CLINICAL IMPLICATIONS OF BASIC RESEARCH

Elizabeth G. Phimister, Ph.D., *Editor*

Teasing the Immune System to Repair the Heart

Jonathan A. Epstein, M.D., Nadia Rosenthal, Ph.D., and Arthur M. Feldman, M.D., Ph.D.

Heart failure is a pandemic disease that affects nearly 6 million persons in the United States and 26 million persons worldwide. Despite the development of new drugs and devices for the treatment of heart failure, the outlook for patients with the disease remains grim. Thus, there was great excitement two decades ago when scientists purported to show for the first time that injection of autologous bone marrow cells into the peri-infarct region of a mouse heart after a myocardial infarction resulted in the generation of new myocardial cells. However, the therapeutic behavior attributed to cells derived from bone marrow or the heart in the early studies has turned out to be illusory. Subsequent trials failed to show even modest survival times for transplanted cells, findings that suggested that they were not acting as stem cells, and numerous foundational studies were retracted. In response to continued scandals and controversy, the underlying mechanism by which injection of “stem cells” into the damaged heart may affect outcomes has been robustly questioned.¹

A recent study by Vagnozzi et al.² provides a new mechanistic explanation as to how the delivery of various cell populations to the heart may enhance the function of cardiac muscle injured by ischemia and subsequent reperfusion. These investigators tested two primary cell populations previously postulated to be stem

cells: fractionated bone marrow mononuclear cells and cardiac mesenchymal cells that express the receptor tyrosine kinase c-Kit (also known as cardiac progenitor cells), both of which were administered either as live cells or as cells that were killed through a freeze–thaw cycle. They also tested zymosan — a yeast-derived protein–carbohydrate complex used to induce sterile inflammation — and used saline as a control (Fig. 1).

When healthy mice received an injection of live or dead mononuclear cells, live or dead cardiac progenitor cells, or zymosan into the heart, the number of activated macrophages increased in the area of injection and then gradually decreased concomitantly with the clearing of cells or zymosan from the heart. Injection of live or dead mononuclear cells, live or dead cardiac progenitor cells, or zymosan also caused a shift in the macrophage subtype: CX3CR1+ macrophages, which are abundant throughout the resting heart, showed a mild increase in number at the periphery of the injection site, whereas CCR2+ macrophages, which are largely absent in the naive state and implicated in adverse inflammatory responses,³ showed robust increases in number. Vagnozzi et al. did not detect either the genesis of new cardiomyocytes or the transdifferentiation of injected mononuclear cells or cardiac progenitor cells into cardiomyocytes

Figure 1 (facing page). A Therapeutic Acute Immune Response in Lieu of “Cell Therapy.”

Vagnozzi et al. tested the mechanism whereby stem-cell injection reportedly ameliorates the effects of ischemia–reperfusion injury (modeling myocardial infarction) in mice. In the absence of any intervention, ischemia–reperfusion injury in the mouse led to the development of an obvious scar within the distribution of the affected coronary artery (Panel A). By contrast, injection of live or dead mononuclear cells (MNCs), live or dead cardiac progenitor cells (not shown), or zymosan into the peri-infarct region 1 week after ischemia–reperfusion injury quickly led to the appearance of CCR2+ macrophages at the injection site, followed by CX3CR1+ macrophage expansion. These responses were associated with functional benefit that persisted for at least 8 weeks after the injection (Panel B). Immunosuppression or macrophage blockade abrogated the beneficial effects (Panel C).

or endothelial cells. However, they observed that injection of zymosan resulted in an increase in the number of endothelial cells at the site of injection but only after the mononuclear cells and cardiac progenitor cells had cleared.

A week after mice were subjected to ischemia–reperfusion injury, injection of mononuclear cells, cardiac progenitor cells, or zymosan into the infarct border zone improved left ventricular contractility and decreased end-systolic volume. To prove that immune activation was necessary for the observed improvements in cardiac function, Vagnozzi et al. abrogated the effects of the cell or zymosan injection by coadministration of a broad-spectrum immunosuppressant or by macrophage depletion. Thus, the observed beneficial effects were apparently produced by selective activation of innate immune responses in a manner that ischemic injury does not achieve on its own. The salutary effects did not rely on active involvement of the injected cells; in fact, zymosan had the most durable effect. Indeed, when the investigators tested mechanical effects *ex vivo* using a forced-lengthening assay on strips of infarcted tissue, they found that the tissue from the mice that had received an injection of zymosan in the heart was most resistant to mechanical force, a finding that underscores the importance of immunoreactivity over any active role of injected materials.

The human heart contains diverse macrophage populations, and heart failure is influenced by an altered myeloid-cell repertoire.³ To pinpoint the respective roles of induced CCR2+ and CX3CR1+ macrophages in cardiac outcome, the investigators used knockout mice to show that cardiac function immediately after ischemia–reperfusion injury was improved in the presence of CCR2+ macrophages and that the presence of CX3CR1+ macrophages is important for later stages of infarct maturation.

Thus, Vagnozzi et al. have provided an explanation for the observed benefits of stem-cell therapy in the treatment of cardiac injury. Their assessment calls into question the continued pursuit of cell-based therapeutic approaches,

which are expensive, difficult to scale, and potentially hazardous. Rather, it underscores the critical role played by the immune system in regulating cardiac homeostasis and repair after an ischemic injury, suggesting that a shift toward immune-directed therapeutic approaches for facilitating cardiac repair, regeneration, or both is in order. Their explanation does not exclude the involvement of other pathways, such as those that regulate apoptosis and autophagy, which also influence the response of the heart to injury.⁴ Given the rapid advances under way in our understanding of immune-cell subtypes and their roles in many human diseases, coupled with the development of small molecules and biologics to manipulate the immune response, tremendous opportunity awaits the cardiovascular research community. On the basis of studies in amphibians, fish, and mammals, it is now clear that the immune response and the specificity of the immune cells that respond in the context of injury determine both the extent and the quality of repair.⁵ The challenge will be determining how to tease the immune system into repairing, restoring, or regenerating the injured heart.

Disclosure forms provided by the authors are available at NEJM.org.

From the Perelman School of Medicine at the University of Pennsylvania (J.A.E.) and the Department of Medicine, Division of Cardiology, Lewis Katz School of Medicine at Temple University (A.M.F.) — both in Philadelphia; the Jackson Laboratory, Bar Harbor, ME (N.R.); and the National Heart and Lung Institute, Imperial College London, London (N.R.).

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