Mechanisms of Cell Therapy for Clinical Investigations
An Urgent Need for Large-Animal Models

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Evidence from experiments performed in animal models and from early-stage clinical trials demonstrates that the contractile performance of hearts with severe ischemic injury can be improved by transplanting a variety of cell types into the injured heart. As a result of these investigations, progenitor cells have emerged as a promising therapeutic agent not only for limiting postinfarction left ventricular (LV) remodeling, but also for improving cardiac performance in hearts with severe LV dysfunction secondary to other diseases such as dilated cardiomyopathy and concentric LV hypertrophy. Some of the most encouraging clinical results were obtained in the Cardiac Stem Cells in Patients With Ischaemic Cardiomyopathy (SCIPIO) trial, which investigated the use of autologous cardiac progenitor cells (CPCs) in patients with severe LV dysfunction undergoing coronary artery bypass graft surgery. Although the small patient population and open-label design preclude definitive mechanistic conclusions, CPC infusion was associated with significant improvements in LV ejection fraction from before treatment to 4 months afterward, and even greater improvement was observed at 12 months. These and other promising results could soon lead to the initiation of more definitive, large-scale, phase 2 clinical trials.

Novel therapies have often been adopted into clinical practice before their mechanisms of action have been completely deciphered, provided that their safety and beneficial effects are reproducible. For the emerging cellular therapy, however, the optimal cell type, dose, timing, and route of administration need to be identified to ensure that cell therapies produce the best possible benefit for each patient while avoiding overtreatment. Many of these variables are likely to depend both on the specific disease state and on complex interactions among numerous mechanisms that function seamlessly in an in vivo system. The availability of clinically relevant large-animal models of LV dysfunction is critical for identifying, characterizing, and quantifying responses to cell therapy.

This issue of Circulation includes a report by Bolli et al that is among the first investigations of cell therapy to be performed in a large-animal model of ischemic cardiomyopathy with a mature LV scar that mimics the ongoing clinical trial. The authors induced infarction in the hearts of swine by ligating a coronary artery for 90 minutes followed by reperfusion, and then they allowed the animals to recover for 3 months before infusing the reopened infarct-related coronary artery with autologous CPCs that expressed c-Kit and had been expanded from samples of atrial tissue collected at the time of infarction. The treatment led to significant improvements in LV chamber and regional contractile function that were accompanied by improvements in myocardial structure and evidence of cardiomyocyte and vascular cell regeneration. Importantly, both the treatment administered and the experimental results closely mimic those reported for the SCIPIO trial, which suggests that this animal model can provide a useful platform for addressing many unmet needs and unanswered mechanistic questions in the field of cardiovascular cell therapy.

In analogy with novel pharmacological treatments, cell therapy has been adopted clinically before the recognition and characterization of their mechanisms of action have been proven. The current work by Bolli et al points to growth and differentiation of the administered CPCs as the prevailing modality of recovery of the infarcted heart. The accumulated results provide critical insights into the fundamental issues currently faced by clinical cell therapy. The major questions include the implementation of the most effective cell type and its dose, timing of delivery, and route of administration. These variables depend on the disease state, the functional competence of the progenitor cell category, and the complex interactions between the cells and the microenvironment of the injured myocardium. Some of these problems are briefly discussed below.

Myocardial Regeneration Secondary to Progenitor Cell Therapy

The impressive results achieved in recent clinical trials and the preclinical trials likely involve a seamless interplay of numerous processes that can be loosely grouped into 2 categories: myocardial protection and myocardial regeneration (Figure). In addition to myocardial regeneration from the differentiation of the engrafted CPCs, protective effects of cell therapy are induced through the release of trophic factors such as vascular endothelial growth factor, insulin-like growth factor, and fibroblast growth factor, which subsequently reduce apoptosis and adverse cardiac remodeling by promoting neovascularization and improved perfusion. Because the experiments presented by Bolli et al were performed in animals with mature infarcts, the observed response to treatment is most likely attributable to regenerative rather than protective mechanisms. The study also provides convincing evidence that the administered CPCs differentiated into functional cardiac cells but...
The Bolli et al study found small regions of viable myocardial tissue by relieving border zone subendocardial ischemia. However, whether these observations in humans that challenged the dogma that the adult heart is a postmitotic organ was by Beltrami et al, who carefully examined the histology of the border zone myocardium in patients dying of acute myocardial infarction. After these original reports, the existence of myocyte turnover in the normal adult heart has been demonstrated by numerous studies such as the recent one by Bergmann et al using carbon-14 pulse-chase analyses that was made possible by above-ground nuclear testing in the 1950s and 1960s. Myocyte turnover in adult mammalian hearts can occur through the proliferation of preexisting cardiomyocytes or the activity of endogenous CPCs. The relative importance of these 2 mechanisms and the myocyte turnover rate in the adult heart remains a matter of intense debate. Once the signaling pathways of specific CPC populations are deciphered, the methods used for cell delivery can be refined to promote cardiomyocyte turnover, leading to more comprehensive activation of mechanisms for cardiac repair.

Myocyte Turnover

The concept of cardiomyocyte turnover in the adult heart is relatively new. The first significant report from direct observations in humans that challenged the dogma that the adult heart is a postmitotic organ was by Beltrami et al, who carefully examined the histology of the border zone myocardium in patients dying of acute myocardial infarction. After these original reports, the existence of myocyte turnover in the normal adult heart has been demonstrated by numerous studies such as the recent one by Bergmann et al using carbon-14 pulse-chase analyses that was made possible by above-ground nuclear testing in the 1950s and 1960s. Myocyte turnover in adult mammalian hearts can occur through the proliferation of preexisting cardiomyocytes or the activity of endogenous CPCs. The relative importance of these 2 mechanisms and the myocyte turnover rate in the adult heart remains a matter of intense debate. Once the signaling pathways of specific CPC populations are deciphered, the methods used for cell delivery can be refined to promote cardiomyocyte turnover, leading to more comprehensive activation of mechanisms for cardiac repair.

Myocardial Protection

The severity of LV dysfunction and remodeling after myocardial infarction is linearly related to the size of the initial infarct. Thus, one of the primary goals for the treatment of postinfarction LV remodeling is to protect the peri-infarct border zone myocardium and to reduce the infarct size. Many reports indicate that apoptosis declines when cell transplantation is performed shortly after an infarct event, and cell transplantation is frequently associated with improved myocardial perfusion and increased border zone myocardial resistance vessel density, which may preserve functional myocardial tissue by relieving border zone subendocardial ischemia. The Bolli et al study found small regions of viable myocardial tissue within the scar that appeared to be derived from the transplanted cells. However, whether these observations were accompanied by significant declines in infarct size was not determined. At present, a fair evaluation of the reduction in LV infarct size in response to a therapy remains controversial. The existing technology for quantification of the extent to which damaged myocytes cannot recover from injury remains controversial. The gold standard for evaluating cardiomyocyte irreversible damage is triphenyltetrazolium staining, which monitors the leakage of dehydrogenase enzymes and cofactors from myocytes, but the technique is performed on explanted hearts and consequently cannot be used to determine the proportion of damaged cells that may recover or are destined to die. Thus, cardiovascular science will benefit from new technologies that can noninvasively track the fate of injured cardiomyocytes, particularly at the border zone, where apoptosis and programmed necrosis are active. Measurements of infarct size reduction can be confounded by the infarct shrinkage and LV dilation that occur during acute-phase postinfarction LV remodeling. Currently, the appropriate quantification of infarct size is obtained by cardiac magnetic resonance imaging and the assessment of the ratio of LV scar surface area to the LV surface area.

Only a small fraction of transplanted cells is retained in the infarcted myocardium, and the survival of engrafted cells is poor; however, cell engraftment and survival can be improved by incorporating the cells into a cardiac patch before administration. One week after transplantation, the engraftment rate was 4-fold greater when the cells were administered in a fibrin patch than when they were injected directly into the injured myocardium. In a swine myocardial infarction model, patches containing human embryonic stem cell–derived vascular cells led to reduced cardiomyocyte apoptosis and to improvements in the recruitment and activation of endogenous CPCs and in border-zone perfusion and contractile function. Similar benefits have been observed after treatment with patches containing vascular cells derived from human induced pluripotent stem cells. The patches can also be modified to contain cytokines that promote the activity of the transplanted cells or induce cytoprotective and regenerative mechanisms in resident cardiac cells. However, clinical application of this patch-based approach requires the development of a practical and minimally invasive delivery method.

In conclusion, the preclinical study reported by Bolli et al demonstrates that the benefits associated with intracoronary
CPC infusion in patients with severe LV dysfunction can be successfully reproduced in swine and consequently that this model may be an appropriate platform for the development of new analytic techniques for comparing the effectiveness of different cell types, doses, and delivery methods and for mechanistic experimental investigations. Their report is also among the first large-animal studies to investigate the response to cell therapy administered late after the initial infarct event when the injured tissue has been replaced by a thin fibrotic scar. Thus, the findings are particularly important for guiding the clinical trials needed to develop and optimize the emerging cellular therapy for cardiac repair.

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