Acute myocardial infarction (MI) triggers healing and compensatory responses that serve to restrain damage and maintain cardiac output in the face of myocyte death. These encompass both local events (e.g., inflammatory cell infiltration, effector cytokines, myofibroblast transformation, and extracellular matrix turnover) and global events (e.g., neurohormonal activation and augmented wall stress) that ultimately result in the generation of a stable infarct scar capable of withstanding distending forces together with variable degrees of left ventricular (LV) enlargement.1-3 The size of the initial infarct and the adequacy of the ensuing wound healing response are both key determinants of the attendant LV dilatation and heart failure. In humans, the degree of LV dilatation after MI is the primary determinant of late mortality.4,5 It was originally proposed that this biphasic tissue macrophage response after MI resulted from the sequential recruitment of Ly6C<sup>hi</sup> and Ly6C<sup>low</sup> monocytes from the circulation into the heart4,5; however, the aforementioned studies did not use flow cytometry strategies that were sufficiently stringent to discriminate between Ly6C<sup>hi</sup>-expressing monocytes vis-à-vis macrophages. Moreover, the sequential monocyte recruitment model was at odds with recent studies demonstrating that Ly6C<sup>low</sup> monocytes do not contribute to the pool of macrophages generated from extravasated monocytes during inflammation, but rather provide vascular surveillance and orchestrate the killing of damaged endothelial cells.16,17 This paradigm also contrasted with studies of skeletal muscle18 and hepatic injury,19 which indicated that reparative Ly6C<sup>low</sup> macrophages derive from local phenotypic switching of proinflammatory Ly6C<sup>hi</sup> monocytes in response to environmental cues. These observations suggested a need for reappraisal of this schema of macrophage infiltration in the post-MI heart.

Accordingly, in this issue of Circulation Research, using an elegant study design, Hilgendorf et al20 advance their previous studies and provide important and definitive insights into both the source and role of proinflammatory and reparative macrophages in the infarcted heart. Using highly stringent flow cytometry approaches, they evaluated post-MI monocyte and macrophage infiltration in chimeric mice deficient for the orphan nuclear hormone receptor nuclear receptor subfamily 4, group a, member 1 (Nr4a1<sup>−/−</sup>) in hematopoietic cells (Nr4a1<sup>−/−</sup> mice); this receptor is essential for the development and survival of Ly6C<sup>low</sup> monocytes.21 Notably, chimeric Nr4a1<sup>−/−</sup> mice still exhibited the biphasic monocyte/macrophage response, despite the demonstrated absence of circulating Ly6C<sup>low</sup> monocytes. When taken together with Ly6C<sup>hi</sup> and Ly6C<sup>low</sup> monocyte fate-mapping experiments, the data indicated that both phases derive from circulating Ly6C<sup>hi</sup> monocytes—an initial proinflammatory Ly6C<sup>hi</sup> monocyte (and macrophage) dominant phase and a delayed Ly6C<sup>hi</sup> monocyte-derived reparative Ly6C<sup>low</sup> macrophage phase.

The microenvironment in the infarcted heart supported phenotypic switching to reparative macrophages exhibiting augmented Nr4a1 expression in wild-type mice, as well as relatively robust local macrophage proliferation in both chimeric Nr4a1<sup>−/−</sup> and wild-type mice. Loss of Nr4a1 in

### Critical Re-Evaluation of the Biphasic Monocyte/Macrophage Infiltrative Response

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**Editorial**

It Takes Two to Tango

Monocyte and Macrophage Duality in the Infarcted Heart

Sumanth D. Prabhu

Immune Cells and Wound Healing Post-MI

Immune cells are critical for local infarct zone remodeling. Both innate and adaptive immune cell types dynamically infiltrate into the infarct, with the highest activity 1 week after MI, followed by a subsequent decline. Following the early appearance of neutrophils, monocytes/macrophages are the predominant cells and display phasic functional heterogeneity that serve to guide proper wound healing. In a seminal report, Swirski et al demonstrated that monocytes are rapidly recruited from a splenic reservoir to the infarcted heart where they accumulate and participate in tissue repair. Subsequent studies from these and other investigators suggested that monocytes/macrophages infiltrate the infarct, with the highest activity 1 week after MI, followed by a subsequent decline. Following the early appearance of neutrophils, monocytes/macrophages are the predominant cells and display phasic functional heterogeneity that serve to guide proper wound healing. In a seminal report, Swirski et al demonstrated that monocytes are rapidly recruited from a splenic reservoir to the infarcted heart where they accumulate and participate in tissue repair. Subsequent studies from these and other investigators suggested that monocytes/macrophages infiltrate the infarct, with the highest activity 1 week after MI, followed by a subsequent decline. Following the early appearance of neutrophils, monocytes/macrophages are the predominant cells and display phasic functional heterogeneity that serve to guide proper wound healing. In a seminal report, Swirski et al demonstrated that monocytes are rapidly recruited from a splenic reservoir to the infarcted heart where they accumulate and participate in tissue repair. Subsequent studies from these and other investigators suggested that monocytes/macrophages infiltrate the infarct, with the highest activity 1 week after MI, followed by a subsequent decline. Following the early appearance of neutrophils, monocytes/macrophages are the predominant cells and display phasic functional heterogeneity that serve to guide proper wound healing.
Spleen
Altered splenic niche?
Immune cell function?

Heart - Acute MI

Therapeutic Targets
Endogenous Triggers?
Inhibitors?

Tissue Digestion
Inflammation

Ly6C<sup>hi</sup> Mo

Ly6C<sup>hi</sup> Mo

Ly6C<sup>lo</sup> Mo

Nr4a1+Ly6C<sup>lo</sup>

Phase 1
Phase 2

Local Mφ proliferation

Mφ Persistence?
Phenotypic Plasticity?

Chronic LV remodeling
Ischemic Cardiomyopathy

Figure. The center box, in black, summarizes the results of the study by Hilgendorf et al<sup>20</sup> and prior work from the authors regarding 2 phases of monocyte (Mo) and macrophage (Mφ) infiltration into the heart after myocardial infarction (MI). Both the initial proinflammatory Ly6C<sup>hi</sup> monocyte (and macrophage) dominant phase (peak ≈3 days) and the delayed reparative Ly6C<sup>lo</sup> macrophage phase (peak ≈7 days) derive from circulating Ly6C<sup>hi</sup> monocytes mobilized from the spleen, the latter phase triggered by the induction of nuclear receptor subfamily 4, group a, member 1 (Nr4a1) and indicative of phenotypic switching. Moreover, macrophages were shown to proliferate locally in the remodeling heart. The boxes in blue put forth 3 conceptual/translational implications raised by these studies related to (1) the identification (and potential therapeutic targeting) of the local environmental factors, as of yet unidentified, that trigger reparative macrophage polarization; (2) the potential impact of sustained local macrophage proliferation and persistence on the pathogenesis of late remodeling and ischemic cardiomyopathy; and (3) the effect of alterations in splenic niches and immune cells on the tissue reparative process. See text for details and further discussion. LV indicates left ventricular.
splenic niches and splenic immune cells can affect monocyte function and mobilization (eg, see Zouggari et al22) in a manner that interferes with the proper orchestration of remote phasic macrophage responses. Indeed, in chronic ischemic heart failure, the spleen undergoes intense white pulp remodeling and exhibits more prominent proinflammatory features such that activated splenocytes that home to the heart induce fibrosis and tissue damage.22 However, whether specifically limiting splenic LyC6hi monocyte mobilization beyond the early post-MI period modulates subsequent long-term pathologic remodeling is unknown.

In summary, the study by Hilgendorf et al20 significantly informs and advances our understanding of the derivation and roles of the monocyte and macrophage phenotypes in the infarcted heart and its relationship to post-MI remodeling. By using chimeric Nr4a1−/− mice and stringent flow cytometry and fate-mapping strategies, they have identified Nr4a1 as a potential molecular regulator of reparative macrophage differentiation and have established that macrophages proliferate and replenish locally in the infarcted heart. Their study adds to the growing body of evidence supporting the signal importance of macrophages, and other immune cell types, in the genesis of both early and late remodeling after MI and gives further credence to the concept that immune cell modulation represents a novel therapeutic avenue for both improving cardiac repair and alleviating heart failure.

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None.

References

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