SURGERY FOR ACQUIRED CARDIOVASCULAR DISEASE

COMPARISON OF THE EFFECTS OF FETAL CARDIOMYOCYTE AND SKELETAL MYOBLAST TRANSPLANTATION ON POSTINFARCTION LEFT VENTRICULAR FUNCTION

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Objectives: Transplantation of fetal cardiomyocytes improves function of infarcted myocardium but raises availability, immunologic, and ethical issues that justify the investigation of alternate cell types, among which skeletal myoblasts are attractive candidates.

Methods: Myocardial infarction was created in rats by means of coronary artery ligation. One week later, the animals were reoperated on and intramyocardially injected with culture growth medium alone (controls, $n = 15$), fetal cardiomyocytes (5×10^6 cells, $n = 11$), or neonatal skeletal myoblasts (5×10^6 cells, $n = 16$). The injections consisted of a 150- μ L volume and were made in the core of the infarct, and the animals were immunosuppressed. Left ventricular function was assessed by echocardiography immediately before transplantation and 1 month thereafter. Myoblast-transplanted hearts were then immunohistologically processed for the expression of skeletal muscle-specific embryonic myosin heavy chain and cardiac-specific connexin 43.

Results: The left ventricular ejection fraction markedly increased in the fetal and myoblast groups from $39.3\% \pm 3.9\%$ to $45\% \pm 3.4\%$ ($P = .086$) and from $40.4\% \pm 3.6\%$ to $47.3\% \pm 4.4\%$ ($P = .034$), respectively, whereas it decreased in untreated animals from $40.6\% \pm 4\%$ to $36.7\% \pm 2.7\%$. Transplanted myoblasts could be identified in all animals by the positive staining for skeletal muscle myosin. Conversely, clusters of connexin 43 were not observed on these skeletal muscle cells.

Conclusions: These results support the hypothesis that skeletal myoblasts are as effective as fetal cardiomyocytes for improving postinfarction left ventricular function. The clinical relevance of these findings is based on the possibility for skeletal myoblasts to be harvested from the patient himself. (J Thorac Cardiovasc Surg 2000;119:1169-75)

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Over the past 20 years, the incidence of heart failure has markedly increased, and its prevalence in patients over 65 years of age is now 10%.¹ As such, it represents one of the greatest challenges in public health for the next century. Standard surgical procedures, such as coronary artery bypass grafting, mitral valvuloplasty, and/or valve replacement, have been used to treat patients with advanced left ventricular dysfunction with good initial results. However, these procedures only prevent further myocardial deterioration and do not act on the underlying disease, which is myocardial fibrosis. At the opposite end of the spectrum, cardiac transplantation provides a radical therapy,

but organ shortage, along with strict eligibility criteria, mandate the search for alternate treatments in cases in which a substantial portion of the myocardium has been irreversibly destroyed.

Among those treatments, cellular transplantation has emerged as a possible means of increasing the number of contractile elements in damaged myocardium. We² and others^{3,4} have previously shown that fetal cardiomyocyte transplantation improves the function of ischemic and globally failing hearts. In a clinical perspective, however, the use of fetal cardiomyocytes raises several issues, including availability, immunogenicity, and ethics. These considerations have led to the search for alternate cell types among which skeletal muscle progenitors (myoblasts, also known as satellite cells) are attractive candidates because (1) they can re-enter the cell cycle, and thus should augment the number of contractile elements in the host myocardium, and (2) they can be used as autografts, thereby avoiding any immunologic conflict. The present study was therefore designed to assess, in a rat model of coronary artery ligation, whether the functional benefits of intramyocardially injected fetal cardiomyocytes on postinfarct function could be equaled by skeletal myoblasts.

Material and methods

Wistar rats were used in this study. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (National Institutes of Health Publication No. 80-23, revised 1985).

Fetal cardiomyocyte isolation procedure. Cardiomyocytes from fetal rat hearts were isolated and purified as previously described.² Twenty-day-old embryos (Iffa Credo, les Oncins, France) were removed, and their hearts were enzymatically digested. The pellet was then resuspended in a culture medium (68% Dulbecco's modified Eagle medium [DMEM], 17% medium 199, 10% horse serum, and 5% calf serum in the presence of 100 IU/mL penicillin, 10^{-7} mol/L triiodothyronine, 100 mg/mL streptomycin, and 10^{-9} mol/L insulin) and diluted to achieve a final concentration of 5×10^6 cells/150 μ L to be used for the intramyocardial injections. This procedure was performed on the day of transplantation and has previously been shown to preserve viability of cells.²

Neonatal myoblast isolation procedure. Primary muscle cell cultures were prepared from newborn Wistar rats (Iffa Credo). The 4 legs were skinned and dissociated enzymatically at 37°C by using first collagenase IA (2 mg/mL for 1 hour; Sigma Chemical Company, St Louis, Mo) and trypsin (0.25% for 20 minutes; Gibco BRL, Life Technologies, Inc, Rockville, Md). The cells were grown in 57% DMEM, 27% Earle's 199 medium, 15% fetal calf serum, and 1% antibiotics

(all components from Gibco). The cells were harvested and frozen on the second day. Twenty-five million cells were prepared from one newborn. A homogeneous stock of cells was prepared on the same day from 20 newborns. The cells from this stock were used throughout the transplantation experiments. On the day of transplantation, the cells were rapidly thawed and washed 3 times in injection medium, which contained 75% DMEM, 24% 199, and 0.5% bovine serum albumin (Fraction V, Sigma). The cell death did not exceed 5%, as assessed by trypan blue staining. Samples of 5×10^6 cells were prepared and kept on ice until transplantation.

After thawing, some samples were plated onto 12-well dishes in growth medium for desmin immunolabeling. Eighteen hours after seeding, the cells were fixed in methanol. Nonspecific labeling was blocked by use of a mixture of 5% horse serum and 5% fetal calf serum in phosphate-buffered saline solution (PBS). The cells were incubated with anti-desmin antibody (clone 33, 1:300, 1 hour; Dako, Trappes, France) and then with Cy3-conjugated anti-mouse immunoglobulin antibody (Jackson, 1:300, 1 hour; Asnières, France). With the use of an inverted microscope, several fields were randomly photographed both under fluorescent illumination and phase contrast. The percentage of myogenic cells was obtained by dividing the total number of cells counted on phase contrast pictures by the total number of desmin-positive cells counted on immunofluorescence pictures.

Myocardial infarction model. Male rats weighing an average of 230 g were anesthetized with ketamine (50 mg/kg body weight administered intraperitoneally) and xylazine (5 mg/kg body weight administered intraperitoneally) and tracheally ventilated. Each heart was exposed through a lateral thoracotomy. The left coronary artery was then identified and occluded proximally by means of a 7-0 polypropylene snare (Ethicon, Inc, Somerville, NJ).

Functional assessment. One week after myocardial infarction and 1 month after cellular transplantation, cardiac function was studied by echocardiography. Left ventricular dimensions and function were assessed by 2-dimensional echocardiography (Sequoia; Acuson Corp, Mountain View, Calif) equipped with a 15-MHz phased-array linear transducer (15L8), allowing a 160-Hz maximal frame rate and specifically designed for cardiac ultrasonic studies in murine models. The probe was positioned on the left anterior side of the chest after the precordium was shaved, and the rat was placed on a warming pad. The heart was imaged in 2-dimensional mode at a minimum depth setting and image size by means of the RES enhanced resolution imaging function in the long-axis view of the left ventricle, taking care to include the mitral and aortic valves and the apex. Care was taken to avoid excessive pressure. When a correct image was obtained with well-defined continuous interfaces for the septum and posterior wall at the higher frame rate, a numeric acquisition was performed on the hard disk of the echocardiographic machine. The following measurements were then performed on-line at end-diastole (at the time of apparent maximal cavity dimension) and at end-systole (at the time of maximum anterior motion of the posterior wall) by use of the cine-loop

Table I. Two-dimensional EF measurements

	Control (n = 15)	Fetal (n = 11)	Myoblast (n = 16)
Left ventricular EF			
Baseline (EF1)	40.6% $\pm$ 4.0%	39.3% $\pm$ 3.9%	40.4% $\pm$ 3.6%
One month (EF2)	36.7% $\pm$ 2.7%	45.0% $\pm$ 3.4%	47.3% $\pm$ 4.4%
Δ EF (EF2–EF1)	–4.0% $\pm$ 11.2%	5.64% $\pm$ 12.9%	6.94% $\pm$ 13.9%
P value (vs baseline)	.19	.086	.034

The baseline between-group difference was a *P* value of .97. All data are given as means $\pm$ SEM.

feature to retrospectively obtain adequate visualization of the following fast-beating hearts (300–400 beats/min): maximal left ventricular long-axis lengths and endocardial area tracings (by using the leading edge method). These data were then used to calculate left ventricular end-diastolic volume (LVEDV) and left ventricular end-systolic volume (LVESV) by using the single-plane area-length method: $EF = LVEDV - LVESV / LVEDV$, where EF is ejection fraction. All measurements were made by one experienced observer who was blinded to the treatment group. Measurements were averaged over 3 to 5 consecutive cardiac cycles. Intraobserver variability was assessed at baseline by 2 sets of measurements in 10 randomly selected rats, and the coefficient of correlation and the standard error of estimate were calculated according to the method of Bland and Altman.⁵

Experimental groups. One week after coronary artery ligation, left ventricular function was echocardiographically assessed. Thereafter, rats (n = 42) were reoperated on through a median sternotomy and randomly allocated to 1 of the following 3 groups. In the control group (n = 15) 150 μ L of culture medium alone (without cells) was injected subepicardially by using a 30-gauge needle in the core of the infarcted area. The fetal group (n = 11) received an equivalent volume supplemented with fetal cardiomyocytes (5×10^6 cells in 150 μ L of culture medium) at the same time point and in the same location. The myoblast group (n = 16) also received an equivalent volume supplemented with neonatal myoblasts (5×10^6 cells in 150 μ L of culture medium). Differences in the incidence of deaths that occurred intraoperatively or thereafter account for the observation that sample sizes were unequal. All animals received cyclosporine (INN: ciclosporin) throughout the study (15 mg $\cdot$ kg^{–1} $\cdot$ d^{–1} administered intraperitoneally; Sandoz Pharmaceutical Corporation, Basel, Switzerland), starting 3 hours before anesthetic induction. One month after the transplantation, left ventricular function was assessed again by means of echocardiography.

Pathology. Twenty-four hours after the last echocardiographic study, the rats were killed by injecting an overdose of ketamine and xylazine. The hearts were removed and rapidly rinsed in PBS. They were snap-frozen in isopentane cooled with nitrogen. Serial sections 8- μ m thick were prepared by use of a cryostat and subsequently processed for standard histology (hematoxylin-eosin staining) and immunohistofluorescence (desmin, myosin, and connexin staining). The fate of the transplanted myoblasts was investigated by looking for the presence of cardiac- or skeletal-specific antigens on serial sections of heart tis-

sue. The sections were rinsed in PBS and fixed with cold methanol for 5 minutes, and the nonspecific labeling was blocked with a mixture of 5% horse serum and 5% fetal calf serum in PBS for 30 minutes. The sections were incubated with the primary antibody for 1 hour at room temperature. We used the mouse monoclonal antibody directed against desmin (1:300, Dako), the mouse monoclonal antibody directed against the embryonic myosin heavy chain (EMHC; a gift of Dr Gillian Buttler-Browne, Paris, France; dilution 1:5), and a polyclonal rabbit anti-connexin 43 antibody (gift of Dr Daniel Gros, Marseille, France; dilution 1:100). After several washes, the sections were incubated with the Cy3-conjugated anti-mouse or anti-rabbit immunoglobulin antibodies (1:200, Jackson) for 1 hour at room temperature. The sections were mounted in PBS-glycerol (1:1).

Data analysis. For statistical analysis, we used SAS procedures (Statistical Analysis System, Cary, NC). Echocardiographic data recorded in the 3 groups before and after myocardial injections were compared by means of 1-way analysis of variance. Within each group, echocardiographic data were compared before and 1 month after myocardial injections by paired *t* tests. Mean EFs at 1 month were compared between groups after adjustment for basal EF. If analysis of variance was significant, groups were compared by using a Scheffé test. Data are given as means $\pm$ SEM.

Results

Characterization of the cell preparation. On the day of injection, desmin immunostaining indicated that approximately 60% of the cells were expressing this muscle-specific marker; approximately 6000 cells were counted.

Functional assessment. Baseline pretransplant echocardiographic data were not different among the 3 groups (Table I). One month later, the major result is that transplantation of either fetal cells or skeletal myoblasts improved EF compared with control hearts (Table I). This was paralleled by a significant increase in LVEDV in control rats (from 0.46 ± 0.02 at baseline to 0.68 ± 0.04 at 1 month, *P* = .0007), whereas remodeling was limited in the transplanted hearts with LVEDV, being 0.45 ± 0.03 at baseline and 0.58 ± 0.06 at 1 month (*P* = .038) in the fetal group and 0.52 ± 0.04 at baseline and 0.6 ± 0.05 at 1 month (*P* = .11) in the myoblast group.

Fig 1. Two-dimensional echocardiographic EF of each group before and 1 month after transplantation.

Comparison of EF at 1 month between groups demonstrated a clear effect of transplantation ($P = .09$); these differences were even more marked after adjustment on baseline EF ($P = .0001$), with EF in myoblast and fetal groups greater than that found in control animals ($P < .05$ [confidence limits = 0.45-21.0] and $P > .05$ [confidence limits = 2.9-19.7], respectively), but without significant differences between the 2 transplanted groups. The salutary effects of cellular transplantation were further supported by the scattergram (Fig 1) plotting 1-month functional outcome versus baseline data. Thus hearts with the largest infarcts and the greatest impairment of EF (baseline values of $<40\%$) were found to benefit most from the transplantation procedure. However, even in cases where the impairment of baseline EF was less pronounced ($>40\%$), the beneficial effects of cellular transplantation were still apparent, as reflected by a stability of EF over time, which contrasted with the marked alterations of this parameters in control hearts (Fig 1). Of note, these post-transplantation functional data were recorded under similar conditions of heart rate (300 ± 19 beats/min, 293 ± 15 beats/min, and 308 ± 22 beats/min in the control, fetal, and myoblast groups, respectively).

Histologic characterization of the transplanted tissue. Multinuclear elongated structures were observed within the injected area 4 weeks after transplantation (Fig 2, A). These structures were seen both in the core and at the borders of the infarcted area, and they were absent from hearts that did not receive the skeletal muscle cells. Immunostaining for EMHC

revealed both small and elongated cells in the infarcted zone of the rats injected with the primary cultures (Fig 2, B). These structures were typical of myotubes and muscle fibers, and they were aligned along either the long or the short axis of the heart. The smallest stained cells were probably committed myoblasts. Desmin staining of serial sections confirmed the muscular nature of the structures for EMHC (Fig 2, C). Desmin staining also revealed the presence of cardiac tissue surrounding the skeletal tissue at the borders of the infarcted zone; this cardiac tissue did not express EMHC. Finally, connexin 43 was expressed by the cardiac cells surrounding the skeletal muscle; however, the myoblasts, myotubes, and skeletal muscle fibers did not express connexin 43 (Fig 2, D). In summary, engrafted myoblasts were identified within and at the borders of the fibrous scar by their positive staining for desmin and EMHC (a specific marker of skeletal myoblasts) and could thus be differentiated from the surrounding host cardiomyocytes, which only stained for desmin (a nonspecific marker of muscular cells).

Discussion

The two major findings of this study are that (1) transplantation of fetal cardiomyocytes improves function of infarcted myocardium, primarily through a reduction of left ventricular remodeling, and (2) this functional benefit can be matched by intramyocardial injection of skeletal myoblasts.

Echocardiographic assessment of function. Because rat hearts beat at a fast rate and because of the limitation of M-mode echocardiography in assessing volumes and performance in infarcted ventricles, we used a 2-dimensional echocardiographic machine, allowing a numeric acquisition of a maximum of 160 images per second and yielding more than 25 heart scans at a heart rate of 300 beats/min. Previous necropsy findings have shown that the left ventricle in rats has an elliptical shape, which justifies that volume measurements were based on an elliptical model of left ventricular geometry.⁶ We used the single-plane area-length method, which has been shown in human infarcted hearts to be more accurate than those relying on M-mode measurements. The echocardiographic method of volume calculation was based on a single plane model, which could underestimate the extent of infarction. This method was preferred to biplane methods, which have not yet been validated in infarcted rats and are difficult to apply in practice because of cavity distortion after large anterior infarction. Thus in this study differences between groups might have only been underestimated.

Fig 2. Photomicrographs of skeletal muscle tissue 4 weeks after transplantation into the myocardial scar. **A**, Elongated multinucleated fibers (*asterisks*) are formed within the scar tissue. These fibers contain central nuclei (*arrows*; hematoxylin and eosin staining). Panels **B**, **C**, and **D** show serial sections of immunohistochemistry staining. **B**, A cluster of skeletal muscle fibers (*asterisk*) surrounded by small myotubes characterized by embryonic myosin heavy chain staining. **C**, The same cluster of skeletal muscle fibers (*asterisk*) and also the surrounding host cardiac tissue (*arrows*) are positive for desmin staining. **D**, The surrounding host cardiac tissue (*arrow*), but not the skeletal muscle fibers (*asterisk*), stain positively for connexin 43. (Original magnification $\times 150$.)

Fetal cardiomyocyte transplantation. The functional benefits of fetal cell transplantation have been well established in myocardial infarction models created by cryonecrosis³ or coronary artery ligation² and in which function was assessed by in vitro Langendorff perfusion studies³ or in vivo echocardiographic measurements.² More recently, these observations made in regionally ischemic preparations have been extended to the setting of global heart failure induced by anthracyclines.⁴ The present data extend these findings mechanistically.

Namely, the fact that intramyocardially injected fetal cardiomyocytes primarily limited left ventricular dilation strongly suggests that the elastic properties of these cells played a major role in the overall improvement of function.⁷ Indeed, this improvement was probably underestimated because of the expectedly limited effects of cell transplantation in rats with initially small infarcts that did not tend to substantially enlarge over time. Conversely, the greatest benefits were seen in those animals with the largest infarcts and the consequently lowest pretransplant EFs, which supports the potential relevance of this procedure to patients with severe ischemic cardiomyopathy. However, apart from

limitation of remodeling, other mechanisms could account for the functional benefits of fetal cardiomyocyte transplantation. For example, these cells have been shown to establish gap junctions with those of the recipient myocardium,⁸ which should allow them to beat synchronously with adjacent cells and thus directly contribute to enhanced contractility. Indeed, analysis of our data in relation with the pretransplant functional status of hearts raises the possibility that a contention mechanism could prevail in cases of initially small infarcts, whereas direct systolic assistance would be additionally involved in large infarcts. It is also conceivable that intracardiac grafts could act as platforms releasing growth factors, angiogenic factors, or both. This hypothesis is indeed supported by two lines of evidence: (1) intramyocardially implanted cardiomyocytes increase the density of capillaries and arterioles⁹ and (2) locally injected cells improve global heart function, although they do not seem to migrate away from the implant sites,^{10,11} which could be consistent with paracrine effects exerted by cell-derived mediators.

A major issue raised by fetal cardiomyocyte transplantation is tracing of the injected cells within the host myocardium. This is due to the fact that fetal car-

diomyocytes lose their characteristic phenotypic patterns over time and become progressively indistinguishable from adult host cardiomyocytes. The intramyocardial injections of genetically labeled cells expressing β -galactosidase activity has been the most common means of identifying them once they have been engrafted into the recipient heart. However, despite immunosuppressive therapy, the expression of β -galactosidase can be highly immunogenic¹² and, through this mechanism, can adversely affect function. Thus to avoid confounding of our echocardiographic measurements by this factor, we made no attempt to label injected fetal cells, assuming that we² and others^{8,7,3} have previously documented their ability to form stable intracardiac grafts.

Skeletal myoblast transplantation. Myoblasts feature several advantages for cellular transplantation, including their capacity to proliferate. Neonatal myoblasts have thus been shown to replicate in the recipient myocardium until 1 week after grafting.¹¹ Furthermore, although most engrafted myoblasts differentiate into mature fibers, some of them might become quiescent established satellite cells that could participate in tissue repair in cases of subsequent ischemic episodes.¹¹

Our data confirm that neonatal skeletal myoblasts can be successfully engrafted in ischemically damaged myocardium, can limit infarct expansion, and can improve 1-month EF compared with values recorded before transplantation, as well as those yielded by non-transplanted control animals at a time-matched study point (ie, 1 month after injection of the culture medium alone). In the present study neonatal myoblasts were used to enhance the efficacy of *in vitro* expansion before implantation. Furthermore, grafting allogenic cells also provided the opportunity to provide cyclosporine immunosuppression under the same conditions as those used in the fetal cardiomyocyte transplantation experiments. This was deemed methodologically important to avoid between-group comparisons of functional outcome to be confounded by the intrinsic effects of cyclosporine on postinfarct myocyte hypertrophy.¹³ However, in a rabbit model of cryonecrosis, Taylor and associates¹⁴ have shown that injections of autologous adult myoblasts were equally successful in improving systolic and diastolic function, as assessed by ultrasonic dimension transducers. In this study a causal relationship between the presence of myoblasts and outcome was clearly established in that function was only found to improve in hearts that were successfully engrafted.

The mechanism by which skeletal myoblast transplantation is functionally beneficial remains elusive. Chiu and coworkers¹⁵ have hypothesized that implant-

ed satellite cells can undergo a milieu-induced differentiation that leads them to acquire a cardiac-like phenotype. This hypothesis is largely based on the observation by these authors¹⁵ and others¹⁴ of structures resembling intercalated disks between transplanted myoblasts. The influence of the cardiac environment on the phenotypic changes of engrafted cells is further suggested by their conversion to fatigue-resistant slow-twitch fibers.^{11,16} However, implanted myoblasts fail to express cardiac-specific myosin heavy chain up to 3 months after transplantation.¹¹ The formation of intercalated disks between cardiac host cells and donor cells labeled with a reporter gene has been unambiguously reported only in the case in which a permanent mouse myogenic cell line (C2C12) was injected into a noninfarcted mouse myocardium.¹⁷ Our present results argue against a classical pathway of electrical coupling of cardiac and skeletal tissue *in vivo* because engrafted myoblasts, identified within and around the fibrotic tissue by their positive staining for the skeletal muscle-specific myosin heavy chain, failed to express connexin 43 on their sarcolemma. Taken together, these data suggest that some structures may develop over time that mechanically tether implanted myoblasts to the adjacent host cardiomyocytes. Conversely, gap junction-supported electrical coupling, which is a prerequisite for transplanted cells to provide synchronized mechanical support, is more unlikely to occur.

Interestingly, the present results show that the lack of such a coupling did not preclude improvement of postinfarct function of myoblast-implanted hearts to the same extent as hearts transplanted with fetal cardiomyocytes. Likewise, the functional benefits of skeletal myoblasts¹⁴ and fetal cardiomyocytes³ have been reported in cryoinjury models in which implanted cells are insulated from the remaining myocardium by scar tissue and have therefore no possibility for establishing connections with host cardiomyocytes. This suggests that skeletal myoblasts could primarily be cardioprotective through their elastic properties, which would oppose the trend for postinfarct fibrous tissue to dilate over time. Because skeletal muscle tissue is formed both in the core and at the borders of the infarcted zone, cardiac, fibrotic, and skeletal tissues are intricately *in vivo*. The production of a muscle tissue of skeletal nature within the infarcted area, however, may change the physical properties of the scar and lead to a better compliance of the cardiac tissue, which could become more resistant to a progressive dilation. Thus transplanted cells would act as a tissue bandaging within the scar tissue. This hypothesis, which is consistent with our observations of limited remodeling in

myoblast-implanted hearts, is also supported by the micromanometric and sonomicrometric findings that these cells are particularly effective in preserving postinfarct diastolic compliance.¹⁸

In conclusion, transplantation of skeletal myoblasts limits postinfarct remodeling of the left ventricle and ultimately results in improved function. This improvement was similar in magnitude to that seen after implantation of fetal cardiomyocytes. Because skeletal myoblast transplantation would be easier to implement in clinical practice, it might thus find a place in the armamentarium of techniques designed to augment function of the failing heart.

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Discussion

Dr Bradley S. Allen (*Oak Lawn, Ill*). I have a question from a clinical standpoint. It seems that what you have done is just thickened the scar so that the ventricle does not dilate. Clinically, most of our cardiologists seem to do the same thing just by opening the artery at the end of an infarct either with tissue plasminogen activator or angioplasty. Do you have a group in which you simply opened up the artery after the infarct, and did you see the same results as when you transplanted cells, which seems much more complicated?

Dr Scorsin. I did it before and, indeed, transplantation of cells results in an improvement of function, irrespective of whether the artery is reperfused or not.

Dr Juan C. Chachques (*Paris, France*). In the last 3 years of our work on an experimental model, we have implanted satellite cells (myoblasts) in small and big animals. We learned that mainly in the rat model it is necessary to correlate the echocardiographic studies with histologic studies because in many cases we do not find cells in the myocardium, even with an improvement in EF. Therefore I recommend that you correlate all your research in small animals with histologic studies of the myocardium because you can improve the EF by another mechanism that is not related to cell implantation. I think that the main conclusions in cellular cardiomyoplasty can be obtained by using big animals.

Dr Scorsin. Thank you for your remarks, Dr Chachques.

Dr Philippe Menasché (*Paris, France*). I would like to follow up briefly on Dr Allen's question. Indeed, the first study that we did, now 5 years ago, was a study with ischemia and reperfusion. At that time, this was actually the first study we had done in showing that transplantation of cells did improve function to the same extent as it did in this particular study, where there was no reperfusion.

Regarding Dr Chachques' comments, it should be noted that in all these animals, the improvement in function assessed by echocardiography was actually correlated with the presence of engrafted cells, which could be identified in all animals. This has also been shown by Doris Taylor by using a slightly different rabbit model, which shows a close correlation between engraftment of cells and improvement of function. Therefore we definitely believe that even if the mechanism by which cells improve function remains elusive, there is a clear correlation between the fact that cells are present in the tissue and the fact that function is improved.