Stem Cell Therapy for Ischemic Heart Disease

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Recent experimental and clinical observations have suggested that cell transplantation could be of therapeutic value for the treatment of heart disease. This approach was based on the idea that transplanted donor cardiomyocytes would integrate with the host myocardium and thereby directly contribute to cardiac function. Surprisingly, the observation that non-cardiomyogenic cells could also improve cardiac function indicates that functional integration of donor cells might not be required to achieve a beneficial effect. More recently, several observations have suggested the presence of a greater than anticipated developmental repertoire in adult-derived stem cells, which, if further validated, would offer unprecedented opportunities for the restoration of cardiac function in diseased hearts. Here, we discuss current issues regarding the potential use of stem cell transplantation for the treatment of ischemic heart disease.

Cell transplantation has emerged as a potential therapeutic intervention for the treatment of heart disease. Initial experimental studies were based on the premise that transplantation of cardiomyocytes might result in increased myocardial mass with concomitant augmentation of systolic function. Preliminary studies demonstrated that single-cell suspensions of fetal cardiomyocytes formed stable grafts when delivered into the ventricular wall of normal or injured adult hearts. The transplanted cells terminally differentiated and expressed many of the molecular and morphological attributes typical of normal adult cardiomyocytes.[1,2] The level of donor cardiomyocyte maturity appears to be crucial for successful transplantation, as fetal cardiomyocytes give the best results.[3] Moreover, intercalated discs comprising fascia adherens, desmosomes, and gap junctions were formed between the transplanted donor cells and host cardiomyocytes. Figure 1 shows an example where fetal donor cardiomyocytes expressing an enhanced green fluorescent protein were transplanted into a recipient heart. Intracellular calcium transient image analyses have recently confirmed that transplanted donor cardiomyocytes were functionally coupled with the host myocardium,[4] suggesting that donor cells were able to participate directly in an electro-mechanical syncytium with the host heart.

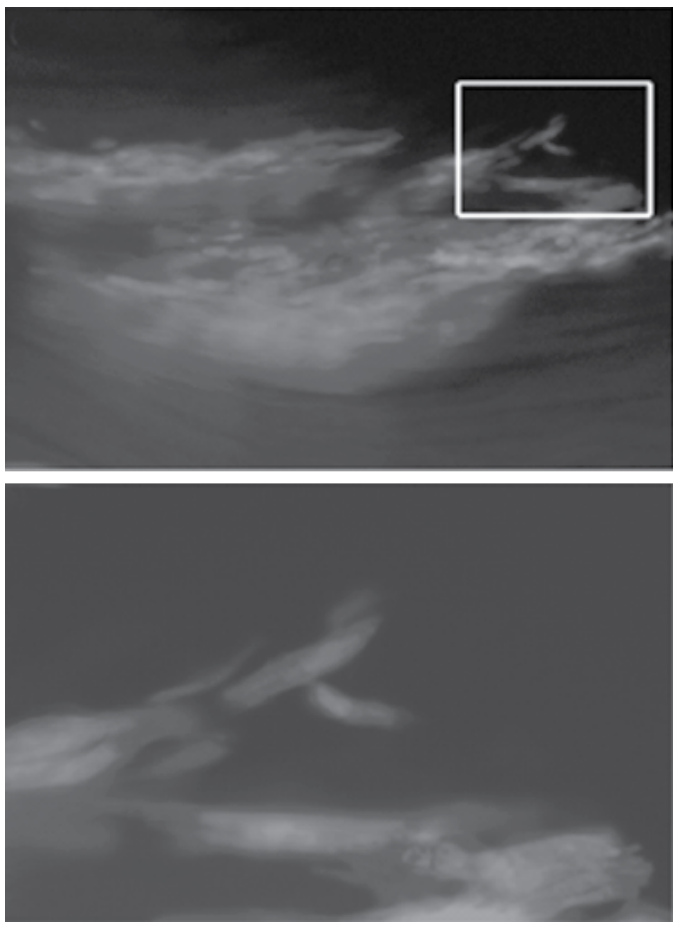

Fig. 1: Example of a fetal cardiomyocyte graft. Donor cells expressing an enhanced green fluorescent protein were transplanted into a normal, syngeneic adult heart. The heart was harvested 14 days later, sectioned on a vibratome (300 mm) and visualized under fluorescent illumination. The boxed region in (a) is shown at higher magnification in panel (b). The magnification in (a) is 63 £ and in (b) is 200 £ . Image courtesy of Michael Rubart (Indiana University School of Medicine, USA).

EMBRYONIC STEM CELLS AS A SOURCE OF DONOR CARDIOMYOCYTES

Embryonic stem (ES) cells are derived from the inner cell mass of pre-implantation embryos and can be cultured indefinitely in an undifferentiated state when maintained under correct culture conditions. Upon differentiation, cells of endo-, ecto- and meso-dermal lineages can be obtained. Cardiomyogenic differentiation in mouse ES cells has been well-characterized.[5] Moreover, two independent groups have isolated human ES cells.[6-8] Subsequent studies revealed that these cells readily differentiated into cardiomyocytes.[9-14] Figure 2 shows the sarcomeric striations showed by cardiomyocytes derived from human ES cells. Although cardiomyocytes obtained from *in vitro*

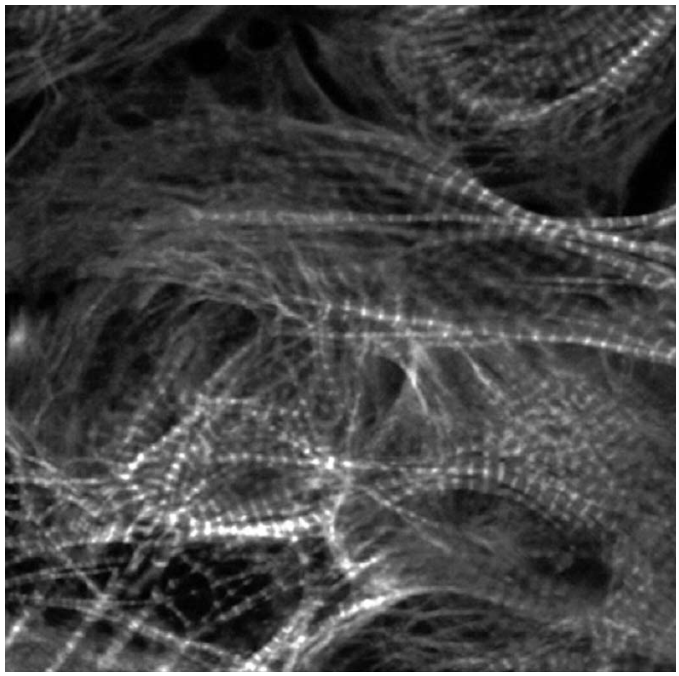

Fig. 2: Sarcomeric banding pattern (stained with phalloidin) in human embryonic-stem-cell-derived cardiomyocytes. The magnification is 63 x. Image courtesy of Robert Passier, Dorien Ward-van Oostwaard and Christine Mummery (Hubrecht Laboratory, The Netherlands).

mouse and human ES cell differentiation tended to resemble early embryonic cardiomyocytes, and failed to acquire adult molecular and morphological characteristics,[9] transplanted mouse ES-derived cardiomyocytes were morphologically indistinguishable from neighboring host cardiomyocytes.[15-17] This observation suggested that appropriate environmental and physiological cues are required for cardiomyocyte maturation and terminal differentiation.

Although the local environment might impart some information for lineage-directed differentiation, transplanted ES cells typically differentiated into multiple cell lineages.[7,18] Moreover, mouse ES cells form teratomas when injected into the hearts of syngeneic recipients (M. Klug and L.J. Field, unpublished). Consequently, therapeutic use of ES cells in the heart would probably require *in vitro* differentiation and purification of donor cardiomyocytes. Embryonic stem cell differentiation *in vitro* is stochastic in nature, and the cardiomyocyte content comprises only 0.5–5.0% of such cultures. To date, several approaches have been developed to enhance cardiomyogenic differentiation. Treatment with growth factors such as transforming growth factor b1, activin-A or 5-aza-20-deoxycytidine increased cardiomyocyte content in differentiating human and murine ES cell cultures.[14,19,20] Gradient-based purification resulted in cell populations containing 70% cardiomyocytes.[14] Alternatively, transgenes with cardiomyocyte-restricted promoters were used to target expression of selectable markers encoding, for example, antibiotic resistance genes or enhanced green fluorescent protein.[16,21] Subsequent purification using antibiotic treatment or fluorescent-activated cell sorting, respectively, resulted in highly purified murine cardiomyocyte cultures suitable for transplantation. The antibiotic selection protocol was directly scalable to bioreactor production, and 2 £ 107 cardiomyocytes were easily obtained from 250 ml culture medium.[22] Thus, ES cells could provide a surrogate source of donor cardiomyocytes for cell-transplantationbased therapies. Therapeutic use of existing ES cell lines would result in the generation of allogenic transplants, and would thus require immune suppression,[23] although some of the reported studies showed that ES or ES-derived cells could be transplanted without immune suppression in the host.[15,20]

SKELETAL MYOBLAST TRANSPLANTATION

Adult skeletal muscle contains a population of myogenic stem cells called myoblasts, which are able to contribute to regenerative growth. These cells can be easily isolated and amplified in an undifferentiated state in culture. When exposed to medium with low serum content, myoblasts exited the cell cycle and fused to form multi-nucleated skeletal myotubes. Similarly, when transplanted into normal or injured hearts, myoblasts differentiated and fused with one another resulting in the formation of patches of vascularized, multi-nucleated myotubes with characteristics typical of skeletal muscle.[24-26] Several studies have shown that the nascent skeletal myotubes were not electrically coupled with the host myocardium.[24,27] This view was supported by the observed absence of cadherin and connexin-43 expression in engrafted skeletal myotubes.[28] Another recent study confirmed again that transplanted skeletal myoblasts contract independently of neighboring cardiomyocytes.[27] Nevertheless, improved ventricular diastolic and systolic function was observed when myoblasts were transplanted into injured rabbit hearts.[26] Similarly, sheep subjected to myocardial infarction and myoblast transplantation showed increased myocardial contractility at one-year post-transplantation.[29] Both skeletal myotubes and viable myoblasts were detected in the infarct scar in these animals. Given the apparent absence of direct coupling between the host myocardium and the preponderance of the nascent skeletal myotubes, the mechanism(s) by which myoblast transplantation enhanced cardiac function is not known.

CARDIOMYOGENIC ADULT STEM CELLS

Studies reported over the past several years have suggested that adult stem cells might have a greater degree of plasticity than was previously appreciated. Several studies have shown that bone-marrow-derived stem cells had apparent cardiomyogenic potential *in vitro* and *in vivo*. For example, treatment of crude bone marrow cell preparations with 5-aza-20-deoxycytidine resulted in the formation of a cell line that, upon further differentiation, gave rise to cells with spontaneous contractile activity and other molecular attributes of differentiated cardiomyocytes.[30] Unfortunately, the expression profile of skeletal-muscle-lineage-determining genes was not monitored in this study, which is a potential concern given the use of 5-aza-20-deoxycytidine and the morphology of the resulting myocytes reported in this experiment. In other studies, bone-marrow-derived hematopoietic stem cells appeared to have cardiomyogenic potential. C-kit-expressing hematopoietic stem cells injected into mouse myocardial infarcts formed myocardium comprising new myocytes as well as endothelial cells and smooth muscle cells.[31] A concomitant improvement of hemodynamic function was observed in the injected animals. Similarly, bone-marrow-derived myocytes and endothelial cells were observed in infarcted mouse hearts following bone marrow reconstitution with sidepopulation (i.e.

highly enriched, CD34 negative, selected on the basis of Hoechst dye staining) hematopoietic stem cells.[32]

Mesenchymal stem cells isolated from the bone marrow also appeared to undergo cardiomyogenic differentiation. For example, multi-potent adult progenitor cells contributed to the forming heart when injected into early blastocysts, although, in the absence of injured myocardium, they did not differentiate into cardiomyocytes when injected into the systemic circulation.[33] Other marrowderived mesenchymal stem cells integrated into the myocardium when transplanted into fetal sheep,[34] normal mice,[35] or pigs with infarcts.[36] In some cases, the integrated cells morphologically resembled the surrounding host cardiomyocytes. Similar experimental approaches suggested that other adult stem cells were capable of cardiomyogenic differentiation, including neuronal stem cells,[37] hepatic stem cells[38] and endothelial progenitor cells.[39,40] Of particular interest was the observation that non-cardiomyocytes isolated from adult hearts also appeared to have cardiomyogenic properties.[41] Interestingly, these cells were isolated using the same criteria that gave rise to the side-population marrowderived hematopoietic stem cells.[31, 32]

Several other studies supported the idea that adult stem cells can migrate into the heart. Cytokine administration promoted bone-marrow stem-cell mobilization with concomitant myocardial restoration with myocytes, arterioles and capillaries, and prevented collagen deposition.[42] Similarly, mouse bone-marrow stem cells injected intravenously contributed to the myocardium and vasculature after coronary artery ligation in rats.[43] The existence of adult cardiomyogenic stem cells with homing capabilities was further supported by gender-mismatched human transplant studies. Some studies examining female hearts that were transplanted into male recipients observed the presence of Y-chromosome-positive cardiomyocytes and vascular components,[44-46] although the reported frequencies of these events differed significantly between different studies. Indeed, other studies using the same technology failed to observe Y-chromosomepositive cardiomyocytes.[47,48] Similar approaches in female patients who underwent bone marrow transplantation with male donor cells revealed the presence of Y-chromosome-positive cardiomyocytes.[49] By contrast, mouse studies suggested that selected hematopoietic stem cells are probably not multi-potent following bone marrow reconstitution.[50]

Although these studies have generated a great deal of enthusiasm, several cautionary notes are in order. While the evidence supporting cardiomyogenic differentiation in some of the studies cited earlier was convincing, others lacked sufficient documentation of cardiomyogenic differentiation. Enthusiasm was further tempered by the observation that stem cell and/or target cell fusion could mediate apparent lineage-switches that were previously attributed to adult stem cell plasticity *in vitro*[51,52] and *in vivo*.[53,54] Despite these caveats, the improvement in cardiac function observed following stem cell transplantation in experimental animals has prompted several clinical studies.

STEM-CELL-INDUCED VASCULARIZATION

It is well-established that endothelial progenitor cells (EPCs) capable of contributing to neovascularization are present in the peripheral circulation in adults. If cultured at sufficiently high density and in the presence of appropriate growth factors, these cells could differentiate into endothelial-like cells that aggregate into capillarylike structures.[55] These EPCs co-existed with hematopoietic stem cells in cord blood and adult bone marrow, and could also be harvested from the peripheral blood after granulocyte colony-stimulating factor (G-CSF) or granulocyte macrophage colony-stimulating factor (GM-CSF) treatment.[56] The observation that endothelial cells of donor-cell genotype populated the lumina of impermeable vascular Dacron grafts implanted in dogs following bone marrow transplantation confirmed that EPCs of bone marrow origin could enter the peripheral circulation.[57] As indicated earlier, both side-population and lin $^{-}$kit^{+} hematopoietic stem cells can contribute to endothelial cell formation following transplantation in mice.[32,33] The contribution of EPCs to neovascularization has also been documented in humans. For example, a female patient who experienced glomerulonephritis after bone marrow transplantation from a male donor showed Y-chromosomepositive renal endothelial cells.[58] Similarly, hostderived endothelial cells were observed in the hearts of male patients who received female hearts.[45]

In vivo stem-cell-derived EPCs enhanced the natural mechanisms of repair of blood vessels after ischemia or vascular obstruction via both angiogenesis and arteriogenesis. The functional consequences of these activities were frequently dramatic. For example, infusion of bone marrow, cord blood or peripheral-blood-derived mononuclear cells enhanced the formation of new vasculature and concomitantly increased perfusion in chronic hindlimb ischemia models.[59-63] Genetically enhanced EPCs expressing either human telomerase or vascular endothelial growth factor showed superior renewal capacity and revascularization activity, respectively.[62,64] Endothelial progenitor cells also enhanced vascularization in injured hearts. Rats injected with cultured EPCs showed a reduction of myocardial infarct size and preserved cardiac function compared with animals injected with culture medium.[65] Similarly, injection of CD34^{+} cells isolated from human peripheral blood after G-CSF treatment, improved angio- and vasculo-genesis in a rat myocardial infarction model.[66] Improved cardiac function was thought to result indirectly from enhanced perfusion (which, in turn, decreased cardiomyocyte apoptosis and reduced myocardial remodeling).

CLINICAL EXPERIENCES WITH STEM-CELL-BASED INTERVENTIONS

The studies reported earlier have prompted several clinical trials aimed at establishing the safety of stem cell transplantation for the treatment of ischemic heart disease. The first studies used skeletal myoblasts.[67] Autologous myoblasts harvested from patients were amplified *in vitro* and injected into the injured myocardium during coronary artery bypass grafting. Contraction and viability, as measured by echocardiography and positron emission tomography, was improved at five months post-delivery. However, these studies in a small number of patients were compromised by the presence of ventricular arrhythmias, requiring concomitant defibrillator implantation. More clinical trials are currently being performed in the USA and Europe. No arrhythmias have been reported to date.

Several studies using bone-marrow-derived progenitors as donor cells have recently been reported. For example, the effect of

mononuclear bone marrow cell transplantation in combination with percutaneous transluminal coronary angioplasty was studied in ten myocardial infarction patients. Donor cells were harvested via density-gradient centrifugation, cultured overnight and delivered intracoronary in the infarct-related vessel during balloon dilatation. Three months after cell delivery, the size of the infarcted region was significantly decreased compared with patients treated with percutaneous angioplasty of the infarct-related artery only, with concomitant improved wall motion.[68] Two other studies isolated bone-marrow-derived mononuclear cells via density gradient centrifugation; the cells were then directly injected into the myocardium via transendocardial catheterization in the absence of surgical revascularization. In one case, the donor cells were injected into areas with decreased contractile activity (i.e. hibernating myocardium) in 14 patients.[69] Significant reduction in total reversible defect and improvement in global left ventricular function within the treatment group was apparent two months after cell delivery, and ejection fraction was improved at four months post-delivery. In the other case, eight patients were treated in a similar way.[70] After a three-month follow-up, patients reported a reduction of anginal episodes, and magnetic resonance imaging analyses showed improved target wall-thickening and -motion, but global ejection fraction was unchanged. Finally, theeffects of intracoronary delivery of circulating bloodderived cells (11 patients) and bone-marrow-derived (nine patients) cells were compared.[71] The circulating cells were harvested from the peripheral circulation and amplified in culture for three days, while the bone marrow cells were isolated by density-gradient centrifugation as described earlier. In both cases, the donor cells were delivered via intracoronary injection at the site of the stent deployment four days earlier. At four months postdelivery, a modest but significant increase in ejection fraction, improved regional wall motion and reduced endsystolic left ventricular volumes were noted with both types of donor cells. Collectively, these studies suggest that intracoronary delivery or direct injection of bone-marrowor peripheral-circulation-derived stem cells after myocardial infarction was safe in humans.

CONCLUDING REMARKS

The basic and clinical studies outlined earlier indicate that myogenic and vasculogenic stem cells can be successfully transplanted into the adult heart. The ability of exogenous or auxiliary EPCs to contribute to the vasculature and to enhance regional blood flow in ischemic cardiac tissue appears to be well-established in experimental animal models. It is also clear from surgical interventions that alleviation of chronic ischemia in patients can rescue both hibernating and at-risk myocardium. Thus, the rationale for stem-cell-based revascularization interventions would appear to be sound. However, there are several issues that need to be further explored. For example, the systemic consequences of EPC cell delivery have not been wellcharacterized. Additionally, although a trend towards functional improvement was noted in the initial EPC clinical transplantation experiments, larger randomized studies are required to establish the superiority of the intervention over traditional treatment.

The cardiomyogenic capacity of adult stem cells appears, thus far, to be somewhat less-established. This is reflected by the highly variable results obtained between different groups using, what appear to be, very similar reagents and approaches. The rigor of assessment of myocardial identity resulting from putative transdifferentiation events was highly variable between studies. The apparent contribution of cell-fusion events further complicates interpretation of putative transdifferentiation events. Additionally, given that these studies reported similar cell markers of putative cardiomyogenic stem-cell preparations compared with those of cells with established EPC activity, the underlying cellular mechanisms responsible for any improved cardiac function observed with cardiomyogenic stem cell transplantation remains to be established in many instances.

Although there remain many unknowns, the application of stem cell therapy for ischemic heart disease presents promising possibilities for both research and therapy (Box 1 and Box 2). Targeted manipulation of genetic pathways might influence cell survival, transdifferentiation and/or function following transplantation in pathological conditions such as ischemia, apoptosis and pressureoverload. The potential positive impact of such interventions on morbidity and mortality in cardiovascular disease is quite exciting.

Box 1 : Cardiomyocyte transplantation: outstanding issues

- Can donor cell viability and/or cell cycle activity be enhanced to permit the generation of grafts with sufficient mass to impact upon systolic function?
- Can revascularization be achieved at a level sufficient to support large grafts?
- Would large grafts form a functional syncytium with the host myocardium?
- Would the presence of naı¨ve or growth-enhanced donor cells present an arrhythmogenic substrate?

Box 2 : Adult stem cells: outstanding issues

- To what extent does cardiomyogenic induction versus cardiomyocyte stem-cell fusion contribute to apparent transdifferentiation events?
- What is the growth potential and viability of adult-stem-cellderived cardiomyocytes?
- To what extent can endogenous cardiomyogenic stem cells be mobilized by cytokine treatment?
- Is the apparent cardiomyogenic phenotype of transplanted cells reflective of intrinsic characteristics or a consequence of *ex vivo* manipulation and/or delivery into a non-physiological niche?

ACKNOWLEDGEMENTS

We thank NHLBI (L.J.F.) and AHA (J.D.D.) for support, and Michael Rubart, Mark Soonpaa, Hidehiro Nakajima and Hisako Nakajima for comments on the manuscript.We also thank our many colleagues working in the field and apologize if any relevant views/studies were inadvertently not included.

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