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Cell therapy of heart failure

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Abstract – Together with angiogenesis and gene therapy, cell transplantation is one of the newest treatments that have been proposed to improve the still grim outcome of patients with cardiac failure. The underlying rationale is that implantation of contractile cells into fibrous post-infarction scars can functionally 'regenerate' these areas. Primarily for practical reasons, autologous skeletal myoblasts have been the first to be tested in a clinical trial but other cell types can be considered, among which bone marrow stromal and hematopoietic stem cells are of particular interest because of their autologous origin and their purported transdifferentiation potential into cardiac and/or endothelial cells. However, several key issues still need to be addressed, including (*i*) the optimal type of donor cells, (*ii*) the mechanism by which cell engraftment improves cardiac function, actively (i.e., by increasing contractility) or passively (i.e. by limiting infarct expansion and remodelling), (*iii*) the optimisation of cell survival, and (*iiii*) the potential benefits of cell transplantation in non-ischaemic heart failure. Parallel to the numerous experimental studies designed to address these issues, initial clinical trials are underway or in preparation and, if properly designed and conducted, should allow to know whether the hopes raised by cellular therapy are met by clinically meaningful improvements in the outcomes of patients with heart failure. ***To cite this article: P. Menasché, C. R. Biologies 325 (2002) 731–738.*** © 2002 Académie des sciences / Éditions scientifiques et médicales Elsevier SAS

heart failure / cell therapy / skeletal myoblasts / bone marrow stem cells / transplantation

Résumé – **Thérapie cellulaire de l'insuffisance cardiaque.** Avec l'angiogenèse et la thérapie génique, la transplantation cellulaire est l'un des traitements les plus récemment proposés pour améliorer le pronostic encore sombre des patients en insuffisance cardiaque. Le rationnel de cette approche est que l'implantation de cellules contractiles dans une cicatrice fibreuse d'infarctus peut y restaurer une certaine fonctionnalité. Pour des raisons essentiellement pratiques, les myoblastes squelettiques autologues ont été les premiers à être évalués dans le cadre d'un essai clinique de phase I, mais d'autres types cellulaires sont également considérés, principalement les cellules stromales de la moelle et les progéniteurs hématopoïétiques, dont l'intérêt est lié, non seulement à leur origine autologue, mais aussi à une possible trans-différenciation en cellules cardiaques et/ou endothéliales. ***Pour citer cet article : P. Menasché, C. R. Biologies 325 (2002) 731–738.*** © 2002 Académie des sciences / Éditions scientifiques et médicales Elsevier SAS

insuffisance cardiaque / transplantation cellulaire / myoblastes squelettiques / cellules médullaires

Version abrégée

Le principe général de la thérapie cellulaire est la recolonisation des cicatrices fibreuses akinétiques post-infarctus par des cellules contractiles, avec l'espoir qu'elles puissent s'intégrer dans le tissu hôte et y restaurer une fonctionnalité. L'approche la plus pragmatique pour parvenir à ce but est la transplantation directe, dans la zone infarcie, de cellules allogéniques ou autologues, naturellement douées de propriétés con-

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tractiles (cardiomyocytes fœtaux, myoblastes squelettiques) ou susceptibles de les acquérir (cellules-souches de la moelle).

La preuve de cette « prise de greffe » a été apportée dans différents modèles expérimentaux d'infarctus. Les cardiomyocytes fœtaux s'intégrant structurellement dans le myocarde receveur, leur identification ultérieure nécessite la transfection des cellules, avant la transplantation, par des gènes codant pour la β-galactosidase, une analyse immuno-histochimique des cœurs explantés visant à détecter l'alpha-actine du muscle lisse, qui n'est normalement présente que dans les cardiomyocytes fœtaux, ou la mise en évidence du chromosome Y après greffe de cellules mâles dans des cœurs d'animaux femelles. Les myoblastes sont plus faciles à repérer, car ils se différencient en myotubes multi-nucléés typiques, qui peuvent être identifiés histologiquement et caractérisés par un marquage positif à des anticorps spécifiques de la myosine du muscle squelettique. Sur le plan fonctionnel, la preuve des bénéfices de la transplantation cellulaire a été apportée par les techniques ex vivo (perfusion de cœur isolé) et in vivo (échocardiographie, sonomicrométrie). Dans leur ensemble, les résultats indiquent que la transplantation de cardiomyocytes fœtaux et de myoblastes squelettiques dans les cicatrices d'infarctus améliore la fonction ventriculaire à la fois globale et régionale, cette amélioration étant étroitement dépendante du nombre de cellules injectées. Enfin, les résultats que nous avons obtenus après un an, à la fois chez le rat et le mouton, sont encourageants, dans la mesure où ils indiquent que l'amélioration fonctionnelle constatée deux mois après la greffe se maintient inchangée à plus long terme. À un an, l'explantation des cœurs permet d'y retrouver les myotubes et l'expression par ces fibres d'une myosine lente est cohérente avec une résistance des cellules musculaires implantées à la fatigue et donc avec leur capacité de supporter une activité mécanique de type cardiaque au long cours.

Compte tenu des problèmes pratiques liés à l'utilisation des cellules cardiaques fœtales, les myoblastes présentent de nombreux avantages : (a) une origine autologue, qui élimine tout problème immunologique et par conséquent toute nécessité d'un traitement immuno-suppresseur ; (b) un fort potentiel prolifératif, permettant d'obtenir un grand nombre de ces cellules à partir d'une petite biopsie musculaire ; (c) une programmation exclusivement myogénique, qui rend quasiment nul le risque tumoral, et (d) une forte résistance à l'ischémie, caractéristique fondamentale compte tenu de l'environnement peu vascularisé dans lequel ils seront implantés. Nos résultats expérimentaux ont montré que, bien que ces myoblastes squelettiques ne communiquent pas avec les cardiomyocytes de l'hôte par les *gap junctions* classiques, ils permettent une amélioration fonctionnelle identique à celle observée après greffe de cellules cardiaques fœtales.

Les cellules souches de la moelle sont également attractives pour deux raisons principales : (a) elles partagent avec les myoblastes la possibilité d'être utilisées comme autogreffes et (b), au contraire de ces cellules musculaires, elles ont une plasticité qui peut conduire à leur différentiation cardiomyogénique et/ou endothéliale. En dépit de l'enthousiasme qu'elles suscitent actuellement, ces cellules souches posent de nombreux problèmes non résolus, qui tiennent autant au concept lui-même qu'à leur technique de préparation. Les problèmes conceptuels sont de trois types : (a) il n'est toujours pas établi de façon certaine que la pluri-potentialité de ces cellules leur permette de se transformer véritablement en cardiomyocytes ; (b) si une telle trans-différentiation se produit néanmoins, il semble qu'elle ne puisse concerner qu'un nombre limité de cellules, ce qui pose le problème du bénéfice fonctionnel qu'on peut en attendre ; (c) enfin, il est vraisemblable que le moment auquel ces cellules sont injectées dans le cœur joue un rôle essentiel dans leur évolution future. En effet, s'il est concevable que des cellules souches de la moelle implantées très précocement après un infarctus trouvent, dans un tissu encore largement ischémique, des signaux susceptibles d'orienter leur différentiation en cellules cardiaques et/ou endothéliales, une telle évolution paraît plus incertaine si l'injection se fait au stade plus tardif de la cicatrice fibreuse. Il existe même alors le risque de voir ces cellules souches médullaires se transformer en fibroblastes. L'utilisation clinique des cellules médullaires concerne donc peut-être davantage les syndromes coronaires aigus que l'insuffisance cardiaque chronique. Du point de vue strictement technique, plusieurs questions se posent également. Elles concernent : (a) le choix des cellules (faut-il injecter l'ensemble des cellules présentes dans un prélèvement médullaire, seulement les cellules mésenchymateuses dites stromales ou une sous-population définie de progéniteurs hématopoïétiques ?), (b) l'éventuel traitement des cellules avant de les implanter dans le myocarde (faut-il injecter la moelle prélevée extemporanément, comme l'a fait récemment une équipe japonaise chez cinq patients, avec des résultats peu convaincants, faut-il cultiver d'abord les seules cellules stromales en présence de composés susceptibles d'induire leur différenciation myogénique, faut-il expandre, avant de les réinjecter, les progéniteurs hématopoïétiques, naturellement présents en très petit nombre, en prenant garde de ne pas leur faire perdre tout ou partie de leur plasticité ?),

(c) les sites de prélèvement (moelle ou sang) et de ré-injection (myocarde ou sang périphérique si l'on admet l'hypothèse – peut-être un peu trop belle pour être totalement vraie – que les tissus lésés émettent des signaux qui vont « attirer » les cellules souches et les conduire à une différenciation aboutissant à une régénération myocardique). Toutes ces considérations n'ont pas pour objet de prôner l'utilisation exclusive des myoblastes squelettiques, qui ne représentent d'ailleurs sans doute qu'une première étape sur une voie encore longue. Elles visent simplement à tempérer l'enthousiasme parfois excessif suscité par les cellules-souches et à insister sur les problèmes qui restent à résoudre pour concilier au mieux attractivité du concept et applicabilité clinique.

Dans la plupart des études expérimentales, ainsi qu'au cours de notre essai clinique chez l'homme, les injections de cellules ont été faites à ciel ouvert, par voie trans-épicardique. Si le contrôle de la vue permet de cibler les zones d'injection, il n'évite pas pour autant un pourcentage élevé de mort cellulaire précoce. Même si une certaine reconstitution du *pool* initial peut être espérée de la multiplication des cellules ayant survécu, il est clair que l'optimisation des bénéfices fonctionnels de la transplantation cellulaire passe par une amélioration des systèmes d'injection actuels. Ces remarques s'appliquent aussi largement aux injections par voie endocardique, même si la précision de cette transplantation « aveugle » bénéficie des progrès récents des systèmes de navigation intraventriculaire. Il faut d'ailleurs remarquer que, si la faisabilité de cette technique est établie, de nombreuses inconnues persistent quant à l'importance de la rétention réelle des cellules dans le myocarde. Quant aux injections directes par voie endocoronaire, elles ont fait l'objet de quelques études expérimentales aux résultats encourageants, mais dans des conditions qui s'apparentent davantage à la pratique de la transplantation cardiaque traditionnelle (injections sur cœur explanté) qu'à celle de la cardiologie interventionnelle de tous les jours.

Les mécanismes par lesquels la greffe de cellules améliore la fonction restent à ce jour incertains. Trois hypothèses, au demeurant non exclusives, peuvent cependant être avancées. Selon la première, le tissu greffé aurait avant tout un effet de contention, limitant la dilatation ventriculaire. Cette hypothèse repose sur la constatation expérimentale fréquente que la transplantation cellulaire limite l'expansion de la zone infarcie. La deuxième hypothèse fait appel aux propriétés, non plus seulement élastiques, mais également contractiles, des cellules, qui agiraient ainsi sur la composante systolique de la fonction cardiaque. Dans le cas des cardiomyocytes fœtaux, la présence de jonctions communicantes rend tout à fait plausible un couplage électromécanique classique entre les cardiomyocytes du receveur et les cellules greffées. Ce mécanisme est plus difficile à imaginer dans le cas des myoblastes squelettiques, qui n'expriment pas ces jonctions, mais permettent toutefois, jusqu'à un an, une amélioration des paramètres de la fonction systolique. Il est concevable que ces myoblastes se contractent mécaniquement, en réponse à la contraction exercée par les cardiomyocytes qui les entourent ou sous l'influence d'un effet de champ électrique généré par ces cardiomyocytes. Enfin, on ne peut exclure que ces cellules myogéniques greffées se comportent comme des plate-formes relarguant des facteurs de croissance angiogénique, contribuant aussi bien à assurer leur propre survie qu'à améliorer la perfusion du myocarde greffé. À ce jour, cependant, nos études expérimentales n'ont pas pu documenter un tel effet angiogénique.

Sur la base de ces multiples données pré-cliniques, nous avons initié un essai de phase 1 dont le premier patient a été opéré le 15 juin 2000. Les critères d'inclusion dans cet essai étaient au nombre de trois : altération de la fonction ventriculaire gauche traduite par un abaissement de la fraction d'éjection à moins de 35%, cicatrice définie d'infarctus, akinétique et métaboliquement non viable et indication de pontage coronaire dans des territoires autres que celui de l'infarctus. En novembre 2001, dix patients répondant à ces critères ont été opérés, selon une procédure en trois étapes : prélèvement à la cuisse, sous anesthésie locale, d'un petit fragment musculaire ; culture de la biopsie, visant à obtenir un nombre élevé de cellules (au moins 400×10^6, dont 50% au moins de myoblastes) ; réimplantation des cellules ainsi obtenues dans la cicatrice fibreuse en de multiples sites, afin de couvrir la totalité de la zone de nécrose. Une analyse détaillée des résultats de cette phase 1 sera prochainement publiée ; nous nous limiterons ici à quelques commentaires généraux.

Les deux critères de jugement principaux étaient la faisabilité et la tolérance. La faisabilité peut être considérée comme parfaitement acquise, puisqu'il a été toujours possible d'obtenir des nombres de cellules bien supérieurs aux chiffres seuils et ce, dans un délai cliniquement pertinent de deux à trois semaines. En ce qui concerne la tolérance, aucune complication spécifiquement liée à la réimplantation des cellules n'a été observée. Le seul effet secondaire vraisemblablement attribuable à la greffe cellulaire a été la survenue, chez quatre patients, d'une tachycardie ventriculaire qui semble avoir un caractère transitoire. Cette dernière conclusion dérive du fait que, chez les quatre patients qui ont reçu un défibrillateur automatique implantable,

l'analyse de la fonction Holter du système ne montre, avec un recul maximum de huit mois, qu'une seule récidive rythmique. Un traitement prophylactique est donc désormais mis en place ; il est fondé sur l'administration d'amiodarone dès la biopsie musculaire et poursuivie pendant trois mois après l'intervention. Par définition, et pour des raisons méthodologiques évidentes, l'efficacité ne peut être, dans cet essai de phase 1, qu'un critère de jugement secondaire. On se limitera donc à indiquer qu'à côté de l'amélioration symptomatique et de l'augmentation de la fraction d'éjection globale qui peuvent seulement traduire les effets bénéfiques de la revascularisation, 60% des segments myocardiques initialement akinétiques et dans lesquels ont été implantées les cellules ont vu leur cinétique s'améliorer sous la forme d'un nouvel épaississement pariétal systolique à l'échocardiographie. Un essai multicentrique randomisé de phase 2, qui va commencer en 2002, devrait permettre de savoir si ces données préliminaires encourageantes sont ou non confirmées sur un plus large collectif de patients.

1. Introduction

Cell transplantation is one of the newest treatment modalities that have been proposed to improve the outcome of patients with heart failure. The already high incidence of this condition (approximately 500 000 new cases per year in the USA) is expected to further increase in the forthcoming years, because of the ageing of the population and the improved post-infarction survival rates resulting from recent therapeutic developments. The mortality of heart failure is also high, as it can reach 60% within one year for patients in New York Heart Association functional class IV and, not unexpectedly, these figures translate into tremendous financial costs, estimated to consume 1–2% of the total health care budget of western countries [1].

Over the past decades, improvements in medical therapy, primarily based on β-blockers, angiotensin-converting enzyme inhibitors and anti-aldosterone, have dramatically improved the prognosis of heart failure and new drugs currently under investigation (e.g., endothelin-1 or angiotensin II receptor antagonists) might have an additional favourable impact on patient outcomes. However, some forms of cardiac failure remain refractory to an optimal medical management, thereby requiring implementation of more aggressive approaches, like ventricular resynchronisation or cardiac surgery. In this setting, operations could so far be categorised as those aimed at 'reshaping' the dilated left ventricle (endocardial patch plasty) or radically replacing it (transplantation). The well-known limitations of these approaches (inconsistent efficacy of remodelling procedures when the scar is akinetic rather than dyskinetic, organ shortage and complications of immunosuppression in the case of cardiac transplantation) justify the search for alternate therapeutic options. In parallel with the development of permanent implantable assist devices that have been shown to improve survival of select end-stage patients but still remain plagued by a high rate of severe complications [2], cell therapy could represent one of these new treatment modalities.

2. Fundamental basis

The overall objective of cell therapy is to repopulate post-infarction scar tissue with contractile cells that can engraft in sufficient numbers to restore functionality in these akinetic areas. Conceptually, this objective can be achieved through three distinct approaches. The first consists of stimulating residual cardiomyocytes to re-enter a mitotic cycle to expand the number of contractile elements. In contrast to the long-standing belief that adult mammalian cardiomyocytes have irreversibly lost the capacity to proliferate, recent clinico-pathological studies [3] have shown that cardiomyocytes of infarcted or failing human hearts had actually retained a capacity of re-entering a cell cycle. The therapeutic applicability of this concept is, however, more than questionable, because the level of proliferation reported in these studies is by far too low to compensate for the loss of cardiomyocytes resulting from a large infarct. Likewise, stimulation of postnatal cardiomyocyte DNA replication has been obtained by expression of transgenes encoding viral oncoproteins or endogenous cellular proteins involved in cell cycle control, but this approach raises major safety issues that also cast serious doubts about its clinical relevance (for a review, see [4]).

The second strategy is based on the transformation of in-scar fibroblasts into contractile cells. This can be theoretically achieved by transfection with the *MyoD* master gene that controls the skeletal muscle differentiation programme. Although this approach has yielded some successful experimental results by allowing MyoD-transfected cardiac fibroblasts to turn to multinucleated myotubes expressing myogenic differentiation markers [5], it is fraught with the major issues still associated with gene therapy, which questions the feasibility of its clinical implementation.

The third approach consists of injecting exogenous contractile cells into the scar. From a clinical standpoint, this 'transplantation' strategy has looked the most realistic and, consequently, has been extensively investigated in the laboratory setting before being tested in the first human trial. Of note, although most of these experiments have focused on ischaemic, *segmental* cardiomyopathies, preliminary studies yet suggest that the putative benefits of cellular transplantation might extend to idiopathic [6] or doxorubicin-induced [7] *globally* dilated cardiomyopathies.

3. Experimental studies

The prerequisite for implanted cells to improve cardiac function is that they feature contractile properties. Fibroblasts [8] or smooth muscle cells [9], for example, can improve post-infarct diastolic performance but not systolic function.

Contractile cells, in turn, can be categorized into *naturally contractile cells* and *cells whose phenotype can be oriented towards a contractile pattern.* These two categories will be reviewed successively.

3.1. Naturally contractile cells

3.1.1. Foetal and neonatal cardiomyocytes

Studies with foetal and neonatal cardiomyocytes have yielded pivotal proof-of-concept experiments by showing, in rodent models of myocardial infarction, that these cells formed stable intracardiac grafts, connected with host cardiomyocytes through gap junctions and improved left ventricular function [10–12]. Whether this improvement is directly related to the material contractile properties or to indirect paracrine effects mediated by some graft-derived 'inotropic' factors has not yet been established. Whatsoever, in a clinical perspective, the transplantation of foetal cells is associated with significant hurdles related to ethics, availability and immunogenicity, so that, in spite of the encouraging results obtained with intracerebral transplantation of brain tissue in patients with Parkinson's disease, emphasis has rather been put on the second variant of intrinsically contractile cells, i.e., skeletal myoblasts.

3.1.2. Skeletal myoblasts

These myogenic precursors (known as satellite cells) normally lie in a quiescent state under the basal membrane of skeletal muscular fibres. In case of injury, they are rapidly mobilized, proliferate and fuse to regenerate the damaged fibres. In a clinical perspective, these cells feature several attractive characteristics: (*i*) an autologous origin that overcomes all problems related to immunogenicity and is a key factor for large-scale clinical applicability, (*ii*) an ability to grow in large numbers from a small biopsy [13], (*iii*) a commitment to a well-differentiated myogenic lineage that makes the risk of tumoreginicity extremely low (in our human trial, none of the NOD–SCID immunodeficient mice injected with human myoblasts has developed a tumour), and (*iiii*) a high resistance to ischaemia, which is a major advantage given the hostile environment (a post-infarct scar) in which they are intended to be implanted.

Analysis of the bulk of experimental data on myoblast transplantation leads to the main following conclusions. Morphologically, the injected myoblasts differentiate into typical multinucleated myotubes that tend to substitute for the postinfarction fibrosis (data on press). Although we and others [14] have failed to show any transdifferentiation of the injected cells into cardiomyocytes, engrafted myotubes co-express fast, skeletal muscle-type but also slow myosin, both in rat [14] and sheep (data on press), thereby suggesting some form of phenotypic adaptation to the myocardial environment (similar observations made after dynamic cardiomyoplasty suggest that stretch and/or repeated electromechanical stimulation might account for this 'reprogramming' of engrafted myoblasts towards expression of a slow fibre pattern). In contrast, however, to foetal cardiomyocytes, engrafted skeletal myotubes do not establish junctions with host cardiac cells. Indeed, cultured skeletal myoblasts express *N*-cadherin and connexin-43 (the major proteins constitutive of fascia adherens and gap junctions and therefore responsible for mechanical and electrical coupling, respectively, in heart tissue) but expression of these proteins is down-regulated following intramyocardial implantation [15].

The functional correlate of these observations is an improvement in left ventricular function, which has now been demonstrated in small and large animal models of myocardial infarction [13, 16–19]. A causal relationship between the engraftment of cells and the functional outcome is supported by the data of Taylor et al. [17], who could only document an improvement in mechanical function in cryoinjured rabbit hearts, where incorporation of autologous myoblasts had been successful. The posttransplant improvement in function (compared with hearts only injected with culture medium alone) is closely related to the number of injected cells [20] and, importantly, seems to be sustained over time, as suggested by our 1-year follow-up data, which show a striking stability of results compared with the 4-month posttransplant study point [21].

This long-term benefit could conceivably be related to the expression of slow myosin by engrafted fibres and the attendant switch of their metabolism towards predominantly oxidative patterns.

The mechanism(s) by which implanted myoblasts improve function still remain largely elusive and are currently the subject of intensive experimental investigations. At least three hypotheses, which are not mutually exclusive, have been put forward.

First, the elastic properties of implanted cells could provide a scaffold strengthening the ventricular wall and subsequently limiting postinfarct scar expansion. This protective effect against excessive remodelling is supported by the experimental observations of reduced endiastolic volumes in cell-transplanted hearts.

Second, a direct contribution to systolic function is marshalled from the previously mentioned observation that intrinsic contractile properties of the cells are a prerequisite for maximal preservation of left ventricular function. This hypothesis is supported by our observations of improved systolic indices on 1-year posttransplant pressure-volume curves in rats [21] and increased velocity gradients (both in systole and in diastole) through the infarcted myoblast-grafted segments, as assessed by Doppler tissue imaging, in sheeps [13]. We acknowledge that whereas in the case of foetal cardiomyocytes, the presence of gap junctions makes plausible a synchronous propagation of electrical impulses between donor and host cardiac cells; such a mechanism becomes unlikely in the case of skeletal myoblasts that lack these junctions. Notably, however, previous experiments from our laboratory have established that, in spite of these morphological differences, foetal cardiomyocytes and skeletal myoblasts provide equivalent functional benefit [22], which suggests alternate non-gap junction-mediated mechanisms of cell-to-cell coupling. It is, for example, conceivable that engrafted myoblasts contract in response to the stretch exerted by the surrounding cardiomyocytes, a hypothesis currently under investigation. Whether the contractile performances of grafted myoblasts could be further improved by a preimplantation engineering with genes encoding critical cardiac-specific proteins involved in excitation–contraction coupling (connexin-43, cardiac-type dihydropyridine membrane receptors) is another area of investigation [23, 24].

The third hypothesis is that transplanted cells behave as platforms releasing growth and/or angiogenic factors. Such a mechanism is currently not supported by our experimental findings that myoblast transplantation fails to increase angiogenesis beyond that seen in control hearts receiving an equivalent volume of cell-free culture medium alone. However, paracrine effects exerted by grafted myoblasts on putative resident cardiac precursor cells [25] and driving them towards full maturation in functionally effective cardiomyocytes cannot be completely excluded.

3.2. Cells orientable towards a contractile phenotype

Cells that can be oriented towards a contractile phenotype can be broadly categorised into embryonic stem cells and bone marrow stem cells.

Embryonic stem cells are addressed in another section of this Symposium and will not be discussed here, except for stressing that there is still a wide gap between the in vitro observation of a few beating cells in a culture dish and the generation of cardiomyocytes in sufficient numbers for improving function of infarcted myocardium without the risk of tumour development due to uncontrolled proliferation.

In contrast, bone marrow stem cells are currently more attractive for two main reasons: their purported pluripotentiality, which could allow them to turn to cardiomyocytes (and/or endothelial cells), and their potential for being used as autografts. Interest in bone marrow stem cells currently relies on studies reporting that these cells can regenerate ischaemic myocardium by differentiating into cardiomyocytes and endothelial cells and subsequently improving postinfarction function [26–28]. As bone marrow cells are also discussed in another separate article, we shall limit our comments to a brief listing of the most clinically relevant issues that still need clarification in the perspective of treating heart failure patients: (*i*) the reality of the ‘cardiac’ transformation of these cells, whether spontaneously following their intramyocardial engraftment or after in vitro exposure to 5-azacytidine, as there is still some controversy about the potential for these cells to express the whole set of cardiac-specific markers; (*ii*) the type of cell yield best suited for this phenotypic switch if it does occur (whole bone marrow, stromal cells, hematopoietic progenitors); (*iii*) the possibility of a scale-up (by in vitro expansion or in vivo mobilisation), which is made necessary by the small percentage of hematopoietic progenitors in the bone marrow population (in the study of Jackson and co-workers [28], cardiomyocytes and endothelial cells derived from the donor stem cells were found at a prevalence of 0.02% and 3.3%, respectively), without altering cell plasticity, and (*iiii*) the optimal timing of administration, as all successful studies with bone marrow stem cells have virtually entailed early post-infarction cell injections [26], but local signals driving implanted cells towards a cardiomyogenic differentiation are likely to differ between a freshly infarcted myocardium, which still harbours a

mix of irreversibly damaged and viable cardiomyocytes, and an old postinfarction scar, almost only made of fibroblasts (the clinical correlates of these two settings would be an acute coronary syndrome and a chronic heart failure, respectively).

Irrespective of the potential clinical use of bone marrow-derived stem cells, a more thorough understanding of their biology might shed some additional light on the mechanisms by which cell transplantation improves function of the infarcted heart. Thus, recent data on cardiac chimerism (where recipient cells were found in transplanted hearts) [29] raise the hypothesis that the beneficial effects of myoblast transplantation might involve paracrine effects whereby myoblast-derived factors (to be identified) could trigger the differentiation of stem cells, either resident in cardiac tissue or homing to sites of injury, towards a cardiomyogenic lineage.

3.3. Routes of cell delivery

So far, cell delivery has usually been accomplished under direct vision, through multiple epicardial injections. Not unexpectedly, however, less invasive percutaneous approaches are currently raising a growing interest. Although reported to be effective [30], direct, non-vector-mediated intracoronary injections still require to be validated and more emphasis is now put on the endoventricular approach, which is made possible by improvements in catheter design and navigation systems. Importantly, however, whereas the technical feasibility of these 'blind' techniques has now been established in animals and in man, we still lack data demonstrating their functional efficacy.

Regardless of the route (and the epi- and endocardial techniques are not mutually exclusive), it is important to stress that the early death rate of implanted cells is extremely high (up to 90%) and likely results from both physical (pressure during injections) and biological (inflammatory reaction, environmental hypoxia, apoptosis) factors. Optimisation of the benefits of cell transplantation thus requires that these factors be satisfactorily handled by various measures like less traumatic delivery systems, concurrent induction of angiogenesis (for example, by injection of transfected myoblasts overexpressing vascular endothelial growth factor [31]) or pharmacological blockade of apoptosis.

4. Early clinical data

Upon approval by the French Regulatory Health Authorities and our Institutional Ethics Committee, we have started a phase-I human trial and the first patient to ever receive intramyocardial injections of his own cultured skeletal myoblasts was then successfully operated on 15 June of that year [32]. Eligibility for inclusion in this trial was based on the following three criteria: (*i*) impairment of left ventricular function (ejection fraction ≤ 0.35), (*ii*) history of myocardial infarct with a residual discrete, akinetic and nonviable scar and (*iii*) indication for concomitant coronary artery bypass grafting in remote, (i.e., different from the transplanted area), viable but ischaemic myocardium.

The protocol involves three distinct steps. First, a muscular biopsy is retrieved from the thigh under local anaesthesia. Second, the minced muscle is grown for 2–3 weeks in the Cell Cultures Laboratory so as to obtain a high (at least 400×10^6 cells) and pure (at least 50% myoblasts) cell yield. The third stage consists of cell re-implantation into the postinfarct scar while bypassable coronary arteries supplying ischaemic areas are concomitantly grafted. Cells suspended in a small volume (4–6 ml) are injected in multiple sites so as to cover the entire area of scar tissue.

The detailed results of this phase I trial will be published soon and this review will limit to some general comments. The *feasibility* of the whole procedure has been clearly established as it has been possible to consistently reach cell numbers well above the target values within the preset time frame (2–3 weeks). Regarding *safety*, we have not seen any perioperative complication related to the procedure. The only adverse event has been the occurrence of ventricular arrhythmias in some patients. Several mechanisms can be considered (re-entry pathways due to inhomogeneous conductive properties of grafted myoblasts and host cardiomyocytes, release of arrhythmogenic by-products by the inflammatory cells clearing dead myoblasts, disorganisation of the supracellular architecture by the injected volume of fluid) and are currently being investigated in the laboratory. *Efficacy* data are much more difficult to interpret because of the small sample size (ten patients) and the lack of a control group. However, with the caveat that a confounding effect of the concomitant revascularisation can never be completely ruled out, we have documented a new-onset systolic thickening in approximately 60% of the cell-implanted scar areas and these encouraging results have paved the way for a phase II efficacy trial that should start soon.

5. Conclusions

Thus, several questions still need to be addressed including (1) the optimal type of donor cells, (2) the

mechanism by which cell engraftment improves cardiac function (i.e., actively by increasing contractility) or passively (i.e., by limiting infarct expansion and remodelling), (3) the optimisation of cell survival, and (4) the potential benefits of cell transplantation in non-ischaemic heart failure. Ongoing experimental studies should hopefully provide some answers to these issues in a reasonably close future and thus allow better delineating the place of cell therapy in the management of patients with severe heart failure.

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