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Temporal patterns of bone marrow cell differentiation following transplantation in doxorubicin-induced cardiomyopathy

Onnik Agbulut[a,*], Marie-Laurence Menot[b], Zhenlin Li[c], Françoise Marotte[a], Denise Paulin[c], Albert A. Hagège[d], Christine Chomienne[b], Jane-Lyse Samuel[a], Philippe Menasché[e]

[a]*Inserm U572, Hôpital Lariboisière, 41, Bd de la Chapelle, 75475 Paris Cedex 10, France*
[b]*Laboratory of Hematopoietic Cell Biology, Inserm E003, Hôpital Saint-Louis, Paris, France*
[c]*University Denis-Diderot, Paris, France*
[d]*Department of Cardiology, Hopital Européen Georges Pompidou, Paris, France*
[e]*Department of Cardiovascular Surgery & Inserm U572, Hopital Européen Georges Pompidou, Paris, France*

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Abstract

Objective: Recent studies have suggested benefits of bone marrow cell transplantation for the regeneration of ischemic cardiac tissue. To extend the potential of cell transplantation, we assessed this treatment in a mouse model of acute nonischemic doxorubicin-induced cardiomyopathy. **Methods:** To allow detection of engrafted cells, we used transgenic mice expressing the nuclear-located LacZ under the control of either desmin or vimentin promoters, which identify muscle lineage and mesenchymal cells, respectively. All transplanted cells were also labeled with the fluorescent dye DIL. One week after the administration of doxorubicin (15 mg/kg), mice were intramyocardially injected with either allogeneic unpurified bone marrow cells (6×10^6 in 30 μl, $n=59$) or purified sca-1^{pos} cells (4×10^5 in 30 μl, $n=22$). In parallel, control normal mice received only unpurified bone marrow cells ($n=28$). Hearts were harvested at serial intervals until 2 weeks after transplantation and analyzed by immunohistochemistry to assess the degree of engraftment and transplanted cell differentiation. **Results:** In control mice, no differentiation of bone marrow cells was detected. In contrast, unpurified bone marrow cells grafted into diseased myocardium featured two successive phases of cell differentiation. The first yielded cells with a mesenchymal phenotype (44.1 ± 10.1 cells/3×10^{-2} mm^3 at 2 days), was transient and lasted 1 week. The second phase was characterized by cells with a muscular phenotype detected in a small number of cells (5.6 ± 2.3 cells/3×10^{-2} mm^3 at 7 days). Two weeks after transplantation, some of these cells appeared phenotypically close to cardiomyocytes, as evidenced by morphology and positive staining for myosin binding protein C, vinculin and myosin heavy chain. In sca-1^{pos} hematopoietic progenitor grafted mice hearts, no transdifferentiation into cardiac cells was detected at any time point. **Conclusion:** These data support the hypothesis of the potential for a myogenic differentiation of bone marrow cells following engraftment in a nonischemic model of global cardiomyopathy. Bone marrow-derived cells amenable to cardiac differentiation are present in total unpurified bone marrow but not in the sca-1^{pos} hematopoietic progenitor cell population. However, the very small number of transdifferentiated cells raises concerns over their functional efficacy.
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Keywords: Cell therapy; Doxorubicin-induced cardiomyopathy; Hematopoietic progenitor cell; Transgenic mice

1. Introduction

Cell therapy is becoming increasingly important as a potential new therapy for patients with advanced heart failure. Although autologous skeletal myoblasts have been the first cells to be used clinically [1] at the completion of extensive laboratory testing [2–9], several investigators argue that the bone marrow could represent the second generation of myocardial cell replacement therapy. This assumption is based on the pluripotentiality of bone marrow stem cells [10]. Bone marrow cell (BMC) can acquire the phenotypic characteristics of their host tissue

*Corresponding author. Tel.: +33-1-4463-1720; fax: +33-1-4874-2315.
E-mail address: onnik.agbulut@larib.inserm.fr (O. Agbulut).

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and are suggested to differentiate into cardiomyocytes and endothelial cells following engraftment into the myocardium [11–18]. So far, this assumption has been supported by experimental data obtained in animal models of myocardial infarction in general shortly after coronary ligation. In contrast, the present study was designed to assess the opportunity of BMC transplantation in a model of nonischemic cardiomyopathy. Nonischemic cardiomyopathy accounts for approximately one-third of the heart failure cases. Unlike ischemic forms which are amenable to palliative procedures like revascularization and remodeling operations, nonischemic cardiomyopathies, once they have reached an end stage of drug refractoriness, can only be treated radically by heart transplantation. In spite of its efficacy, this operation remains associated with major hurdles including organ shortage, complications of immunosuppression and late graft vasculopathy so that novel less invasive approaches like cell therapy would represent significant clinical advances for this category of heart failure patients.

In the present study, doxorubicin-injured mice hearts were transplanted with either unpurified BMCs or sca-1^{pos} hematopoietic progenitor cells. The aim of this study was to determine the capacity of these cells to transdifferentiate to cardiac cells in a nonischemic cardiac setting.

2. Methods

2.1. Animals

All experiments were carried out in accordance 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 Institute of Laboratory Animal Resources, Commission on Life Science, National Research Council, and published by the National Academy Press, revised 1996. In this study, C57BL6 mice were used as recipients. Desmin-LacZ or vimentin-LacZ transgenic mice were used as donors. These transgenic mice bear a transgene in which the DNA 5′ regulatory sequences of either the desmin gene or the vimentin gene were linked to a reporter gene coding for Escherichia coli beta-galactosidase (β-gal). To introduce a nuclear localisation signal (NLS), a sequence from SV40 virus T antigen gene coding for PKKKRKV was added at the beginning of the LacZ gene [19,20].

2.2. Collection of BMCs

To isolate BMCs, the mice were anaesthetised with intraperitoneal administration of ketamine hydrochloride (50 mg/kg) and bone marrow of femurs was flushed with 2 ml medium using a 21G needle. Afterwards, cells were washed two times with RPMI (Life Technologies, Cergy Pontoise, France) medium. Cells (40×10^6 cells/per mice) were divided into three parts for: cell characterization (10×10^6 cells), culture (10×10^6 cells) and transplantation (20×10^6 cells).

2.3. Characterization of BMCs

Freshly isolated BMCs (5×10^5 cells/per analysis) were incubated for 30 min in the dark with phycoerythrin-conjugated monoclonal antibodies against mouse c-kit (Becton Dickinson, USA), thy-1 (Becton Dickinson) and a FITC-labeled monoclonal antibody against sca-1 (Becton Dickinson). Isotype-identical antibodies served as controls (IgG_1-phycoerythrin, IgG_{2b}-phycoerythrin and IgG_{2a}-FITC, Becton Dickinson). Cytometric analysis were carried out using a fluorescence-activated flow cytometer (FACS Calibur, Becton Dickinson). Each analysis included 10 000 events.

2.4. Purification of sca-1^{pos} cells

Sca-1^{pos} cells were obtained using magnetic beads coated with monoclonal antibody against sca-1 (Miltenyi Biotec, Auburn, CA, USA). After incubation with this antibody, the cells were positively selected using the magnetic-activated cell separation magnetic bead system (Miltenyi Biotec). The purity of cell preparation was controlled by flow cytometry after staining of cells with FITC-conjugated monoclonal antibody against sca-1 (Becton Dickinson).

2.5. Culture of BMC

BMCs and sca-1^{pos} cells were diluted in MyeloCult (Stem Cells Technologies, Vancouver, Canada) culture medium at a final concentration of 5×10^5 cells/ml and then seeded in polystyrene tissue culture dishes with or without 5-azacytidine (10 μM, final concentration) and incubated in 5% CO_2 at 37 °C. The medium was changed once a week. After centrifugation, non-adherent cells present in the medium were resuspended in 1 ml fresh MyeloCult prior to addition to the initial culture dishes. 5-Azacytidine was added to the culture dishes every week for only 24 h.

2.6. Transplantation of BMCs

Doxorubicin-treated mice ($n=81$) received a single dose of doxorubicin at 15 mg/kg i.p., 1 week before transplantation. This regimen causes clinical symptoms of heart failure, which correlated with a 20% decrease in shortening fraction, as assessed by echocardiography, carried out as described previously [21]. In parallel, untreated mice ($n=28$) were used as controls. All mice were treated by FK-506 (2.5 mg/kg/d, i.p., Fujisawa Healthcare, Deerfield, USA) the day before surgery and daily until sacrifice. These mice were subdivided into two subgroups: BMCs

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treated ($n=59$ doxorubicin-treated, $n=28$ nondoxorubicin-treated mice) and sca-1^{pos}-treated ($n=22$ doxorubicin-treated mice). After anaesthesia (ketamine, 50 mg/kg) and tracheal ventilation, the mice heart was exposed through a left lateral thoracotomy. Three intramyocardial injections (10 μl each) were carried out with the use of a Hamilton syringe into the equator of the left ventricular free wall, so that a total volume of 30 μl was injected into each heart. The chest was closed with 6-0 silk sutures. Before transplantation, BMCs or sca-1^{pos} isolated either from desmin-LacZ or vimentin-LacZ mice were incubated at 37 °C for 5 min and then at 4 °C for 15 min with the fluorescent dye 1,1-dioctadecyl-3,3,3,3-tetramethyl indocarbocyanine perchlorate (DiI-Ac-LDL, Molecular Probes, Eugene, OR, USA) at a final concentration of 1 μg/ml. After two washes in RPMI medium, BMC and sca-1^{pos} cells were resuspended at a final concentration of 2×10^6 cells/10 μl and 1.3×10^5 cells/10 μl, respectively, and stored on ice until use.

2.7. Pathology

Two ($n=49$), 7 ($n=37$) and 14 ($n=23$) days after transplantation, the animals were sacrificed. The hearts were harvested and then rapidly frozen in liquid nitrogen cooled isopentane. Heart cryosections (5 μm thick) were processed for histological (hematoxylin–eosin, X-gal) and immunohistological techniques. The research for the transplanted cells was primarily based on the presence of β-gal activity detected using X-gal coloration.

2.7.1. X-gal coloration

The sections were fixed with 1% paraformaldehyde for 10 min at 4 °C. β-gal staining was carried out overnight at 37 °C with the following reagents: 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 2 mM $MgCl_2$ and 1 mg/ml 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (Life Technologies). For in vitro experiments, the cells were fixed with methanol and β-gal positive cells were then detected using X-gal coloration (see above) at serial intervals.

2.7.2. Immunofluorescence

The sections were treated with 5% bovine serum albumin for 20 min and then incubated with the primary antibody for 1 h at room temperature. The primary antibodies which we used in this study are as follows: anti-vinculin (1:100, mouse monoclonal (mAb), Sigma–Aldrich Chimie, Saint Quentin Fallavier, France), anti-sarcomeric myosin heavy chain (1:20, mAb, Developmental Studies Hybridoma Bank, University of Iowa City, IA, USA), anti-myosin binding protein C (1:200, rabbit polyclonal (pAb), kindly provided by L. Carrier, Paris, France), anti-caveolin 1α (1:50, pAb, Santa Cruz Biotechnology, CA, USA). Binding of primary antibodies was detected by incubating the sections for 1 h, with FITC-conjugated anti-mouse IgG (1:40) or anti-rabbit IgG (1:40). Sections were finally washed as above and mounted in Vectashield medium. Triple-labeling, DAPI, fluorescein and Texas Red images were made using a DMRB Leica microscope equipped with epifluorescence optics. Digital images, were transferred to a computer equipped with Vision Explorer software (Graftek Imaging, Austin, TX, USA).

3. Results

In this study, we used desmin-LacZ and vimentin-LacZ transgenic mice as donors. The main advantage of these two strains is that desmin-β-gal cells allow to specifically identify muscle lineage while vimentin-β-gal cells permit to track the mesenchymal cell types (i.e. fibroblasts, endothelial, smooth muscle and myeloid cells). Fig. 1 demonstrates localization of β-gal activity in vimentin-LacZ and desmin-LacZ mice hearts. In vimentin-LacZ hearts, the expression of β-gal is restricted around larger vessels and some capillaries (Fig. 1A). In desmin-LacZ hearts, the expression of β-gal is detected in numerous cardiomyocytes (82±3%) but not in all (Fig. 1B).

3.1. Characterization of BMCs

We quantified the percentage of sca-1^{pos}, thy-1^{pos} and c-kit^{pos} cells in unpurified BMCs. Data were similar regardless of the donor (data not shown) and experiments. Freshly isolated BMCs and sca-1^{pos} (purity>85%) cells were cultured to characterize the in vitro phenotype of these cells. Cell cultures were carried out in the presence or absence of 5-azacytidine. In the unpurified BMCs population, vimentin-β-gal+ cells were first detected at 2 days (<2%) after culture and the number of these cells continuously increased over time (11.6±2% at 6 weeks). The amount of vimentin-β-gal+ cells was much more important in the presence of 5-azacytidine and reached 26.6±2.3% at 6 weeks of culture (Fig. 2A,C). The same results were obtained with sca-1^{pos} cells (Fig. 2C). In the unpurified BMCs population, desmin-β-gal+ cells were not detected before 4 weeks in culture and the number of these cells was limited. In the presence of 5-azacytidine, the number of these cells increased and at 6 weeks, 10.6±4.5% cells did express β-gal (Fig. 2B,C). In contrast, in the sca-1^{pos} cell population, only in the presence of 5-azacytidine could a few (0.2%) desmin-β-gal+ cells be detected at 6 weeks in culture (Fig. 2C). Put together, our in vitro data indicate that: (1) 5-azacytidine amplified cell differentiation towards the myogenic and mesenchymal phenotypes, and (2) the percentage of cells induced into a myogenic lineage in response to 5-azacytidine was much more important in unpurified BMCs than in the sca-1^{pos} cell population.

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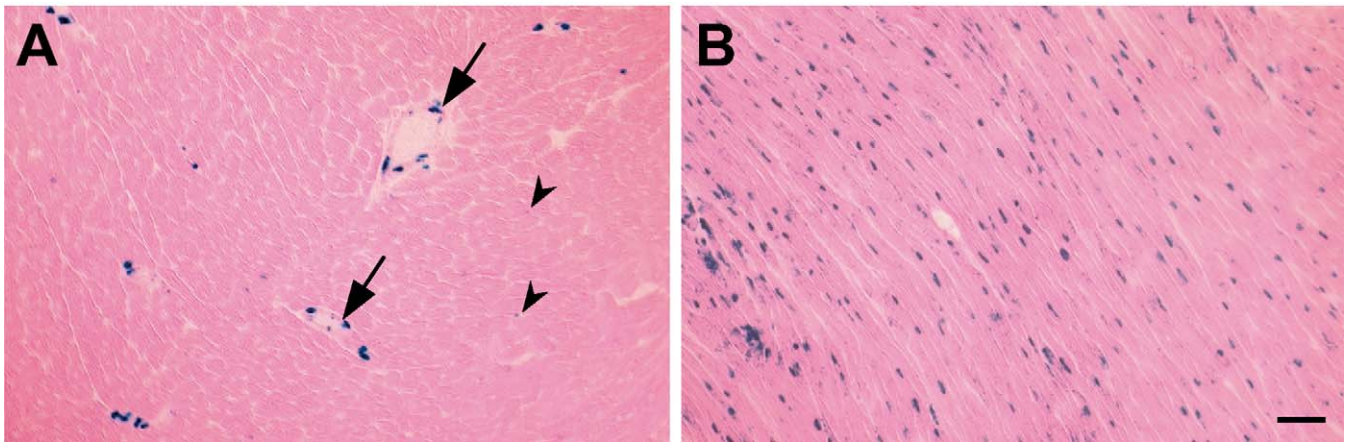

Fig. 1. Detection of β-gal activity in vimentin-LacZ (A) and desmin-LacZ (B) mice hearts. Frozen sections of 6-week-old mice hearts stained with X-gal. Note the presence of vimentin promotor activity in larger vessels (arrows) and in some capillaries (arrowheads). Desmin promotor activity was detected in the majority of cardiomyocytes. β-gal activity is restricted to nucleus. Bar: 50 μm.

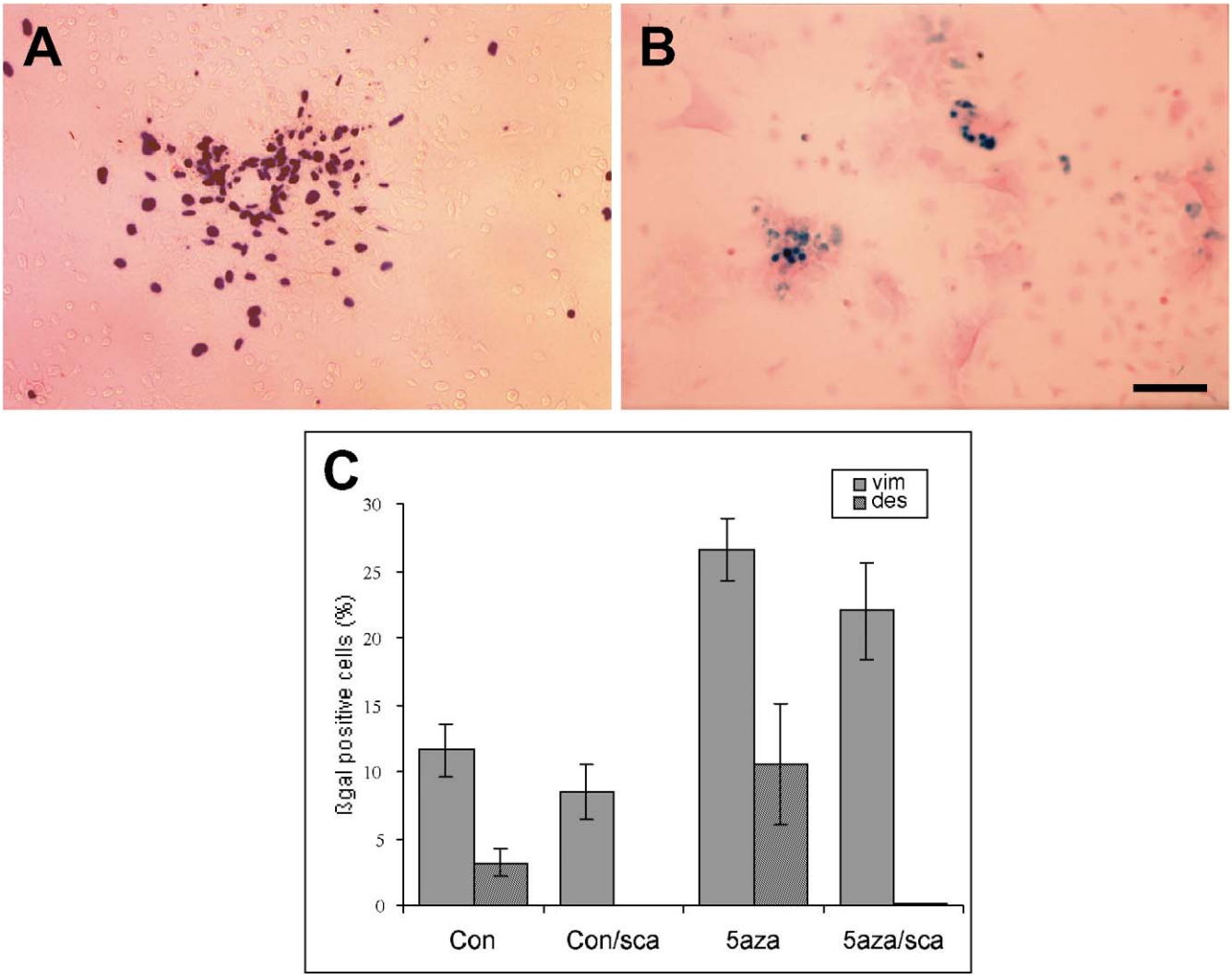

Fig. 2. In vitro differentiation of BMCs. Vimentin-LacZ (A) and desmin-LacZ (B) mice unpurified BMCs were cultured for 42 days in the presence of 5-azacytidine. Note the presence of β-gal+ cells in culture. (C) The percentage of β-gal+ cells in unpurified BMCs and sca-1^{pos} cells (sca) population in culture (data are mean±S.E.M. of 3–7 independent cultures). The cells were cultured for 42 days with (5aza) or without (Con) 5-azacytidine. Vim: vimentin-β-gal+; des: desmin-β-gal+ cells. Data are expressed as mean±S.E.M., $n \geq 4$. Bar: 100 μm.

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3.2. Phenotypical characterization of transplanted cells

Injection of unpurified BMCs (6×10^6 in 30 μl) into normal myocardium failed to result in any expression of β-gal activity regardless of the timing of assessment (data not shown). One week after the administration of doxorubicin, mice were grafted with unpurified, freshly isolated BMCs. Two days after transplantation, numerous vimentin-β-gal+ cells (44.1 ± 10.1 cells/3×10^{-2} mm^3) could be identified in all grafted hearts (Fig. 3A). The number of vimentin-β-gal+ cells decreased over time and at 14 days, only a few cells (2.4 ± 2.4 cells/3×10^{-2} mm^3) still demonstrated this phenotype (Fig. 3C, Fig. 4). In addition, vimentin-β-gal+ cells were detected in 50% of grafted hearts at 7 days and in 20% of grafted hearts at 14 days after transplantation. In contrast, desmin-β-gal+ cells could not be detected before 7 days after transplantation (Fig. 3D–F). At this time point, a small number of cells (5.6 ± 2.3 cells/3×10^{-2} mm^3) started to express β-gal in 80% (seven out of nine hearts) of grafted hearts (Fig. 3E, Fig. 4). In keeping with these temporal patterns, numerous scattered DIL-labeled cells were found 2 days after transplantation (red fluorescence, Fig. 3G) while only a few of them could still be identified, particularly at the border of the injection area, 2 weeks later (Fig. 3I). It should be noted that most transplanted cells were found along the needle tracts, particularly at the border of the injection area, as detected by β-gal positive staining and the presence of DIL-positive cells. However, positive cells were also occasionally detected throughout the heart.

By immunohistochemistry, DIL-positive cells were also found to stain positively for vinculin (Fig. 5A–C), sarcomeric myosin heavy chain (Fig. 5D–F) and myosin binding protein C (Fig. 5G–I). DIL-positive cells were also positively labeled by antibodies against caveolin-1α, thereby suggesting formation of new vessels in the myocardium (Fig. 5J–L).

In contrast with the previous findings, neither vimentin-β-gal+ nor desmin-β-gal+ cells were identified in the 22 hearts transplanted with sca-1^{pos} cells, regardless of the

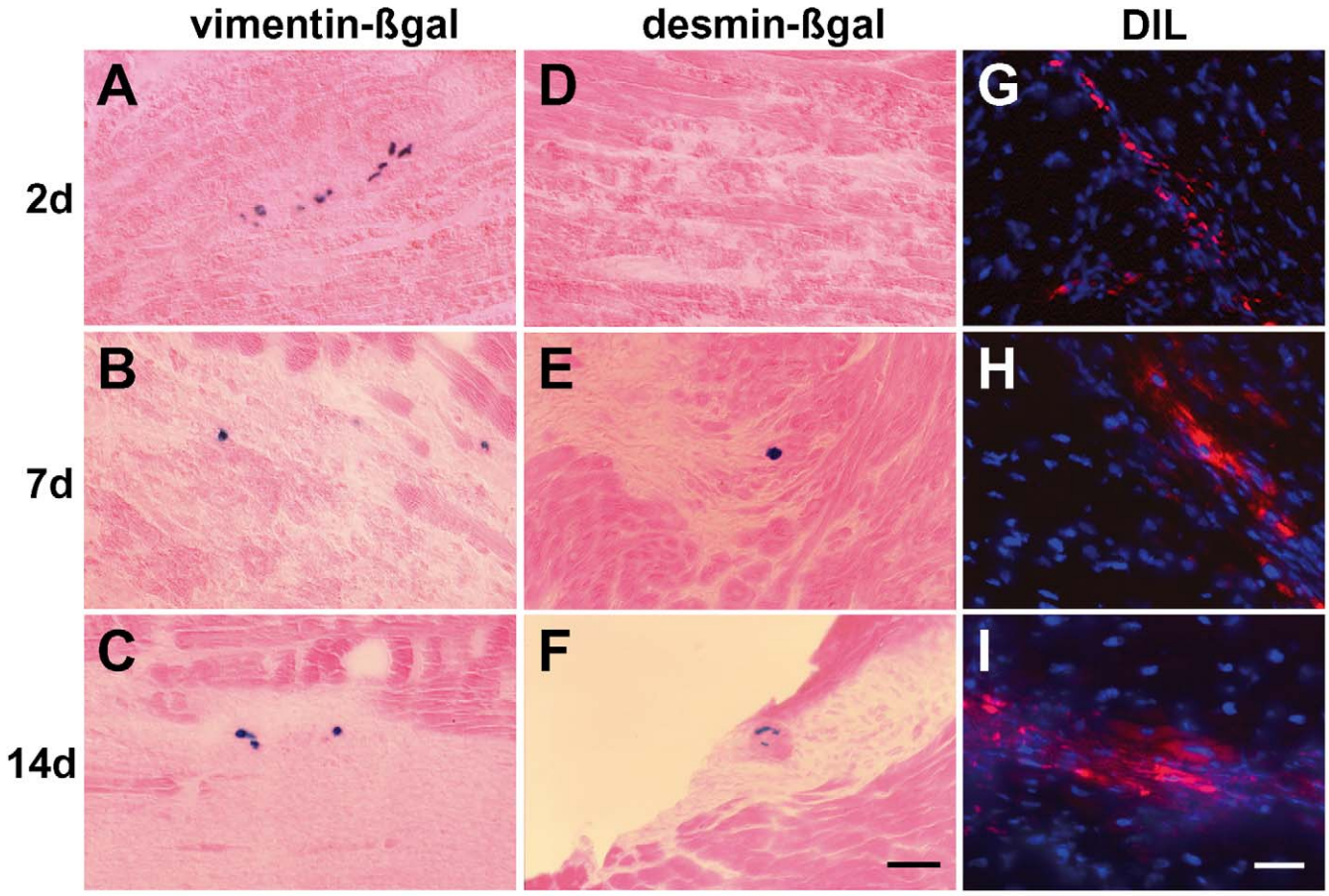

Fig. 3. Detection of grafted cells into myocardium of doxorubicin-treated mice. One week after administration of doxorubicin, mice were intramyocardially injected with unpurified BMCs. β-gal (blue, A–F) and DIL (red fluorescence, G–I) staining of heart section 2 (A, D, G), 7 (B, E, H) and 14 (C, F, I) days after transplantation. Note vimentin-β-gal+ (A–C), DIL+ cells (G–I) at 2, 7 and 14 days after transplantation whereas few desmin-β-gal+ cells (D–F) were detected 7 and 14 days after transplantation. Bar: 50 μm (A–F); 25 μm (G–I).

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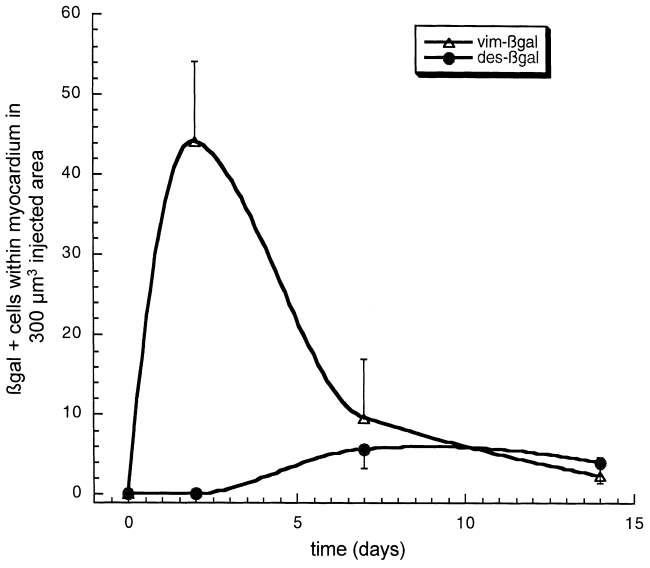

Fig. 4. Time course analysis of vimentin-β-gal+ and desmin-β-gal+ expression in unpurified BMC grafted hearts. Note the earlier and transient expression of vimentin in transplanted cells. Desmin-β-gal+ cells were first observed at 7 days after transplantation. The number of β-gal+ cells were counted in 3×10^{-2} mm^3 injection areas. Data are expressed as mean±S.E.M., $n>6$.

time of analysis. Likewise, immunohistochemical analysis did not reveal any evidence for cardiac transdifferentiation of the sca-1^{pos} grafted cells.

4. Discussion

The major finding of the present study is that implantation of total unpurified bone marrow into nonischemically injured myocardium is associated with some cardiomyogenic and endothelial transdifferentiation whereas purified hematopoietic progenitor sca-1^{pos} cells do not show any evidence for transdifferentiation. However, the very small number of transdifferentiated cells in the unpurified BMC population raises a major scale-up issue which renders the clinical relevance of the procedure questionable.

It is generally admitted that heart failure develops when a critical number of cardiomyocytes has been irreversibly lost. The rationale of cell therapy is thus to repopulate the damaged areas with new cells so as to improve function. Not unexpectedly, these exogenously supplied cells must possess contractile properties to make the procedure successful and, for this reason, cell replacement therapy has, so far, primarily relied on fetal (and neonatal) cardiomyocytes and skeletal myoblasts. In small and large animal models of myocardial infarction, these cell types have been shown to form stable intramyocardial grafts and to concomitantly improve left ventricular function, both at the regional and global levels [4,5,7,22–24]. The potential therapeutic benefits of this novel approach have been strengthened by the encouraging results of a phase I human trial of autologous skeletal myoblast transplantation in patients with severe postinfarction left ventricular dysfunction [1].

In parallel, a great deal of interest is paid to the use of bone marrow as a source of cells. BMCs share with skeletal myoblasts the advantage of a possible retrieval from the patient himself, thereby avoiding the availability and immunological issues associated with allografts. However, in contrast to myoblasts which, once engrafted, remain committed to a skeletal muscle lineage, BMCs have the purported advantage of a transdifferentiation potential which would allow them to acquire a cardiomyogenic phenotype, either following appropriate preimplantation processing or directly under the influence of their new myocardial environment. Should such a switch really occur, it would have the major advantage, among others, to induce expression of connexin 43 by the transdifferentiated cells. Propagation of the electrical impulses through gap junctions should, in turn, result in transplanted cells beating synchronously with those of the host tissue, thereby allowing them to actively participate in pump function while reducing the risk of arrhythmias. So far, several studies have reported on the successful use of BMC transplantation into diseased myocardium. These studies differed in the type of injected cells (i.e. total unpurified BMC, mesenchymal cells, hematopoietic progenitors) and the selected end point (angiogenesis vs. restoration of function) [11–18]. However, they shared in common the use of both small and large animal models of myocardial infarction. To the best of our knowledge, the present study is the first to assess the cardiomyogenic transdifferentiation potential of BMCs in a nonischemic model of global cardiomyopathy. This condition accounts for approximately half of the cases of heart transplantation [25]. The nonischemic mouse model, which we chose in this study, features a doxorubicin-induced acute global cardiomyopathy. The validity of this model has been established by previous studies [21,26–28] and its clinical relevance stems from the high incidence of anthracyclin-induced heart failure in patients receiving chemotherapy for malignant blood diseases [29]. Basically, doxorubicin, as administered in the present protocol, results in important abnormalities including loss of weight, pulmonary edema and reduced fractional shortening, as measured by echocardiography. These changes correlate to a high rate mortality in the drug-treated animals. In our model, cells were grafted 7 days after administration of doxorubicin, a time by which the drug is almost completely eliminated from plasma and cardiac tissue [30–32], thereby reducing the risk of toxic graft death. The detection of allografts was primarily based on the use of transgenic mice expressing the nuclear-located LacZ under the control of either vimentin or desmin promoters. This model provides a highly accurate means of tracking the differentiation patterns of the injected cells, which turn blue (β-gal

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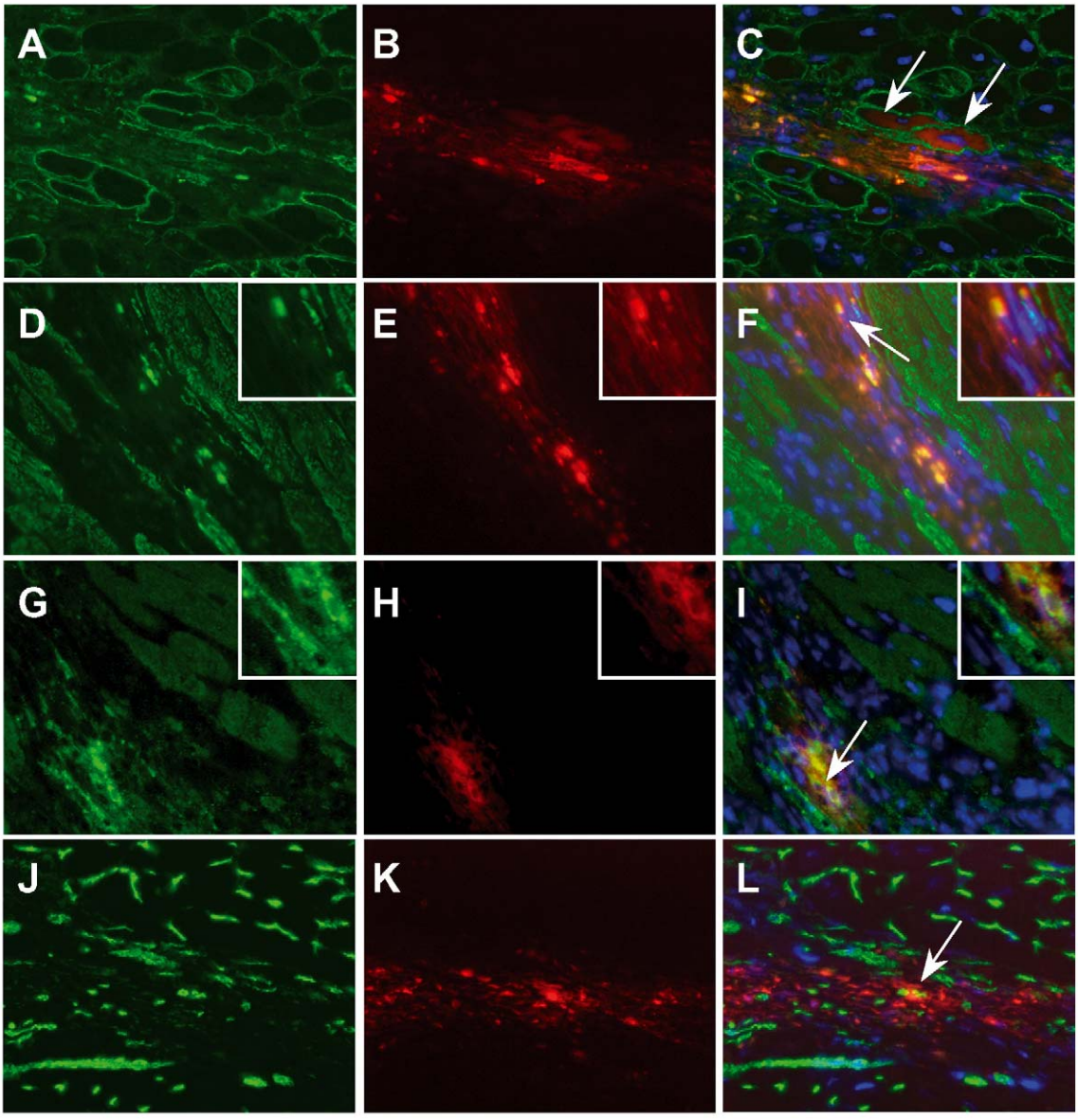

Fig. 5. Immunofluorescent detection of transdifferentiated BMCs into cardiac cells. Two weeks after BMC treatment, the heart sections stained with: (A,C) anti-vinculin; (D,F) anti-MyHC; (G,I) anti-myosin binding protein C and (J,L) anti-caveolin 1α antibodies (green fluorescence). Dil+ (red fluorescence, B,E,H,K) cells revealed BMCs present into myocardium. Combined (C,F,I,L) red and green fluorescence, and DAPI-stained nuclei (blue) in images were made. Arrows indicate BMC-derived cardiac cells. Inserts show higher magnification (×2) of transdifferentiated BMCs in these areas. Bar: 25 μm.

activity) as they commit to a mesenchymal or muscular lineage (vimentin+ and desmin+ strains, respectively). We first attempted to graft total, unpurified bone marrow because it is the simplest and most clinically appealing scenario in the context of cellular therapy. Once engrafted into diseased myocardium, these unpurified BMCs featured two successive phases of cell differentiation. A few days following the injections, many cells were found to be vimentin+, thereby suggesting a mesenchymatous transformation. This phase, however, was transient and was followed by the appearance of desmin+ cells reflecting a switch to a muscular phenotype. Although this sequence may reflect the stepwise differentiation of a single population of cells, we cannot rule out the possibility that a population of cells in the unfractionated bone marrow undergoes a relatively early mesenchymal differentiation (vimentin-expressing cells) while a separate population undergoes muscle differentiation on a slower time scale (desmin-expressing cells). The presence of transplanted cells was double-checked by performing additional experiments in which BMCs were labeled with DIL, a membrane-permeable dye, before transplantation. DIL+ cells were also found in the grafted myocardial areas. Whereas their positive staining for vinculin (a marker of costameres) and MyHC (a marker of thick filaments) did not allow to distinguish between skeletal and cardiac muscle, their

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round-shaped morphology, with a large central nucleus, was actually very similar to that of native cardiomyocytes and, more importantly, these DIL-tagged cells stained positively for myosin binding protein-C, a highly specific marker of cardiac tissue [33,34]. One issue with DIL is that this dye can be released by grafted cells upon their death and subsequently be taken up by the neighbouring host cardiomyocytes. This hypothesis, however, is unlikely because: (1) the number of DIL+ cells was much smaller than would have been expected if the dye had marked cardiac cells of the recipient myocardium, (2) DIL+ cardiomyocytes were not observed before 7 days after transplantation whereas most cell death occurs during the first 24 h post transplantation and (3) DIL+ cardiomyocytes were not observed in the myocardium of control mice after DIL-labeled-BMC transplantation. In addition, double immunolabeling showed that DIL+ cells also stained positively for caveolin 1α, a marker of endothelial cells [35–37], which is consistent with the finding of vimentin+ cells during the early posttransplantation period.

Of note, however, cardiomyogenic/endothelial differentiation of the injected BMCs only involved a very small number of them. This is likely due to the fact that pluripotent BMCs only comprise a minor fraction (1–2%) of the total bone marrow pool, which questions the functional benefits of such an engraftment. In a clinical perspective, optimization of the procedure would likely require to scale up the number of pluripotent cells, either by in vitro expansion or by in vivo cytokine-induced mobilization. In the former setting, our in vitro data support the efficacy of 5-azacytidine in amplifying the differentiation potential of BMCs but the effects of this demethylation agent on a wide variety of genes may raise clinically relevant safety concerns.

When a purified population of sca-1^{pos} progenitor cells was injected, no differentiation of any type could be identified. This contrasts with the previous findings that these cell fractions regenerate infarcted myocardium through formation of new cardiomyocytes, smooth muscle and endothelial cells [15,38]. As phenotypic changes in engrafted progenitors are likely to occur in response to cues provided by the target organ, it is conceivable that signals emitted by doxorubicin-injured myocardium are less effective that those originating from ischemic tissue. Furthermore, our finding of a greater degree of differentiation following injections of total bone marrow also suggests that cell types other than pure progenitors may be more sensitive to environmentally dictated changes of fate. This view is indeed consistent with the reported benefits of injecting unfractionated mononuclear BMCs [39,40] so as to combine the regenerative potential of the various subpopulations (hematopoietic stem cells, endothelial precursors, stromal cells) which make up this cell type.

In conclusion, the present data show that the concept of cardiomyogenic transdifferentiation of BMCs, which has been previously established in ischemic heart models can possibly be extended to the setting of nonischemic cardiomyopathy. However, they also demonstrate that transplantation of total unpurified bone marrow, although appealing because of its practicality, is only associated with a minimal rate of transdifferentiation, which is unlikely to be of any clinical relevance. Thus, the full therapeutic exploitation of the potential benefits of bone marrow stem cell transplantation still requires identification and expansion of yet-to-be-defined pluripotential cell subpopulations as well as a clearer understanding of the signals that can drive them along the cardiomyogenic differentiation pathway.

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