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Persistent Inflammation, Stem Cell–Induced Systemic Anti-Inflammatory Effects, and Need for Repeated Stem Cell Injections: Critical Concepts Influencing Optimal Stem Cell Strategies for Treating Acute Myocardial Infarction and Heart Failure

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The therapeutic potential of stem cell treatment for patients with either acute myocardial infarction (AMI) or heart failure (HF) began >15 years ago. Many preclinical studies have been published demonstrating stem cell administration improves myocardial function in animal models of AMI and of HF. Parallel results were found in many small phase 2 clinical trials. However, the results of more recent, larger, and statistically more powerful trials can, at best, be described as "disappointing."

The status of the results of clinical trials of stem cell administration to patients with HF was summarized in 2013 by Sanganalmath and Bolli.[1] They concluded " . . . to date, no cell therapy has been conclusively shown to be effective in patients with HF."[1] This conclusion has not changed with time.[2,3] Similar conclusions are applicable to clinical trials of AMI. Although smaller trials found stem cells improve myocardial outcomes, pivotal trials have not definitively improved their prospectively identified primary end points.[4,5]

It is informative to consider 2 recent examples of altered outcomes when positive results in small phase 2 studies drive initiation of pivotal trials. Thus, the C-CURE (Cardiopoietic Stem Cell Therapy in Heart Failure)/CHART-1 (Congestive Heart Failure Cardiopoietic Regenerative Therapy) trials both injected cardiopoietic stem cells (autologous bone marrow–derived mesenchymal stem cells [MSCs] treated with "cardiogenic cocktail") transendocardially in patients with ischemic cardiomyopathy. The smaller C-CURE trial (48 patients) demonstrated significantly improved myocardial function,[6] leading to the larger pivotal CHART-1 trial (271 patients).[7] This trial demonstrated that the cardiopoietic stem cells failed to improve patients' clinical status or myocardial function compared with control. A similar example is found in the CADUCEUS (Cardiosphere-Derived Autologous Stem Cells to Reverse Ventricular Dysfunction)/ALLSTAR (Allogeneic Heart Stem Cells to Achieve Myocardial Regeneration) trials, in which cardiac-derived stem cells ("cardiospheres") were administered into the infarct-related coronary artery in patients after AMI. Positive results in the initial small CADUCEUS trial (31 patients)[8] led to the ALLSTAR trial (142 patients),[9] which demonstrated cell therapy did not improve the trial's primary end point.

Do all of these less than encouraging results of the larger, statistically more powerful, clinical trials mean that stem cell delivery for AMI and HF does not hold promise? Or might there be another explanation for why definitive efficacy has not as yet been demonstrated. This question bears directly on why the study from Bolli's group, published in this issue of *Journal of the American Heart Association* (*JAHA*),[10] is of major significance.

Many reasons have been postulated as contributing to these negative clinical trial results, including the type of stem cell used, the mode of administration, and the dose.[4] Each of these undoubtedly contribute, probably importantly, to the discouraging results. We have been intrigued, in particular, by the fact that virtually all clinical trials testing the efficacy of stem cells in cardiac disease, including the more recent larger trials, were designed with 2 basic assumptions.

First, each study design used *direct delivery of the stem cells* to the myocardium, by either intracoronary or transendocardial injection. The concept intrinsic to this delivery strategy is that, to be effective, the stem cells must *reside in the myocardium.* Once so situated, it was hypothesized they would transdifferentiate into functioning cardiac myocytes or, through paracrine activities, either exert a panoply of healing effects and/or stimulate resident cardiac stem cells to expand and contribute to myocardial function. Second, all

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these studies used *a single injection of stem cells*. This assumes that a single stem cell injection would either "cure" the disease processes causing progressive myocardial dysfunction or produce a prolonged beneficial myocardial effect persisting over 6 months or a year (the usual time of termination of most of the clinical trials).

We believe that these 2 assumptions are flawed and, by influencing the study designs, have limited the ability of each study to adequately test the concept that stem cells improve myocardial function. Our speculation derives from recent preclinical studies that provide newly appreciated insights into both the mechanisms contributing to the pathophysiological characteristics of the disease processes that are being treated and the mechanisms by which stem cells could improve myocardial outcomes. These insights are imbedded in 3 mechanistic concepts.

1. A long-term, inappropriate, and excessive inflammatory process contributes importantly to the progressive myocardial deterioration that occurs after AMI and that occurs in patients with HF.

The concept that the progressive deterioration of cardiac function seen in both AMI and HF is in part caused by an excessive persistent inflammatory response has a compelling experimental basis supporting its validity.[11,12] For example, in the setting of AMI, there is a subgroup of patients whose progressive deterioration in left ventricular function is not caused solely by the AMI-induced magnitude of myocardial damage, but involves other mechanisms.[13] Chronic inflammation has been postulated as being one of these mechanisms responsible for progressive myocardial dysfunction in AMI, and also in HF.[11–15]

2. Systemic anti-inflammatory effects of MSCs constitute one of the major mechanisms by which MSCs may improve left ventricular function.

Any myocardial benefit provided by stem cell administration is undoubtedly not caused by repopulating the damaged myocardium with new myocytes, but rather by paracrine activities with a diverse array of beneficial effects.[12,16,17] Prominent among these are marked *systemic* anti-inflammatory effects, activities most firmly established for MSCs.[12,13,15] These systemic anti-inflammatory effects are induced by intravenous administration of MSCs and constitute an important mechanism by which MSCs improve myocardial function in murine models of AMI and ischemic cardiomyopathy.[15] Use of the intravenous route of administration provides, in addition to efficacy, a practical advantage. Intravenous injection can be accomplished safely and inexpensively and can deliver MSCs repeatedly, all of which provides the setting for appreciating the importance of the article published in this issue of *JAHA* by Bolli and colleagues.[10]

3. Need for repeated administration of stem cells.

It is unclear why certain patients/animals, after developing an inflammatory response to acute injury (ie, myocardial injury caused by AMI or by other causes), do not resolve the inflammatory response but transition to a persistent inflammatory state. We postulated 2 potential mechanisms that could predispose to this.[13]

First, compelling experimental data demonstrate that genetic mutations and polymorphisms in genes encoding components of the innate immune system increase expression of many cytokines associated with activation of inflammation.[18] Such abnormalities lead to a diverse array of clinical syndromes, specifically, those categorized as *autoinflammatory*. Although data are lacking linking such genetic abnormalities to myocardial disease, if present, a triggering event, such as an AMI, could lead to expression of the abnormal gene(s), or to epigenetic alterations, leading to chronic inflammation and to progressive myocardial deterioration. Such a mechanism suggests a possible parallel relationship to diseases characterized as autoinflammatory.

Second, pathogens associated with *chronic infection* induce expression of multiple inflammatory genes, with the number of pathogens ("pathogen burden") with which an individual has been infected (indicated by pathogen seropositivity) incrementally increasing risk of AMI and death.[19] Pathogen-induced chronic inflammatory activity might also contribute to the progressive myocardial dysfunction occurring in subgroups of patients with AMI and HF.

Whatever the cause, *although the chronic inflammation existing in certain subgroups of patients might be transiently suppressed by a single injection of a therapeutic agent, it will almost certainly not be curative. These patients probably eventually experience recrudescence of the inflammation, with continuing long-term myocardial damage.*

The concept of the need for repeated administration of stem cells is elegantly advanced by the article by Bolli et al.[10] This group hypothesized that, in the setting of ischemic cardiomyopathy, greater stem cell efficacy would be achieved by injecting the cells multiple times, thereby providing more prolonged exposure of diseased myocardium to the paracrine actions of the cells than could be achieved by a single injection.

In their first series of studies, they demonstrated that cardiac progenitor cells (c-kit positive), administered 3 times, 35 days apart, into the left ventricular cavity of rats with 30-day-old myocardial infarctions improved myocardial function more than did a single injection. Similar results were observed in mice in which cardiac mesenchymal cells were injected.[20] The authors recognized, however, that because the single-dose group received only one third of the total number of cells given to the 3-dose group, they could not distinguish between whether greater efficacy was caused by repeated treatments or by the higher total number of cells.

This question was addressed in the present study.[10] The investigators injected identical total doses of cells in the single and multiple injection groups. Multiple injections still improved myocardial function more than a single injection, demonstrating it is the *repeated treatment* with cells that accounts for the greater improvement in myocardial function that occurs with multiple injections.

This article has one serious limitation–the number of rats in each subgroup is small. Thus, although this study cannot be considered as proving unequivocally that repeated administration of stem cells is superior to a single injection, the study was performed carefully and its conclusions are intuitively consistent with what we know of the disease mechanisms operative in chronic HF. The results indicating the superiority of multiple injections therefore need be carefully considered in the design of new stem cell trials.

Conclusions

On the basis of the considerations previously addressed, it seems unwarranted to conclude that the discouraging data of recent large clinical trials of stem cell treatment of patients with AMI or with HF indicate stem cell therapy is unlikely to benefit myocardial dysfunction. A more accurate perspective, it seems, is to recognize that it has taken >15 years of studying and testing stem cell efficacy in patients with AMI and with HF to learn the best treatment strategies to test their potential efficacy. Thus, fundamental changes in both our mechanistic understanding of the diseases we are treating and in the mechanisms by which stem cells exert their beneficial effects have occurred.

We now, as depicted in the Figure, appreciate the following: (1) the role of chronic systemic inflammation as an important contributor to progressive myocardial deterioration in both AMI and HF, (2) the critical importance of *systemic* anti-inflammatory activities of MSCs and that such anti-inflammatory activities are achieved by intravenous administration (providing the opportunity to repeatedly administer MSCs safely and inexpensively), and (3) the probability that the processes leading to a chronic inflammatory state in these 2 conditions will not be cured by a single administration of stem cells but will require repeated administrations over time. Although the preclinical data supporting these concepts are strong, they still have to be considered as hypotheses in need

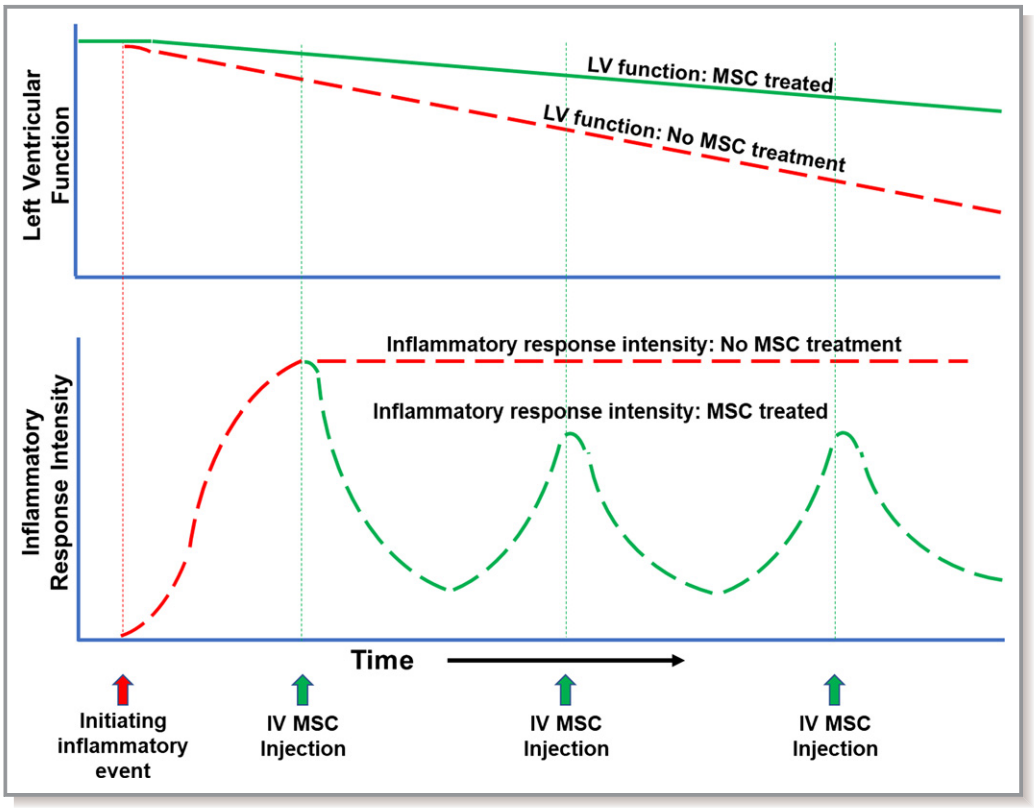

Figure. Illustration of the concepts that persistent inflammation is a key contributor to the progressive myocardial dysfunction occurring in patients with acute myocardial infarction and with heart failure. The relevant underlying disease processes leading to the persistent inflammation, and thereby to progressive left ventricular (LV) dysfunction, are not "cured" by a single injection of stem cells, but necessitate repeated injections. IV indicates intravenous; and MSC, mesenchymal stem cell.

of definitive clinical testing. It is our opinion, however, that if these mechanistic insights are used in the design of future pivotal clinical trials, we will acquire, for the first time, definitive data that will allow us to determine the validity of the hypothesis that stem cell therapy improves left ventricular function in patients with AMI and in those with HF.

Disclosures

Epstein has equity in CardioCell and is on its Board of Scientific Advisors. The remaining authors have no disclosures to report.

References

1. Sanganalmath SK, Bolli R. Cell therapy for heart failure: a comprehensive overview of experimental and clinical studies, current challenges, and future directions. *Circ Res*. 2013;113:810–834.
2. Fisher SA, Doree C, Mathur A, Taggart DP, Martin-Rendon E. Cochrane Corner: stem cell therapy for chronic ischaemic heart disease and congestive heart failure. *Heart*. 2018;104:8–10.
3. Lipinski MJ, Luger D, Epstein SE. Mesenchymal stem cell therapy for the treatment of heart failure caused by ischemic or non-ischemic cardiomyopathy: immunosuppression and its implications. *Handb Exp Pharmacol*. 2017;243:329–353.
4. Henry TD, Moyé L, Traverse JH. Consistently inconsistent-bone marrow mononuclear stem cell therapy following acute myocardial infarction: a decade later. *Circ Res*. 2016;119:404–406.
5. Micheu MM, Dorobantu M. Fifteen years of bone marrow mononuclear cell therapy in acute myocardial infarction. *World J Stem Cells*. 2017;9:68–76.
6. Bartunek J, Behfar A, Dolatabadi D, Vanderheyden M, Ostojic M, Dens J, El Nakadi B, Banovic M, Beleslin B, Vrolix M, Legrand V, Vrints C, Vanoverschelde JL, Crespo-Diaz R, Homsy C, Tendera M, Waldman S, Wijns W, Terzic A. Cardiopoietic stem cell therapy in heart failure: the C-CURE (Cardiopoietic stem Cell therapy in heart failURE) multicenter randomized trial with lineage-specified biologics. *J Am Coll Cardiol*. 2013;61:2329–2338.
7. Bartunek J, Terzic A, Davison BA, Filippatos GS, Radovanovic S, Beleslin B, Merkely B, Musialek P, Wojakowski W, Andreka P, Horvath IG, Katz A, Dolatabadi D, El Nakadi B, Arandjelovic A, Edes I, Seferovic PM, Obradovic S, Vanderheyden M, Jagic N, Petrov I, Atar S, Halabi M, Gelev VL, Shochat MK, Kasprzak JD, Sanz-Ruiz R, Heyndrickx GR, Nyolczas N, Legrand V, Guédès A, Heyse A, Moccetti T, Fernandez-Aviles F, Jimenez-Quevedo P, Bayes-Genis A, Hernandez-Garcia JM, Ribichini F, Gruchala M, Waldman SA, Teerlink JR, Gersh BJ, Povsic TJ, Henry TD, Metra M, Hajjar RJ, Tendera M, Behfar A, Alexandre B, Seron A, Stough WG, Sherman W, Cotter G, Wijns W; CHART Program. Cardiopoietic cell therapy for advanced ischaemic heart failure: results at 39 weeks of the prospective, randomized, double blind, sham-controlled CHART-1 clinical trial. *Eur Heart J*. 2017;38:648–660.
8. Malliaras K, Makkar RR, Smith RR, Cheng K, Wu E, Bonow RO, Marbán L, Mendizabal A, Cingolani E, Johnston PV, Gerstenblith G, Schuleri KH, Lardo AC, Marbán E. Intracoronary cardiosphere-derived cells after myocardial infarction: evidence of therapeutic regeneration in the final 1-year results of the CADUCEUS trial (CArdiosphere-Derived aUtologous stem CElls to reverse ventricular dySfunction). *J Am Coll Cardiol*. 2014;63:110–122.
9. Henry T. Presentation at AHA. 2018. Intracoronary ALLogeneic Heart STem Cells to Achieve Myocardial Regeneration - ALLSTAR. 11/15/2017. Anaheim, California, US.
10. Tang X-L, Nakamura S, Li Q, Wysoczynski M, Gumpert A, Wu W, Hunt G, Stowers H, Ou Q, Bolli R. Repeated administrations of cardiac progenitor cells are superior to a single administration of an equivalent cumulative dose. *J Am Heart Assoc*. 2018;7:e007400.
11. Van Tassell BW, Toldo S, Mezzaroma E, Abbate A. Targeting interleukin-1 in heart disease. *Circulation*. 2013;128:1910–1923.
12. Epstein SE, Luger D, Lipinski MJ. Paracrine-mediated systemic anti-inflammatory activity of intravenously administered mesenchymal stem cells: a transformative strategy for cardiac stem cell therapeutics. *Circ Res*. 2017;121:1044–1046.
13. Westman PC, Lipinski MJ, Luger D, Waksman R, Bonow RO, Wu E, Epstein SE. Inflammation as a driver of adverse left ventricular remodeling after acute myocardial infarction. *J Am Coll Cardiol*. 2016;67:2050–2060.
14. Kelkar AA, Butler J, Schelbert EB, Greene SJ, Quyyumi AA, Bonow RO, Cohen I, Gheorghiade M, Lipinski MJ, Sun W, Luger D, Epstein SE. Mechanisms contributing to the progression of ischemic and nonischemic dilated cardiomyopathy: possible modulating effects of paracrine activities of stem cells. *J Am Coll Cardiol*. 2015;66:2038–2047.
15. Luger D, Lipinski MJ, Westman PC, Glover DK, Dimastromatteo J, Frias JC, Albelda MT, Sikora S, Kharazi A, Vertelov G, Waksman R, Epstein SE. Intravenously delivered mesenchymal stem cells: systemic anti-inflammatory effects improve left ventricular dysfunction in acute myocardial infarction and ischemic cardiomyopathy. *Circ Res*. 2017;120:1598–1613.
16. Peng Y, Pan W, Ou Y, Xu W, Kaelber S, Borlongan CV, Sun M, Yu G. Extracardiac-lodged mesenchymal stromal cells propel an inflammatory response against myocardial infarction via paracrine effects. *Cell Transplant*. 2016;25:929–935.
17. Phinney DG, Prockop DJ. Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair–current views. *Stem Cells*. 2007;25:2896–2902.
18. Kastner DL, Aksentijevich I, Goldbach-Mansky R. Autoinflammatory disease reloaded: a clinical perspective. *Cell*. 2010;140:784–790.
19. Epstein SE, Zhu J, Najafi AH, Burnett MS. Insights into the role of infection in atherogenesis and in plaque rupture. *Circulation*. 2009;119:3133–3141.
20. Tokita Y, Tang XL, Li Q, Wysoczynski M, Hong KU, Nakamura S, Wu WJ, Xie W, Li D, Hunt G, Ou Q, Stowers H, Bolli R. Repeated administrations of cardiac progenitor cells are markedly more effective than a single administration: a new paradigm in cell therapy. *Circ Res*. 2016;119:635–651.

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