Calling on Reserves
Granulocyte Colony Stimulating Growth Factor in Cardiac Repair

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Tissue injury elicits a regenerative response designed to restore its function through replacement of damaged cells. Such repair is thought to involve lineage-specific, tissue-specific progenitor cells that have been identified in most tissues, including the heart. The effectiveness of this repair ranges from minimal to complete, depending, among other factors, on the involved organ and the age of the individual in question, with certain organs such as the brain or the heart thought to have very low repair potential. Furthermore, little role has been ascribed to bone marrow–derived progenitors in adult tissue repair. Recent studies, however, have challenged both of these paradigms. The notion that the heart is a terminally differentiated organ without the capability of self-renewal has been challenged by the discovery of cardiac-specific progenitor cells residing in the myocardium. However, it is unclear how much functional effect such repair processes have and whether they can be effectively stimulated with pharmacological or biological therapies.

The beneficial effects observed after transplantation of various cell types into the myocardium in animal models of acute myocardial infarction (AMI) have led to the initiation of clinical studies even though the mechanism of these effects remains uncertain. The cell types tested in the setting of acute myocardial ischemia have ranged from unfractionated to different fractions of bone marrow–derived or peripheral blood mononuclear cells delivered by percutaneous intramyocardial or intracoronary injections. Essentially all studies have suggested some biological effectiveness, although in all cases, this conclusion is tempered by the uncontrolled nature and the small size of the trials. Nevertheless, the suggested benefits of cell therapy in AMI settings led Ince et al to perform the study reported in the current issue of Circulation.

Instead of carrying out injections of one or another cell population, the authors hypothesized that mobilization of bone marrow–derived multipotent cells with a granulocyte colony stimulating factor (G-CSF) may lead to improvement in cardiac perfusion and function. Fifty patients with an AMI and single-vessel coronary artery disease treated with primary angioplasty and stent implantation in the infarct-related artery were randomized to twice-daily injections of G-CSF at a dose of 5 μg/kg during the first 6 days after myocardial infarction (MI) or no additional treatment in addition to standard post-MI therapy. The 2 treatment groups were comparable with regard to various clinical parameters, including infarct size and baseline left ventricular function.

As expected, G-CSF treatment was associated with a significant leukocytosis, with the white blood cell count (WBC) reaching $56.1 \times 10^9 \pm 15.9 \times 10^9/\mu L$ in the treatment group 6 days after MI. The number of CD34+ mononuclear cells, the cell fraction thought to be potentially involved in myocardial repair, was increased $\approx 20$-fold in the treatment group. Such elevation of WBC is normally considered a bad prognostic indicator in AMI patients, with the extent of elevation correlating with increased post-MI mortality. Remarkably, despite a very significant WBC and CD34+ count rise in G-CSF–treated patients, C-reactive protein levels or the levels of various cytokines in the blood were no higher in the treatment group than in the controls.

Functional assessment at 35 days and 4 months after MI demonstrated improved left ventricular wall thickening and wall motion in the infarction zone both at rest and under dobutamine stress. Importantly, $^{18}$F-deoxyglucose positron emission tomography, although showing comparable uptake in the infarct and noninfarct zone at baseline, demonstrated a significantly greater uptake in the infarct territory 4 months later in the treatment group, which suggests a greater recovery of the territory at risk. Finally, coronary angiography 6 months after G-CSF treatment showed comparable in-stent restenosis rates between the groups.

This well-conducted, randomized trial of G-CSF therapy in the setting of AMI provides important novel insights into the potential role of cell therapy while raising new and interesting questions about our assumptions regarding the pathophysiology of AMI injury and the role of bone marrow in the recovery process. Although multiple functional end points examined in this trial are suggestive of the functional benefit of G-CSF treatment, they are by no means definitive. For example, most of the benefit of the treatment is attributable as much to an increase in various functional measures in the G-CSF group as to a decrease in these measures in the control group. Such deterioration in the ejection fraction and wall motion score is not normally observed in patients undergoing primary angioplasty within the time frame of AMI onset examined in this trial. To the contrary, an increase in these parameters is normally expected. This anomaly, plus the
small size of the trial, makes it difficult to draw definitive conclusions regarding the functional impact of G-CSF therapy.

Another caveat to be considered here is the lack of placebo treatment in the control group. In one recent placebo-controlled study of G-CSF–mediated mobilization of progenitor cells after MI, there was no difference in recovery between the treatment group and the control group. It is also noteworthy that G-CSF therapy was not associated with any increase in complications. In particular, there was no increase in serum levels of 2 of the most prominent inflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), in response to G-CSF. In contrast, an earlier trial using granulocyte macrophage colony stimulating factor (GM-CSF) showed elevated intracoronary TNF-α levels after treatment with this cytokine. It is possible, therefore, that increased cardiac levels were missed in the current study because no plasma samples were taken from the coronary arteries. Equally important is the demonstration of no significant increase in the in-stent restenosis rate after G-CSF treatment, given a recent report of a dramatic increase in a small study of G-CSF therapy in the setting of intracoronary bone marrow cell injection.

Assuming that some benefits of G-CSF therapy, as suggested in this study, will stand the test of larger randomized, placebo-controlled trials, what are the potential mechanisms that account for this? G-CSF is a hematopoietic cytokine produced by monocytes, fibroblasts, and endothelial cells that plays a role in neutrophil production and acts at different stages of myeloid cell development. The current approach to mobilizing multipotent bone marrow cells with G-CSF originated in the hematological practice, where the cytokine is used to collect granulocytes from donors for blood banking and allograft therapy. Typically, the G-CSF–mobilized cells are characterized by the presence of CD34 cell surface marker, which is thought to identify cells that possess a certain degree of differentiation plasticity. In the setting of AMI, various populations of CD34+ cells that express early cardiac, endothelial, and muscle markers are mobilized into the peripheral blood, potentially in response to endogenous G-CSF, which has been shown to be significantly elevated in AMI patients.

However, the role played by CD34+ cells in tissue repair/regeneration in general and in myocardial infarction in particular is highly controversial. Initial reports suggesting transdifferentiation of bone marrow cells into specific terminally differentiated organ cells, such as cardiac myocytes or brain neurons, have been followed by reports of cell fusion and by claims of no meaningful cell-cell transformation taking place. On balance, it seems unlikely, given the present state of evidence, that either bone marrow cell transdifferentiation into cardiac myocytes, given its very low frequency, or cell fusion can explain the striking functional benefits reported in animal studies or in the current trial. The study by Ince et al did not provide evidence for incorporation of circulating CD34+ cells into the myocardium, and the authors have not attempted to isolate and label a fraction of the CD34+ cells to follow their fate. Recently, this approach has been used to demonstrate that CD34+ cells obtained after bone marrow aspiration in post-MI patients do not localize to the myocardium after an intravenous injection but that some myocardial retention (1.3% to 2.6%) is observed after an intracoronary injection. Interestingly, myocardial retention increases to 14% to 36% after an intracoronary injection of a CD34+–enriched cell population. Therefore, it would have been very interesting to find out whether G-CSF therapy promotes cell targeting to the AMI territory, given its ability to markedly increase systemic CD34+ cell levels.

The increased 18F-deoxyglucose uptake in the G-CSF treatment group seen in the current study suggests some recovery of the territory at risk. This may be the consequence of the enhanced neovascularization in response to cytokine therapy, antiapoptotic effects of therapy, or both. With the evidence of direct bone marrow cell participation in new vessel growth remaining fairly weak, recent attention has been focused on potential paracrine effects of cell therapy. The various cell populations used in AMI settings are a rich source of cytokines, and their administration, especially by an intramyocardial approach, may be viewed as a large bolus of the cytokine mix. Thus, in an animal model, Akt-1–transfected mesenchymal progenitor cells can protect the heart via a paracrine mechanism.

Another possible explanation for the recovery is myocardial regeneration by resident cardiac progenitor cells. Indeed, such cells, negative for blood lineage marker (Lin-) and positive for the stem cell markers c-Kit and Sca-1, have been shown to be capable of differentiation into cell lines with phenotypic characteristics of cardiac myocytes, smooth muscle cells, and endothelial cells, respectively. It is possible, therefore, that the delivery of various cell types to the heart, either directly or stimulated by G-CSF therapy, provides the source of cytokines that stimulate these cells to differentiate into myocytes and endothelial cells, thereby potentiating the repair process.

Yet another possibility is that the tissue preservation observed may be secondary to an antiapoptotic effect of G-CSF. Indeed, G-CSF has been shown to have direct antiapoptotic effects on cardiac myocytes that can significantly affect infarct healing, resulting in smaller infarcts. Abrogation of G-CSF signaling in mice that expressed a cardiac-restricted dominant-negative STAT3 gene abolished the beneficial effects of G-CSF on infarct healing.

In summary, the trial by Ince et al provides tantalizing evidence for a meaningful biological effect of G-CSF therapy on cardiac perfusion and function after AMI. It is not clear whether this effect is due to the stimulation of bone marrow release of a particular cell type, stimulation of myocardial tissue-resident cardiac progenitor cells, or, indeed, direct tissue effects of the drug itself. The small size of the trial, the lack of placebo treatment in the control group, and the absence of any attempts to address the potential mechanisms of G-CSF action make it impossible to draw any definitive conclusions as to the efficacy of the treatment. Important safety concerns relating to the exposure of the recently injured heart to increased levels of inflammatory cells remain and will require much larger trials to resolve. Nevertheless, this trial will serve as an important step in the further development of new AMI treatment modalities.
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