

# Heart Cell Transplantation Improves Heart Function in Dilated Cardiomyopathic Hamsters

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**Background**—Little is known about the effect of heart cell transplantation into the dilated cardiomyopathic myocardium.

This study was designed to evaluate the effect of heart cell transplantation into dilated cardiomyopathic hamsters.

**Methods and Results**—Ventricular heart cells were isolated from 4-week-old BIO 53.58 hamsters and cultured for 2 weeks before transplantation. The cells were labeled with bromodeoxyuridine (BrdU) before transplantation for identification. Adult hamsters (17 weeks old) were used as recipients. Heart cells ( $4 \times 10^6$  cells) or culture medium was transplanted into the left ventricular free wall (transplantation and control groups, respectively,  $n=12$  each). Sham-operated hamsters ( $n=12$ ) underwent the surgery but not the transplantation. Cyclosporine A was administered subcutaneously to all hamsters daily after the operation. Four weeks after the transplantation, heart function was evaluated with the use of a Langendorff preparation. Histology showed severe focal myocardial necrosis in all groups. BrdU-stained tissue was found at the cell transplantation sites. The transplanted hearts had greater ( $P<0.001$ ) developed pressures at all balloon volumes and improved dP/dt (transplantation  $915 \pm 253$  versus control  $453 \pm 120$  and sham  $530 \pm 187$  mm Hg/s,  $P<0.001$ , balloon volume of  $15 \mu\text{L}$ ). No differences in ventricular function were found between control and sham-operated hamsters.

**Conclusions**—The transplanted ventricular heart cells formed cardiac-like tissue in cardiomyopathic myocardium and improved its contractile function. (*Circulation*. 2000;102[suppl III]:III-204-III-209.)

**Key Words:** cells ■ transplantation ■ cardiomyopathy

Cell transplantation, which involves the injection of suspended cultured cells into myocardial scar tissue, offers a new approach to restore impaired heart function. Cultured fetal and neonatal cardiomyocytes,<sup>1-4</sup> smooth muscle cells<sup>5</sup> and satellite cells<sup>6,7</sup> have been transplanted into myocardial scar tissue. The implanted cells survived and formed muscle at the transplanted site and improved heart function by preventing scar dilation or expansion. According to the registry of the International Society for Heart and Lung Transplantation in 1998,<sup>8</sup> patients with cardiomyopathy and coronary artery disease constitute the majority of heart transplantation patients. Until now, cell transplantation research has focused on improving the function of the regionally impaired myocardium, such as after a myocardial infarction. The effects of cell transplantation on global heart failure, especially in dilated cardiomyopathy, are unknown.

In this study, cultured ventricular heart cells were transplanted into the left ventricular free wall of adult cardiomyopathic hamsters. The hearts with transplanted cells, in comparison to the control and sham hearts, had improved heart function as measured by a Langendorff heart preparation. This improvement we attributed to the transplanted tissue in the cardiomyopathic myocardium.

## Methods

### Experimental Animals

The Animal Care Committee of the Toronto Hospital approved all procedures performed on the test animals in this study. Experimental animals used were BIO 53.58 hamsters (BIO Breeders, Fitchburg, Mass). Male hamsters, weighing 100 g, were used. The donor heart cells were obtained from 4-week-old BIO 53.58 hamsters. Seventeen-week-old BIO 53.58 hamsters were used as recipients. All experiments were performed according to the Guide to the Care and Use of Experimental Animals of the Canadian Council on Animal Care and the Guide for the Care and Use of Laboratory Animals (NIH publication 85-23, revised 1985).

The animals were divided into 3 groups: cell transplantation group ( $n=12$ , cultured heart cells transplanted), control group ( $n=12$ , culture medium transplanted), and sham group ( $n=12$ , similar chest surgery without transplantation).

### Heart Cell Isolation, Culture, and Identification

Heart cells were isolated by means of an enzymatic digestion method from 4-week-old BIO 53.58 hamsters.<sup>9</sup> The hamsters were euthanized. Their hearts were excised and washed with PBS (NaCl, 136.9 mmol/L; KCl, 2.7 mmol/L;  $\text{Na}_2\text{HPO}_4$ , 8.1 mmol/L;  $\text{KH}_2\text{PO}_4$ , 1.5 mmol/L; pH 7.3). The left ventricle was minced and incubated in 10 mL PBS containing 0.2% trypsin, 0.1% collagenase, and 0.02% glucose for 30 minutes at 37°C. After repetitive pipetting of the digested myocardial tissue, we isolated the heart cells. The cells in the supernatant were transferred into a tube containing 20 mL of cell

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culture medium (Iscove's modified Dulbecco's medium [Gibco Laboratory, Life Technologies] containing 10% FBS, 0.1 mmol/L  $\beta$ -mercaptoethanol, 100 U/mL penicillin, and 100  $\mu$ g/mL streptomycin). The tube was centrifuged at 600g for 5 minutes at room temperature. The cell pellet was resuspended in the cell culture medium and evenly divided into two 10-cm-diameter dishes. The heart cells were subcultured once during the 2 weeks of culturing before transplantation.

The cultured cardiomyocytes were identified immunohistochemically with monoclonal antibodies against myosin heavy chain (Vector Laboratory), troponin I (Spectral Diagnostics Inc), and myosin light chain (Biogenesis), as previously described.<sup>2,5</sup>

### Identification of Transplanted Cells

To identify the transplanted cells in the recipient myocardium ( $n=10$ ), the cultured heart cells were labeled with the thymidine analogue 5-bromo-2'-deoxyuridine (BrdU) for 2 days before transplantation. When the cells reached 50% confluence, 25  $\mu$ L of 0.4% BrdU solution was added to the culture dishes and incubated 48 hours before cell transplantation. To determine the BrdU labeling efficiency, 3 dishes were randomly selected and immunohistochemically stained for BrdU. The BrdU-labeled cells were transplanted as described below. One day ( $n=2$ ), 1 week ( $n=2$ ), 2 weeks ( $n=2$ ), 3 weeks ( $n=2$ ), and 4 weeks ( $n=2$ ) after transplantation, the hamsters were killed and the hearts were examined. BrdU in the cells was identified by an immunohistochemical staining technique as previously described.<sup>5</sup> After staining, the samples were covered with a crystal mount and photographed.

### Heart Cell Preparation and Transplantation

#### Cell Preparation for Transplantation

The cultured heart cells were washed 3 times with PBS to remove dead cells. The cells were then detached from the cell culture dish and separated by adding 0.05% trypsin in PBS to the culture dish for 2 minutes. After the addition of 10 mL of culture medium, the cell suspension was centrifuged at 580g for 3 minutes. The cell pellet was then resuspended in the culture medium at a concentration of  $100 \times 10^6$  cells/mL culture medium; 0.04 mL of cell suspension or culture medium was transplanted into each heart.

#### Cell Transplantation

BIO 53.58 strain hamsters were anesthetized with ketamine (20 mg/kg body wt IM), followed by an intraperitoneal injection of pentobarbital (30 mg/kg body wt). The anesthetized hamsters were intubated, and positive-pressure ventilation was maintained with a Harvard ventilator (model 683). The respiratory rate was set at 60 cycles/min, with a tidal volume of 1.5 mL of oxygen-supplemented room air. The heart was exposed through a 3-cm left lateral thoracotomy. The heart cell suspension was injected, with a tuberculin syringe, into the left ventricular anterior free wall of each of the test hamsters. Control animals were injected with culture medium into the same site as the cell-transplanted animals. The muscle layer and skin incision were closed with 5-0 vicryl sutures. Animals in the sham group were operated on without transplantation. The hamsters then recovered from surgery in a warm environment and were electrocardiographically monitored for 4 hours. Penlong XL (150 000 U/mL benzathine penicillin G and 150 000 U/mL procaine penicillin G) was given intramuscularly (0.2 mL/each hamster) every 3 days and buprenorphine (0.01 to 0.05 mg/kg body wt) was given subcutaneously every 8 to 12 hours for the first 48 hours after the surgery. Cyclosporine A, at a dose of 5 mg/kg body wt per day, was administered subcutaneously to all groups after surgery. The hamsters were housed in cages with filter tops.

### Myocardial Function Study

Four weeks after cell transplantation, heart function was evaluated in all groups by means of a Langendorff perfusion apparatus. The hamsters were anesthetized, and heparin sodium (100 U) was administered intravenously. The heart was quickly isolated and perfused in a Langendorff apparatus with filtered Krebs-Henseleit

buffer (mmol/L: NaCl, 118; KCl, 4.7;  $\text{KH}_2\text{PO}_4$ , 1.2;  $\text{CaCl}_2$ , 2.5;  $\text{MgSO}_4$ , 1.2;  $\text{NaHCO}_3$ , 25; and glucose, 11; pH 7.4) equilibrated with 5% carbon dioxide and 95% oxygen. A latex balloon was passed into the left ventricle through the mitral valve and connected to a pressure transducer (model p10EZ; Viggo-Spectramed) and differentiator amplifier (model 11-G4113-01; Gould Instrument System Inc). After 30 minutes of stabilization, the balloon size was increased by 0.005-mL increments from 0.005 mL to 0.025 mL by the addition of a saline solution. Coronary flow was measured in triplicate by timed collection in the empty beating state. After measuring coronary flow for 1 minute, heart rate, systolic and diastolic left ventricular pressures, and maximum and minimum dP/dt were recorded at each balloon volume. Developed pressure was calculated as the difference between the systolic and diastolic pressure. After completion of the measurements, the hearts were arrested by perfusion of 5 mL 20% KCl solution. The passive diastolic pressure in the arrested hearts was recorded at each balloon volume in 0.005-mL increments from 0.005 mL to 0.035 mL by the addition of saline solution. After evaluation of heart function, the hearts were weighed and fixed with 10% formaldehyde for histologic studies.

### Measurement of Left Ventricular Chamber Volume

The left ventricular volume was measured by the techniques of Pfeffer and associates<sup>10</sup> and Jugdutt and Khan.<sup>11</sup> Briefly, the hearts were fixed in left ventricular distension (30 mm Hg) with 10% phosphate-buffered formalin solution and then cut into 1-mm-thick sections after 2 days. Each heart yielded 6 slices. The sections were photographed and quantified by computerized planimetry (Jandal Scientific Sigma-Scan). Both the apical and the basal aspects of each section were measured. The mean area of the left ventricle in each section was calculated. The total left ventricular wall volume was calculated as the sum of left ventricular mean area in each section, and then multiplied by 1 mm.

### Histology and Electron Microscopy

#### Electron Microscopy of Transplanted Heart Cells

Four weeks after transplantation, the transplanted hearts ( $n=3$ ; randomly selected from 12 transplanted hearts) were fixed in 1% glutaraldehyde in phosphate buffer. The samples were postfixated with 1% osmium tetroxide, dehydrated in graded ethanol (50%, 70%, 90%, and 100%), polymerized in propylene oxide at 60°C overnight, sectioned, and scoped in a Phillips 201 transmission electron microscope.

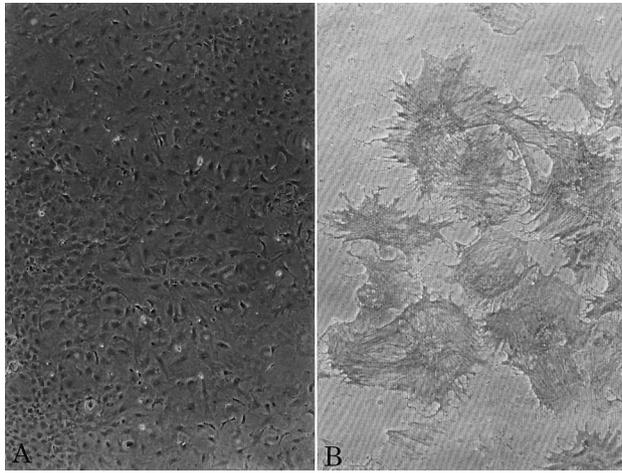
#### Histologic Studies of Transplanted Heart Cells

The heart sections were fixed in 5% glacial acetic acid in methanol, embedded in paraffin, and cut into 10- $\mu$ m-thick slices. The slices were stained with hematoxylin and eosin as described in the manufacturer specifications (Sigma Diagnostics). Slices were also immunohistochemically stained for BrdU, troponin I, and myosin light chain.

### Statistical Analysis

All results are presented as mean  $\pm$  SD; otherwise SAS System software was used for all analysis (SAS Institute). Comparisons of continuous variables between  $>2$  groups were performed by a 1-way ANOVA. If the  $F$  ratio from the ANOVA was significant, a Duncan's multiple-range  $t$  test was used to specify differences between the groups. The critical  $\alpha$ -level for this analysis was set at  $P < 0.05$ .

Ventricular function data were evaluated for the 3 groups by ANCOVA, with intraventricular volume used as the covariance and systolic, diastolic, developed pressure, and maximum and minimum dP/dt as dependent variables. The main effects were group, volume, and interaction between group times volume. If a significant difference was identified, multiple pairwise comparisons were performed to specify between-group differences.



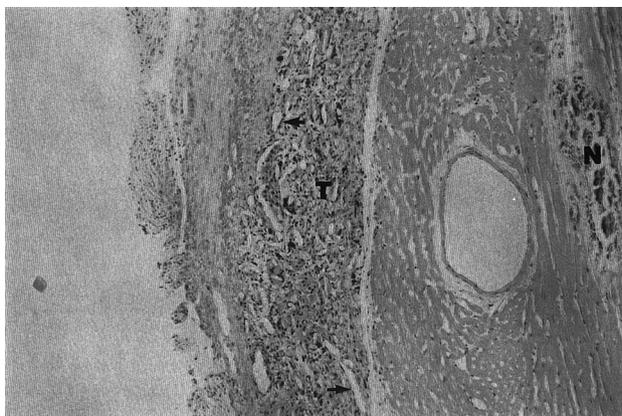
**Figure 1.** Photomicrographs of cultured ventricular heart cells (A, magnification  $\times 40$ ) that stained positively for myosin heavy chain (B, magnification  $\times 200$ ).

### Results

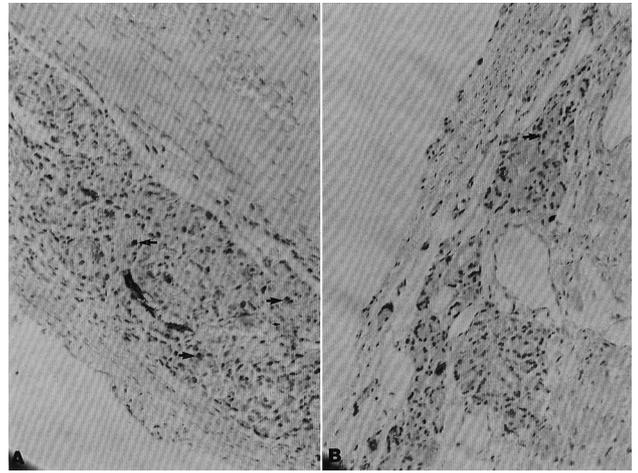
Heart rates, coronary flows, and heart–body weight ratios were not significantly different between the sham, control, and transplantation groups.

Immediately before transplantation, the heart cell culture contained  $95.4 \pm 1.7\%$  ( $n=5$ ) cardiomyocytes, as identified by positive immunohistochemical staining for myosin heavy chain (Figure 1, A and B).

Four weeks after transplantation, severe focal myocardial necrosis was present in the host myocardium of all groups. The transplanted cells formed cardiac-like tissue in the dilated myocardium of the transplantation group (Figure 2). The heart cells labeled with BrdU (labeling efficiency  $61.3 \pm 5.5\%$ ,  $n=3$ ) formed cardiac-like tissue and stained positively for BrdU 1, 2, 3, and 4 weeks after transplantation (Figure 3, A and B). The cardiac-like tissue identified by BrdU-labeling appeared to decrease in size with time. This tissue stained positively for troponin I and myosin light chain (Figure 4, A and B). Blood vessels were apparent in the



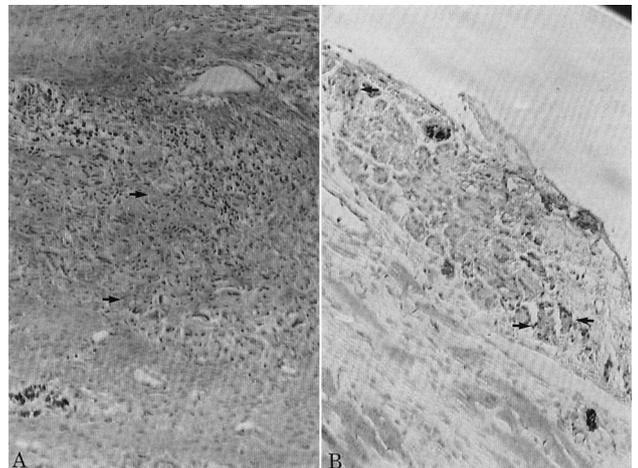
**Figure 2.** Photomicrograph of ventricular heart cells 4 weeks after transplantation into dilated cardiomyopathic heart stained with hematoxylin and eosin (magnification  $\times 100$ ). Transplanted cells formed cardiac-like tissue (T) that contained many primitive vessels (arrow); dilated cardiomyopathic myocardium had foci of severe necrosis (N).



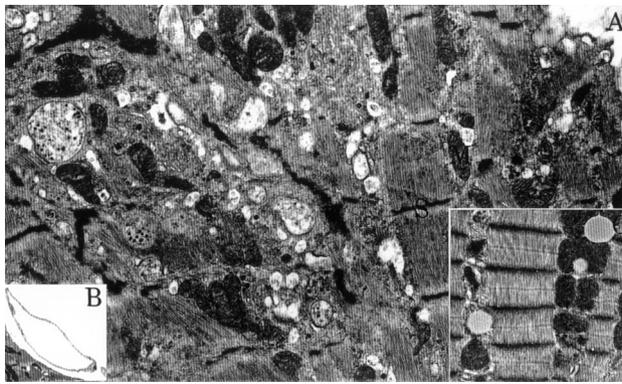
**Figure 3.** Identification of transplanted BrdU-labeled cells. Cardiomyopathic myocardium at 2 (A) and 4 weeks (B) after transplantation was stained with hematoxylin and immunohistochemically for BrdU (magnification  $\times 200$ ). Transplanted cells stained positively for BrdU (arrows).

transplanted tissue. Some lymphocytes infiltrated the transplanted tissue. Under electron microscopic visualization, the cardiac-like tissue formed by transplanted cells showed many of the components of normal myocardium including sarcomeres. The blood vessels lacked pericytes and basement membranes (Figure 5, A through C). No newly formed cardiac tissue was present in control and sham hearts.

Langendorff measurement of left ventricular function demonstrated significantly greater ( $P<0.001$ ) systolic and developed pressures, and dP/dt in the transplanted hearts compared with the control and sham hearts (Figure 6). The transplanted hearts also had significantly greater ( $P<0.001$ ) diastolic pressure at equivalent balloon volume than control and sham-operated hamsters (Figure 7, A and B). No differences in systolic and developed pressures, dP/dt, and diastolic pressure between the control and sham group were found. The left ventricular chamber volume of the transplanted hearts at 30 mm Hg of passive diastolic pressure was



**Figure 4.** Photomicrographs of muscle tissue formed by transplanted heart cells stained immunohistochemically for troponin I (A) and myosin light chain (B) (magnification  $\times 200$ ). Arrows indicate positively stained cells.

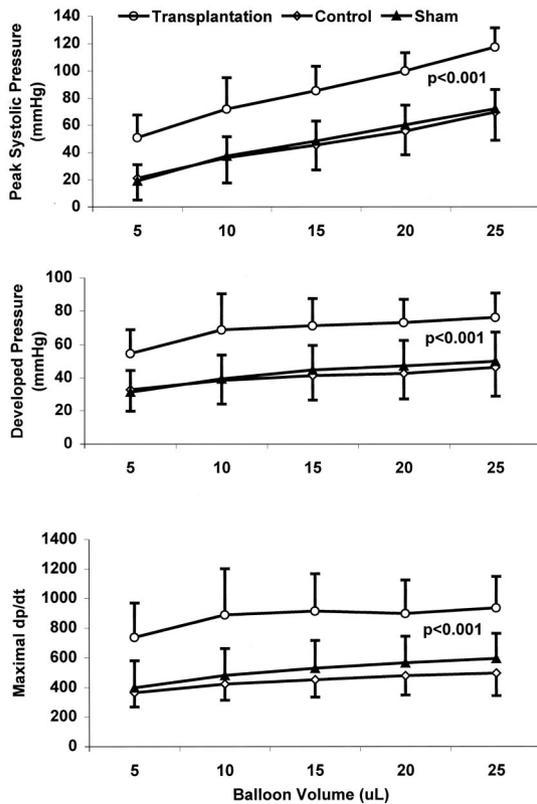


**Figure 5.** Electron micrographs of transplanted heart cells. A (magnification  $\times 17\,920$ ), Transplanted cell contained sarcomeres (S) and mitochondria (M). B (magnification  $\times 5360$ ), Primitive blood vessels that lack pericytes and basement membranes. C (magnification  $\times 11\,920$ ), Normal myocardium of noncardiomyopathic hamster.

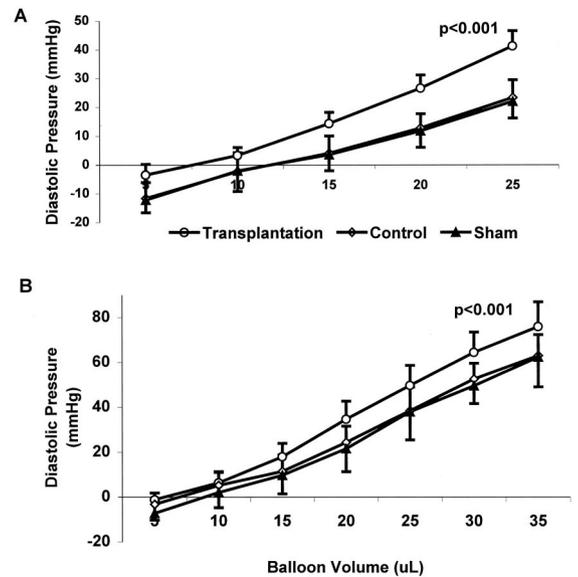
$58.38 \pm 7.71 \mu\text{L}$ , which was significantly ( $P < 0.001$ ) smaller than control ( $98.97 \pm 13.27 \mu\text{L}$ ) and sham ( $93.98 \pm 8.27 \mu\text{L}$ ) hearts (Figure 8).

### Discussion

Significant progress has been achieved during the past 10 years in cell transplantation-related technology and in the



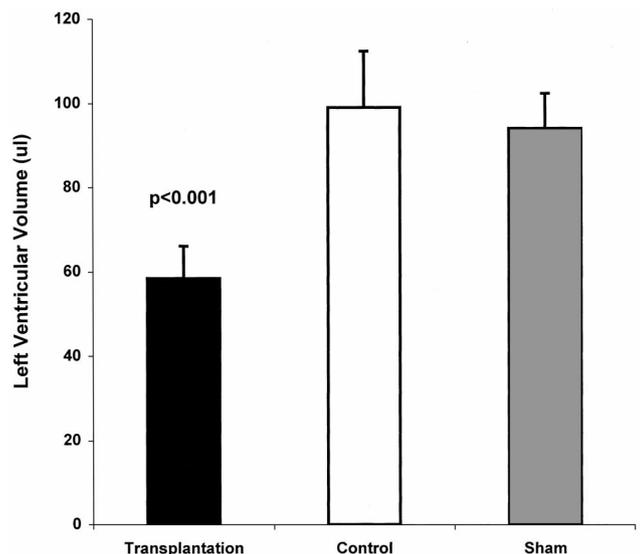
**Figure 6.** Peak systolic and developed pressures and maximal  $dP/dt$  of left ventricle of transplanted, control, and sham hearts with increasing balloon volumes. Results are expressed as mean  $\pm$  SD. Peak systolic pressure, developed pressure, and maximal  $dP/dt$  in transplantation group were significantly higher than those of control and sham groups (both  $P < 0.001$ ). There was no significant difference between control and sham groups.



**Figure 7.** End-diastolic pressure of left ventricle of transplanted, control, and sham hearts with increasing balloon volumes in beating heart (A) and arrested heart (B). Transplanted hearts had significantly higher end-diastolic pressures than those of control and sham hearts ( $P < 0.001$ ). There was no significant difference between control and sham hearts.

development of efficient and relatively safe immunosuppressive regimens. Recently, cell transplantation research has focused on improving heart function in the regionally impaired myocardium, such as after myocardial infarction. However, the effect of cell transplantation on global heart failure, especially in dilated cardiomyopathy, is unknown.

The myocardial environment is important for the survival of the transplanted cells. Ideally, this should provide conditions as close as possible to the original physiological situation from which the cells were harvested or cultured.



**Figure 8.** Left ventricular chamber volume at 30 mm Hg of end-diastolic pressure. Chamber volume in transplanted heart was significantly smaller than that of control and sham hearts ( $P < 0.001$ ). There was no significant difference between control and sham groups.

Successful engraftment will be dependent on neovascularization, which provides nutrients necessary for survival. The dilated cardiomyopathic myocardium, with its normal coronary circulation, may provide a better milieu for the transplanted cells to survive than the scar of the infarcted myocardium.

In 1998, Scorsin et al<sup>12</sup> reported that cell transplantation improved heart function in mice with doxorubicin-induced heart failure. One month after transplantation, echocardiography showed greater fractional shortening in the cell-transplanted hearts than the control and sham-operated hearts. Since the transplanted cells were not detected in the host myocardium, the mechanism of improvement of heart function was attributed to paracrine effects such as secretion of growth factors.

In this study, we evaluated the effects of cell transplantation into the dilated cardiomyopathic myocardium of BIO 53.58 hamsters. In these hamsters, hereditary abnormalities of cardiac and skeletal muscles are inherited as autosomal recessive trait. Two hamster strains, BIO 14.6 and BIO 53.58, have been used to study dilated cardiomyopathy. The BIO 14.6 strain develops characteristic pathological changes: cardiac myolysis at 30 to 40 days of age, cardiac hypertrophy at  $\approx 150$  days of age, cardiac dilatation at  $\approx 250$  days of age, and frank congestive failure at  $\approx 1$  year of age. Unlike BIO 14.6 hamsters, BIO 53.58 hamsters do not develop myolysis or hypertrophy before dilatation, have a significantly shorter life span, and demonstrate reduced cardiac function at an earlier age than BIO 14.6 hamsters. BIO 53.58 hamsters gradually develop a dilated cardiomyopathy, which is characterized by enlarged cardiac chambers and thin ventricular walls with spotty cellular myocytolysis. The cardiomyopathy is apparent at  $\approx 10$  weeks of age and becomes clinically apparent at 17 weeks of age.<sup>13–20</sup> Therefore, BIO 53.58 hamsters provide a good model of dilated cardiomyopathy. We used 4-week-old BIO 53.58 hamster cardiomyocytes as transplant donors. The growth of the cultured hamster heart cells was dependent on the size of the heart from which the cells were isolated. Four-week-old hamsters were selected because the size of the heart was sufficient to yield enough heart cells for subsequent culturing.

Four weeks after transplantation, the transplanted cardiomyocytes survived and formed a cardiac-like tissue that stained positively for BrdU, troponin I, and myosin light chain. The BrdU-labeled cardiac-like tissue gradually decreased in size with time after transplantation. We attributed the decrease in tissue size, at least in part, to immunorejection. The BrdU-labeled cardiac-like tissue appeared to be smaller than non-BrdU-labeled tissue 4 weeks after transplantation. Since BrdU decreases the rate of cell proliferation, the difference could be caused by a lower proliferation rate of labeled cells in the host myocardium compared with transplanted non-BrdU-labeled heart cells obtained from 4-week-old hamsters. The arrangement of the transplanted heart cells was disorganized compared with that of the host myocardium. Electron microscopy showed that the transplanted cardiac-like tissue had all the structures of normal myocardium, such as sarcomeres, mitochondria, and glycogen, but the

capillaries lacked pericytes and basement membranes. By light microscopy, the transplanted tissue was more vascular than the surrounding tissue.

The reason for improvement of heart function was not determined in this study. The hamster heart cells did not contract in culture, and we were unable to determine whether they contracted in the host myocardium. We attributed the improvement of diastolic function to increased wall thickness, which prevented ventricular dilation. The diastolic pressure of the transplanted hearts rose more rapidly and reached physiological levels at lower left ventricular chamber volumes than the control and sham hearts. This could, theoretically, restrict the diastolic filling of the cell-transplanted hearts. However, the transplanted hearts did develop higher developed pressure and thus did not dilate as much as the control and the sham hearts. The improved diastolic function was reflected in the improved systolic performance (systolic pressure, developed pressure, and  $dP/dt$ ) of the heart. The rate increase of the intraventricular pressure ( $dP/dt$ ) indicated improvement in the force-velocity relation and hence improvement of the inotropic state of transplanted myocardium. Another possible mechanism could be the elastic properties of contractile apparatus of the transplanted heart cells preventing fibrotic scar stretching and ventricular enlargement. The release of growth factors, which were not assessed in this study, might have provided positive effects on myocardial contractility and angiogenesis.

A limitation of the study is that syngeneic animal or autologous cells were not used to avoid immunorejection of the transplantation. Syngeneic hamsters are not commercially available. Since the BIO 53.58 hamsters begin to develop a dilated cardiomyopathy at  $\approx 10$  weeks of age, adult hamster heart cells could not be cultured and used for autotransplantation. Another limitation is that we used Langendorff perfusion apparatus to assess ventricular function after transplantation. The Langendorff perfusion apparatus provides reasonable data about heart function. Unfortunately, the hearts were not ejecting. We also have used echocardiography to evaluate the function of the ejecting heart. Although echocardiography provides interesting information, we have found it difficult to control preload, afterload, and pulse rate in the intact animal, and these data are subject to wide variability.

In summary, transplantation of cultured ventricular heart cells into the cardiomyopathic left ventricular free wall of the BIO 53.58 hamster formed cardiac-like tissue and improved myocardial contractile function.

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