.3 kg) (p = 0.005). Four weeks after transplantation, the BMC- and HC-transplanted pigs tended to be heavier than controls, although the differ-

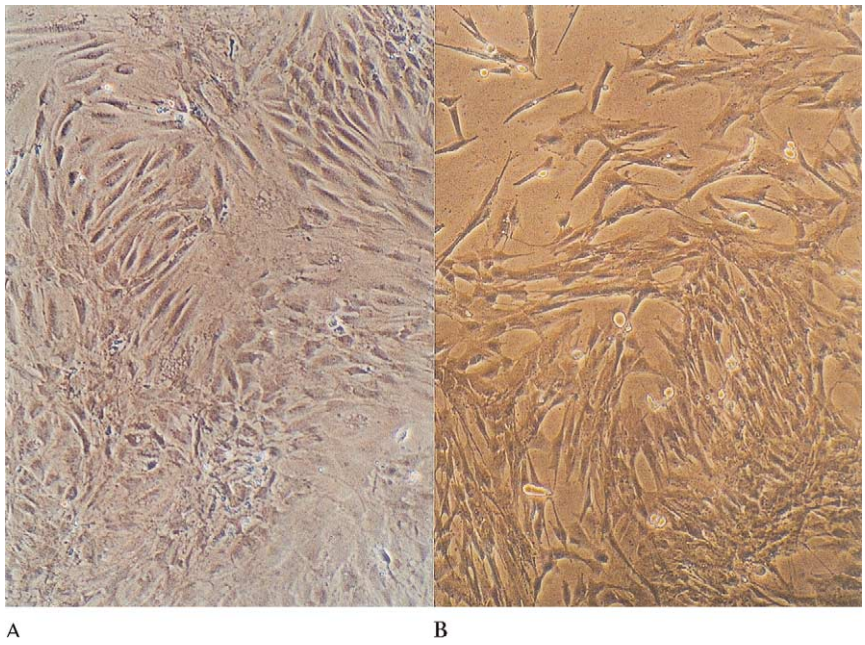


Fig 1. Photomicrographs of cultured (A) bone marrow cells and (B) heart cells before transplantation. (×200 before 44% reduction.)

ence was not significant (BMC transplant, 47.3 ± 1.3 kg; HC transplant, 47.3 ± 2.9 kg; control, 42.6 ± 1.5 kg; $p = 0.2$).

Regional Myocardial Perfusion by ^{99m}Tc-MIBI SPECT

Pre- and posttransplantation ^{99m}Tc-MIBI SPECT scans were performed on each animal, and results compared between time points and between the control, BMC- and HC-transplanted groups. Bone marrow cell- and HC-transplanted pigs demonstrated increased ^{99m}Tc-MIBI SPECT perfusion values 4 weeks after transplantation, whereas control pigs demonstrated decreased values as the scar matured (Fig 2C). Perfusion values were greater in both BMC- and HC-transplanted groups than in control pigs ($p = 0.033$), but no difference was noted between the BMC-transplanted and HC-transplanted hearts ($p = 0.8$).

End-Systolic Elastance and Preload-Recruitable Stroke Work

From the pressure–volume loops constructed 4 weeks after cell transplantation, the end-systolic pressure–volume relation and the LVSW–EDV relation were plotted. The group and group × LV volume interactive effects were evaluated by analysis of covariance. There was a significant group × EDV interactive effect on LVSW ($p < 0.001$), with an increased slope of the LVSW–EDV relation in the HC-transplanted pigs, indicating that preload-recruitable stroke work was greater in the HC-transplanted groups than in the controls (Fig 3A). There was a significant group effect on the end-systolic pressure–volume relation ($p < 0.001$), which was shifted to the left in the BMC- and HC-transplanted hearts, compared with controls (Fig 3B).

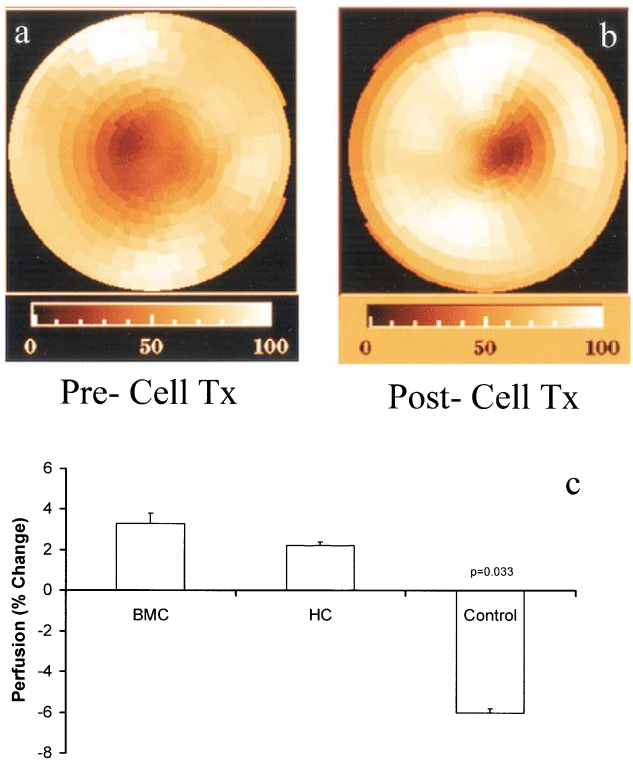
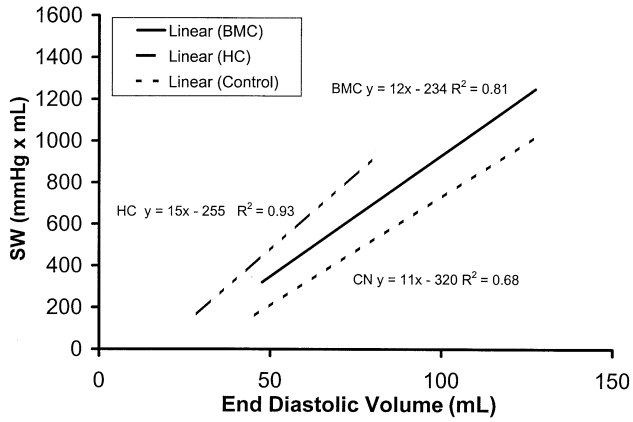
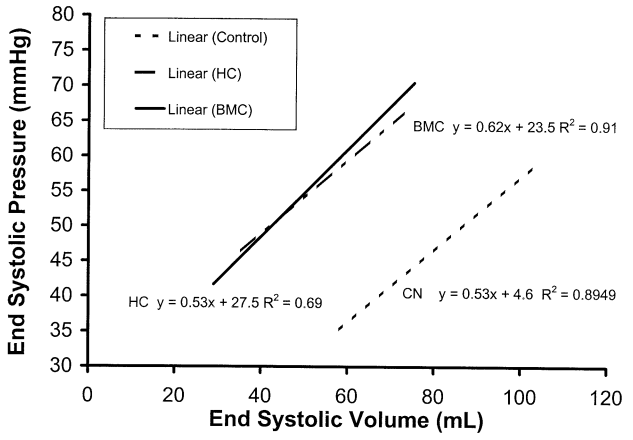


Fig 2. A representative polar map of left ventricular perfusion, generated by ^{99m}Tc-sestamibi single-photon emission tomography (^{99m}Tc-MIBI SPECT), (a) before and (b) 4 weeks after bone marrow cell transplantation. (c) Changes in infarct zone perfusion scores, evaluated by ^{99m}Tc-MIBI SPECT, in the bone marrow cell (BMC) transplanted, heart cell (HC) transplanted, and control pigs. Perfusion scores decreased over the 4-week period after transplantation in the control animals, but increased to a similar extent in both the cell transplanted groups (p = 0.033). (Tx = transplantation.)



A



B

Fig 3. (A) Preload-recruitable stroke work (PRSW, the slope of the stroke work–end-diastolic volume relation) 4 weeks after transplantation of bone marrow cells, heart cells, or culture medium alone (controls). A significant interactive effect of group × end-diastolic volume on stroke work was noted ($p < 0.001$). *PRSW was greatest in the heart cell-transplanted pigs. (B) The end-systolic pressure–volume relation. Transplantation of bone marrow cells or heart cells shifted the end-systolic pressure–volume relation to the left* ($p < 0.001$). *(*BMC = *bone marrow cells;* CN = *control;* HC = *heart cells;* SW = *stroke work.)*

Morphometric Studies

In the center of the infarct zone, the thickness of the scar area was significantly greater in the two transplant groups than in the controls (BMC transplant 6.4 ± 1.2 mm; HC transplant 6.4 ± 1.0 mm; control 3.4 ± 0.3 mm; $p = 0.008$). Scar areas in the BMC- and HC-transplanted groups were lower than those of the controls (as % of LV free wall; BMC transplant 9.6% ± 2.8%; HC transplant 13.2% ± 1.2%; control 18.4% ± 2.1%; $p = <0.01$ for both BMC and HC versus control, $p = 0.14$ for BMC versus HC).

Histologic Studies

Histologic evaluation of myocardial sections stained with hematoxylin and eosin demonstrated engraftment of both BMCs (Fig 4A) and HCs (Fig 4D) in the cell-transplanted groups, with greater cellularity in the scar than the control hearts. In this model of transplantation of autologous BMCs and HCs, the histologic evidence of rejection was minimal at 4 weeks.

A proportion of the cultured BMCs and HCs were prelabeled with BrdU before transplantation, and BrdU-labeled cells were identified within the infarct zone 4 weeks after transplantation of either BMCs (Fig 4B) or HCs (Fig 4E). In some sections from BMC-transplanted hearts, BrdU-labeled cells had been incorporated into newly formed blood vessels. Sections stained for troponin I demonstrated troponin I positive cells in the transplanted scar in both the BMC-transplanted hearts (Fig 4C) and the HC-transplanted hearts (Fig 4F) 4 weeks after transplantation.

Comment

We have previously reported that allogeneic fetal rat cardiomyocytes can alter the remodeling and improve the function of a cryoinjured rat heart [15]. However, these allogeneic cells were eventually destroyed by rejection [16]. Autologous cell transplantation may permit a durable effect on postinfarction LV function without the immunosuppressive regimen required for maintenance of allotransplants in vivo. Histologic examination in the two autologous cell-transplanted groups in this study revealed minimal evidence of rejection at the sites of transplantation. We were therefore able to avoid the confounding effects of rejection and eventual destruction of the transplanted cells on the outcomes studied. In the absence of a supply of universally tolerated, nonimmunogenic stem cells, autotransplantation is likely to remain the most clinically practical strategy by which cell transplantation is applied to humans.

In the same porcine model of LAD infarction used in our current study, we have previously demonstrated that transplantation of autologous HC induced angiogenesis and resulted in greater end-systolic elastance and preload-recruitable stroke work than transplantation with culture medium alone [8]. Heart cells were isolated from endomyocardial biopsies under fluoroscopic guidance, a technique routinely performed for posttransplant monitoring for rejection, but which is not without potential complications and discomfort to the patient. Skeletal myoblasts may also be transplanted, but must also be biopsied and cultured for several weeks before reimplantation [10].

The use of BMCs, which may easily harvested from the iliac crest, may represent an attractive alternative to the transplantation of HCs or skeletal myoblasts. Particularly when induced with 5-azacytidine to differentiate into cardiomyocyte-like cells, BMCs have demonstrated encouraging results in rat models [12].

In this study, both BMCs and HCs induced myogenesis, with BrdU-labeled cells appearing to have a cardiomyocyte-like histologic appearance. We have previously reported that HC transplantation induces angiogenesis [2], and that expression of a vascular endo-

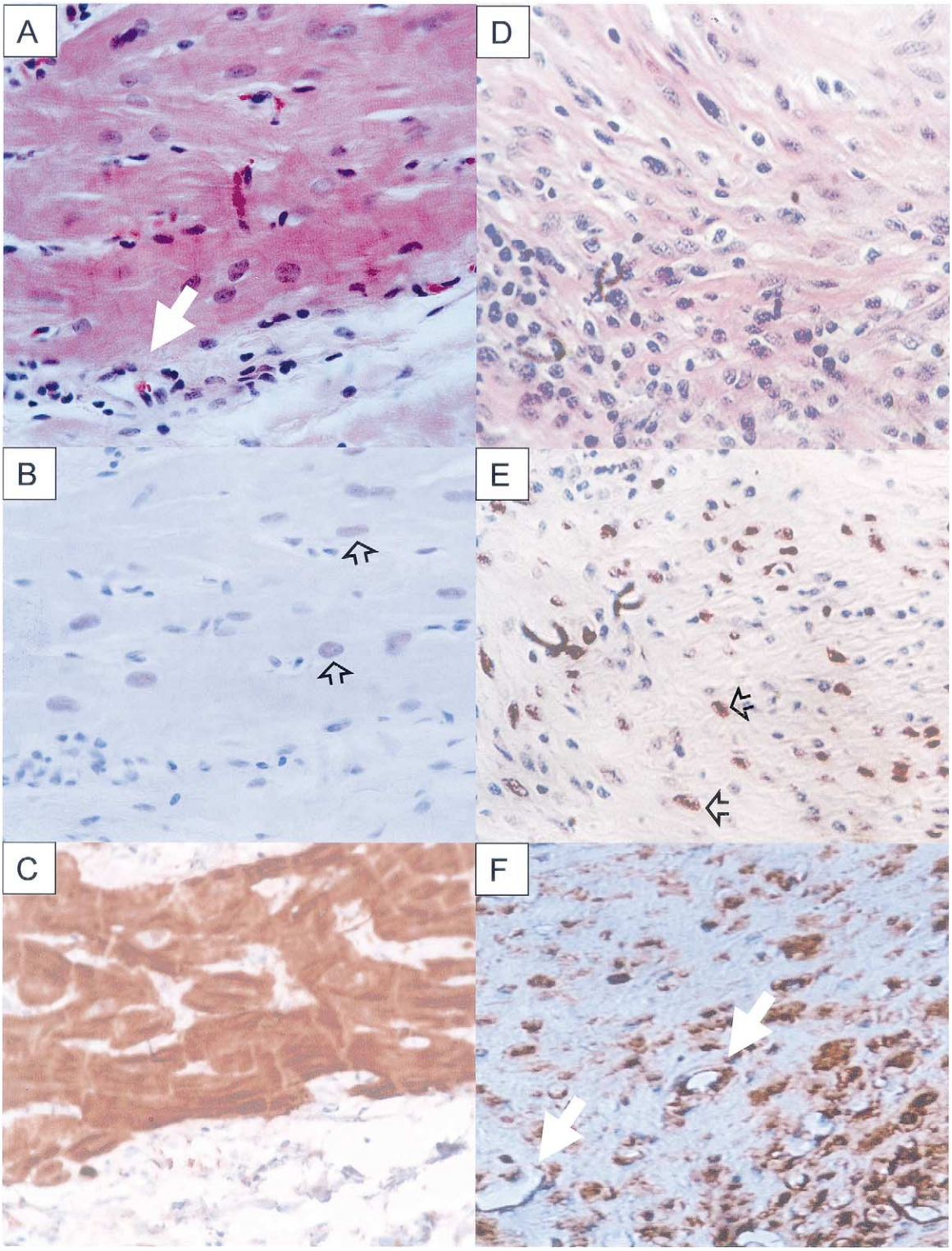


Fig 4. Photomicrographs of representative sections of a pig heart transplanted with bone marrow cells (A–C) or heart cells (D–F). Sections were stained with hematoxylin & eosin (A, D), antibodies against bromodeoxyuridine (BrdU) (B, E), or antibodies against troponin I (C, F). Both bone marrow cells and heart cells engrafted within the infarct zone. BrdU-positive cells in the bone marrow cell-transplanted hearts and the heart cell-transplanted hearts are indicated by arrows *(B, E). In both the bone marrow cell- and heart cell-transplanted hearts, troponin I-positive cells were also noted in the infarct zone.* Arrows *indicate blood vessels in the transplanted scar (A, F). (×200 before 20% reduction.)*

thelial growth factor transgene in the transplanted cells can significantly increase angiogenesis and regional perfusion in the infarct zone [9]. Neovascular ingrowth was similarly noted in the HC-transplanted pigs in this study. Bone marrow cell transplantation also resulted in angiogenesis, and in some sections in the BMC-transplanted hearts, BrdU-labeled cells were identified in the endothelial and smooth muscle cell layers of vessels in the transplanted zone. This finding suggests that the BMCs may differentiate not only into a cardiomyocyte-like phenotype, but also into smooth muscle and endothelial cell phenotypes, depending on the local microenvironment. We have noted a similar phenomenon when endothelial cells are transplanted into a cryoinjured rat heart [6], apparently participating in the structural organization of the newly formed vessels. The transplanted HCs, which were primarily troponin I-positive cells, did not appear to behave in a similar fashion. The mixed culture of HCs included 6% ± 4% endothelial cells, which might have been incorporated into newly formed vessels, but because only 10% of this subset of cells would have been prelabeled with BrdU, it is likely that their numbers were simply too low to be noted in the histologic sections of the HC-transplanted hearts.

The importance of the local extracellular milieu in determining the fate of the transplanted BMCs is also implied in the requirement for incubation with 5-azacytidine to induce a cardiomyocyte-like phenotype before transplantation into our rat cryoinjury model, which results in a relatively avascular, acellular scar with fewer environmental cues to guide differentiation of the transplanted cells. In our current study, LAD coronary occlusion resulted in a significant but not completely uniform infarct, with areas of surviving native cardiomyocytes. These native cells may have influenced the cardiomyogenic differentiation of the transplanted BMCs.

Although load-insensitive global measures of LV systolic function and myocardial performance were greater in both the BMC- and HC-transplanted pigs, we did not, in this study, demonstrate contraction of the engrafted cells. The mechanism by which transplanted cells may influence systolic function without having been demonstrated to form an electrical syncytium with the native myocardium remains unclear. Synchronous contraction may not be required, however, to improve systolic function. By altering scar geometry and the transmission of wall tension, the transplanted cells may favorably influence regional function. We are currently studying the effect of cell transplantation on the extracellular matrix, as it may be a potential mediator of its effects on ventricular function.

Both HCs and BMCs have advantages when transplanted into infarcted hearts. Transplanted HCs form myocyte-like cells that have been demonstrated to form gap junctions linking them to each other [17]. Bone marrow stromal cells may have the capacity to differentiate into all of the cell types required to repair the injured heart.

A number of limitations in this study remain to be addressed in future experiments. The transplanted cells were injected into the center of the infarct, and smaller aliquots of cells were deposited in four positions around the periphery of the infarct. The effect of local implantation on global function has yet to be fully explained, although we have previously reported that transplantation of a large number of cells at a single site can improve function even in a globally dysfunctional, cardiomyopathic hamster heart [18, 19]. In addition, it seems probable that a significant proportion of the transplanted cells do not survive, although the exact percentage is unknown and is likely to vary with the model studied. The optimal number of cells to induce angiogenesis, improve function, and yet not to induce arrhythmias is unclear, but early clinical experience suggests that these previously theoretical issues have assumed greater urgency.

This research was supported by Dr Li's research grants from the Heart and Stroke Foundation of Ontario and the Canadian Institutes of Health Research. Dr Li is a Career Investigator of the Heart and Stroke Foundation of Ontario.

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DISCUSSION

DR WILLIAM L. HOLMAN (Birmingham, AL): I think that is very exciting work, and I have two questions. First, did you make any attempt to revascularize the region that you infarcted? In an actual clinical situation in a cardiac operating room, presumably you would be using some sort of coronary artery grafts in that area. How do you think that revascularization will affect the engraftment of cells? In particular, do you think that angiogenesis, which seems to be a little bit better in the bone marrow stromal cells, will be perhaps negated by the improvement in blood flow?

And then the second question: I didn't hear any mention of the number of cells that you actually injected from each one of these cultures. Were the numbers comparable? In the pictures you showed of the isolated myocytes and the bone marrow cells, the bone marrow stromal specimen appeared to have many more cells. Do you think that that the number of cells implanted had any effect on your outcome?

DR MICKLE: Unfortunately, I will not be able to answer your questions concerning the clinical usefulness of cell transplantation since I am a chemist.

In our porcine studies, we did not attempt to revascularize the infarct region at the time of cell transplantation. Both the heart cells and bone marrow stromal cells survived without revascularization. We found evidence of extensive angiogenesis induced by the transplanted cells. Therefore, it is possible that cell transplantation can be accomplished without any ancillary procedure to induce angiogenesis.

The number of cells injected in the heart was different in the two groups. We injected 20 million heart cells and 100 million bone marrow cells. This difference was because over the 4-week period to culture the cells, we were able to culture only 20 million cells from the heart biopsies. The beneficial effect of cell transplantation is probably related to the dose injected. The optimal dose has not been fully identified. Further clinical trials will likely be required to define the optimal dose of each cell type in humans.

DR ABDULAZIZ AL-KHALDI (Montreal, Quebec, Canada): I would like to ask two short questions, mainly concerning the heart cell arm of your study. After culturing the cells that you obtained from the myocardial biopsy and before implanting them, did you evaluate the proportion of these cells that were actually cardiomyocytes and whether there were other components of the myocardium such as fibroblast, endothelial cell, or vascular smooth muscle in these cultures?

My second question: Do you think that your cultured cardiomyocytes proliferate without being transformed? Have you looked at whether they actually transformed during the culture process? Because, in our own experience and the experience of others in the field who culture cardiomyocytes, it is difficult beyond 2 to 3 weeks to keep these cell viable or induce them to divide in culture without causing a transformation.

DR MICKLE: Many researchers have isolated rod-shape cardiomyocytes as a myocardial model to investigate heart problems. These cells have organized sarcomeres and link with intercalated discs. The heart cells we used in our current study are different from rod-shape cardiomyocytes. We isolated cells from myocardial biopsies and the isolated cells were cultured in a culture dish. The cells de-differentiated and grew in culture. We define these as heart cells because most of them (74% prior to transplantation) stained positively with antibodies against troponin-I. However, these cells do not have sarcomeres, but they have contractile proteins, which are different from myofibroblasts. We also identified 6% endothelial cells in the cell population we implanted as stained with antibodies against factor VIII.

DR MARK MARBEY (Kalamazoo, MI): It looked from your video as if the injection of the cells was superficial, and I am wondering when you later harvested the specimens whether the cells migrated throughout the myocardium or whether you were evaluating only the epicardial area where you injected the cells?

DR MICKLE: We attempted to inject the cells into the midmyocardium, but as you saw on the video, there was a bleb that formed under the epicardium. Our histologic studies did not suggest that the cells migrated outside the region of the injection. It is likely that a proportion of the cells injected did not survive and that the surviving cells remained within the infarct region.

DR TODD K ROSENGART (Evanston, IL): Congratulations on your group's work in helping to pioneer this field. To what extent do you believe that angiogenesis contributes to your observed results, and to what extent would other cell types produce a similar response? If angiogenesis is playing a significant role, how important is it to be using myocytes as opposed to endothelial progenitor cells, bone marrow cells that are going to differentiate into other cell types, or even something as presumably inert as fibroblasts?

DR MICKLE: I believe that angiogenesis is extremely important to the benefits achieved by cell transplantation. For this technology to work and have long-term benefits, angiogenesis is essential to keep the cells alive. Each cell type has a different effect. Endothelial cells, for example, will not improve function, but will induce angiogenesis in a transmural scar. In a transmural scar, smooth muscle cells have a significant angiogenic effect and also improve function, but it is unlikely that they contract in vivo. It is possible that the cells affect the matrix and perhaps restore matrix function, and in this way improve regional function. However, I believe that the long-term survival of the cells requires that they induce a profound angiogenesis.

In our previous studies using a cryonecrosed-derived

transmural scar in an adult rat model, we demonstrated that only muscle cells will restore regional and global function. Both endothelial cells and fibroblasts engrafted in the infarcted region, but did not improve function. However, skeletal myoblasts, smooth muscle cells, heart cells, and bone marrow mesenchymal stem cells all improved function.

DR GINO GEROSA (Padua, Italy): Were those bone marrow cells CD 34 positive? Did you have any chance to identify them?

DR MICKLE: We did not identify the cells with antibodies against CD34. No porcine-specific antibodies were available.

The Society of Thoracic Surgeons: Thirty-ninth Annual Meeting

Mark your calendars for the Thirty-ninth Annual Meeting of The Society of Thoracic Surgeons, which will be held in San Diego, California, January 31–February 2, 2003. The program will provide in-depth coverage of thoracic surgical topics selected to enhance and broaden the knowledge of practicing thoracic and cardiac surgeons. Traditional abstract presentations as well as topic-specific ancillary sessions and courses will make up the continuing medical education opportunities that will be offered at the Thirty-ninth Annual Meeting.

Advance registration forms, hotel reservation forms, and details regarding transportation arrangements, as well as the complete meeting program, will be mailed to Society members. Also, complete meeting information will be available on The Society's Web site located at http://www.sts.org. Nonmembers wishing to receive information on attending the meeting may contact The Society's Secretary, Gordon F. Murray.

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