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Defining macrophages in the heart one cell at a time

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Abstract

Macrophages in the heart have dual roles in injury and repair after myocardial infarction, and understanding the two sides of this coin using traditional “bulk cell” technologies has been challenging. By combining genetic fate-mapping and single-cell transcriptomics, a new study reveals how distinct macrophage populations expand and diverge across the healthy heart and after infarction.

Macrophages are fundamental to maintaining tissue homeostasis and mounting host defense against pathogens[1]. The tissue macrophage pool originates from both “resident” macrophages that are embryonically seeded and maintained through self-renewal, and from monocytes that are recruited from the blood and differentiate in response to their local tissue environment[2]. Regardless of their origin, these mononuclear phagocytes are remarkably plastic, and their functional phenotypes in tissues vary over the course of pathological processes. This is exemplified by the highly dynamic changes in macrophage number and phenotype in the heart after myocardial infarction (or heart attack)[3]. Cardiac macrophages mediate both the initial inflammatory response to injury and tissue repair, and imbalance in either of these responses leads to adverse cardiac remodeling and cardiovascular-specific outcomes[4,5]. A recent study[6] used single cell RNA sequencing (RNA-seq) to perform high-resolution mapping of the myeloid compartment of the mouse heart under normal conditions and following myocardial infarction. By combining this powerful technique with fate mapping strategies, Dick et al. revealed the cellular and functional heterogeneity of monocyte, macrophage and dendritic cells in the heart, and delineated how these various myeloid subpopulations change over the course of disease. This is an exciting illustration of how single-cell technologies can help discriminate unique immune cell subpopulations in tissues that shift or emerge in response to tissue perturbations or disease. Such understanding has the promise to help guide new interventional strategies to block or enhance the actions of distinct cell subpopulations, including inflammatory and tissue reparative macrophages in the heart.

Classic experimental approaches to defining macrophage tissue subpopulations and their dynamics in health and disease have relied on a limited number of cell surface markers. There has been little consensus in the field on the markers that distinguish resident and

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monocyte-derived macrophages, and in certain contexts, these macrophage subsets can be difficult to distinguish[7,8]. Single cell RNA-seq overcomes these limitations by allowing unbiased determinations of cell population substructures based on the most prominently expressed gene sets. By combining this strategy with *Cx3cr1* fate mapping and depletion approaches, Dick et al identified four distinct macrophage populations in the healthy adult mouse heart including one resident population that was maintained independently of blood monocytes (TIMD4^{+}LYVE1^{+}MHCII^{lo}CCR2^{-}), one that was partially replaced (TIMD4^{-}LYVE1^{-}MHCII^{hi}CCR2^{-}) and two that were fully replaced (CCR2^{+}MHCII^{hi} subsets) by blood monocytes. In these newly defined populations, *Timd4* (phosphatidylserine receptor TIMD4) and *Ccr2* expression alone were able to identify resident and recruited monocyte-derived macrophage populations, respectively. The transcriptomes of the four macrophage populations also revealed unique functional signatures, suggesting that they take on distinct roles in maintaining normal tissue homeostasis. For example, transcriptome pathway analysis revealed that genes defining the *Timd4* cluster were related to endocytosis, lysosome function, angiogenesis and regeneration. homeostatic and regenerative, while the *Ccr2* and *Isg* clusters were enriched in genes involved in inflammatory pathways, including respiratory burst, response to interleukin-12, interferon-γ and type I interferons. These studies not only reveal new markers for defining macrophage subpopulations, but also a hierarchy of resident and monocyte-derived macrophage contributions to functional roles in the healthy myocardium.

After a myocardial infarction, the number and heterogeneity of the myeloid population in the heart increases dynamically. It is in this setting that the power of single cell RNA-seq becomes a major advantage. Using a mouse model of myocardial infarction induced by surgical ligation of the left anterior descending coronary artery, Dick et al. show that in addition to the four macrophage subsets already described, seven new myeloid cell populations were identified in the heart. More than 60% of the macrophages profiled in the heart after infarction fell into these new clusters, whose distinct transcriptional signatures suggest that different functional processes (e.g., inflammation, tissue repair) are being carried out by distinct macrophage subtypes. Furthermore, although the four macrophage populations detectable under non-pathologic conditions were unchanged in their transcriptome, the resident macrophage pool was markedly depleted in the cardiac infarct zone within 2 days of infarction. In the peri-infarct zone, this population underwent proliferative expansion, but still had not returned to baseline levels at 28 days post-infarction. Notably, although resident macrophages comprised only 2–5% of total cardiac macrophages within the infarct zone in the week after infarction, their depletion revealed essential reparative functions. Selective elimination of the resident macrophage population using an inducible *Cx3cr1*-based strategy showed no impact on infarct size or cardiac function one week following infarction, but worsened long-term infarct healing and increased cardiac hypertrophy and fibrosis after four weeks. These experiments indicate that despite the high numbers of monocyte-derived macrophages in the heart after ischemic injury, resident cardiac macrophages have non-redundant, cardioprotective roles that are not adopted by monocytes recruited from the circulation.

Initial studies in human cardiac tissue suggest that similar macrophage subpopulations exist in humans as in mice, but this is an area that will require more studies. Based on CCR2 and

MHC-II expression, Dick et al identified three macrophage subpopulations in cardiac tissue from subjects with cardiomyopathies. In particular, the CCR2^{-}MHC-II^{hi} subset expressed many genes found in the murine *Timd4* cluster, including *TIMD4*, *LYVE1*, *CD163* and *IGF1*, which could be used to classify resident macrophages. CCR2 defined recruited macrophages and this population was divided into MHC-II^{hi} and MHC-II^{lo}, which correlated with the *Isg* and *Ccr2* clusters found in mice. Further studies will be needed to understand the temporospatial dynamics of these human macrophage subsets in the healthy heart and after myocardial infarction, but this work has provided an initial roadmap. This study also provides the foundation for a plethora of new areas of exploration. For example, in both humans and mice, how do these newly identified macrophage populations in the heart fluctuate across normal physiologic conditions, and do they change with age, lifestyle factors (e.g., stress and exercise), and other cardiac pathologies? How do local microenvironmental cues regulate the emergence, diversity and size of these macrophage pools, and what are the spatial dynamics of these populations in the myocardium? Are there specific macrophage subpopulations that may be targetable to reorient excessive inflammation or aberrant tissue repair? Many of these questions will require combining single cell approaches with classic and emerging technologies, such as fate mapping, immunofluorescence microscopy, flow cytometry-based approaches (e.g., Cite-Seq, cytometry by time of flight (CyTOF)], and spatial transcriptomics and nanotechnology-based approaches) to achieve higher temporal, spatial and functional resolution. Although such translational studies may be quite challenging, the potential reward is hefty given that myocardial infarction is a leading cause of death worldwide.

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