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EDITORIAL COMMENT

Macrophages Remember When Your Heart Was Broken*

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The immune system is essential for the proper functioning of the heart under normal and physiological conditions but especially in response to myocardial injuries such as ischemia, reperfusion, genetic cardiomyopathies, and nonischemic injury, including pressure overload and neurohormonal stimulation. Although the precise mechanisms and specific cell types responsible for these essential roles are poorly understood, recent work has indicated that cardiac-resident macrophages are essential in the protection of cardiac structure and function in response to stress.

In a paper recently published in *JACC: Basic to Translational Science*, Hayashi et al[1] demonstrated that cardiac immune cells, including macrophages and dendritic cells, respond to a single dose of 300 mg of isoproterenol (ISO) by up-regulation of the programmed death-1 (PD-1)/programmed death ligand (PD-L) axis. The PD-1/PD-L axis is an immune checkpoint with an important role in inhibiting immune responses and promoting self-tolerance. Accumulating evidence has shown that inhibition of the PD-1/PD-L pathway promotes an effective immune response against cancer cells. Hayashi et al[1] injected a single dose of ISO to model takotsubo cardiomyopathy, also known as broken-heart syndrome. The investigators showed that the PD-1/PD-L axis restrains the acute inflammatory response and helps normalize cardiac function after a single dose of ISO. They further showed that mice that did not express PD-1 (PD-1-knockout mice) responded to ISO with increased serum levels of troponin I, a greater inflammatory score index, and a delayed inflammatory response. Moreover, wild-type mice treated with PD-L1-blocking antibodies showed a 40% increase in mortality and increased myocardial inflammation after ISO injection. These results highlight immune checkpoints as key regulators of acute myocardial inflammation and cardiac repair following injury. However, how the activation of immune checkpoints regulates the cardiac response to subsequent stressor stimulus, including inflammatory processes regulated by resident immune cell cross-talk, was unknown.

In a follow-up study published in this issue of *JACC: Basic to Translational Science*, Tiwary et al[2] demonstrate that the immune response to a single dose of ISO helps protect the heart from the damaging effects of a second exposure to ISO 1 week later. The investigators show that a second dose of ISO resulted in diminished tissue damage, cardiomyocyte death, and preservation of cardiac structure and function, indicating that the first dose of ISO induced some level of cardiac protection. From a pharmacologic perspective, such protection could be the result of tachyphylaxis and the desensitization of β_1-adrenergic receptors in cardiac tissue. However, the investigators demonstrate that the inotropic and chronotropic responses of the heart to ISO were maintained, indicating that ISO pretreatment did not blunt the heart's β_1-adrenergic responsivity. Therefore, disease tolerance was associated with the cardioprotective effect of subsequent exposures to ISO and highlights the ability of the heart to develop protective mechanisms against collateral tissue damage to recurrent injuries.

What could be the mechanism of protection induced by the first ISO stimulus? Tiwary et al[2]

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demonstrate that the initial administration of ISO resulted in acute inflammation characterized by the accumulation of neutrophils, transitioning monocytes, and macrophages. After a second administration with ISO, the hearts of pretreated mice showed no increase in the number of $CD45^+$ leukocytes, while control mice showed a substantial increase in the number of immune cells after a single dose of ISO. Importantly, the protective effect of an initial ISO administration to a subsequent dose of ISO lasted multiple weeks. These results suggest that cardiac immune cells could confer cardiac protection.

Which immune cells might be mediating these protective effects? In previous work published by the same group, Hayashi et al[1] demonstrated that a single injection of ISO increases the population of $CD64^+LY6C^{low/-}$ macrophages as well as $CD4^+$ and $CD8^+$ T cells. When these cells were incubated with necrotic cardiomyocytes, the percentage of macrophages and CD4 and CD8 T cells expressing the PD-1/PD-L axis increased. Thus, either macrophages or T cells could be the cellular source of protection induced by exposure to ISO. In the present study, Tiwary et al[2] show that, at least in part, macrophages protect the heart against a second exposure to ISO. The investigators show that mice in which the cardiac macrophages were depleted between the first and second doses of ISO using liposomal clodronate had higher lethality compared with phosphate-buffered saline–loaded liposomes between ISO doses. Furthermore, the clodronate liposome–induced macrophage depletion was associated with increased troponin I levels, reinforcing the finding that macrophages are at least partly responsible for the cardioprotective effect of a first ISO injection, although the absolute level of troponin release was substantially lower compared with a first ISO injection.

The notion that macrophages protect the heart against stressful stimuli has been shown to involve antifibrotic and proangiogenic effects. Various groups, including our own, have demonstrated that resident macrophages hold off the development of heart failure following pressure overload.[3] The protective effects of macrophages are typically inferred on the basis of the experimental depletion of macrophages via administration of liposomal clodronate, antibodies that block the macrophage colony-stimulating factor receptor, or through genetic approaches in which macrophage subsets express a diphtheria toxin receptor. Similar to the present study, depletion of macrophages has been shown to aggravate disease in ischemic injury, pressure overload, neurohormonal stimulation, and genetic forms of cardiomyopathy. Notably, the nature of the protective effect varies among studies, which can be attributed to the differences in disease pathophysiology, animal models, and modes of macrophage depletion. Certainly, most strategies used to deplete macrophages are too broad and lack specificity to determine the role of specific subsets of macrophages, as identified by single-cell sequencing studies. To what extent the protective effects conferred by macrophages arise from resident or from monocyte-derived macrophages is not clear from the present work. In addition, the effector mechanisms responsible for the protective effects of cardiac macrophages are unclear but likely include secreted factors and their unique ability to remove dead cardiomyocytes and debris.

Ultimately, the loss of cardioprotection provided by ISO during macrophage depletion suggests that these cells must express mediators involved in cardiac preconditioning after tissue injury. Currently, it remains unknown which signaling pathways could be involved in this protective process. Considering the nonselectivity of the beta-adrenergic agonist ISO, one possibility could be the involvement of immune cells expressing β_2-adrenergic receptors. Indeed, Grisanti et al[4] showed that deletion of the β_2-adrenergic receptor on bone marrow–derived immune cells resulted in enhanced mortality and cardiac rupture after myocardial infarction (MI). However, the potential role of resident macrophages expressing β_2-adrenergic receptor in the initiation of immune cell preconditioning is unknown. Importantly, these findings indicate that recruited macrophages, at least in cardiac remodeling induced by MI surgery, can exert protective functions. Along the same line, Grune et al[5] showed that the depletion of all macrophage subsets (Csf1r inhibition) or only recruited macrophages ($Ccr2^{-/-}$ mice) increased ventricular tachycardia and fibrillation after myocardial infarction in the setting of hypokalemia. Clearly, the dichotomy of resident macrophages being protective and monocyte-derived ones being proinflammatory (analogous to M2 and M1 phenotypes in culture) is an overly simplistic viewpoint, and a better understanding of what signals allow macrophages to develop protective mechanisms is needed.

Currently, it is unclear how long-lasting the protective effects will be. Tiwary et al[2] found that the cytoprotective mechanisms related to prior exposure to ISO injection were partially lost 5 weeks later. However, in functional terms, the effects promoted by ISO preconditioning were still sufficient to preserve myocardial homeostasis. Furthermore, the underlying mechanism of protection is unclear beyond the involvement of macrophages and potentially other immune cells. Could it be that macrophages

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develop some sense of memory after a bad experience? Recent studies have coined the term *memory macrophages*, whereby macrophages undergo epigenetic and metabolic changes in response to a stimulus, resulting in altered behavior of macrophages after a second stimulus.[6] Tiwary et al[2] did not formally investigate the development of memory macrophages, nor did they assess the induction of epigenetic or metabolic changes within macrophages. Nevertheless, the notion that macrophages alter the response to a second stimulus suggests that some entrainment must have happened. Identification of the precise mechanisms and how entrainment of macrophages can influence disease outcomes are important areas for future study.

To what extent the findings of the present study are translatable to patients is unclear. On the basis of Tiwary et al's[2] findings and previous research, one possible therapeutic strategy could involve the modulation of macrophages to improve disease outcomes. Future studies will need to replicate these findings, assess if other stimuli might also invoke some memory in macrophages, and identify the mechanisms by which immune cells confer protection from repeated injury.

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