EDITORIAL COMMENT

Wanted! The Best Cell for Cardiac Regeneration*

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Despite the development of novel pharmacological strategies and electrophysiological and surgical treatment regimens, heart failure remains one of the major causes of mortality in the western world. Heart failure can develop as a consequence of myocardial infarction despite revascularization procedures. Ischemia-induced death of cardiac myocytes results in scar formation and reduced contractility of the ventricle. Cell therapy for the regeneration of contractile tissue or the improvement of vascularization was proposed as a novel therapeutic option. Several distinct cell types were used in experimental and first clinical pilot trials. The crucial question currently is: which cells are best suited for cardiac regeneration and vascularization? Obviously, the answer to this question is complex. Practical and safety issues as well as the different types of patients we have to treat have a major impact. Moreover, the different routes of cell delivery, either by intravenous/intracoronary infusion or intramyocardial injection, can yield different outcomes.

See page 458

At first, cell therapy trials were initiated using skeletal myoblasts, which were isolated from muscle biopsies. Skeletal myoblasts were injected into the myocardium during open-heart surgery or by injection catheter. Although skeletal muscle cells improved heart function in animal models and in a pilot trial (1), the enthusiasm was dampened by the occurrence of life-threatening arrhythmias, which were most likely caused by the lack of connexin expression and the failure of skeletal muscle cells to couple to neighboring cardiac myocytes (2).

Stem cells are a second option to be used for cardiac regeneration. Although embryonic stem cells are by nature more versatile than their adult counterparts, increasing evidence suggests that stem and progenitor cells of the adult organisms also have a capacity to give rise to several lineages and may be suitable for regenerative medicine (3). The best-established source is the bone marrow. Bone marrow contains the hematopoietic stem cells, which have been used for stem cell transplantation for more than 25 years in hematolgy. These cells are characterized by the human marker proteins CD34 or the more immature marker protein CD133. Mesenchymal stem cells also can be isolated from bone marrow. The group of Verfaillie succeeded in isolating multipotent adult progenitor cells, which differentiate, at the single cell level, not only into mesenchymal cells but also into cells with visceral mesoderm, neuroectoderm, and endoderm characteristics in vitro (4). Bone marrow-derived cells (hematopoietic stem cells, endothelial progenitor cells, mesenchymal stem cells, or side-population cells) were shown to express endothelial and/or cardiomyogenic markers (5) and to profoundly increase functional recovery after myocardial infarction (6–8).

In this issue of the Journal, Agbulut et al. (9) report on a side-by-side comparison of two different cell types: purified CD133+ hematopoietic progenitor cells and skeletal myoblasts. Both cell types were injected into the infarcted myocardium 10 days after ligation of the left coronary artery of immunodeficient rats and elicited similar improvement in global left ventricular function as assessed by echocardiography and left ventricular pressure-volume loops one month after transplantation. Despite the significant functional improvement induced by both cell types, the authors failed to detect engrafted CD133+ hematopoietic progenitor cells by immunohistochemistry, whereas skeletal myoblasts showed robust engraftment. These findings appear to be consistent with recent reports questioning the capacity of purified hematopoietic progenitor cells to differentiate into cardiac myocytes when directly injected into the infarcted myocardium after permanent coronary occlusion (10,11), whereas unselected expanded bone marrow cells robustly engrafted in a rabbit model of myocardial cryoinjury (12). The question, however, remains how the injected CD133+ cells mediate improved left ventricular function despite the obvious lack of robust engraftment into the infarcted area. Surprisingly, Agbulut et al. (9) did not observe any differences in capillary density in the border zone of the infarcts in culture medium-injected control animals compared with either CD133+ cells or skeletal myoblast-injected animals, suggesting that enhanced angiogenesis does not contribute to improved left ventricular function after the injection of CD133+ cells or skeletal myoblasts. These findings contrast with previous reports documenting a significant increase in capillary density after intravenous infusion of CD34+ bone marrow-derived hematopoietic cells (13) or both intravenous infusion or injection of blood-derived progenitor cells (7,14) immediately after experimentally induced myocardial infarction. This discrepancy might be explained by the different cell types studied or by the timing of cell transplantation after induction of myocardial infarction: whereas the researchers in those studies documenting increased capillary density after the transplantation of progenitor cells applied cell therapy immediately after the induction of ischemia, in the present study, the injection of cells into the infarcted area was...
performed 10 days after coronary artery ligation. Thus, it seems likely that 10 days after permanent occlusion of a coronary artery, the microenvironment in the infarcted and in the border zone does not favor cell transplantation-induced neovascularization.

The failure to detect increased neovascularization in the present study leaves two potential mechanisms to explain the beneficial effects of cell transplantation on left ventricular function: interference with scar formation by altering extracellular matrix formation within the healing myocardial infarction and/or the recruitment of cardiac resident stem cells by the release of progenitor cell-derived mediators. However, none of these mechanisms has yet been formally tested to occur after progenitor cell transplantation. The rescue of reversibly damaged host cardiomyocytes appears to be rather unlikely given that cardiac myocyte death is completed 10 days after permanent coronary artery occlusion. In support of a cytokine-mediated paracrine effect are previous studies showing that skeletal myoblasts, although robustly engrafted into infarcted myocardium, remain functionally isolated (2), do not electrically couple, and remain lineage-restricted, e.g., do not express cardiomyocyte-specific markers (9). Further studies will have to address whether, for cytokine-mediated paracrine effects to become operative, it is essential that transplanted cells robustly engraft or whether a transient retention of transplanted cells within the infarcted area is sufficient to mediate functional improvement, as suggested by the present study, where engrafted CD133 progenitor cells could not be detected one month after injection.

**CLINICAL IMPLICATIONS**

How do the reported observations translate into clinical practice? First of all, the present study attempted to mimic a subchronic state of myocardial infarction. As such, it does not readily apply to clinical attempts to use progenitor cell transplantation in patients with acute myocardial infarction. The two published studies reporting a beneficial effect of transplantation of bone marrow-derived or blood-derived progenitor cells used an intracoronary route of application of progenitor cells in patients with reperfused acute myocardial infarction (15,16). Importantly, ischemic but non-necrotic cardiac tissue up-regulates a variety of cardioprotective but also progenitor cell-attracting and survival-promoting chemokines and transcription factors such as vascular endothelial growth factor, stromal cell-derived factor-1, and hypoxia-inducible factor-1α (17). Thus, the local environment within the reperfused infarcts with islands of viable myocardium and release of cellular survival factors may greatly favor recruitment but also survival and potentially engraftment of progenitor cells transplanted via an intrarterial route into the infarct area. In contrast, it is difficult to envisage that progenitor cells that are directly injected into necrotic tissue with a completely destructed syncytium and deprived of blood flow-delivering oxygen and nutrients will receive any cues or signals to differentiate into cardiac myocytes or vascular cells. As such, it is also not surprising that recent studies in mice with permanent occlusion-induced myocardial infarction and direct intranecrosis-injected CD34+ hematopoietic progenitor cells did not observe any substantial numbers of engrafted or differentiated cells bearing markers of cardiac myocytes (11), but the injected cells rather adopted mature hematopoietic fates (10).

The present study, however, is more applicable to clinical attempts to improve heart function in semichronic myocardial infarction. Although scar formation is clearly not completed after 10 days of a non-reperfused myocardial infarction (18), skeletal myoblasts transplanted by intramyocardial injection appear to have a survival and engraftment advantage compared with CD133 progenitor cells during the healing phase of myocardial infarction. Nevertheless, the observed functional improvement supports recent preliminary clinical data showing improved regional perfusion and contractile function after intramyocardial injection of CD133 progenitor cells during bypass surgery in patients with 4- to 12-week-old myocardial infarction (19). However, it should be kept in mind that these patients simultaneously underwent bypass surgery that provided an oxygen supply and nutrient blood flow to the infarcted myocardial area that was transplanted with progenitor cells. Finally, the two other studies reporting beneficial effects on regional left ventricular function after intramyocardial injection of bone marrow-derived mononuclear cells selected patients with evidence of hibernating myocardium by the electromechanical mapping technique (20,21). Thus, despite the presence of chronic myocardial ischemia and infarction, the regions injected with progenitor cells contained viable myocardium and, thus, may have provided a more favorable microenvironment for injected cells compared with the nonreperfused 10-day-old infarctions treated in the present study.

In summary, although the authors should be congratulated for a thorough experimental head-to-head comparison of different cell types currently under clinical investigation for enhancing cardiac repair after myocardial infarction, careful interpretation of the results should be used when translating these experimental findings into clinical practice. Encouragingly, both skeletal myoblasts as well as CD133 hematopoetic progenitor cells appear to be associated with the improvement of left ventricular function after experimentally induced myocardial infarction. Nevertheless, the quest for the best (adult) cell for cardiac regeneration is still ongoing; if there is any regeneration in its pure sense at all or only modifications in the infarct healing process. As it is with many pioneering studies, careful examinations not only will provide conclusive answers but may leave even more open questions. Finally, it should not be dismissed that clinical practicability and, most importantly, safety will ultimately determine the potential benefit of novel thera-
peutic approaches aiming at regenerating infarcted myocar-
dium in patients with ischemic heart disease.

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REFERENCES

2. Leobon B, Garcin I, Menasche P, Vilquin JT, Audinat E, Charpak S.
Myoblasts transplanted into rat infarcted myocardium are functionally
isolated from their host. Proc Natl Acad Sci USA 2003;100:7808–11.
3. Hirschi KK, Goodell MA. Hematopoietic, vascular and cardiac fates
cardiac muscle and vascular endothelium by adult stem cells. J Clin
transplantation of autologous endothelial progenitor cells for thera-
8. Assmus B, Schachinger V, Teupe C, et al. Transplantation of
Progenitor Cells and Regeneration Enhancement in Acute Myocardial
by autologous intracoronary mononuclear bone marrow cell transplan-
10. Lee SH, Wolf PL, Escudero R, Deutsch R, Jamieson SW, Thistleth-
waite PA. Early expression of angiogenesis factors in acute myocardial
stem-cell transplantation for myocardial regeneration. Lancet 2003;
361:45–6.
13. Perin EC, Dohmann HF, Borovevic R, et al. Transendocardial,
autologous bone marrow cell transplantation for severe, chronic isch-
in ischemic myocardium by intramyocardial autologous bone marrow