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EDITORIAL COMMENT

Cardiac Macrophages, Reactive Oxygen Species, and Development of Left Ventricular Dysfunction*

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After cardiac injury, monocytes and macrophages play a complex, yet central role in tissue injury, repair, and long-term adverse cardiac remodeling. In some circumstances, monocytes and macrophages have been shown to have profound effects that go beyond promoting repair, but can directly promote regeneration of lost tissue (1). For example, amphibians and neonatal mammals have a remarkable regenerative capacity, and if "neonatal" monocytes and macrophages are depleted, the regenerative capacity is abolished (2–6). Similarly, in adult mammals where regeneration is limited, nonspecific depletion of all monocytes and macrophages after myocardial infarction (MI) leads to poor scar formation, left ventricular dysfunction, and myocardial rupture (7,8). These data indicate that monocytes and macrophages are central to the repair/regenerative process across species. However, excessive numerical expansion of monocytes and macrophages within the myocardium also impairs infarct healing, and strategies used to limit the excessive expansion have been proven beneficial (9–11). As a result, factors such as developmental stage, mechanism of injury, and strategies used to target either monocytes or macrophages all influence whether monocytes and macrophages are viewed as protective or pathologic, which has limited therapeutic advancements in this area.

The initial development of macrophage polarization into defined functional groups was an important early concept that structured and informed our understanding of how macrophages could behave in vivo. However, much of the data that support polarization were primarily based on in vitro evidence where different culture conditions induced the 2 main subsets; either a classical (M1, pro-inflammatory) or alternative (M2, anti-inflammatory) pattern of chromatin remodeling, transcription factor usage, and gene expression profiles was proposed (12). Recent studies have added considerable depth to this initially dichotomous understanding to develop a concept that favors a broad continuous spectrum of activation states, each associated with diverse surface marker expression and functionality in vivo (13,14). Importantly, what we have come to appreciate in the last few years is that bone marrow–derived blood monocytes and resident tissue macrophages are, in fact, different lineages of cells. Many tissue macrophage populations are derived from embryonic development, self-renew in tissue, and may themselves be heterogeneous in cell surface markers and functions (15). Thus, conceptualizing recruited monocytes, monocyte-derived macrophages, and resident tissue macrophages as functionally unique populations has become a prism that we must view previous, current, and future studies through.

In this issue of *JACC: Basic to Translational Science*, Mongue-Din et al. (16) discovered a novel reactive oxygen species pathway that could direct macrophage polarization post-MI. The authors found that nicotinamide adenine dinucleotide phosphate oxidase-4 (Nox4),

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which predominantly generates hydrogen peroxide, is upregulated in heart tissue post-MI. Cardiomyocyte-specific *Nox4* overexpression increased survival rates and improved cardiac function, which are favorable effects seen in both ischemia-reperfusion and completed infarct mouse models. The authors also showed that *Nox4* overexpression was associated with increased expression of CD206 on cardiac macrophages which suggested that macrophages may have been polarized toward an "anti-inflammatory (M2) phenotype" because CD206 is a marker of this subset. These data suggested that *Nox4* activity in cardiomyocytes may have therapeutic potential.

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Although the separation of monocyte influx and numerical expansion of resident cardiac macrophages was not assessed in this study, it would be interesting to speculate. One of the most fascinating aspects of the study was their observations of cardiac macrophage abundance at baseline. The authors found that overexpression of *Nox4* within cardiomyocytes increased the number of cardiac macrophages, and that this was associated with increased expression of the monocyte chemokine CCL2. Because resident cardiac macrophages seed the heart during early embryonic development and do not require signaling through the CCL2 receptor (CCR2) (17,18), these data suggest that monocytes may be recruited to the myocardium before injury, and thereafter they differentiate into tissue macrophages. Although future studies are required to determine whether the cardiomyocyte Nox4 targets monocytes or resident cardiac macrophages directly, increased macrophage density correlated to improved outcomes post-MI, despite the presumed peripheral source of those cells. This is in contrast to other more inflammatory models, such as in the setting of hyperlipidemia, where there is also increased cardiac macrophage density before ischemic injury; however, cardiac injury and adverse remodeling are amplified post-MI (10).

It is also interesting to speculate how the local cardiac microenvironment shapes both the composition of cardiac macrophages (embryonic-derived vs. recruited monocytes), and also how the function may be altered by reactive oxygen species. To truly understand whether macrophage polarization is altered in vivo, more advanced techniques, such as single-cell RNA-sequencing technology coupled with epigenetic analyses will be required to determine whether fully differentiated macrophages have changed their polarization state. These data harken back to the age-old question: which is more critical, the ontogeny "nature" or the tissue environment "nurture"? The reality is rather complex; it appears that neither ontogeny nor polarization alone dictate the function of macrophages. For instance, primitive yolk-sac derived macrophages, fetal liver monocytes, and adult monocytes can all colonize an empty alveolar macrophage niche and develop into functional alveolar macrophages, indicating that the tissue environment instructs a diverse group of myeloid cells to adopt a similar, if not identical, alveolar macrophage fate (19). However, when those subsets compete against each other, it is the fetal monocytes that outcompete the others, suggesting this particular lineage is more capable of receiving instructive signals from the tissue (in this case it was shown to be granulocyte-macrophage colony-stimulating factor) than the other lineages. Detailed analyses of macrophage-specific enhancers reveal that both lineage- and tissue-specific transcription factors control chromatin specification in tissue resident macrophages (20). Therefore, it is likely the primary factor that dictates macrophage fate and function is the tissue environment; however, that can be modified by macrophage ontogeny—the balance of which is context and organ-specific. A promising future field of study can be the investigation of macrophage activation from different lineages under disease condition (e.g., infection and tissue injury), making use of traditional phenotypic methods and lineage tracing models. These studies would eventually decipher the nature versus nurture question of macrophage function.

In summary, the role of macrophages in MI is multifaceted, governed by their diverse developmental origin and a myriad of unknown, local cardiac-specific stimuli. Beyond the interesting protective aspects of *Nox4* in the post-MI setting, the study of Mongue-Din et al. (16) provides a glimpse into the potential contribution of cardiomyocyte *Nox4* pathway to altering cardiac macrophage density before ischemic injury.

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