

Immunomodulation Is the Key to Cardiac Repair

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Because the fundamental defect in both acute myocardial infarction (MI) and ischemic cardiomyopathy is myocyte loss, stem cell therapy to regenerate lost myocardium is conceptually appealing. Multiple clinical trials have been performed in patients with acute MI and chronic ischemic heart failure using a variety of cell types, including bone marrow stem cells, mesenchymal stem cells (MSCs), and cardiac resident stem cells.¹ However, the results from clinical and preclinical studies have been variable, with generally modest benefits in human trials, and disappointingly low levels of cell persistence, engraftment, and cardiomyocyte differentiation. Emerging evidence suggests that rather than the generation of new cardiac tissue, the cardioprotective effects observed with cell therapy may be in large part secondary to secreted paracrine factors that enhance endogenous reparative pathways.¹ These factors may include proteins, RNA, bioactive lipids, and metabolites, and the clustering of these factors into exosomes may facilitate their delivery.

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The low levels of stem cell engraftment observed in these studies suggest that a harsh cardiac microenvironment may compromise cell survival and reparative potential. Importantly, both acute MI and chronic heart failure are characterized by heightened inflammation. Acute MI induces abrupt and massive cardiomyocyte death that triggers intense sterile inflammation to clear necrotic cells, followed by a reparative phase of inflammation resolution, neovascularization, and scar formation.² Ischemic cardiomyopathy is accompanied by inappropriately sustained chronic inflammation, with expansion of innate^{3,4} and adaptive⁵ immune cells and the elaboration of proinflammatory cytokines that promote adverse left ventricular remodeling. Attenuation of the cardiac inflammatory response may, therefore, represent a fruitful approach to enhance the efficacy of cell therapy in these diseases.

In this context, MSCs are particularly attractive as a therapeutic. In addition to being multipotent cells capable of differentiating into a variety of cell types, MSCs are also immunomodulatory and can suppress neutrophil, dendritic cell, T-cell, and natural killer (NK) cell activation, while promoting

regulatory T-cell proliferation and anti-inflammatory macrophage polarity.^{6,7} Moreover, in contrast to other stem cell types, MSCs can evade immune system detection, and as such, autologous and allogeneic MSCs have been reported to have comparable therapeutic efficacy.⁸ To date, most clinical and experimental studies of MSC therapy have used direct tissue delivery using intramyocardial or intracoronary injection, with the idea that local delivery would enhance tissue retention and cell engraftment.⁶ Nonetheless, cell persistence and engraftment are demonstrably low.^{1,6} This suggests that mechanisms aside from MSC differentiation contribute to beneficial responses and that direct myocardial delivery may not be required if the induced reparative mechanisms are systemic rather than local in nature.

In this issue of *Circulation Research*, Luger et al⁹ report a fascinating study exploring the therapeutic efficacy of MSCs delivered via intravenous injection in mice with reperfused acute MI and with ischemic cardiomyopathy. The authors used hypoxia preconditioned human MSCs (hMSCs) given 24 hours after MI (acute MI studies) or at 4 and 7 weeks after MI (ischemic cardiomyopathy studies). Their results indicate that while intravenously administered hMSCs ($1-2 \times 10^6$ cells) homed to the acutely infarcted and cardiomyopathic heart and remained viable for at least 3 weeks, cardiac retention was quantitatively low compared with other organs, such as the spleen, liver, and kidney, indicating preferential distribution of hMSCs systemically. Despite widespread distribution to tissues aside from the heart, single-dose hMSC treatment after acute MI ameliorated late left ventricular structural remodeling in mice with large infarcts and improved long-term left ventricular systolic function when given in repeated doses to mice with ischemic cardiomyopathy. Although tissue-level changes (eg, fibrosis, neovascularization, hypertrophy) were not explored, these benefits of hMSCs were associated with global anti-inflammatory effects: (1) fewer splenic and cardiac NK cells and cardiac neutrophils in mice after MI and (2) fewer splenic neutrophils and a reduction in the splenic neutrophil to lymphocyte ratio in mice with ischemic cardiomyopathy. Finally, antibody-mediated NK cell depletion prior to MI also reduced splenic and cardiac neutrophil infiltration and subsequent adverse left ventricular remodeling.

Taken together, these results have important implications for the field of cell therapy (Figure). Specifically, rather than cell engraftment and differentiation, systemic immunomodulation and resultant tempering of inflammation may be of greater importance in the cardioprotective benefits of MSC therapy. Moreover, local cardiac delivery was not required to improve cardiac remodeling and function, consistent with a previously published phase I trial using intravenous allogeneic MSCs in humans after acute MI.¹⁰ The data also support using a repeated administration cell therapy approach, as has been advocated by other groups,¹¹ via the readily accessible

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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(*Circ Res.* 2017;120:1530-1532.)

DOI: 10.1161/CIRCRESAHA.117.310954.)

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Circulation Research is available at <http://circres.ahajournals.org>

DOI: 10.1161/CIRCRESAHA.117.310954

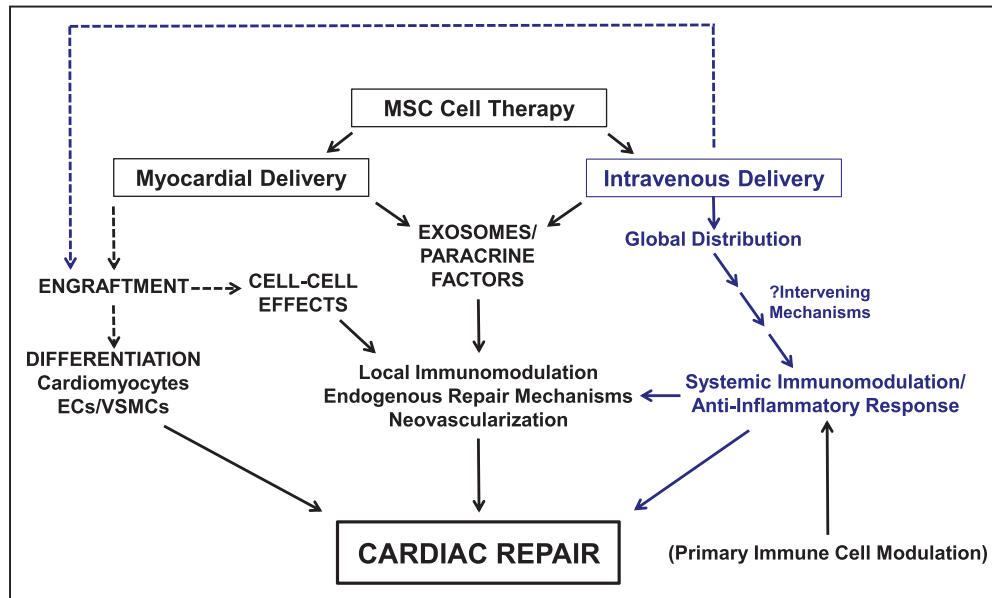


Figure. Potential mechanisms underlying the reparative effects of mesenchymal stem cells (MSCs), with the implications of new findings elucidated by the study of Luger et al⁹ in blue. MSCs are thought to induce repair predominantly by the secretion of paracrine factors (including those clustered in exosomes) that induce a variety of local effects, including the modulation of inflammation, activation of endogenous repair mechanisms, and vascular growth. Because cell engraftment rates have been generally reported to be low, MSC differentiation into cardiomyocytes and other cell types (eg, endothelial cells [ECs] and vascular smooth muscle cells [VSMCs]) is thought to play a more minor role (dashed arrows). Intravenous delivery of MSCs results in a global distribution of cells that includes the heart, but with the majority of MSCs residing in organs aside from the myocardium. Through as-of-yet poorly defined mechanisms, this produces systemic (and local) immunomodulation and anti-inflammatory responses that in turn augment cardiac repair. Primary targeting of specific proinflammatory immune cell subsets, either as an adjunct to MSC therapy or as a stand-alone approach, may further augment systemic anti-inflammatory effects and subsequent cardiac repair.

intravenous route to sustain reparative responses over the long term. Such a paradigm would greatly facilitate the practical application of MSC therapy in humans.

Beyond the implications for cell therapy, the work of Luger et al⁹ also underscores the importance of immune cell modulation as a crucial factor for inducing cardiac repair after MI (Figure). Their study supports a specific role for the suppression of NK cells, and NK-mediated modulation of neutrophil activation and survival,¹² as underlying immune-related mechanisms for the benefits of intravenous hMSCs. Beyond this therapeutic context, however, several studies have established important pathophysiological roles for a variety of immune cell populations in regulating cardiac repair after MI (reviewed in Prabhu and Frangogiannis²) and in driving pathological remodeling in ischemic cardiomyopathy.³⁻⁵ In addition to resident immune cell populations, the spleen seems to be a particularly important source of inflammatory cells that infiltrate and engender local responses in the infarcted and failing heart.³ Moreover, experimental studies have established that direct targeting of specific innate and adaptive immune cell populations (eg, infiltrating macrophages, CD4⁺ T-cells)^{4,5,13} in these pathologies, in the absence of any accompanying stem cell therapy, can have profound effects on cardiac repair and remodeling at the chamber and tissue level. These observations, taken together with the results of Luger et al,⁹ suggest that direct immune cell modulation, either as an adjunct to stem cell therapy or as a primary approach, may represent a high-yield therapeutic strategy for producing robust reparative responses in the heart.

Importantly, although these study results are exciting, the mechanisms linking hMSCs to the modulation of inflammation were not explored in detail, and there are several extant possibilities that can be considered. First, efferocytosis is known to induce potent anti-inflammatory immune responses during infarct repair.^{2,13,14} Is there a role for efferocytosis of apoptotic hMSCs by tissue phagocytes in the production of immunomodulation? Second, as noted earlier, secreted paracrine factors are thought to be a primary mechanism for the beneficial effects of cell therapy.¹ What are the specific paracrine factors secreted by hMSCs in the various tissues and how do they specifically impact immune cell activation and function? Third, the classification used in this study of immune cells being either myeloid or lymphoid belies the cellular complexity of the immune system. What specific and specialized innate and adaptive cell subpopulations are impacted by hMSCs and how do they uniquely contribute to immunomodulation? Fourth, the authors demonstrated greater retention of intravenously delivered murine MSCs (as compared with hMSCs) in both the heart and spleen post-MI. Based on these observations, would there be more effective and durable immunomodulation if autologous MSCs are used? Fifth, are there specific cytokines and chemokines that may be adjunctively targeted during MSC therapy to enhance anti-inflammatory and regenerative responses? In this regard, we have recently reported that tumor necrosis factor- α modulates *in vitro* differentiation of cardiac stem cells in a tumor necrosis factor- α receptor-dependent manner¹⁵; specific proinflammatory signaling pathways may have analogous effects in MSCs that in turn impact reparative

efficacy. Answering these and other relevant questions will require further study to include expanded cell controls and MSC tracking, genetically modified transplanted MSCs and mouse recipients, more sophisticated immune cell markers, and the examination of tissue-level pathological remodeling.

These issues notwithstanding, this provocative study by Luger et al⁹ adds to the emerging evidence that the interrelationship between stem cells and immune cells should occupy a more prominent position under the aegis of reparative therapeutics and that effective immunomodulation may be a key element for successful cell therapy-mediated cardiac repair. We eagerly await the results of future studies examining the mechanistic links between stem/progenitor cells and the immune system and the clinical implications of the same for the treatment of human disease.

Sources of Funding

This work was supported by National Institutes of Health (NIH) grant 1R01HL125735 and a VA Merit Award I01BX002706 (both to S.D. Prabhu).

Disclosures

None.

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KEY WORDS: Editorials ■ cell therapy ■ immunomodulation ■ inflammation ■ mesenchymal stem cells ■ myocardial infarction

Circulation Research

JOURNAL OF THE AMERICAN HEART ASSOCIATION



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Circ Res. 2017;120:1530-1532

doi: 10.1161/CIRCRESAHA.117.310954

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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