

Stem Cell Therapy for Ischemic Heart Disease: Beginning or End of the Road?

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Despite improvements in emergency treatment, myocardial infarction is often the beginning of a downward spiral leading to congestive heart failure. Other than heart transplantation, current therapeutic means aim at enabling the organism to survive with a heart that is working at a fraction of its original capacity. It is therefore no surprise that cardiac stem cell therapy has raised many hopes. However, neither the ideal source and type of stem cell nor the critical cell number and mode of application have been defined so far. Early reports on myocardial repair by adult bone marrow stem cells from rodent models promoted an unparalleled boost of clinical and experimental cell therapy studies. The phenomenon of stem/progenitor cell-induced angiogenesis in ischemic myocardium has ever since been reproduced by numerous groups in a variety of small and large animal models. Myogenesis, however, is an altogether different matter. Many of the initial clinical studies were fueled by the suggestion that early hematopoietic stem cells have a plasticity high enough to enable cross-lineage differentiation into cells of cardiomyocyte phenotype, but the initial enthusiasm has largely faded. The myogenic potential of stroma cell-derived mesenchymal stem cells is much better documented in animal models, but transfer to the clinical setting faces a variety of obstacles. In clinical pilot trials, we and others have demonstrated the feasibility and safety of administering progenitor cells derived from autologous bone marrow to the myocardium of patients with ischemic heart disease. Clinical efficacy data are still rare, but the few controlled trials that have been completed uniformly show a tendency towards better heart function in cell-treated patients. This review is an attempt to describe the scientific basis for cardiac cell therapy from the point of view of the clinician, focusing on problems that arise with beginning translation into the clinical setting.

Key words: Heart; Stem cells; Infarction; Regeneration; Myocardial ischemia

INTRODUCTION

Stem cells are unspecialized cells that renew themselves for long periods of time and can be induced to become cells with a specialized function. The traditional definition requires the capacity for asymmetric cell division (i.e., the stem cell divides into one stem cell and one differentiated cell), while a typical progenitor cell gives rise to two differentiated daughter cells. Whereas embryonic stem cells are uncommitted and pluripotent in their differentiation capability, adult stem cells are believed to differentiate only into specialized cells of the organ or tissue they are derived from. The function of adult stem cells appears to be maintenance and repair of their tissue of origin. Various types of progenitors and stem cells with myocardial regenerative potential have been derived from skeletal muscle and myocardium as well as different hematopoietic cell sources, including bone marrow (BM), peripheral blood (PB), and umbili-

cal cord blood (UCB). Understanding of adult/somatic stem cells has been upset by recent experimental data indicating that adult stem cells derived from hematopoietic tissue can give rise to nonhematopoietic cells such as cardiomyocytes, hepatocytes, endothelial, and epithelial cells. Initially this was interpreted to represent trans-differentiation of hematopoietic stem cells by crossing lineage boundaries, the so-called “stem cell plasticity” (24). Alternatively, the existence of nonhematopoietic stem cells or even more immature multipotent types of stem cells in the various transplanted cell sources, as well as the phenomenon of fusion of transplanted cells with resident cells in the damaged organ, has been taken into consideration (6,12). To date the mechanisms underlying adult stem cell-mediated organ regeneration are not clear.

In the following, we will discuss the pathophysiological background of ischemic heart failure and the rationale for the employment of adult stem cells to treat myocardial

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ischemia. We will further highlight information on contractile muscle-derived regenerative cells as well as adult stem cells from hematopoietic tissue to build a basis for a critical discussion of the ongoing clinical trials.

ISCHEMIC HEART DISEASE

Despite a better understanding of its etiology, the prevalence of ischemic heart disease remains exceedingly high in industrialized countries and is on the rise in developing countries. Risk factors for coronary atherosclerosis have long been established, but it remains unclear whether there is one unifying mechanism by which atherosclerotic narrowing of the coronary arteries develops. In many patients, sudden rupture of the endothelial surface of a localized atherosclerotic plaque leads to thrombus formation with acute occlusion of the coronary vessel. The resulting myocardial ischemia induces immediate biochemical changes and loss of myocardial contractility. Irreversible necrosis of cardiomyocytes takes about 30 min to develop and further extends with time for several hours. Thanks to improvements in primary and secondary prevention as well as therapeutic interventions, both incidence and mortality of acute myocardial infarction (AMI) recently decreased, but the number of individuals suffering AMI is still estimated to exceed 1.5 million per year in the US and 2 million per year in Western and Central Europe. Many patients develop diffuse atherosclerotic disease of the entire coronary artery tree, and repeated episodes of AMI may result in severely impaired myocardial contractility and heart failure, often described as “ischemic cardiomyopathy.” The most problematic consequence—besides the clinical symptoms of angina pectoris—is a net loss of contractile tissue. The myocardium mainly consists of terminally differentiated cells without a clinically relevant potential for regeneration, although the existence of cardiac stem cells has recently been indicated. Hence, large numbers of cardiomyocytes that were subject to necrotic or apoptotic cell death cannot sufficiently be replaced by new contractile cells. Instead, remodeling processes ultimately lead to diffuse interstitial myocardial fibrosis or formation of a transmural fibrous scar. Without invasive treatment, survival of patients with myocardial infarction and considerably reduced left ventricular contractility [e.g., left ventricular ejection fraction (LVEF) <30%] is less than 40% after 5 years, which illustrates the urgent need for novel therapeutic measures. Surgical or interventional restoration of blood supply to ischemic myocardium effectively treats angina, prevents myocardial infarction, improves function of the remaining viable myocardium, but viability and function of necrotic myocardium cannot be restored with current therapeutic means.

CARDIAC CELL THERAPY

The ultimate goal of any cardiac cell therapy approach is the reconstitution of contractile tissue in functionally relevant quantities. The neo-tissue must be integrated in the myocardial syncytium, and blood supply to this tissue must also be secured.

Initial reports on experimental cell therapy for myocardial tissue repair described the implantation of differentiated cells (i.e., cardiomyocytes) or defined progenitor cells (i.e., skeletal myoblasts or EPC) (29,30,60). Due to their ease of isolation, cells from hematopoietic tissue are currently considered the best available source of “true” adult stem cells (18). Embryonic stem cells have been used experimentally in the context of myocardial cell therapy, but will not be reviewed here. A very intriguing concept is the existence of cardiac stem cells that appear to be present in the adult mammal myocardium (59). Theoretically, such cells could be induced to proliferate and regenerate the myocardium *in situ*. Alternatively, they could be isolated, expanded, and turned into cardiomyocytes *ex vivo*, and be reimplanted where needed (4,8). The various aspects of myocardial cell therapy have elsewhere been reviewed in great detail (9,41,45). Here we will briefly portray various cell types that are considered to be relevant for clinical application today, and discuss some of the problems arising in the process of rapid bench-to-bedside translation.

Contractile (Progenitor) Cells

Among the first cells that were implanted in experimental models of myocardial infarction were fetal and neonatal *cardiomyocytes*. After direct injection into postinfarction scar tissue, they survived, appeared to be integrated into the myocardial syncytium as evidenced by formation of intercalated disks and expression of specific gap-junction proteins, and led to improvement of myocardial contractility (25). Even though the initial reports were promising, cardiomyocyte transplantation is unlikely to reach clinical significance soon. Neonatal rodent cardiomyocytes can be isolated and cultivated, but adult cardiomyocytes in higher mammals have virtually no capacity for proliferation in cell culture. Theoretically, allogenic or xenogenic cardiomyocytes could be obtained from donors, but would be subject to rejection, unless immunosuppression would be induced as in any other organ transplant patient. Moreover, transplanted cardiomyocytes are as susceptible to ischemia as the native cardiomyocytes. Implanted in ischemic myocardium, they are therefore prone to succumb to tissue hypoxia just as the native host cells did. Cardiomyocytes spontaneously produce action potentials, albeit slowly. In fact, transplanted cardiomyocytes have been shown to act as

pacemakers after implantation in dog hearts once the native conduction system was destroyed (50).

In contrast, *smooth muscle cells* do not contract spontaneously in vitro or after implantation in the heart. They can be isolated from intestine, blood vessel wall, or genitourinary organs, and may readily be expanded in cell culture. Their potential usefulness for restoration of contractile tissue following AMI has been demonstrated in rodent models (26), but they are currently not as intensively studied as other cell types.

The largest body of knowledge has most likely been collected regarding *skeletal myoblasts*. These so-called “satellite cells” reside in the periphery of skeletal muscle fibers and serve to regenerate skeletal muscle tissue following injuries. Skeletal myoblasts can be readily isolated from a small muscle biopsy; in humans the thigh musculature is commonly used. The isolation process involves mechanic destruction and enzymatic digestion of myofibers, and the myoblasts are collected and enriched by filtration and plating. No specific surface marker-based cell selection is necessary. There is usually some fibroblast contamination, which can be sufficiently controlled by frequent culture replating, and purity of the final product should exceed 80%. Myoblasts have a robust proliferation capacity and multiply in high-serum concentration for numerous passages without changes in phenotype. Once the serum concentration in the medium is lowered, they rapidly differentiate and fuse to form multinucleated myotubes.

Besides their intrinsically preprogrammed differentiation in a myocyte phenotype, their most intriguing quality is resistance to hypoxia, and it has been assumed that myoblasts are able to survive in ischemic myocardium better and longer than cardiomyocytes. Doubt remains, however, as to their ability to functionally integrate into the myocardial syncytium (i.e., to express cardiac-specific connexins and form functioning gap junctions with surrounding viable cardiomyocytes). In principle, undifferentiated myoblasts can express connexin 43 (Cx43), the predominant gap-junction protein in ventricular myocardium, and at least some of the myoblasts appear to be able to form functioning cell–cell communications with cocultured cardiomyocytes in vitro (46). Once they have differentiated and formed myotubes in conventional 2D culture, the Cx43 expression is markedly downregulated but may persist to some degree.

On the other hand, when myoblasts are grown and differentiate in 3D culture under mechanical stimulation such as longitudinal strain, Cx43 expression is preserved or even upregulated. The situation in vivo after implantation in infarcted myocardium is naturally more difficult to assess. Many reports indicated that skeletal myotubes are not morphologically integrated in the host

myocardium; instead they appear to form distinct islets in postinfarct tissue. Cx43 expression in transplanted myoblasts has been described in several animal models, but has not been detected in patients who underwent myoblast injection and postmortem histology studies.

In a careful study of myoblast transplantation in mice, Rubart et al. found that the majority of the intramyocardial myoblasts/myotubes are functionally isolated from the surrounding myocardium, and suggested that the remaining cells connect with host cardiomyocytes as a result of cell fusion (49). Their observations might not only explain the conflicting results of other studies, but also provide an explanation of a significant clinical problem: the durations of calcium transients recorded from intramyocardial skeletal myoblasts were heterogeneous compared with those in neighboring host cardiomyocytes, which may interfere with the propagation of excitation across the ventricular myocardium and pose the heart at risk of ventricular arrhythmia. Rhythm disturbances have indeed been observed in several patients who underwent skeletal myoblast implantation. Whereas the very first patient who underwent myoblast implantation together with a coronary artery bypass operation (CABG) in a pioneering undertaking by Menasche and colleagues had an uneventful postoperative course (31), several other patients developed life-threatening ventricular arrhythmia a few days after the procedure. Surgical patients were still under close observation and could be treated, but elsewhere patients had undergone transcatheter myoblast implantation with early discharge from the hospital and could not be saved.

Therefore, at least in well-structured trials, clinical myoblast implantation for heart failure is currently limited to patients who have an automatic defibrillator device implanted. As to the efficacy of myoblast implantation, numerous small- and large-animal studies have shown a significant improvement of left ventricular contractility. Results of the early clinical pilot trials were also encouraging, but results of large-scale controlled clinical trials are not yet available.

Marrow, Blood, and Cord Blood Cells

Blood and bone are attractive, readily accessible sources of stem cells. These cell reservoirs harbor various populations of candidate regenerative cells including hematopoietic progenitor cells (HPC) (30) and hematopoietic stem cells (HSC), endothelial progenitor cells (EPC) (29), mesenchymal stem cells (MSC) (18,45,60), and multipotent adult progenitor cells (MAPC) (9,41).

In the context of cardiac cell therapy, the term “adult stem cells” usually refers to the application of mixed cell populations obtained from marrow, peripheral blood, or cord blood. Other sources of cells with the capacity

for asymmetric division and plasticity such as fatty tissue or pancreas have, so far, only occasionally been used in myocardial regeneration attempts. Contradictory findings regarding the regenerative potency of these cells are considered to be, at least in part, due to the heterogeneity of stem cells present in different sources and preparations.

Within bone marrow, at least two distinct populations of progenitor and stem cells have so far been clearly recognized. These are (i) CD34⁺/45⁺/133⁺ HPC including a small fraction of HSC as the traditional source of hematopoietic progeny, and (ii) CD34⁺/45⁺/133⁻ MSC with the capacity to renew marrow stroma, support hematopoiesis, and to differentiate into various mesodermal cell types including connective tissue (chondrocytes, osteocytes), adipocytes, and myocytes (43,44). The phenotype of in vitro cultured MSC shows a typical combination of marker molecules CD73 (SH3, SH4), CD90 (Thy1), and CD105 (SH2) with a lack of the hematopoietic stem cell markers CD34 and CD133. The homogeneous expression of the otherwise endothelial-specific molecule CD146 (Muc18) may allow for speculations about a certain relation between MSC and the endothelial lineage. In fact, almost complete acquisition of endothelial phenotype and function under optimized culture conditions has recently been reported (38).

A minute subpopulation of cells that copurify with MSC has been originally described as mesodermal progenitor cell and, due to the wide spectrum of differentiation, renamed as MAPC (47). These CD34⁺/133⁺/Flk1⁺ cells have been postulated to comprise the principle endothelial progenitor in human postnatal BM (48). Human MAPC can differentiate into adipocytes, osteoblasts, EC, and hepatocytes in vitro and, after injection into the tibialis anterior muscle of NOD/SCID mice in vivo, into myocytes (33).

Endothelial cells can definitively be derived from bone marrow (27) but a conclusive description of an endothelial stem cell in adult bone marrow is still a matter of debate. CD133⁺/VEGFR2⁺ BM-derived cells are currently the most widely used functional EPC (60). Putative BM-derived progenitors of endothelial cells have been originally recovered from normal peripheral blood as CD34⁺/45⁺ cells (1). The relation between these cells and circulating endothelial cells could not be clarified so far (54).

Various types of cells related to the endothelial lineage have thus far been described in most hematopoietic and nonhematopoietic tissues. The differences in frequency, phenotype, and function of these endothelial lineage cells clearly need to be analyzed in greater detail. The reactivity of several of these cell types with CD133 and the coexpression of CD133 on the greater part of CD34⁺ HPC and HSC have been taken as a strong argu-

ment in favor of positive selection to enrich adult SC for clinical use.

In Vivo Myocardial Regeneration by Adult Stem Cells

Ideally, a stem cell that has been implanted into infarcted myocardium would give rise to new blood vessels and new contractile cells (i.e., angiogenesis and myogenesis). Proliferation and differentiation would be guided by local humoral and/or cellular control mechanisms, and no neoplastic growth or differentiation in unwanted noncardiac cell types would occur. So far, neither the ideal source and type of SC nor the critical cell number and mode of application have been defined.

In 2001, two experimental studies of myocardial repair by adult stem cells from hematopoietic sources after experimental myocardial infarction promoted an unparalleled boost of clinical and experimental regenerative SC therapy studies. They also mirror the variety of adult SC used for myocardial repair. Kocher et al. found that systemic intravenous infusion of purified human CD34⁺ cells from G-CSF mobilized PB (MPB) can improve rat heart function by generating new blood vessels within the infarct area ("neovasculogenesis") (21). Orlic et al. reported on both neovasculogenesis and transdifferentiation of transplanted cells into cardiomyocytes by either using intramyocardial application of mouse BM-derived Lin^{NEG}/c-Kit⁺ SC or cytokine-induced SC mobilization (35,36).

Many of the initial clinical pilot trials were fueled by the suggestion that early hematopoietic stem cells have a plasticity high enough to enable differentiation into contractile cells of cardiomyocyte phenotype. In fact, such cells were considered to be able to truly regenerate infarcted myocardium by promoting both neoangiogenesis and neomyogenesis. The initial enthusiasm, however, has largely faded. While in situ neoangiogenesis induction by hematopoietic cells, associated with functional improvements, is consistently observed, it proved difficult to find corroborating evidence for true cardiomyocyte differentiation. In fact, two independent groups reported early in 2004 that they did not detect any meaningful evidence of cardiomyocyte differentiation of hematopoietic stem cells in mouse models that were designed to confirm the earlier findings (3,34).

In contrast, the myogenic potential of stroma cell-derived mesenchymal stem cells is much better documented. Several years ago, Wakitani et al. reported the in vitro development of myogenic cells from rat bone marrow mesenchymal stem cells exposed to the DNA demethylating agent 5-azacytidine (62), and Makino et al. isolated a cardiomyogenic cell line from murine bone marrow stromal cells that were treated with 5-azacytidine and screened for spontaneous beating (28). Those cells connected with adjoining cells, formed myotube-

like structures, and beat spontaneously and synchronously. They expressed various cardiomyocyte-specific proteins, had a cardiomyocyte-like ultrastructure, and generated several types of sinus node-like and ventricular cell-like action potentials.

When isogenic marrow stromal cells are implanted in rat hearts, they appear to become integrated in cardiac myofibers, assume the histologic phenotype of cardiomyocytes, express connexins, and form gap junctions with native cardiomyocytes (7,63). Again, pretreatment with 5-azacytidine is believed to facilitate differentiation towards a cardiomyocyte phenotype *in vivo* (5). Human mesenchymal stem cells derived from the marrow of volunteers have also been injected in hearts of immunodeficient mice, and again it was observed that they assume cardiomyocyte morphology and express various cardiomyocyte-specific proteins (57).

Taken together, there is quite convincing evidence that mesenchymal stem cells derived from marrow stroma may assume a myocyte-like phenotype under certain conditions, while hematopoietic stem and progenitor cell types are primarily involved in angiogenic processes. Nevertheless, numerous questions remain to be answered before cell therapy for neovascularization of ischemic myocardium can become clinical routine. First, is the angiogenic potential of human cells sufficient for relevant neovascularization of ischemic myocardial tissue? Second, what are the best surface markers of human adult stem cells with angiogenic potential? Third, how are such cells best delivered to ischemic myocardium? Fourth, what is the molecular mechanism of stem cell endothelial differentiation? Fifth, are there risks of cell-induced neovascularization that outweigh the potential benefit? Finally, can the natural angiogenesis-inducing capacity of adult stem cells, which is obviously not sufficient in many situations, be further augmented?

CELL SURVIVAL

When cells are injected into ischemic myocardium, it is likely that most of them do not survive. The cell preparation process is usually well tolerated. Skeletal myoblasts or other *ex vivo* expanded cells, as well as processed primary cells, normally have viability rates higher than 90%. It is often argued that injection of cell suspension through a needle or a catheter significantly compromises cell viability. In our experience, this is not the case. Even injection through a long cardiac catheterization device has very little effect on cell survival. Once the cells have entered the myocardial interstitium, however, many of them appear to succumb to necrotic or apoptotic death. Its magnitude is difficult to determine, but the suggested survival rate over days or weeks ranges between 0.1% and 10%. The causality is proba-

bly multifactorial. First of all, the ischemic myocardium is obviously a hostile environment, due to local hypoxia, acidosis, lack of substrates, and accumulation of metabolites. Skeletal myoblasts are known to be quite resistant to ischemia, but little is known about the energy requirements of hematopoietic or mesenchymal stem cells. Second, necrotic myocardium is subject to infiltration with phagocytic cells that remove cell debris and initiate the scarring process. Even though the stem/progenitor cells used clinically are autologous, many are probably lost in this "clean-up" process. Third, the mechanic forces that are present in the myocardium may play a role. Transmural pressure is high during systole, and there are shear forces between contracting myofibers and layers. Again, a skeletal myoblast might be able to tolerate this, but a marrow cell is certainly not well equipped to withstand such stress. Fortunately, the rate of cell death can be slowed by targeted manipulation of the cells to be injected. Transfection of marrow stromal cells with a gene encoding for the antiapoptotic protein AKT has been shown to greatly improve cell survival and regenerative capacity upon injection in infarcted myocardium. Pretreatment of endothelial progenitor cells with eNOS-enhancing substances also appears to have a beneficial conditioning effect. Simple hypoxic preconditioning prior to cell injection might also help, because it has been shown to activate both antiapoptotic and NO-related signaling pathways.

DETERMINING FUNCTIONAL EFFECTS

To the clinician, the functional effects of myocardial cell therapy matter so much more than histologic evidence of neoangiogenesis or neomyogenesis. The reliability of left ventricular function measurements in experimental models depends both on the animal model and on the method used. Much of the basic experimental work on cell therapy for myocardial regeneration has been done in mouse models. The main reasons for that are the availability of immunodeficient mice (SCID mice) that allow for the use of human cell transplants with immunologic response, the use of GFP, lac-Z, or otherwise labeled cells from genetically modified animals, and the ready availability of antibodies and nucleic acid probes for detailed expression analysis *in situ*. The problem, however, is the functional analysis. Echocardiographic as well as magnetic resonance imaging of mouse hearts are indeed possible, but given the tiny dimensions and tremendously high heart rate, reliable determination requires highly experienced investigators. The availability of athymic rats allows for xenogenic transplantation of human cell populations without T-cell-mediated acute rejection, and functional studies are much easier. Serial echocardiographic analysis of global LV function and dimensions can be performed with

good reproducibility. Moreover, cardiac catheterization is feasible, and by using a multielectrode electromagnetic conductance catheter supplied with a pressure transducer, LV pressure–volume curves can be recorded. Those allow for detailed functional analysis of myocardial contractility and relaxation properties.

The rabbit as a model for myocardial cell transfer experiments is being used quite rarely. There is no clear advantage with respect to functional analysis compared with the rat, and the problems with antibodies and other tools for expression profiling and histology are notorious. Thus, truly reliable analysis of myocardial function following cell therapy requires the use of a large-animal model (i.e. porcine, canine, or ovine). Here, both echocardiography and MR imaging allow for serial studies of global as well as regional myocardial contractility in the specific area of interest. In acute experiments or set-ups with chronic instrumentation, very reliable sonomicrometry data can be obtained following cardiac implantation of ultrasound crystals. Scintigraphic perfusion scans and coronary angiography can be performed. A major disadvantage of many large-animal models is the deficiency of antibodies against stem cell surface markers, and the inevitable immunresponse when xenogenic (i.e., human) cells are used.

The baseline result of most of the published work is that there is some improvement of global and—if appropriate—regional contractility following adult stem/progenitor cell implantation in ischemic myocardium. This has been described for skeletal myoblasts, unfractionated mononuclear bone marrow cells, stroma cell-derived cell lines, and cells selected using markers of hematopoietic stem cells or endothelial progenitor cells. The improvement of global left ventricular contractility is usually in the range of 5% to 20%, and the size of the infarct area is somewhat smaller on histology than in untreated animals. In several small animal studies, a reduction of postinfarct mortality has also been observed. One particularly important lesson learned from large-animal experiments is the notion that one should aim at injecting cells that work primarily via angiogenesis induction into the infarct border zone, rather than in the center of the infarct area. Only by doing so a relevant improvement of myocardial contractility and perfusion is likely to be induced (13). When primarily contractile cells (i.e., myoblasts) are used in sufficiently vascularized tissue, this may be less important. To date, no clear advantage of one cell type over another can be seen. Several studies attempted a direct comparison between two or more different cell populations (i.e., skeletal myoblasts vs. bone marrow cell, or marrow cells vs. endothelial progenitor cells) but the data are not conclusive yet. The same is to be said regarding the dose–response relationship of a

given cell population, as well as the host-related issues such as the infarct size or the interval between myocardial infarction and cell treatment.

CLINICAL APPLICATION

Skeletal Myoblasts

Translation of experimental cell therapy approaches for myocardial regeneration into the clinical setting has just begun. Whether the first clinical pilot trials were initiated too early remains subject to very controversial debate. The earliest clinical application was reported by Menasche and colleagues (31). A patient with significantly reduced LV contractility following myocardial infarction had to undergo coronary artery bypass grafting (CABG) to optimize myocardial blood supply. Two weeks before, a muscle sample was obtained from the thigh, and skeletal myoblasts were cultivated and grown for several passages. During the bypass operation, these cells were directly injected into the infarcted myocardium. The patient tolerated the procedure well, and a distinct improvement of wall thickening and motion in the area of cell injection was noted postoperatively. This procedure has been repeated worldwide ever since, and the reports quite uniformly describe a mild improvement in contractility. Analogous to the experimental experience, however, the myofibers that originated from the transferred myoblasts appear to survive in the infarct tissue but do not fully integrate into the myocardial syncytium (see above). The CABG operation represents a unique opportunity to directly access the infarcted myocardium, but at the same time does it conceal the effects of the cell therapy. Analysis of regional contractility may give some hints regarding the cell-induced functional improvement, but, inevitably, there is always some overlap with the effects of myocardial revascularization.

Recently, procedures have been developed that enable the cardiologist to deliver cells directly to the myocardium as a stand-alone treatment. By using a novel cardiac catheterization device, the infarcted myocardium can be quite precisely located based on its electrophysiologic properties. A map of the endocardial surface of the LV myocardium is then constructed that clearly depicts the localization of the infarct tissue. A needle is advanced into through the endocardial layer into the myocardium at a preset depth, and the cell suspension is injected. This procedure has been performed in patients with severely impaired LV function, and, again, a mild improvement of LV contractility was noted, together with some relief of the symptoms of ischemic heart failure. The myoblast injection, however, appears to result in a transient period of electrical instability a few days

after the injection, which repeatedly leads to sustained ventricular arrhythmia (see above).

Bone Marrow Cells

While clinical skeletal myoblast transfer is mainly limited to patients with chronic heart failure, cell therapy with bone marrow- or blood-derived cells may evolve in a novel treatment option for both chronic ischemic heart disease and acute myocardial infarction. In either situation, the angiogenic potential of certain adult stem/progenitor cell types is probably the key to functional improvements, whereas true neomyogenesis is, at present, rather unlikely. Around 2001 a number of clinical trials were initiated, and among the first published was the work by Hamano et al. (14), who injected bone marrow mononuclear cells intramyocardially during a CABG operation, Tse et al. (58), who used a catheter-based system for direct intramyocardial delivery of mononuclear cells, and our own group, who injected a purified population of AC133++ bone marrow cells, again in conjunction with a CABG operation (54). Several other groups have reported similar studies (10,11,42).

What these trials have in common is that patients with chronic ischemic heart disease have been treated. The most recent myocardial infarction in those patients usually dates back several weeks, months, or even years. In the infarcted or chronically ischemic myocardium, a substantial net loss of contractile tissue mass has occurred, increased collagen deposition has led to a more or less pronounced, diffuse, or localized scar formation, and blood supply to the myocardium remains impaired although there may have been some collateral vessel growth. Usually, the ischemic myocardium in those patients is not a complete transmural fibrous scar, which would eventually progress into a left ventricular aneurysm, but still vital cardiomyocytes are dispersed within the fibrous network. Theoretically, such "hibernating" cardiomyocytes can be rerecruited for contractile work once sufficient supply of oxygen and nutrients has been reestablished, and stem/progenitor cell-induced growth of microvessels in the infarct border zone may thus translate into improved myocardial contractility. Nevertheless, it should be kept in mind that the chances to resuscitate hibernating cells in a functionally relevant fashion are likely to decrease with time. The longer the interval between myocardial infarction and cell treatment is, the smaller the chance to achieve a beneficial effect becomes. This notion, however, is largely intuitive, and presently not supported by clear-cut data.

The situation in acute myocardial infarction studies is fundamentally different. Here, the onset of myocardial ischemia is usually between several hours and a few days ago. Typically, a patient is admitted with acute

chest pain and ECG shows signs of myocardial ischemia. Lab tests indicate the onset of myocardial necrosis and liberation of cardiomyocyte-specific intracellular proteins (i.e., CK-MB, troponin) following loss of cell membrane integrity. If possible, the blocked coronary artery is immediately reopened by emergency catheterization, balloon dilation, and stent placement. The extent of myocardial necrosis and thus the impairment in contractility largely depends on the time that has passed until the infarct vessel was reopened. There is no way to predict the ultimate infarct size in a given patient. Ideally, cardiomyocyte necrosis is completely prevented because the coronary artery has been quickly reopened. Analogous to the chronically hibernating myocardium, acutely ischemic cardiomyocytes can still be vital but have temporarily lost much of their capacity for contractile work (myocardial stunning). Tissue infiltration with inflammatory cells is beginning, but fibrous scarring has not yet occurred. In this situation, direct injection of cell suspension into the weakened myocardium is prohibitive, but infusion of stem/progenitor cells into the reopened coronary artery is currently being evaluated. So far, mainly bone marrow mononuclear cell preparations have been used in such trials. A few days after the onset of myocardial infarction, a second cardiac catheterization is performed and the cell suspension is injected into the infarct vessel while blood is temporarily interrupted by balloon inflation. Pilot studies have demonstrated feasibility and safety of this approach, and controlled efficacy trials are currently on the way. In one of the first of those trials, there was a difference in LV ejection fraction of 6% at 6 months follow-up between 30 patients who received intracoronary cell injection and 30 patients who only had standard infarct treatment. On average, LVEF improved by 6.7% in cell-treated patients compared with immediately after myocardial infarction, whereas LVEF in control patients remained largely unchanged (64). Other clinical trials based on the same principle are currently being undertaken, but it is too early to make a definitive judgment about long-term functional efficacy and possible side effects.

Stem Cell Mobilization

Another, fundamentally different, approach aims at circumventing any invasive procedure for cell delivery while minimizing the interval between the onset of myocardial infarction and cell therapy by mobilizing marrow cells using cytokines such as granulocyte stimulating factor (G-CSF). The idea is that stem/progenitor cells mobilized from marrow will be attracted to the ischemic heart and initiate regeneration events or at least modulate remodeling processes. The physiologic equivalent of such reasoning may be the mobilization of EPC in

patients with acute myocardial infarction or various degrees of congestive heart failure (16,52,61). Because SC mobilization did not result in a favorable outcome in a nonhuman primate model of myocardial infarction, this practicable pharmacological approach has been viewed with some restraint (37). Despite safety of G-CSF mobilization with or without consecutive apheresis and favorable short-term results of intracoronary infusion of G-CSF MPB in patients with myocardial infarction after coronary stenting, an unexpectedly high rate of in-stent restenosis has been observed in one trial with G-CSF (19). Moreover, restenosis has at least been mentioned in another (TACT) trial for adult SC therapy for peripheral occlusive artery disease with unfractionated BM (56). In a third study in 12 patients with intractable angina the administration of G-CSF was associated with two acute myocardial infarctions and one cardiac death (17). Nevertheless, several other clinical pilot studies are currently on the way.

SUMMARY

The fundamental mechanisms of myocardial tissue repair based on adult progenitor or stem cells remain unclear. Despite the surge in cell therapy-related publications, the “facts” that are largely undisputed are few. (i) Contractile cells or their immediate progenitors, obtained from whatever source, can be transferred into ischemic myocardium, and some of them survive. In many experimental models, this cell transfer indeed leads to an improvement of left ventricular function. (ii) Cell types belonging to the hematopoietic–angiogenic complex, obtained from any source, can enhance the vascularization of ischemic tissue, including infarcted myocardium. Again, in many experimental models, this is associated with some improvement of heart function. Having accepted the limitations of our current knowledge, it seems appropriate to state that adult stem/progenitor cell therapy for myocardial tissue repair holds exceedingly great promise. We are, however, just at the beginning of the road.

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REFERENCES

- Asahara, T.; Murohara, T.; Sullivan, A.; Silver, M.; van der Zee, R.; Li, T.; et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 275:964–967; 1997.
- Assmus, B.; Schächinger, V.; Teupe, C.; Britten, M.; Lehmann, R.; Döbert, N.; et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction-(TOPCARE-AMI). *Circulation* 106:3009–3017; 2002.
- Balsam, L. B.; Wagers, A. J.; Christensen, J. L.; Kofidis, T.; Weissman, I. L.; Robbins, R. C. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. *Nature* 428:668–673; 2004.
- Beltrami, A. P.; Barlucchi, L.; Torella, D.; Baker, M.; Limana, F.; Chimenti, S.; Kasahara, H.; Rota, M.; Musso, E.; Urbanek, K.; Leri, A.; Kajstura, J.; Nadal-Ginard, B.; Anversa, P. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell* 114:763–776; 2003.
- Bittira, B.; Kuang, J. Q.; Al-Khaldi, A.; Shum-Tim, D.; Chiu, R. C. J. In vitro preprogramming of marrow stromal cells for myocardial regeneration. *Ann. Thorac. Surg.* 74: 1154–1160; 2002.
- Camargo, F. D.; Chambers, S. M.; Goodell, M. A. Stem cell plasticity: From transdifferentiation to macrophage fusion. *Cell Prolif.* 37:55–65; 2004.
- Chedrawy, E. G.; Wang, J. S.; Nguyen, D. M.; Shm-Tim, D.; Chiu, R. C. J. Incorporation and integration of implanted myogenic and stem cells into native myocardial fibers: Anatomic basis for functional improvements. *J. Thorac. Cardiovasc. Surg.* 124:584–590; 2002.
- Dawn, B.; Stein, A. B.; Urbanek, K.; Rota, M.; Whang, B.; Rastaldo, R.; Torella, D.; Tang, X. L.; Rezazadeh, A.; Kajstura, J.; Leri, A.; Hunt, G.; Varma, J.; Prabhu, S. D.; Anversa, P.; Bolli, R. Cardiac stem cells delivered intravascularly traverse the vessel barrier, regenerate infarcted myocardium, and improve cardiac function. *Proc. Natl. Acad. Sci. USA* 102:3766–3771; 2005.
- Forrester, J. S.; Price, M. J.; Makkar, R. R. Stem cell repair of infarcted myocardium—an overview for clinicians. *Circulation* 108:1139–1145; 2003.
- Fuchs, S.; Satler, L. F.; Kornowski, R.; Okubagzi, P.; Weisz, G.; Baffour, R.; et al. Catheter-based autologous bone marrow myocardial injection in no-option patients with advanced coronary artery disease—a feasibility study. *J. Am. Coll. Cardiol.* 41:1721–1724; 2003.
- Fuchs, S.; Kornowski, R. K.; Weisz, G.; Satler, L. F.; Smits, P. C.; Okubagzi, R.; et al. Transendocardial autologous bone marrow cell transplantation in patients with advanced ischemic heart disease: Final results from a multicenter feasibility study. *J. Am. Coll. Cardiol.* 43:A99; 2004.
- Goodell, M. A. Stem-cell “plasticity”: Befuddled by the muddle. *Curr. Opin. Hematol.* 10:208–213; 2003.
- Hamano, K.; Li, T. S.; Kobayashi, T.; Hirata, K.; Yano, M.; Kohno, M.; Matsuzaki, M. Therapeutic angiogenesis induced by local autologous bone marrow cell implantation. *Ann. Thorac. Surg.* 73(4):1210–1215; 2002.
- Hamano, K.; Nishida, M.; Hirata, K.; et al. Local implantation of autologous bone marrow cells for therapeutic angiogenesis in patients with ischemic heart disease: Clinical trial and preliminary results. *Jpn. Circ. J.* 65:845–847; 2001.
- Herreros, J.; Hagege, A. A.; Vilquin, J. T.; Desnos, M.; Abergel, E.; Pouzet, B.; et al. Autologous intramyocardial injection of cultured skeletal muscle-derived stem cells in patients with non-acute myocardial infarction. *Eur. Heart J.* 24:2012–2020; 2003.
- Hill, J. M.; Zalos, G.; Halcox, J. P. J.; Schenke, W. H.; Waclawiw, M. A.; Quyyumi, A. A.; Finkel, T. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N. Engl. J. Med.* 348:593–600; 2003.
- Hill, J. M.; Paul, J. D.; Powell, T. M.; McCoy, P.; Dunbar, C. E.; Horne, M.; Csako, G.; Cannon, R. O. Efficacy and risk of granulocyte colony stimulating factor administra-

- tion in patients with severe coronary artery disease. *Circulation* 108:478; 2003.
18. Honold, J.; Assmus, B.; Lehman, R.; Zeiher, A. M.; Dimmeler, S. Stem cell therapy of cardiac disease: An update. *Nephrol. Dial. Transplant.* 19:1673–1677; 2004.
 19. Kang, H.-J.; Kim, H.-S.; Zhang, S.-Y.; Park, K.-W.; Cho, H.-S.; Koo, B.-K.; et al. Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: The MAGIC cell randomised clinical trial. *Lancet* 363:751–756; 2004.
 20. Kawada, H.; Fujita, J.; Kinjo, K.; Matsuzaki, Y.; Tsuma, M.; Miyatake, H.; et al. Nonhematopoietic mesenchymal stem cells can be mobilized and differentiate into cardiomyocytes after myocardial infarction. *Blood* 104:3581–3587; 2004.
 21. Kocher, A. A.; Schuster, M. D.; Szabo, M. J.; Takuma, S.; Barkhoff, D.; Wang, J.; et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat. Med.* 7: 430–436; 2001.
 22. Kogler, G.; Sensken, S.; Airey, J. A.; Trapp, T.; Müschen, M.; Feldhahn, N.; et al. A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential. *J. Exp. Med.* 200:123–135; 2004.
 23. Kondo, M.; Wagers, A. J.; Manx, M. G.; Prohaska, S. S.; Scherer, D. C.; Beihack, G. F.; Shizuru, J. A.; Weissman, I. L. Biology of hematopoietic stem cells and progenitors: Implications for clinical application. *Annu. Rev. Immunol.* 21:759–806; 2003.
 24. Korbly, M.; Estrov, Z. Adult stem cells for tissue repair—a new therapeutic concept? *N. Engl. J. Med.* 349: 570–582; 2003.
 25. Li, R. K.; Jai, Z. Q.; Weisel, R. D.; Mickle, D. A.; Zhang, J.; Mohabeer, M. K.; Rao, V.; Ivanov, J. Cardiomyocyte transplantation improves heart function. *Ann. Thorac. Surg.* 62:654–661; 1996.
 26. Li, R. K.; Jai, Z. Q.; Weisel, R. D.; Merante, F.; Mickle, D. A. Smooth muscle cell transplantation into myocardial scar tissue improves heart function. *J. Mol. Cell. Cardiol.* 31:513–522; 1999.
 27. Lin, Y.; Weisdorf, D.; Solovey, A.; Heibel, R. P. Origins of circulating endothelial cells and endothelial outgrowth from blood. *J. Clin. Invest.* 105:71–77; 2000.
 28. Makino, S.; Fukuda, K.; Miyoshi, S.; Konishi, F.; Kodama, H.; Pan, J.; et al. Cardiomyocytes can be generated from marrow stromal cells in vitro. *J. Clin. Invest.* 103:697–705; 1999.
 29. Masuda, H.; Asahara, T. Post-natal endothelial progenitor cells for neovascularization in tissue regeneration. *Cardiovasc. Res.* 58:390–398; 2003.
 30. Menasche, P. Cellular transplantation: hurdles remaining before widespread clinical use. *Curr. Opin. Cardiol.* 19: 154–161; 2004.
 31. Menasche, P.; Hagege, A. A.; Scorsin, M.; Pouzet, B.; Desnos, M.; Dubac, D.; et al. Myoblast transplantation for heart failure. *Lancet* 357:279–280; 2001.
 32. Menasche, P.; Hagege, A. A.; Vilquin, J. T.; Desnos, M.; Abergel, E.; Pouzet, B.; et al. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. *J. Am. Coll. Cardiol.* 41:1078–1083; 2003.
 33. Muguruma, Y.; Reyes, M.; Nakamura, Y.; Sato, T.; Matsuzawa, H.; et al. In vivo and in vitro differentiation of myocytes from human bone marrow-derived multipotent progenitor cell. *Exp. Hematol.* 31:1323–1330; 2003.
 34. Murry, C. E.; Soonpaa, M. H.; Reinecke, H.; Nakajima, H. O.; Rubart, M.; Pasumarthi, K. B.; et al. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature* 428:664–668; 2004.
 35. Orlic, D.; Kajstura, J.; Chimenti, S.; Jakoniuk, I.; Anderson, S. M.; Li, B.; et al. Bone marrow cells regenerate infarcted myocardium. *Nature* 401:701–705; 2001.
 36. Orlic, D.; Kastura, J.; Chimenti, S.; Limana, F.; Jakoniuk, I.; Quainti, F.; et al. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc. Natl. Acad. Sci. USA* 98:10344–10349; 2001.
 37. Orlic, D.; Arai, A. E.; Sheikh, F. H.; et al. Cytokine mobilized CD34+ cells do not benefit rhesus monkeys following induced myocardial infarction. *Blood* 100:A28–A29; 2002.
 38. Oswald, J.; Boxberger, S.; Jørgensen, B.; Feldmann, S.; Ehringer, G.; Bornhäuser, M.; Werner, C. Mesenchymal stem cells can be differentiated into endothelial cells in vitro. *Stem Cells* 22:377–384; 2004.
 39. Peichev, M.; Naiyer, A. J.; Pereira, D.; Zhu, Z.; Lane, W. J.; Williams, M.; et al. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. *Blood* 95:952–958; 2000.
 40. Pelosi, E.; Valtieri, M.; Coppola, S.; Botta, R.; Gabbianelli, M.; Lulli, V.; et al. Identification of the hemangioblast in postnatal life. *Blood* 100:3203–3208; 2002.
 41. Perin, E. C.; Geng, Y. J.; Willerson, J. T. Adult stem cell therapy in perspective. *Circulation* 107:935–938; 2003.
 42. Perin, E. C.; Dohmann, H. F.; Borojevic, R.; Silva, S. A.; Sousa, A. L.; Mesquita, C. T.; et al. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. *Circulation* 107:2294–2302; 2003.
 43. Pittenger, M. F.; Mackay, A. M.; Beck, S. C.; Jaiswal, R. K.; Douglas, R.; Mosca, J. D.; et al. Multilineage potential of adult human mesenchymal stem cells. *Science* 284:143–147; 1999.
 44. Pittenger, M. F.; Martin, B. J. Mesenchymal stem cells and their potential as cardiac therapeutics. *Circ. Res.* 95: 9–20; 2004.
 45. Rafii, S.; Lyden, D. Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. *Nat. Med.* 9:702–712; 2003.
 46. Reinecke, H.; MacDonald, G. H.; Hauschka, S. D.; Murry, C. E. Electromechanical coupling between skeletal and cardiac muscle: Implications for infarct repair. *J. Cell Biol.* 149:731–740; 2000.
 47. Reyes, M.; Lund, T.; Lenvik, T.; Aguiar, D.; Koodie, L.; Verfaillie, C. M. Purification and ex vivo expansion of postnatal human marrow mesodermal progenitor cells. *Blood* 98:2615–2625; 2001.
 48. Reyes, M.; Dudek, A.; Jahagirdar, B.; Koodie, L.; Marker, P. H.; Verfaillie, C. M. Origin of endothelial progenitors in human postnatal bone marrow. *J. Clin. Invest.* 109: 337–346; 2002.
 49. Rubart, M.; Soonpaa, M. H.; Nakajima, H.; Field, L. J. Spontaneous and evoked intracellular calcium transients in donor-derived myocytes following intracardiac myoblast transplantation. *J. Clin. Invest.* 114:775–783; 2004.

50. Ruhparwar, A.; Tebbenjohanns, J.; Niehaus, M.; Mengel, M.; Irtel, T.; Kofidis, T.; Pichlmaier, A. M.; Haverich, A. Transplanted fetal cardiomyocytes as cardiac pacemaker. *Eur. J. Cardiothorac. Surg.* 21:853–857; 2003.
51. Schmeisser, A.; Strasser, R. H. Phenotypic overlap between hematopoietic cells with suggested angioblastic potential and vascular endothelial cells. *J. Hematother. Stem Cell Res.* 11:69–79; 2002.
52. Shintani, S.; Murohara, T.; Ikeda, H.; Ueno, T.; Honma, T.; Katoh, A.; et al. Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. *Circulation* 103:2776–2779; 2001.
53. Solovey, A.; Lin, Y.; Browne, P.; Choong, S.; Wayner, E.; Heibel, R. P. Circulating activated endothelial cells in sickle cell anemia. *N. Engl. J. Med.* 337:1584–1590; 1997.
54. Stamm, C.; Westphal, B.; Kleine, H. D.; Petzsch, M.; Kitzner, C.; Klinge, H.; et al. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. *Lancet* 361:45–46; 2003.
55. Strauer, B. E.; Brehm, M.; Zeus, T.; Köstering, M.; Hernandez, A.; Sorg, R. V.; et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation* 106:1913–1918; 2002.
56. Tateishi-Yuyama, E.; Matsubara, H.; Murohara, T.; Ikeda, U.; Shintani, S.; Masaki, H.; et al. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: A pilot study and a randomised controlled trial. *Lancet* 360:427–435; 2002.
57. Toma, C.; Pittenger, M. F.; Cahill, K. S.; et al. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation* 105:93–98; 2002.
58. Tse, H. F.; Kwong, Y. L.; Chan, J. K.; Lo, G.; Ho, C. L.; Lau, C. P. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. *Lancet* 361:47–49; 2003.
59. Urbanek, K.; Quaini, F.; Tasca, G.; Torella, D.; Castaldo, C.; Nadal-Ginard, B.; Leri, A.; Kajstura, J.; Quaini, E.; Anversa, P. Intense myocyte formation from cardiac stem cells in human cardiac hypertrophy. *Proc. Natl. Acad. Sci. USA* 100:10440–10445; 2003.
60. Urbich, C.; Dimmeler, S. Endothelial progenitor cells: Characterization and role in vascular biology. *Circ. Res.* 95:343–353; 2004.
61. Valgimigli, M.; Rigolin, G. M.; Fucili, A.; Della Porta, M.; Soukhornovskaia, O.; Malagutti, P.; et al. CD34+ and endothelial progenitor cells in patients with various degrees of congestive heart failure. *Circulation* 110:1209–1212; 2004.
62. Wakitani, S.; Saito, T.; Caplan, A. I. Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. *Muscle Nerve* 18(12):1417–1426; 1995.
63. Wang, J. S.; Shum-Tim, D.; Galipeau, J.; Chedrawy, E.; Eliopoulos, N.; Chiu, R. C. J. Marrow stromal cells for cellular cardiomyoplasty: Feasibility and potential advantages. *J. Thorac. Cardiovasc. Surg.* 120:999–1006; 2000.
64. Wollert, K. C.; Meyer, G. P.; Lutz, J.; et al. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: The BOOST randomised controlled clinical trial. *Lancet* 364:141–148; 2004.