Cardiac macrophage biology in the steady-state heart, the aging heart, and following myocardial infarction

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Abstract

Macrophages play critical roles in homeostatic maintenance of the myocardium under normal conditions and in tissue repair after injury. In the steady-state heart, resident cardiac macrophages remove senescent and dying cells and facilitate electrical conduction. In the aging heart, the shift in macrophage phenotype to a proinflammatory subtype leads to inflammaging. Following myocardial infarction (MI), macrophages recruited to the infarct produce both proinflammatory and anti-inflammatory mediators (cytokines, chemokines, matrix metalloproteinases, and growth factors), phagocytize dead cells, and promote angiogenesis and scar formation. These diverse properties are attributed to distinct macrophage subtypes and polarization status. Infarct macrophages exhibit a proinflammatory M1 phenotype early and become polarized toward an anti-inflammatory M2 phenotype later post-MI. Although this classification system is oversimplified and needs to be refined to accommodate the multiple different macrophage subtypes that have been recently identified, general concepts on macrophage roles are independent of subtype classification. This review summarizes current knowledge about cardiac macrophage origins, roles, and phenotypes in the steady state, with aging, and after MI, as well as highlights outstanding areas of investigation.

INTRODUCTION

Macrophages were first identified by Ilya Ilyich Mechnikov in 1882 and belong to the vertebrate first-line defense system against infection and injury.[1] With the advent of genetic fate mapping and tracing techniques (cell reporter lines, parabiosis, bone marrow transplant, and intravital microscopy), our understanding of macrophage physiology has been revolutionized over the past decade. We now know that macrophages reside in all organs in the steady state.[2] Tissue resident macrophages persist from embryogenesis into adulthood and minimally rely on monocyte infiltration for renewal, with the exception of skin and gut macrophages that depend on monocyte entry to maintain numbers.[3,4] In response to infection or injury, circulating monocytes are mobilized to inflamed tissue and differentiate

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into macrophages, which constitute the majority of the macrophage population during the acute inflammatory phase.[5]

In the steady state, tissue resident macrophages exert homeostatic functions, including defending against infection and removing senescent or damaged cells. Moreover, macrophages exhibit distinct organ and tissue-specific physiological functions. For instance, skin macrophages participate in regulating salt-dependent extracellular volume and blood pressure homeostasis.[6] In adipose tissue, macrophages generate catecholamines to sustain adaptive thermogenesis and promote insulin resistance by nuclear receptor co-repressor–dependent mechanisms.[7,8] Peritoneal macrophages orchestrate migration of immunoglobulin A-producing B cells to the intestine, where they play a key role in the early response to pathogens.[9] Macrophages are also involved in erythrocyte removal and iron recycling in the liver, synaptic pruning and normal brain development, and hematopoietic control in the bone marrow and spleen.[10–12]

In addition to maintaining equilibrium, the macrophage plays an indispensable role in response to injury, including myocardial infarction (MI) both in the presence and absence of reperfusion. The importance of macrophages during post-MI remodeling has been high-lighted by studies in which depletion of macrophages by clodronate liposomes compromises cardiac repair in mouse MI models.[13,14] Following MI, macrophages can secrete proinflammatory, anti-inflammatory, proangiogenic, or proreparative factors; can phagocytize dying cells; and can directly interact with other cell types to orchestrate the repair response.[15,16] The diverse functions of macrophages are partially attributed to their different phenotypes and polarization status. Macrophage polarization is a process by which macrophages exhibit vastly different gene expression profiles and functions in response to extremes in environmental signals. Post-MI macrophages show a proinflammatory M1 phenotype early and an anti-inflammatory M2 phenotype later, with these phenotypes playing distinct and even opposite roles.[17,18] In this review, we will discuss current literature regarding cardiac macrophage origins, roles, and phenotypes in the steady state, the aging heart, and post-MI, as well as emphasize outstanding areas of investigation to complete our understanding of macrophage polarization in the heart.

MONOCYTE/MACROPHAGE MARKERS

Monocytes and macrophages have been assessed by multiple approaches using a variety of markers to label cells and cell subtypes. Table I provides a comprehensive list of monocyte and macrophage markers that have been used. Ly6C/Gr-1 is expressed in rodents, but not in humans, whereas all other markers in Table I are expressed in both rodents and humans. Single-marker labeling is commonly used in experiments with immunohistochemistry, immunoblotting, or immunofluorescence approaches. One underappreciated concept is the fact that the marker used to identify cell type by itself has biological functions. For instance, the most commonly used macrophage marker F4/80 has proinflammatory properties and can induce antigen-specific regulatory T cells (Tregs).[33] Distinct gating strategies using flow cytometry can delineate monocyte and macrophage origin and subset types based on marker expression patterns. Table II summarizes current gating strategies to discriminate distinct blood and cardiac monocyte and macrophage phenotypes under steady state and after injury.

In addition, the Macrophage Community Website (www.macrophages.com)[51] and the Immunological Genome Project (www.immgen.org) provide excellent macrophage database resources.

MACROPHAGES IN THE STEADY STATE HEART

Macrophage origins

The earlier dogma that macrophages are exclusively derived from circulating monocytes generated by the bone marrow and spleen has been challenged.[52] In the past decade, a growing body of literature demonstrates that tissue resident macrophages, in the brain, spleen, liver, lung, bone marrow, kidney, pancreas, peritoneum, and heart are established prenatally, persist throughout the life span, and self-renew locally.[53,54] In the steady state, resident cardiac macrophages in mice are reported to account for approximately 5%–10% of nonmyocytes in the heart.[43,55] Resident macrophages adopt a spindle-like shape and intermingle closely with myocytes, endothelial cells, and fibroblasts.[43] Genetic fate mapping and lineage-tracing studies reveal that the vast majority of resident cardiac macrophages originate from embryonic yolk sac and fetal liver progenitors (Fig 1).[19] Replenishment occurs at the rate of about once per month via proliferation.[19,43] In terms of subpopulations, CCR2+ macrophages are replenished by blood monocyte recruitment and local proliferation, whereas CCR2− macrophages are repopulated largely by local proliferation (Fig 1).[19]

Macrophage roles

Cardiac macrophages in the healthy state closely resemble alternatively activated anti-inflammatory M2 macrophages, expressing a plethora of M2-designated markers.[5,56] This is logical, as M2 macrophages promote tissue rebuilding after injury and thus help to re-establish homeostasis. In terms of cell physiology, resident macrophages can engulf fluorescently labeled bacteria, indicating the capacity to phagocytose dying cells.[43] The Nahrendorf et al. recently revealed a novel function for macrophages in the healthy mouse heart. Using specific macrophage reporter lines in combination with optical clearing techniques and confocal microscopy, they for the first time demonstrated that macrophages are abundant in the atrioventricular (AV) node, and these AV nodal macrophages intervene with cardiomyocytes through connexin-43-containing gap junctions to accelerate myocyte repolarization and electrical conduction.[57] This is supported by the observation that deleting connexin-43 in macrophages delays AV conduction, and macrophage deletion induces AV block.[57] Therefore, the macrophage is involved in myocardial conduction, representing a novel target to treat cardiac arrhythmias. Although this study only evaluated the steady state, it raises interesting questions as to whether macrophages are involved in arrhythmia generation after injury and whether macrophages help to regulate myocyte contraction under normal conditions.

Macrophage phenotypes

In the steady state, resident cardiac macrophages are heterogeneous in origin. Different laboratories have divided macrophages into subpopulations using different markers. Four populations expressing varying levels of Ly6C and major histocompatibility complex class II (MHCII) have been identified in the mouse heart.[58] Of these populations, the Ly6C−CCR2−

population comprises the vast majority, which originates from the yolk sac and contains MHCII^{high} and MHCII^{low} subsets. The third (Ly6C$^+$CCR2$^-$) and the fourth (Ly6C$^+$CCR2$^+$) subsets are both derived from hematopoiesis.[19,58,59] The exact roles for these macrophage subsets are incompletely understood. MHCII^{high} cardiac macrophages more efficiently present antigen to T lymphocytes, whereas MHCII^{low} cells have higher phagocytic capability. CCR2$^+$ macrophages express high levels of NLRP3-inflammasome associated genes, implying a proinflammatory role for this subtype.[19]

Cardiac macrophages in mice can also be divided into 4 populations based on CX3CR1 and MHCII expression: CX3CR1$^-$MHCII$^-$, CX3CR1$^-$MHCII$^+$, CX3CR1$^+$MHCII$^-$, and CX3CR1$^+$MHCII$^+$.[31] Molawi *et al.* demonstrated that almost all macrophages at birth were CX3CR1$^+$MHCII$^-$, and with age, there was a progressive increase in MHCII$^+$ cells and a decrease of CX3CR1$^+$ population, leading to a more even distribution of these cell populations by adulthood.[31] Combined, the above studies showed the existence of MHCII$^-$ and MHCII$^+$ macrophages. However, the question about the relationship between CCR2 and CX3CR1 lineages remains to be addressed.

MACROPHAGES IN THE AGING HEART

Macrophage origins

Aging is a major risk factor for cardiac morbidity and mortality. Cardiac aging is characterized by myocardial sarcopenia, hypertrophy, vascular hyperpermeability, inflammation, fibrosis, and mild cardiac physiology impairment.[60–62] In mice, blood pressure does not increase with age, and thus changes in the heart due to age can be attributed to direct changes on the myocardium rather than alterations in ventricular pre-load or after-load.[63] In mouse studies, young (<9 months), middle-aged (12–15 months), old (18–24 months), and senescent (>26 months) mice are commonly used to define different age groups.[47,63] We have previously reported that the number of cardiac macrophages in mice increases beginning at about 18 months, and numbers positively correlate with age.[47,63,64] By genetic fate mapping and parabiotic approaches, Molawi *et al.* have reported that with age, self-renewal of resident cardiac macrophages in mice declines, and blood monocytes increasingly contribute to the cardiac macrophage population.[31] Although this study used young mice (2- to 9-month old),[31] it is likely that in the aging heart, macrophages derive from both mechanisms (self-renewal and blood monocyte differentiation) (Fig 1).

Macrophage roles

Aging involves an upregulation in the basal inflammatory response, a process termed inflammaging.[65,66] The macrophage is a key contributor, evidenced by increased numbers of cardiac macrophages and enhanced levels of proinflammatory molecules such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, matrix metalloproteinases (MMPs), and chemokine C-C motif ligand-2 (CCL2)/monocyte chemoattractant protein-1 during cardiac aging.[67] Macrophages produce MMP-9 and CCL2, both of which positively correlate with the increase in LV dimensions, indicating a role for macrophages in cardiac aging.[67]

Immunosenescence refers to the gradual deterioration of the immune system with age, with concomitant higher incidences of infection, neoplasia, autoimmune, and cardiovascular diseases, as well as a worse prognosis after infection or injury.[68–70] Compared to young controls (10–12 week old), splenic macrophages from 18–20 month old mice exhibit reduced responses to a variety of proinflammatory or anti-inflammatory stimuli, indicative of age-induced desensitization.[71] Old macrophages also display impaired phagocytic capacity and reduced production of nitric oxide and hydrogen peroxide.[72,73] Macrophages from old mice produce higher levels of immunosuppressive prostaglandin E2, which contributes to dysregulated immune function.[74] Taken together, aging induces immune senescence to increase the susceptibility to and poor prognosis after cardiovascular disease. [75]

Macrophage phenotypes

How the different subpopulations of resident cardiac macrophages in the steady state change with age in mice has been evaluated. Using flow cytometry, our laboratory has shown that there is a linear increase in cardiac proinflammatory M1 (F4/80$^+$CD206$^-$) macrophages and a decrease in anti-inflammatory M2 macrophages (F4/80$^+$CD206$^+$) with age.[47] This is prevented by MMP-9 deletion, indicating that MMP-9 modifies aging related macrophage polarization.[47] The fact that in vitro MMP-9 alone activates young macrophages to an M1/M2 mid-transition phase implies that other unknown factors contribute to in vivo age-induced macrophage M1 polarization.[47] Increased inflammatory macrophages during cardiac aging may be a result of exaggerated monocyte recruitment, alterations in monocyte fate specification, or changes in resident macrophage behavior. The precise functions of aging associated M1 and M2 macrophages and how many additional macrophage phenotypes there are need to be determined.

MACROPHAGES IN THE MI HEART

Macrophage origins

Following MI, abundant blood monocytes infiltrate the ischemic and border regions and differentiate into macrophages, which is the major source of infarct macrophages during the first 7 days post-MI (Fig 2).[43] Immediately on exposure to ischemia, resident cardiac macrophages begin to die and are almost completely removed by 24 h post-MI in mice; and their numbers begin to recover by day 4 post-MI. After the acute phase of monocyte recruitment in the first 2 weeks post-MI, resident macrophages in the infarct regain independence from blood monocytes and can proliferate locally.[43] This is perhaps caused by the differentiation of local progenitor or stem cells into tissue resident macrophages.

Remote nonischemic myocardium also exhibits alterations in inflammation and macrophage numbers after MI, albeit with lower and delayed changes compared to the infarct area. We have previously shown that the remote region has more inflammation than the infarct region at day 28 post-MI, indicating a secondary inflammatory response that occurs at a late time point and distant location.[76] Sager *et al.* recently reported that macrophage numbers in the mouse remote myocardium increased 2.9-fold at 8 weeks after MI, which resulted from both local macrophage renewal and blood monocyte recruitment (Fig 2).[77] More importantly,

inhibition of monocyte extravasation into the cardiac tissue by silencing 5 endothelial cell adhesion molecules (Icam1, Icam2, Vcam1, E-selectin, and P-selectin) decreased macrophage numbers and improved cardiac physiology, suggesting that macrophages contribute to adverse remodeling of the remote myocardium.[77] In this study, the authors also revealed that mechanical strain, the deformation of the heart, could elicit macrophage proliferation.[77]

During the acute inflammatory phase, the majority of macrophages recruited to the ischemic area derive from the differentiation of peripheral blood monocytes, which stem from 2 sources: bone marrow and spleen. The following paragraphs provide detailed information on these sources.

Peripheral blood monocytes—Mouse blood monocytes are heterogeneous and have 2 subsets based on Ly6C expression. Ly6C^{hi} monocytes are inflammatory and express large amounts of chemokine C-X-C motif receptor (CCR) 2 and low chemokine C-X3-C motif receptor (CX3CR) 1 level; and Ly6C^{low} monocytes low CCR2 and high CX3CR1.[17] In the steady state, 50%–60% of circulating mouse monocytes are Ly6C^{hi}CCR2^{high}CX3CR1^{low}CD62 L^{+}, and have a relatively short life span.[15] Ly6C^{low} monocytes arise from Ly6C^{hi} cell conversion, instead of from different progenitors.[78,79] Likewise, the circulating monocyte pool in humans is heterogeneous and divided into 3 phenotypes based on CD14 and CD16 expression. CD14^{++}CD16^{-} monocytes, which resemble mouse Ly6C^{hi} monocytes, account for 80%–90% of total circulating monocytes and are proinflammatory. CD14^{+}CD16^{++} cells are anti-inflammatory and resemble mouse Ly-6C^{low} population in terms of function.[40,80] The third population of CD14^{++}CD16^{+} cells has proinflammatory features and can secrete proinflammatory TNF-α after exposure to lipopolysaccharide (LPS).[81]

Bone marrow hematopoiesis—Circulating monocytes derive from hematopoietic stem cells (HSCs) residing in the bone marrow. HSCs sequentially differentiate into common myeloid progenitors, granulocyte macrophage progenitors, macrophage monocyte progenitors, common monocyte progenitors, and ultimately monocytes in the bone marrow, a process termed hematopoiesis.[82,83] Numerous housekeeping cells, including mesenchymal stem cells, endothelial cells, CD169^{+} macrophages, nerve cells, and osteoblasts reside in the hematopoietic niche and regulate blood cell production. They produce growth factors and fate-regulating signals such as granulocyte colony-stimulating factor, angiopoietin-1, chemokine C-X-C motif ligand (CXCL) 12, and stem cell factor.[83–85] CXCL12 facilitates quiescent HSC retention in the bone marrow.[86] Lower CXCL12 levels after MI lead to the liberation of HSCs from the bone marrow niche, which then migrate to the spleen and differentiate into monocytes that are subsequently recruited to the infarct.[87] CCR2 and β-adrenergic receptor signaling cascade also mediate monocyte mobilization from the bone marrow to circulation (Fig 2).[87,88]

Spleen extramedullary hematopoiesis—The spleen is an additional reservoir for monocytes that actually outnumbers their equivalents in circulation and contributes to the blood monocyte pool. The spleen can generate new monocytes by a process called extramedullary hematopoiesis.[89,90] Splenic monocytes are located in the subcapsular red

pulp of the spleen and resemble circulating counterparts.[15] Splenic hematopoiesis occurs during embryogenesis and in disease settings but not in the steady state after birth. Post-MI, the spleen produces sufficient monocytes to enter the blood and infiltrate the ischemic myocardium (Fig 2). This process is at least partially angiotensin II-dependent and can be attenuated by angiotensin converting enzyme inhibitors.[91,92] Extramedullary hematopoiesis shrinks the mouse spleen weight by 50% and depletes the number of splenic monocytes by 24 h post-MI.[15] IL-1β, IL-3, and granulocyte macrophage colony-stimulating factor (GM-CSF) can modulate the production of splenic monocytes.[90] More importantly, abrogation of extramedullary monocytopoiesis in mice exacerbates adverse cardiac remodeling and heart failure progression, indicating that spleen-derived monocytes are essential for post-MI cardiac repair.[90]

Macrophage roles

As early as 30 min following MI, blood monocytes infiltrate the infarct, initially outnumbering neutrophils.[93,94] Recruitment of monocytes is dependent on activation of the CCL2/CCR2 signaling pathway.[95,96] On arrival at the tissue, these monocytes begin to differentiate into macrophages. Some monocytes, however, do not undergo differentiation; these monocytes serve similar tissue roles as their macrophage counterparts.[97] Delineation between monocytes and macrophages in mice has been shown based on the expression of F4/80/I-A^b/CD11c. Monocytes are (F4/80/I-A^b/CD11c)^{low}, whereas macrophages are (F4/80/I-A^b/CD11c)^{high}.[97] CD64 and myeloid epithelial reproductive tyrosine kinase (MERTK) can also distinguish cardiac monocytes from cardiac macrophages, with macrophages expressing both CD64 and MERTK and monocytes expressing CD64 and not MERTK.[19,98] In the mouse MI model, macrophages are in the infarct peak at days 5–7 after MI.[35,45,99] MI patients show similar but delayed kinetics of macrophage infiltration compared to rodents. Timely reperfusion reduces leukocyte numbers accumulated in the infarct, shifts the peak of the innate immune response earlier, and blunts the adaptive immune response.[45]

Although inflammation is essential for orchestrating post-MI cardiac repair, timely resolution is necessary for favorable cardiac repair. Following MI, there is a burst of acute inflammation over the first 5 days. After this period, inflammation gradually wanes. Targeting monocyte recruitment to attenuate inflammation is protective by enhancing myocardial repair.[77,100] Phagocytosis of apoptotic myocytes and neutrophils by macrophages is a prerequisite for the resolution of inflammation. Impaired macrophage phagocytic capacity prolongs inflammation and impedes post-MI cardiac repair.[101]

Macrophages play pivotal roles in the post-MI wound healing response. Macrophage depletion compromises wound healing and accelerates adverse remodeling, and adoptive transfer of activated macrophages improves cardiac repair.[13,14,102,103] Similarly, clinical findings demonstrate that patients with high inflammatory CD14$^+$CD16$^-$ blood monocyte counts at the onset of MI have larger cardiac dilation at follow-up, and the peak levels of CD14$^+$CD16$^-$ monocytes negatively correlate with the extent of myocardial salvage.[104,105] The macrophage coordinates each phase of the remodeling process, including the acute inflammatory, reparative, and maturation phases. Macrophage roles include: (1) secreting an

extensive array of inflammatory cytokines, chemokines, growth factors, and MMPs to regulate inflammation and degrade the extracellular matrix; (2) phagocytizing dead cell and tissue debris to clean up the wound; (3) producing proangiogenic and proreparative factors (eg, vascular endothelial growth factor and transforming growth factor [TGF]-β1) to facilitate neoangiogenesis and scar building; and (4) presenting antigen to lymphocytes to induce an adaptive immune response.[58] Neonate mice depleted of macrophages lose their myocardial regenerative capacity,[106] and these reparative macrophages are embryonic and not monocyte-derived.[41] These findings indicate that macrophages may mediate myocardial regeneration in the neonatal heart. This field is controversial, and the results need to be further validated. The multi-functional capacity of macrophages is, at least partially, attributed to different cell polarization phenotypes.

Macrophage phenotypes

Macrophages demonstrate high plasticity and adaptability, both in vitro and in vivo. They can adopt differential phenotypes in response to varying stimuli or when residing in varying environments. Macrophages have been classified into classically activated (M1) and alternatively activated (M2) subsets.[107] Macrophage subsets are further divided based on the in vitro stimuli to which they are exposed. For instance, M1 macrophages can be divided into M1a if stimulated with toll-like receptors or M1b subsets if stimulated with high-mobility group protein B1.[58] Subsets also have distinct cell physiology; for example, M1b is less phagocytic than M1a.

M2 macrophages are further subdivided into M2a if stimulated with IL-4 or IL-13 and M2b if stimulated with immune complexes in combination with IL-1β and M2c if stimulated with IL-10, TGF-β, or glucocorticoids.[17,108] M2a and M2c macrophages are primarily responsible for coordinating adaptive immune response, whereas M2b macrophages suppress inflammation.[58,109] More recently, an M4 phenotype has been proposed to describe monocytes exposed to CXCL4.[110] Moreover, different phenotypes can mutually convert under in vitro conditions. For example, M1 macrophages could switch to the M2 phenotype after stimulation with pro-M2 factors, and vice versa.[111] Although macrophage classification and conversion concepts are based on in vitro stimulation responses, the current literature borrows from this nomenclature to define in vivo stimulated macrophages. This complicates communication in the field, as the M1 in vivo stimulated macrophage is much different than macrophages stimulated in vitro by one or a few stimuli.

Macrophages in the infarcted heart are heterogeneous. M1 macrophages dominate at days 1–3 post-MI, whereas M2 macrophages are the major cell at days 5–7 post-MI in the mouse heart.[45] Proinflammatory M1 macrophages secrete cytokines, chemokines, growth factors, and MMPs to help clear the cell debris and degrade extracellular matrix (Table III).[18] However, the prolonged presence of M1 macrophages can lead to expansion of infarct size and impede the resolution of inflammation and scar formation.[100] In contrast, anti-inflammatory M2 macrophages are proreparative.[18] M2 macrophages can produce anti-inflammatory, proangiogenic, and proreparative factors (eg, IL-10, vascular endothelial growth factor, and TGF-β1) and engulf apoptotic cells to facilitate neoangiogenesis and scar repair (Table III). Shifting the balance from M1 to M2 macrophages improves myocardial

repair and function post-MI.[118–121] Likewise, our laboratory has shown that MMP-9, MMP-28, and IL-10 regulate post-MI cardiac remodeling by affecting the M1/M2 balance. [35,112,122–125]

Macrophage polarization mechanisms

The exact mechanisms regarding post-MI in vivo macrophage polarization remain poorly understood. Nahrendorf et al. previously identified sequential infiltration of 2 distinct monocyte subsets into the ischemic heart.[97] Ly6C^{high} monocytes predominate at days 1–4 post-MI due to selective expansion, whereas cardiac Ly6C^{low} cells dominate from days 5 onward due to increased migration capacity. Using genetically modified mice, they also showed that early Ly6C^{high} subset recruitment relies on CCR2, whereas later Ly6C^{low} accumulation depends on CX3CR1 (Fig 3). In addition, Ly6C^{high} monocytes can differentiate into Ly6C^{low} monocytes during the reparative phase and proliferate locally.[44,81] Ly6C^{high} monocytes are proinflammatory, phagocytic, and proteolytic; in contrast, Ly6C^{low} monocytes are anti-inflammatory, proangiogenic, and proreparative.[97] The monocyte time course reported by Nahrendorf et al. is completely consistent with the time course of macrophages reported by Yan et al.[45] As both Ly6C^{high} and Ly6C^{low} cells circulate in the blood, and recruitment is the major source of infarct macrophages, it is reasonable to conclude that infarct proinflammatory M1-like and anti-inflammatory M2-like macrophages are derived from blood Ly6C^{high} and Ly6C^{low} monocytes, respectively. This hypothesis, however, ignores the impact of the local microenvironment on macrophage polarization. Inflammatory monocytes can switch their phenotype to an anti-inflammatory subset and further differentiate into M2-like macrophages in models of skeletal muscle injury and allergic skin.[126,127] Peritoneal macrophages acquire features of pulmonary macrophages after adoptive transfer to the lung.[128] These findings highlight a deterministic role for the microenvironment in guiding polarization of monocytes and macrophages. The infarct microenvironment is filled with early pro-M1 mediators (eg, IFN-γ and GM-CSF) and later pro-M2 factors (eg, IL-10 and TGF-β1), which likely direct macrophage polarization.[112,129] However, the short life span of post-MI monocytes (~20 h) suggests that phenotypic conversion has to be very rapid if it occurs in the MI setting. Alternatively, conversion may account for a small percentage (~20%) of the macrophage pool. Although it is likely that the 2 mechanisms combined determine macrophage polarization (Fig 3), these ideas need to be validated in future experiments. Table III lists known characteristics of MI-associated M1 and M2 macrophages. Additional studies are warranted to systematically identify pathway networks that coordinate post-MI macrophage polarization.

FUTURE DIRECTIONS IN OUR UNDERSTANDING OF POST-MI MACROPHAGE POLARIZATION

The M1 and M2 nomenclature has been helpful for appreciating the heterogeneity of macrophages. The M1/M2 paradigm, however, was originally based on the in vitro stimuli used, surface marker expression, and production of inflammatory associated factors. For instance, LPS + IFN-γ induce macrophage production of proinflammatory Ccl3, IL-1β, IL-6, and TNF-α; and thus, this macrophage is termed M1. IL-4 elicits macrophages to produce anti-inflammatory Cd206, Arg1, Fizz1, and Ym1, namely M2 macrophage markers.

[35] The main limitation with this nomenclature system is that the simple in vitro setting does not reflect the complex in vivo microenvironment. In the ischemic heart, there is a complex mixture of both pro-M1 and pro-M2 stimuli. Defining M1 or M2 phenotype based only on 1 marker (eg, CD206), or even a combination of several M1/M2 markers, does not reflect the in vivo situation. One simple example is that CD206 could not distinguish pre-MI resident vs post-MI M2 macrophages, as both express high levels of CD206.[46] Second, it is arbitrary to force in vivo data onto an in vitro M1/M2 spectrum.[130] Frequently, there is a mixture of M1 or M2 markers that may not follow the simplified in vitro pattern. Macrophages overall may have more total M1 markers, while displaying divergence in particular markers (eg, less TNF-α or IL-10). If only TNF-α and IL-10 are measured and are lower in the comparison group, one might conclude these cells were M2, which would not be accurate based on the other M1 markers. Third, one assumption this classification system makes is that all stimuli induce macrophages to the same phenotype. For example, although LPS + IFN-γ and GM-CSF both trigger an M1 phenotype, transcriptional profiles induced by these 2 stimuli vary, indicating that M1 does not equal M1.[131,132] In view of the limitations stated previously about the current M1/M2 polarization paradigm, we discuss here 3 outstanding areas of investigation needed to better understand macrophage polarization in the post-MI LV.[109]

(1) The polarization phenotypes of cardiac macrophages at day 0 (before MI) and at varying time points post-MI (eg days 1, 3, and 7) need to be systematically mapped. We propose that there are likely differences in cell phenotypes along the post-MI continuum that span beyond the simple M1/M2 paradigm. As mentioned previously, although day 1 and day 3 macrophages have similar M1 phenotypes in terms of some markers, they are likely different in terms of transcriptional programs and cell physiology. In addition, individual cell phenotypes at a given time may be different. At day 3 post-MI, for example, M1 macrophages may also be heterogeneous, reflecting the exact cytokine and chemokine environments they are exposed to on entry into the infarct region, an environment that is in rapid flux over the first days post-MI. Therefore, we need to know the continuous phenotypes across the time course of MI and the variability across individual cell phenotypes at the same time. The first thing we need to know is what markers distinguish phenotypes. This could be addressed by globally examining transcriptional profiles of macrophages isolated from different time point post-MI using RNA sequencing. Flow cytometry could further distinguish individual cell phenotypes.

The use of a novel nomenclature system on the basis of post-MI time when macrophages are activated may be a better way to define macrophage phenotypes. For instance, cM(MI-D1) could be used to denote cardiac macrophages at day 1 post-MI. This system could be used for in vitro macrophages stimulated by different factors as well. cM(IL-4) represents resident cardiac macrophages stimulated with IL-4. The advantage of this classification system is that we can more clearly distinguish what cell type is under examination.

(2) Computational models mimicking post-MI macrophage polarization have not been established.[109] Mathematical algorithms can provide a means to predict outcomes that integrate complex in vivo factors at molecular, cellular, organ, and systemic levels as well as reduce complexity.[133] Algorithms for macrophage physiology have recently been established for some biological processes, such as the acute inflammatory response, chronic

wound inflammation, cholesterol efflux, tumor, and iron release.[134–139] These models do not incorporate macrophage polarization nor have models been developed to describe macrophages in the infarcted heart. Our team has previously developed cellular models of macrophage polarization and myocardial remodeling on a limited scale.[140,141] A more complete computational map that includes macrophage activation factors, signaling network, and phenotypic information is warranted. Building these algorithms requires the building of an initial framework to ensure the establishment of optimum models.[142]

Macrophage polarization has been defined for the most part by single-stimulus responses; we need to examine how macrophages respond to mixed stimuli. Initial models could focus on short-term in vitro treatment of macrophages with different factors known to regulate macrophage polarization and post-MI remodeling. The structure of the computational algorithm could be based on previously known pathways in conjunction with bridging these inputs to specific downstream genes, secreted proteins, and cell physiology outputs.[109,142] Conversion of these initial models into logic-based distinct equations will provide a window for simulations with other key players that may be identified to build on the existing framework.[142] The in silico integration of complex data sets can help define key trigger point responses, combined with bioinformatics analysis to provide a more comprehensive evaluation. Subsequent model iterations could incorporate comprehensive evaluations of transcriptome and secretome profiles, allowing inference of novel players in these processes. Computational models generated for post-MI macrophage polarization could be used in the future to understand cardiac remodeling patterns, which would allow predictions of new therapeutic interventions to be tested, validated, and refined.

(3) There is a need to know how to modulate endogenous and exogenous targets to generate predictable macrophage polarization subsets.[109] Interfering with endogenous signaling cascades will tell us whether the developed models have successfully defined the key drivers of macrophage polarization and accurately dissected their roles in cardiac remodeling. Similarly, modifying exogenous pathways will tell us whether modifying phenotypes could affect outcomes in a predictable manner. Imitating effects of individual components and combinations on macrophage polarization will provide a systematic picture of the in vivo complexity.

CONCLUSIONS

Our understanding of macrophage ontogeny, polarization, and cell physiology has greatly expanded over the past decade. Basic and pre-clinical studies have shown the promising potential of targeting macrophages to prevent adverse cardiac remodeling and physiological deterioration in the post-MI LV. With emerging knowledge of the beneficial and detrimental functions of macrophages, future studies can be aimed at targeting specific detrimental functions while preserving beneficial roles. Establishing the progression of post-MI macrophage polarization and signaling patterns will provide mechanistic insight into how macrophages coordinate cardiac repair and help us identify novel intervention targets. Developing predictable computational models that incorporate the macrophage phenotype continuum will help to achieve this goal.

Acknowledgments

Abbreviations

AV	atrioventricular
CCL	chemokine C-C motif ligand
CCR	chemokine C-C motif receptor
CXCL	chemokine C-X-C motif ligand
CX3CR	chemokine C-X3-C motif receptor
ECM	extracellular matrix
GM-CSF	granulocyte macrophage colony-stimulating factor
HSC	hematopoietic stem cell
IFN	interferon
IL	interleukin
LPS	lipopolysaccharide
MERTK	myeloid epithelial reproductive tyrosine kinase
MHCII	major histocompatibility complex class II
MI	myocardial infarction
MMPs	matrix metalloproteinases
TGF-β1	transforming growth factor-β1
TNF	tumor necrosis factor

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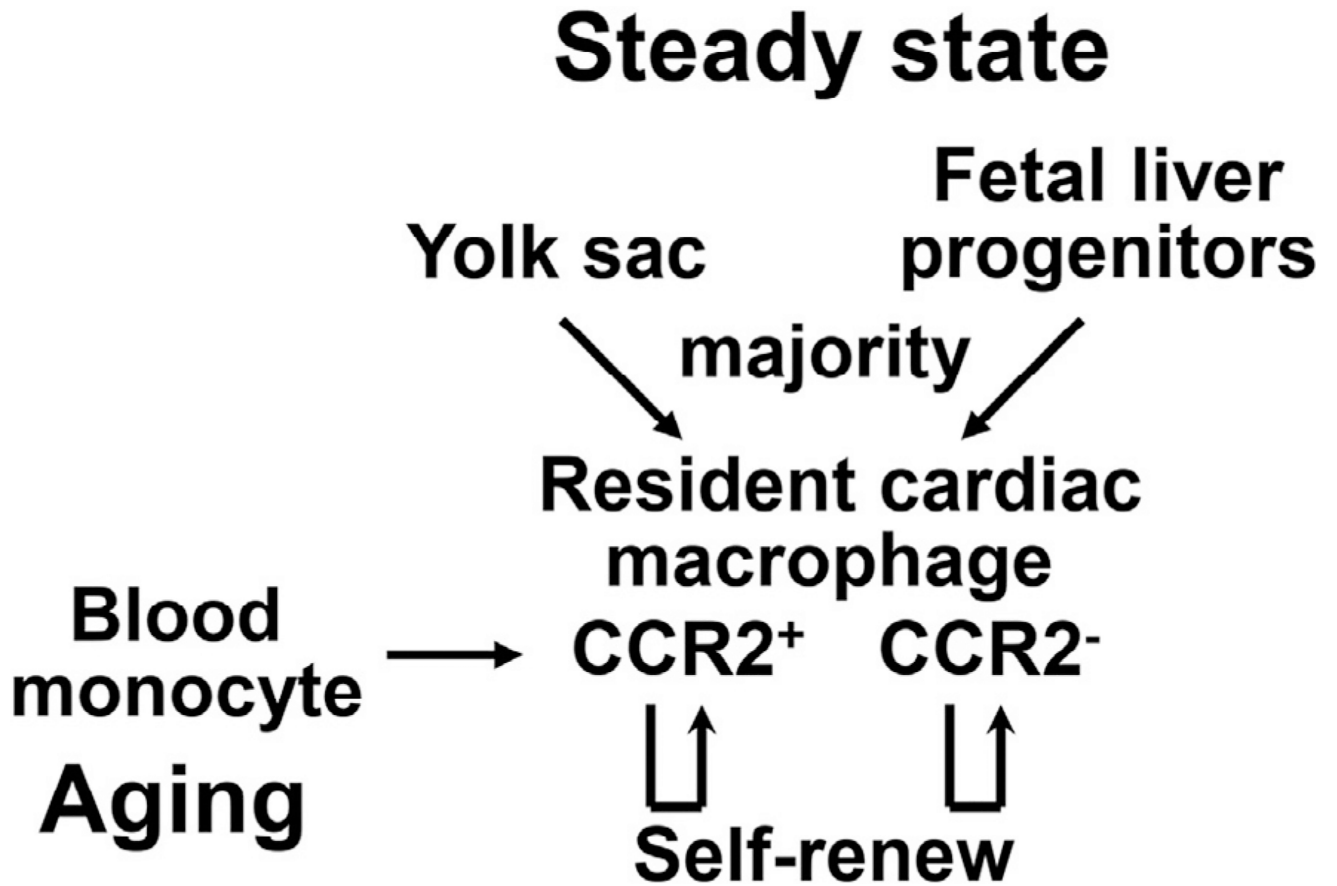
Fig 1.
Macrophage origins in the steady-state heart and the aging heart. In the steady state, the vast majority of resident cardiac macrophages originate from the yolk sac and fetal liver progenitors, with minimal dependence on blood monocytes as a source. In terms of subpopulations, CCR2$^+$ macrophages are replenished by blood monocyte recruitment and local proliferation, whereas CCR2$^-$ macrophages are repopulated largely by local proliferation. With age, self-renewal of resident cardiac macrophage declines, and blood monocytes increasingly contribute to the cardiac macrophage population.
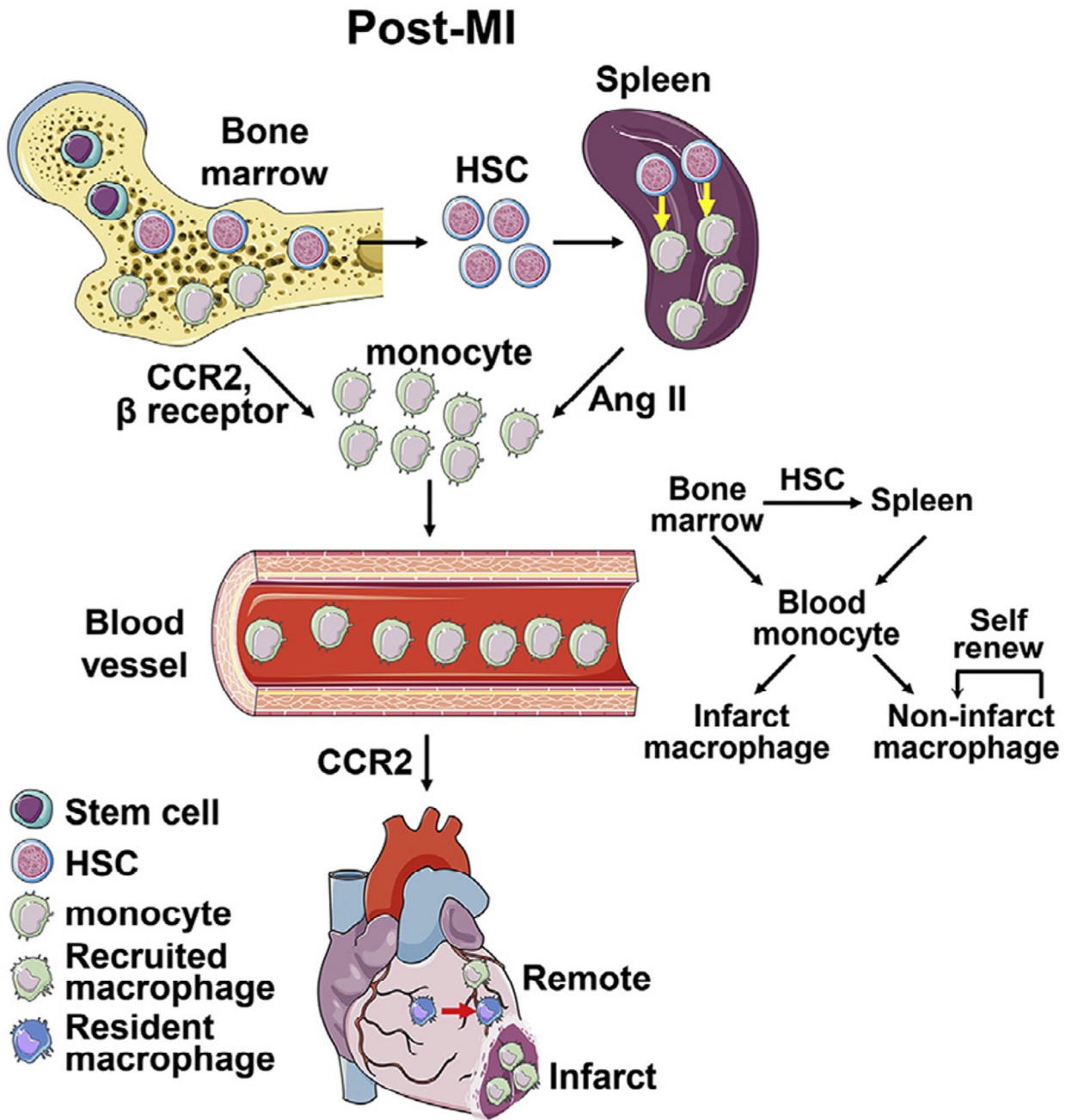
Post-MI

Fig 2.
Macrophage origins in the postmyocardial infarction (MI) heart. Following MI, bone marrow hematopoiesis and extramedullary hematopoiesis by the spleen produce abundant numbers of monocytes, which translocate to the circulation and are recruited to the ischemic heart. In the infarct area, the vast majority of macrophages in the first 3 days are derived from recruitment of blood monocytes, and the renewal of resident macrophages is trivial; in contrast, in the nonischemic remote myocardium, macrophages arise from both local renewal of resident macrophages and recruitment of blood monocytes. The images of cells and organs were obtained from Servier Medical Art (www.servier.com).

Post-MI

Ly6C^high monocyte   Ly6C^low monocyte

CCR2   CX3CR1

M1-like   M2-like

IFN-γ, GM-CSF   IL-4, IL-10, IL-13, TGF-β1
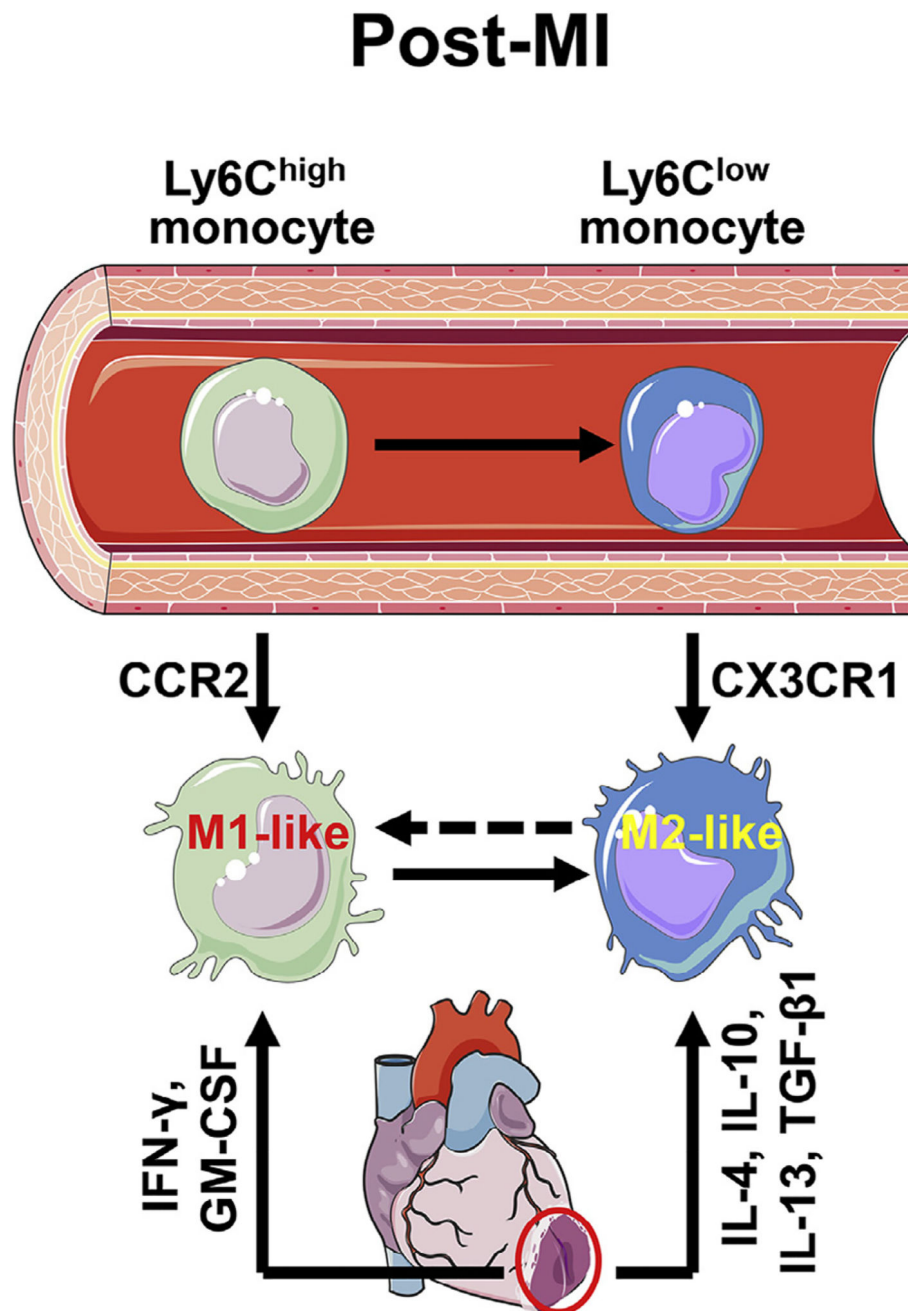
Fig 3.
Proposed post-MI macrophage polarization mechanisms. The integration of 2 mechanisms determines the polarization status of macrophages in the MI heart. (1) M1 and M2 macrophages originate from circulating Ly6C^{high} and Ly6C^{low} monocytes, respectively; Recruitment of Ly6C^{high} monocytes depends on CCR2 signaling, whereas recruitment of Ly6C^{low} monocytes is CX3CR1 dependent; and (2) the mix of pro-M1 and pro-M2 factors existing in the MI myocardium orchestrates macrophage polarization status. MI, myocardial

infarction. The images of cells and organs were obtained from Servier Medical Art (www.servier.com).

Table I

A list of known monocyte and macrophage markers

Marker	Location	Expressed by	Cell physiological functions	References
CCR2/CD192	Cell surface	Monocytes, macrophages	Mediates Ly6C^{high} monocyte recruitment and migration	19,20
CD11 b/ITGAM	Cell surface	Monocytes, macrophages, neutrophils, NK cells	Couples with CD18 to form integrin $\alpha_M\beta_2$ (also named Mac1 or complement receptor 3) to initiate immune responses	21,22
CD14	Cell surface	Human monocytes	Mediates toll-like receptor 4 activation and production of IFN-β	23,24
CD16/FCGR3	Cell surface	Human monocytes	Binds to the Fc portion of IgG antibodies, antigen presentation, anti-inflammatory cytokine production	25
CD64/FcγR1	Cell surface	Monocytes, macrophages	Antibody-dependent phagocytosis, recognizes the Fc region of IgG	19,26,27
CD68/macrosialin	Endosomal/lysosomal compartment, cell surface	Monocytes, macrophages	Antigen processing and presentation, binds to oxidized low-density lipoprotein	28
CD163	Cell surface, secreted (soluble)	Macrophages, neutrophils	Hemoglobin/haptoglobin scavenger receptor, anti-inflammatory	29,30
CX3CR1	Cell surface	Monocytes, macrophages	Mediates Ly6C^{low} monocyte recruitment, inhibits proliferation of local macrophages	31,32
F4/80/EMR1	Cell surface	Macrophages	Promotes proinflammatory factor production, induces antigen-specific efferent Treg cells	33
Galectin 3/Mac2	Cell surface, secreted	Macrophages	Induces monocyte-macrophage differentiation, interferes with dendritic cell fate decision, regulates T cell apoptosis, inhibits B-lymphocyte differentiation into plasma cells	34
Ly6C/Gr-1[*]	Cell surface	Monocytes	A specific marker for proinflammatory monocytes	19
Mac3	Cell surface	Macrophages	A glycoprotein	35,36
MERTK	Cell surface	Macrophages, phagocytes	Mediates phagocytosis, increases migration	19,37
MHCII	Cell surface	Macrophages, dendritic cells, B cells	Mediates antigen presentation	38

[*] Ly6C/Gr-1 is expressed in rodents, but not in humans, whereas all other markers in Table I are expressed in both rodents and humans.

Table II

Gating strategies to label blood and cardiac monocytes and macrophages

Gating strategy	Cells labeled	Species	References
Monocytes			
Ly6C^{high}CCR2^{high}CX3CR1^{low}CD62 L$^+$	Classical monocytes	Mouse	[15]
Ly6C^{low}CCR2^{low}CX3CR1^{high}CD62L$^-$	Nonclassical monocytes	Mouse	[15]
CCR2$^+$Ly6C^{high}	Inflammatory blood monocytes	Mouse	[39]
CD14$^+$CD16$^-$, CD14$^+$CD16$^+$	Blood monocytes	Human	[40]
B220$^-$F4/80$^+$CD115$^+$Ly6C$^-$, B220$^-$F4/80$^+$CD115$^+$Ly6C$^+$	Blood monocytes	Mouse	[40]
MHCII^{low}CCR2$^+$	Cardiac monocytes	Mouse	[41]
CD11 b$^+$F4/80$^-$Ly6G$^-$Ly6C^{high}, CD11 b$^+$F4/80$^-$Ly6G$^-$Ly6C^{low}	Monocytes	Mouse	[42]
Lineage$^-$CD11 b$^+$F4/80^{low}Ly6C$^+$	Cardiac monocytes	Mouse	[43]
CD11 b$^+$CD11C$^-$MHCII$^-$CD68$^-$Ly6C^{low}, CD11 b$^+$CD11C$^-$MHCII$^-$CD68$^-$Ly6C^{high}	Blood and cardiac monocytes	Mouse	[44]
Macrophages			
CD45$^+$CD11 b$^+$F4/80$^+$CD206$^-$	M1 macrophages	Mouse	[45]
CD45$^+$CD11 b$^+$F4/80$^+$CD206$^+$	M2 macrophages	Mouse	[45]
CD45$^+$CD11 b$^+$F4/80$^+$Ly6C^{low}	Resident cardiac macrophages	Mouse	[43]
CD11 b$^+$F4/80$^+$CD206$^+$	Alternatively activated macrophages	Mouse	[46]
CD11 b$^+$F4/80$^+$CD64$^+$Ly6C$^+$MHCII$^{+/-}$	M1 like macrophages	Mouse	[42]
CD11 b$^+$F4/80$^+$CD64$^+$Ly6C$^-$MHCII$^{+/-}$	M2 like macrophages	Mouse	[42]
F4/80$^+$CD86$^+$	M1 macrophages	Mouse	[14]
F4/80$^+$CD206$^+$	M2 macrophages	Mouse	[14,47]
F4/80$^+$CD206$^-$	M1 macrophages	Mouse	[47]
CD45$^+$CD68$^+$	Cardiac, blood, and spleen macrophages	Rat	[48]
CD11 b$^+$F4/80$^+$CD68$^+$Ly6C^{low}, CD11 b$^+$F4/80$^+$CD68$^+$Ly6C^{high}	Monocyte-derived cardiac macrophages	Mouse	[44]
CD14$^+$CD64$^+$MERTK$^+$F4/80$^+$CX3CR1$^+$MHCII$^-$, CD14$^+$CD64$^+$MERTK$^+$F4/80$^+$CX3CR1$^+$MHCII$^+$, CD14$^+$CD64$^+$MERTK$^+$F4/80$^+$CX3CR1$^-$MHCII$^-$, CD14$^+$CD64$^+$MERTK$^+$F4/80$^+$CX3CR1$^-$MHCII$^+$	Resident cardiac macrophages	Mouse	[31]
F4/80$^+$CD11 b$^+$Ly6C^{low}, F4/80$^+$CD11 b$^+$Ly6Cmedium, F4/80$^+$CD11 b$^+$Ly6Cl^{high}	Alternatively activated macrophages	Mouse	[49]
CD45$^+$CD11 b$^+$F4/80$^+$Ly6C$^-$MHCII^{high}, CD45$^+$CD11 b$^+$F4/80$^+$Ly6C$^-$MHCII^{low}, CD45$^+$ CD11 b$^+$F4/80$^+$Ly6C$^+$MERTK$^+$CD206$^+$, CD45$^+$ CD11 b$^+$F4/80$^+$Ly6C$^+$MERTK$^-$CD206$^-$	Cardiac resident macrophages	Mouse	[19]
CD45$^+$F4/80$^+$MHC-II^{low}CCR2$^-$, CD45$^+$F4/80$^+$MHC-II^{high}CCR2$^-$	Cardiac resident macrophages	Mouse	[41]
CD45$^+$F4/80$^+$MHCII^{high}CCR2$^+$	Monocyte-derived cardiac macrophages	Mouse	[41]
CD14$^+$CD16$^+$CD163$^+$CD204$^+$CD206$^+$CD209$^-$	Anti-inflammatory M2c macrophages	Human	[50]

Table III

Characteristics of MI-associated proinflammatory and anti-inflammatory macrophages[17,45,58,107,112–117]

	Proinflammatory	Proinflammatory
Stimuli	GM-CSF, IFN-γ, TNF-α, IL-1β	Hydrogen sulfide, IL-4, IL-10, IL-13, IL-33, TGF-β1, M-CSF
Transcription factors	AP-1, HIF-1α, IRF3, IRF5, NF-κB, STAT1	c-Maf, c-Myc, IRF4, JMJD3, KLF4, PPAR-γ, STAT3, STAT6
Markers	CCL2 (MCP1), CCL3 (MIP1a), CCL4 (MIP1b), CCL5 (RANTES), CCL7, CCL8, CCR2, CD80, CD86, CXCL1, CXCL2, CXCL6, CXCL8 (IL-8), CXCL9, CXCL10, CXCL11, CXCL16, IL-1β, IL-6, IL-12, IL-23, iNOS, MHCII, RNS, ROS, S100a8, S100a9, TNF-α	Arg1, CCL1, CCL16, CCL17, CCL18, CCL22, CCL24, CXCL13, CXCL17, CXCL22, CXCL24, CXCR1, CXCR2, CD163, CD206 (MRC1), CD280 (MRC2), Cd301a (Clec10a, Mgl1), Cd301 b (Mgl2), Dectin-1, Fizz1 (Retnla, Relmα), IL-10, PGE2, Spp1 (osteopontin), Stabilin1, TGF-β1, VEGF, Ym1 (Chi3l3)
Cell physiology	Proinflammation; proteolysis; phagocytosis of debris; antigen presentation to lymphocytes	Anti-inflammation and resolution of inflammation; phagocytosis of apoptotic cells; pro-angiogenesis; ECM production and scar formation

Abbreviations: AP-1, activator protein 1; *HIF-1α*, hypoxia-inducible factor-1α; *IRF*, interferon-regulatory factor; *NF-κB*, nuclear factor-κB; *STAT*, signal transducer and activator of transcription; *KLF4*, Kruppel-like factor 4; *PPAR-γ*, peroxisome proliferator-activated receptor-γ; *iNOS*, induciblenitric oxide synthase; *RNS*, reactive nitrogen species; *ROS*, reactive oxygen species; *M-CSF*, macrophage colony-stimulating factor; *Arg1*, arginase 1; *CXCR*, C-X-C chemokine receptor; *Fizz1*, found in inflammatory zone1; *VEGF*, vascular endothelial growth factor.