Right ventricular failure secondary to chronic overload in congenital heart diseases: Benefits of cell therapy using human embryonic stem cell–derived cardiac progenitors

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Objective: Despite the increasing incidence of right ventricular (RV) failure in adult patients with congenital heart disease, current therapeutic options are still limited. By contrast to left-heart diseases, cell-based myocardial regeneration applied to the right ventricle is poorly studied, even though it may be a therapeutic solution. As human embryonic stem cell–derived cardiac progenitors seem to be good candidates owing to their proliferation capacity, our aim was to assess, in a large animal model of overloaded RV dysfunction, the feasibility and effects of such a cell therapy.

Methods: Human MesP1⁺/SSEA-1⁺ cardiogenic mesodermal cells were administered using multiple intramyocardial injections 4 months after a surgical procedure mimicking the repaired tetralogy of Fallot, and their effects were observed 3 months later on hemodynamic, rhythmic, and histologic parameters.

Results: All pigs (sham n = 6, treated n = 6) survived without complication, and cell therapy was clinically well tolerated. Although functional, contractility, and energetics parameters evolved similarly in both groups, benefits regarding arrhythmic susceptibility were observed in the treated group, associated with a significant decrease of peri-myocyte fibrosis (5.71% ± 2.49% vs 12.12% ± 1.85%; *P* < .01) without interstitial fibrosis change (5.18% ± 0.81% vs 5.49% ± 1.01%). Such a decrease could be related to paracrine effects, as no human cells could be detected within the myocardium.

Conclusions: Cell therapy using intramyocardial injections of human MesP1⁺/SSEA-1⁺ cardiogenic mesodermal cells seems to have benefits regarding overloaded RV tissue remodeling and arrhythmic susceptibility, but this mode of administration is not sufficient to obtain a significant improvement in RV function. (J Thorac Cardiovasc Surg 2015;149:708-15)

See related commentary on pages 715-7.

Supplemental material is available online.

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Supported by the Association Française contre les Myopathies, grants numbers 12063, 13056, and 13968, Evry, France; the Agence Nationale pour la Recherche, grant number ANR-06-MRAR-041-03 Cardiostem, Paris, France; and Field of Major Interest STEM Pole 2008-2010, IDF Paris, France.

Disclosures: Authors have nothing to disclose with regard to commercial support.

Received for publication June 27, 2014; revisions received Oct 7, 2014; accepted for publication Nov 16, 2014; available ahead of print Jan 10, 2015.

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0022-5223/\$36.00

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<http://dx.doi.org/10.1016/j.jtcvs.2014.11.033>

Right ventricular (RV) failure remains a major problem in the long-term follow-up of patients with congenital heart disease, leading to impairment of functional status, severe arrhythmias, and premature death.¹ This RV dysfunction occurs in young adults as a consequence of chronic pressure and volume overload secondary to procedures that had early success, in patients with either 2 ventricles in which the right ventricle was abnormal (eg, tetralogy of Fallot [TOF]), or was the dominant or systemic ventricle (eg, hypoplastic left heart syndrome).² In these patients, more than 50% have a risk of heart failure after age 30 years.¹ Moreover, RV function is a major prognostic factor of pulmonary artery hypertension, regardless of etiology.³ Consequently, management of RV failure was identified as a priority in cardiology research.⁴

By contrast to left ventricular failure, clinical management of patients with RV failure remains challenging: drugs or resynchronization give variable benefits,⁵ and surgical procedures such as valve replacement or repair may delay RV failure, but may fail to improve altered RV function. At the end-stage of heart failure, cardiac transplantation is mandated, but its application is restricted by the availability

Abbreviations and Acronyms

DNA	= desoxuribose nucleic acid
ECG	= electrocardiogram
ESC	= embryonic stem cell
HD	= high-density
PVS	= programmed ventricular stimulation
RV	= right ventricular
TOF	= tetralogy of Fallot

of donor organs. Cell-based myocardial repair may be an alternative approach. Human embryonic stem cell (ESC) engraftments were successfully attempted in left ventricular myocardium after ischemic injuries.⁶ Regarding the right ventricle, cell therapy using myoblastic⁷ or cord-blood stem cells^{8,9} has been attempted but yielded poor results.

Treating RV failure, in contrast to ischemic left ventricle, by cell therapy, has to take into account not only the postoperative scarred area, but also the specific geometry and physiologic processes of the right ventricle. Indeed, RV chronic overload alters the entire RV myocardium.^{10,11} This extensive alteration requires a substantial number of cells to treat, and potentially induce a functional improvement of, the RV myocardium. Among multiple cell types considered as potential sources of cardiac progenitors, some did establish a functional coupling with the host myocardium.^{6,12,13} ESCs derived from the inner mass of the blastocyst possess this capacity and are able to proliferate, differentiate in vivo into mature cardiac myocytes, and repopulate significant regions of the damaged myocardium.

A proof of concept has been reported in a nonhuman-primate model of infarcted myocardium.^{6,13} In one of these models,¹³ the maturation and differentiation of cardiac progenitors into ventricular myocytes seemed to be optimal in areas composed of both fibrosis and damaged cardiac fibers. Instances in which such a structural remodeling constitutes a favorable environment for stem-cell grafting has been observed in patients with overloaded RV dysfunction,¹⁴ suggesting that cardiac progenitor cell therapy may be applied in this indication.

The purpose of our study was to evaluate the feasibility and effects of a cell therapy using human ESC-derived mesodermal cardiogenic cells in our porcine model of chronic overloaded RV dysfunction.¹⁰ The impact of this treatment on hemodynamic and rhythmic parameters was evaluated throughout the follow-up; the fate of cardiac progenitors and the RV structural remodeling were assessed.

METHODS

Experimental Design

Twelve Landrace male piglets were studied in accordance with European Union regulations (Directive 86/609 EEC). This study was approved by the French Ministry of Agriculture (approval No. B92-019-01) and the Committee on Ethics of Animal Experiments

CEEA26 CAPSud. Animals underwent an electrocardiogram (ECG) and hemodynamic evaluation at 3 points in the experiment: baseline, and 4 and 7 months of follow-up. The surgical procedure mimicking repaired TOF was performed after the baseline step. Briefly, an enlargement of the RV outflow tract by a polytetrafluoroethylene patch, excision of 1 pulmonary valve leaflet, and a pulmonary artery banding were performed.¹⁰ The rhythmic risk was evaluated throughout the cell-therapy period. Histologic analyses were performed on each animal at the end of the procedure.

Cardiac Progenitor Characteristics and Implantation into the RV Myocardium

Human MesP1⁺ (mesoderm posterior 1)/SSEA-1⁺ (stage-specific embryonic antigen-1; CD15) cardiac progenitor cells derived from the HUES-24 (human embryonic stem) cell line were used. Briefly, HUES-24 cells were treated with 100-ng/ml Wnt3a for 24 hours and then with 10-ng/ml BMP2 for 3 days; cells were sorted with a biotin anti-CD15 antibody (Exbio, CliniSciences, Nanterre, France) and antibiotin conjugated beads (Miltenyi Biotec, Paris, France). RNAs extracted from both CD15⁺ and CD15⁻ cells were submitted to reverse transcriptase. Complementary DNAs were run in a real-time polymerase chain reaction, as described elsewhere.¹³ Cell characteristics are presented in Figure 1. The selection excludes undifferentiated or early neural stem cells, and no teratoma or proliferative cell foci formation was detected on explanted heart, lung, or liver using scanner multislices imaging (Somatom Definition Flash, Siemens Healthcare, St. Denis, France).

Cell transplantation was performed 4 months after surgery through a right thoracotomy approach. Animals received either medium containing mesodermal cardiogenic cells (treated group: n = 6) or vehicle alone (sham group: n = 6). The total bolus (10⁷ cells) was injected into the RV free wall at 20 separate injection sites; 20 other injections were made in a high-density (HD) area of 1 cm² identified by nonabsorbable sutures, using a 28-gauge needle (3 mm deep, 25 μ l/site) connected to a mesotherapy pistol (DHN-2, Techdent, Sallanches, France). Hydrocortisone (1 mg/kg) was injected intravenously before closing, to reduce inflammation. All animals were immunosuppressed by tacrolimus by mouth (0.3 mg/kg/day, plasmatic level: see Table 1).

ECG and Rhythm Study

A 12-lead surface ECG was recorded, and QRS duration was analyzed.¹⁰ An insertable recorder (Reveal, Medtronic France SAS, Boulogne-Billancourt, France) was implanted subcutaneously under the left scapula at the time of cell implantation, to record the heart's rhythm until the end of follow-up. This cardiac monitor was programmed to record tachycardia >200/minutes, during at least 6 complexes. As a last step, a stored ECG was collected by percutaneous interrogation, and a programmed ventricular stimulation (PVS) was carried out with a quadripolar catheter inserted into the RV apex through the femoral vein.

Standard clinical PVS protocols were employed, including application of single, double, and triple extrastimuli of increasing prematurity until reaching the RV refractory period, after a sequence of 8 conditioning stimuli. The heart was then challenged 3 times with a sequence of 8, followed by a single extrastimulus at compulsory rhythms of 100, 120, and 150/minutes. If no ventricular tachycardia was induced, this procedure was repeated to apply 3 challenges with double, and if necessary triple, extrastimuli. The PVS was considered positive if these challenges produced sustained ventricular tachycardia (>30s) or ventricular fibrillation.

Hemodynamic Study

Quantification of RV overload and contractile performance of RV myocardium were assessed by the conductance catheter technique. Briefly, the conductance catheter was inserted in the right ventricle through the

FIGURE 1. Characteristics of Wnt3a/BMP2 treated cells sorted with an anti-CD15 antibody. Data are expressed as the ratio of gene expression in CD15⁺ cells versus the CD15⁻, after normalization with GAPDH for Goosecoid (*Gsc*), brachyury (*T*), *MesP1*, *Tbx6*, *Nkx2.5*, and *Isl1* (A). Staining of CD15⁺ cells plated for 12 hours on fibronectin-coated coverslips with an anti-MesP1 antibody (Bioss Inc, Woburn, Mass) and Dapi (DNA, nuclei) (B); bar = 50 μ m. DNA, Desoxuribose nucleic acid.

femoral vein, under fluoroscopic guidance; a balloon was inflated in the caudal vena cava to obtain inflow occlusion.¹⁰ The effective stroke volume was calculated as the difference between the total stroke volume and the measured volume of the pulmonary regurgitation. The effective ejection fraction was calculated as the ratio between effective stroke volume and end-diastolic volume. The RV contractile status was examined using the end-systolic pressure-volume relationship, slopes of which (E_{max}) were computed during vena cava occlusion. The systolic pressure-volume area, correlated linearly with ventricular oxygen consumption/beat, was calculated as the area enclosed by the end-systolic and end-diastolic pressure-volume relationships and the systolic pressure-volume trajectory; it represents the total mechanical energy consisting of both external work (or stroke work) and mechanical potential energy.¹⁵

Detection of Engrafted Cells in the Host Myocardium

Indirect immunofluorescence labeling was performed on free wall and HD-area frozen sections using anti-human nuclei antibodies (EMD Millipore, Molsheim, France) to detect progenitor cells. Genomic DNA was prepared from RV free wall and HD-area pieces using the DNeasy Blood & Tissue Kit (Qiagen, Venlo, Netherlands). Samples were submitted to polymerase chain reaction analysis, to quantify the presence of human cells using amplification of human ALU sequences using TaqMan (Life Technologies SAS, Saint Aubin, France).¹⁶ The negative control DNA was isolated from sham RV pieces. The positive control DNA was isolated from atrial human samples (Kremlin Bicêtre Ethics Committee, protocol 04-26).

Histologic Analysis and Immunohistochemistry

After heart explantation, a gross morphologic examination was performed, and the RV area was estimated by planimetric measurements.

RV free wall and HD-area tissue samples were fixed in 4% formalin and embedded in paraffin. Sections (3- μ m thick) were stained with Picrosirius Red F3BA, hematein/eosin, or an anti-von Willebrand factor (1/600, Dako France SAS, Les Ulis, France). Pictures were recorded at 20X, and analyses were conducted by 2 independent blinded investigators. Collagen quantification was performed on 6 sections (20 fields/section) per animal, expressed as a percentage of total tissue, and its distribution (interstitial and peri-myocyte) was analyzed. Myocyte diameters were determined by measuring the short axis of 30 cells/field (20 fields/animal). All measurements were performed with ImageJ software. Anatomic pathologists determined the tissue inflammatory status and evaluated the neoangiogenesis after von Willebrand-factor immunostaining. To assess the remodeling evolution of our animal model, we included in our data set new analyses of samples issued from animals described in our previous study,¹⁰ named nonoperated 4 months and operated sham 4 months (Figures E1 and E2).

Statistical Analysis

Analyses were performed with statistical softwares GraphPad (Prism 5, GraphPad Software, <http://www.graphpad.com>) and R (version 3.1.0; R Foundation, www.r-project.org). Data were tested for normality and expressed as mean $\pm$ SD, or median and range after the result of the Shapiro-Wilk test. The 2 groups (sham and treated) were compared by 1-way analysis of variance for repeated measures (baseline, 4 months, 7 months) including post hoc analysis by Fisher protected least significant difference test when appropriate, or by nonparametric test for repeated measures (Friedman test with replication and Wilcoxon matched-pair signed-rank test) within groups, and Mann-Whitney *U* test between groups. For histologic data, a Mann-Whitney *U* test or a Kruskal-Wallis analysis of variance (Dunn's post hoc analysis) were applied. A *P* value <.05 (2-sided) was considered significant.

RESULTS

Reproducibility of the Experimental Model and Time Evolution of the RV Dysfunction

Clinical, electric, and hemodynamic characteristics of the animal population described in this study were not significantly different from the previously described population¹⁰ at 4 months postoperative, confirming the reproducibility of our experimental model. No significant difference was established between the groups at baseline, 4, or 7 months.

At 7 months postoperative, sham animals presented no complications, gained weight, and showed no clinical signs of heart failure. Heart rate was stable from 4 to 7 months. On ECG, the QRS duration continuously increased indicating the progression of the RV dysfunction. Barometric overload was maintained from 4 to 7 months at a similar level (Table 1): RV end-systolic pressure remained stable at the last follow-up as well as the peak pressure, which was at 50% of systemic pressure. In contrast, end-systolic and end-diastolic volumes continued to progress. Functional parameters such as RV end-diastolic pressure, central venous pressure, effective ejection fraction, and cardiac index remained stable (Figure 2, A). Regarding the myocardial contractility, E_{max} values showed a slight but not significant decrease at the last step (Figure 2, B); in parallel, energetic parameters including stroke work and pressure-volume area did not change (Figure 2, C).

TABLE 1. Population, ECG, and intracardiac pressures and volumes in sham and treated groups at each step of experimentation

Group			Follow-up			
	Baseline		At 4 months		At 7 months	
	Sham n = 6	Treated n = 6	Sham n = 6	Treated n = 6	Sham n = 6	Treated n = 6
Population characteristics						
Age (d)	69.3 ± 11.8	75.0 ± 7.9	201.2 ± 7.1*	194.8 ± 8.1*	303.7 ± 16.3*,†	289.2 ± 12.3*,†
Weight (kg)	21.0 ± 3.6	21.0 ± 1.5	55.9 ± 8.0*	58.9 ± 6.2*	73.1 ± 8.1*,†	71.5 ± 11.9*,†
Body length (cm)	67.0 ± 4.9	65.5 ± 4.6	92.5 ± 4.0*	92.2 ± 4.9*	102.3 ± 4.3*,†	103.5 ± 10.3*,†
Body surface area (m ²)	0.62 ± 0.08	0.61 ± 0.04	1.20 ± 0.11*	1.22 ± 0.09*	1.44 ± 0.11*,†	1.43 ± 0.19*,†
Tacrolimus plasmatic level (ng/ml)			6.0 ± 1.6	5.9 ± 3.2	5.7 ± 3.8	6.0 ± 3.3
ECG						
Heart rate (beats/min)	116 ± 4	118 ± 14	91 ± 18*	97 ± 11*	84 ± 20*	84 ± 11*
QRS duration (ms)	59.7 ± 7.5	59.7 ± 6.9	72.3 ± 3.2*	71.0 ± 9.9*	85.2 ± 9.0*,†	82.0 ± 7.5*,†
Intracardiac pressures						
Mean aortic blood pressure (mm Hg)	58 ± 9	66 ± 9	85 ± 17*	85 ± 11*	80 ± 11*,†	74 ± 8*,†
Systolic aortic blood pressure (mm Hg)	77 ± 12	85 ± 10	103 ± 18*	104 ± 15*	100 ± 11*,†	90 ± 9*,†
Central venous pressure (mm Hg)	1.3 ± 1.0	1.5 ± 0.8	4.8 ± 2.0*	4.7 ± 1.9*	5.3 ± 3.0*	4.8 ± 2.1*
RV pressure (mm Hg)						
Peak	20.3 ± 5.3	22.3 ± 1.6	50.5 ± 27.2*	59.2 ± 14.1*	44.4 ± 13.9*	56.8 ± 11.7*
End-systolic	11.3 ± 3.7	13.2 ± 1.2	42.5 ± 25.8*	49.2 ± 9.3*	37.0 ± 14.6*	50.8 ± 12.6*
End-diastolic	1.5 ± 1.5	1.7 ± 0.8	5 ± 2.9*	5.0 ± 3.2*	6.5 ± 3.7*	5 ± 2.5*
Ratio systolic pressure RV/Aorta (%)	27 ± 8	27 ± 5	47 ± 17*	57 ± 10*	45 ± 12*	64 ± 15*
Intracardiac volumes						
End-diastolic volume (ml)	91 ± 26	85 ± 15	166 ± 46*	148 ± 24*	192 ± 55*,†	208 ± 59*,†
End-systolic volume (ml)	45 ± 19	39 ± 9	80 ± 39	67 ± 20	83 ± 37*	103 ± 36*
Stroke volume (ml)	44 ± 10	42 ± 5	69 ± 5*	71 ± 1*	95 ± 25*	87 ± 20*
Fraction of regurgitation (%)			22 ± 9	18 ± 3	15 ± 8	18 ± 3

ECG, Electrocardiogram; RV, right ventricle. * $P < .05$ versus corresponding group at baseline. † $P < .05$, corresponding group at 7 months versus 4 months.

Cell Therapy: Clinical Tolerance, Hemodynamic Effects, and Rhythmic Impact

Transmyocardial injections were performed after a similar length of time after the operation in each group (120 ± 9 days in treated, 132 ± 5 days in sham). Only transient arrhythmias were observed at the time of needle penetration, and they were fully resolved after injections. No death occurred as a result of the cell injection procedure, or during the follow-up, and no severe adverse effects occurred. Immunosuppression therapy was well tolerated. During the postinjection period, animals gained weight (Table 1) and showed no clinical signs of either heart failure or immune rejection. No difference between treated and sham groups was observed in the RV dysfunction evolution (Table 1). Functional characteristics, contractility parameters, and energetics similarly evolved after either placebo or cell injections (Figure 2).

On ECG, the QRS duration continuously increased, in similar fashion in both the sham and treated groups (Table 1). Despite this risk factor of arrhythmia, all treated animals showed normal long-duration Holter recordings, and no ventricular arrhythmia could be induced by PVS. In contrast, sinus pauses >2 seconds were detected on long-duration Holter in 2 sham animals. In one of them, a nonsustained ventricular tachycardia was induced twice after a single extrastimulus at a compulsory rhythm of

120/min and a ventricular fibrillation had occurred after PVS. In the other animal, the PVS led to a transitory ST depression that appeared after a sequence of 8, followed by the single extrastimulus at a compulsory rhythm of 150/min, suggesting an alteration of the myocardial reserve. This arrhythmic susceptibility supports the evolution capacities of our model in accordance with clinical findings.

Fate of Injected Human Cells and Their Effect on RV Tissue Remodeling

All animals were euthanized about 3 months after injections (94 ± 12 days in treated, 103 ± 11 days in sham). Human cardiogenic mesodermal cells or cardiac cells have been sought in the RV free wall and HD area using 2 different techniques. At the cellular level, immunolabelings against human nuclear antigen did not reveal the presence of human cells. Similarly, at the DNA level, polymerase chain reaction analyses of treated animal samples did not establish the presence of human ALU sequences, thus indicating with a more sensitive approach, the lack of human cell survival at 3 months (data not shown).

Regarding the histologic analysis, human cell injections did not modify either RV area (115.1 ± 17.3 cm² in treated, 117.6 ± 7.9 cm² in sham) or myocyte diameters (23.2 ± 1.2 μm in treated, 22.2 ± 2.4 μm in sham). No inflammatory infiltrates were observed in either group, in

FIGURE 2. Hemodynamic parameters issued from pressure-volume loop measurements. A, RV functional parameters: cardiac index and effective ejection fraction; B, contractility index: $E_{\max}$ value; and C, energetics parameters: stroke work and pressure volume area. Values at baseline, 4 months (4 m), 7 months (7 m), for treated (black, $n = 6$) and sham (white, $n = 6$) animals, are plotted. * $P < .05$; PVA, Pressure volume area.

either the free wall or HD area. The vascular density determined by von Willebrand-factor immunolabeling was equivalent in both groups in either the free wall or HD area (data not shown), indicating the absence of neoangiogenesis due to human cells. Regarding fibrosis, human cells induced an important decrease compared with sham animals ($10.53\% \pm 2.53\%$ vs $17.72\% \pm 2.01\%$; $P < .01$) (Figure 3, A and D). This diminution was mainly due to the drop of the peri-myocyte fibrosis, whereas the interstitial fibrosis was similar (Figure 3, B-D).

DISCUSSION

Herein, we describe the first attempt of cell therapy using cardiogenic mesodermal cells issued from human ESCs in a porcine model of overloaded RV dysfunction that mimics congenital diseases. Feasibility, good tolerance, and a beneficial impact on arrhythmic susceptibility and RV fibrosis were demonstrated.

Our model of RV dysfunction on a large animal¹⁰ is reproducible and mimics, by its evolution, alterations observed in patients with a repaired TOF before a decompensated RV failure.¹⁷ Indeed, despite an increasing dilation, the porcine RV adaptive response is maintained in

association with depolarization anomalies, translating the alteration of the RV function at an early stage of the disease. These functional observations are associated with a structural remodeling evolution.

When new analyses of samples issued from animals described in our previous study¹⁰ are included, the RV area seems to be significantly enlarged under combined overload (Figure E1, A). This enlargement is associated with a marked increase of the cardiac myocyte diameter (Figure E1, B). Similarly, fibrosis of the overloaded RV is greater, compared with normal right ventricle (Figure E2). This increase involves only the peri-myocyte fibrosis; the interstitial fibrosis remains stable. These anomalies reproduce the pathology before the depletion of the compensatory mechanisms^{14,18} leading to the first symptoms in patients and alteration of the ejection fraction.¹⁹

One safety concern in cell therapy is the risk of arrhythmia. Generally, this risk is attributable to not only cells but also the delivery mode and underlying heart disease.²⁰ Engrafted cells might encounter significant difficulties in forming electromechanical junctions with the host myocardium, thus becoming an arrhythmogenic

FIGURE 3. Total fibrosis (A) and its distribution in interstitial (B) and peri-myocyte (C) localization after cell therapy. Values at 7 months for treated (black, $n = 6$) and sham (white, $n = 6$) animals are plotted with lines indicating median. D, Representative longitudinal tissue sections from sham and treated (7 months) animals after Picosirius red; bar = 100 μm . $^{**}P < .01$.

substrate.^{6,21} Additionally, intramyocardial injections could contribute to ventricular arrhythmia because of local tissue injuries, independently of the cell type injected.^{7,20} However, in the 3 studies reporting cell therapy applied to the right ventricle,⁷⁻⁹ all used multiple intramyocardial injections, and none described any arrhythmic susceptibility.

In our study, those injections did not lead to immediate sustained arrhythmia. Moreover, as in patients,²² our experimental model itself presents risk factors for severe ventricular arrhythmia¹⁰: QRS prolongation, RV dilation, and myocyte hypertrophy. Despite these risk factors, which are similar in the 2 groups, only the sham animal group presented, during the postinjection period, an arrhythmic susceptibility detected with 2 different techniques, suggesting that treated animals could be protected against arrhythmia by ESC-issued cardiogenic mesodermal cell injections. Evidential support for this beneficial effect

may come from the normalization of the RV fibrosis (Figure E2), as described in other experimental models.^{23,24} However, in our study, this complete fibrosis regression did not lead to a shortening of QRS duration, suggesting that other factors contribute to depolarization anomalies, such as cellular electrophysiology alterations observed on action potentials in our model.¹⁰

Stem cell-derivative engraftment is now recognized to significantly decrease fibrosis in the heart after ischemic left ventricle injury using mesenchymal²⁵ as well as embryonic stem cells.^{6,13,26} Conversely, for mesenchymal stem cells acting in a paracrine fashion on fibrosis,²⁵ ESC-derived cardiac progenitors are able to differentiate and repopulate fibrosis areas.^{6,13,26} In our study, the absence of human cardiac progenitors in treated animals suggests that such cells have additional effects, which would explain the RV fibrosis normalization and the protective effect against arrhythmia. Indeed, a paracrine

effect of MesP1⁺/SSEA-1⁺-secreting vascular endothelial growth factor-A has been shown in a recent model of limb ischemia.²⁷ In addition, these paracrine effects, mediated through vascular endothelial growth factor, may result from a modulation of local inflammation,²⁸ and/or, by analogy with mesenchymal stem cells, the secretion of soluble factors acting directly on matrix metalloproteinases and matrix metalloproteinase endogenous inhibitor production by cardiac fibroblasts.²⁵

The reason for the lack of grafted MesP1⁺/SSEA-1⁺ cardiac progenitors within the myocardium in our study remains unclear, whereas the RV structural remodeling constituted a favorable environment to cell engraftment.^{10,13} Blin and colleagues¹³ indicated successful engraftments of the same cell type at 2 months after transmyocardial injections.¹³ Our results underline the long-term survival failure of such cells. One explanation may be the immune rejection of ESCs.²⁹ Herein, the immunosuppressive regimen was efficient, as plasmatic tacrolimus levels revealed therapeutic values as recommended in human after organ transplantation, and as no inflammatory infiltrates were detected during histologic analyses. Another reason could be related to a hypoxic environment of grafted cells. Since RV outflow-tract diseases were not corrected, the RV chronic pressure overload was maintained, leading to a diminished anatomic capillary vascular reserve³⁰ that limited the blood supply to engrafted cells and affected cardiac progenitor survival.³¹

In contrast to the results of cell therapies applied to ischemic models, no functional improvement was observed, despite the fibrosis normalization. As RV outlet tract diseases were not corrected, benefits of the fibrosis decrease might not have been sufficient to balance the deleterious effects of combined overload and lead to a detectable functional improvement. Regarding cell therapies applied to the right ventricle, only Davies and colleagues⁹ reported an enhancement of systolic RV function 1 month after injections of human cord-blood stem cells into neonatal pressure-loaded RV myocardium. However, these findings need to be interpreted cautiously, because a significant RV function improvement was observed, though to a lesser degree, without stem cell transplantation, suggesting that the improvement may reflect homeometric adaptation.³² Others^{7,8} did not find any RV systolic functional improvement. Overall, these results underline the difficulties and the specificity of the cell therapy treatment necessitated by RV failure, compared with ischemic left ventricular dysfunction.

CONCLUSIONS

Cell therapy using human MesP1⁺/SSEA-1⁺ cardiogenic mesodermal cells applied to an overloaded right ventricle is feasible in a large animal model, well tolerated, and has

beneficial effects on fibrosis and arrhythmic susceptibility. Next objectives are, first, to improve the long-term survival of ESC-derived cardiac progenitors using more-protective delivery modes and prosurvival factors³³; and second, to promote their migration and differentiation into the RV myocardium.

The authors thank Alain Chapel for ALU sequence analyses; Nicolas Raymond for his excellent histologic work; Dr Dorfmueller and Dr de Montpreville for anatomic-pathology expertise; Dr Stos for his technical assistance; and the Surgical Research Staff: Benoit Decante, Bruno Baudet, Frédéric Seccatore, and Anaëlle Le Breton for technical assistance and animal care.

We greatly acknowledge the Surgical Research Laboratory and Microscopy Facility of the Centre Chirurgical Marie Lannelongue and the Bligny Farm.

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EDITORIAL COMMENTARY

Myocardial regeneration for chronic heart failure: Not as easy as it sounds

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See related article on pages 708-15.

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Disclosures: Author has nothing to disclose with regard to commercial support.

Received for publication Dec 5, 2014; accepted for publication Dec 6, 2014; available ahead of print Jan 23, 2015.

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J Thorac Cardiovasc Surg 2015;149:715-7

0022-5223/\$36.00

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<http://dx.doi.org/10.1016/j.jtcvs.2014.12.021>

Virginie and colleagues¹ describe the absence of functional benefit from injections of human embryonic stem cell-derived (hESC) cardiac progenitors into chronically volume overloaded piglet right ventricles. Further, the authors report 0 persistence of injected cells in the right ventricle myocardium, no neoangiogenesis, no myocardial regeneration, and quantitatively no effect on interstitial myocardial fibrosis. They did report a statistically insignificant reduction in arrhythmogenicity as well as a roughly 50% decrease in perimycocyte fibrosis in the cell-treated animals compared with the sham injected hearts. The significance of this is unclear, because both chronic ventricular overloading and ischemic necrosis result in interstitial fibrosis leading to dysfunction. It is yet another failed animal

FIGURE E1. Evolution of the right ventricle area (A) and cardiac myocyte diameters (B) during right ventricle chronic overload. Representative transverse tissue sections from nonoperated and operated sham (4 months), and operated sham (7 months) animals after Picrosirius red; bar = 100 µm (C). * $P < .05$, ** $P < .01$.

FIGURE E2. Evolution of the total fibrosis (A) and its distribution in interstitial (B), and peri-myocyte (C) localization during right ventricle chronic overload and after cell therapy. The 4 months data set shows results of new analyses of samples issued from animals described in our previous study.¹⁰ Values at 4 months (4 m) and 7 months (7 m), for nonoperated and operated sham (black) and treated (gray) animals are plotted with lines indicating median. Fibrosis was significantly reduced in treated animals, back to the control level, assessing its complete regression. D, Representative longitudinal tissue sections from nonoperated and operated sham (4 months), and operated sham (7 months) animals after Picrosirius red; bar = 100 µm. Kruskal-Wallis analysis of variance (Dunn's post hoc test); * $P < .05$, ** $P < .01$.