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Review

Macrophage heterogeneity in myocardial infarction: Evolution and implications for diverse therapeutic approaches

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SUMMARY

Given the extensive participation of myeloid cells (especially monocytes and macrophages) in both inflammation and resolution phases post-myocardial infarction (MI) owing to their biphasic role, these cells are considered as crucial players in the disease pathogenesis. Multiple studies have agreed on the significant contribution of macrophage polarization theory (M2 vs. M1) while determining the underlying reasons behind the observed biphasic effects; nevertheless, this simplistic classification attracts severe drawbacks. The advent of multiple advanced technologies based on OMICS platforms facilitated a successful path to explore comprehensive cellular signatures that could expedite our understanding of macrophage heterogeneity and plasticity. While providing an overall basis behind the MI disease pathogenesis, this review delves into the literature to discuss the current knowledge on multiple macrophage clusters, including the future directions in this research arena. In the end, our focus will be on outlining the possible therapeutic implications based on the emerging observations.

INTRODUCTION

Myocardial infarction (MI)-induced mortality has multifactorial origins, with heart failure (HF) and sudden cardiac death being the key causes.[1–4] Coronary atherosclerotic plaque rupture is the most common cause of MI. It results in thrombus formation in the coronary artery, which obstructs blood circulation in the heart, thereby inducing cardiomyocyte death and myocardial tissue damage. The resulting infarct becomes a region of mechanical weakness, which requires scar deposition to prevent membrane rupture and preserve cardiac function. Following MI, innate immune cells such as neutrophils, monocytes, and macrophages play key role in the initial inflammatory response and subsequent wound healing (as ventricular remodeling) in the damaged heart. Although this remodeling process is necessary to prevent early mortality following MI, adverse remodeling overtime due to maladaptive immune cell behavior at both the infarct site and remote myocardium altered ventricular size and shape as well as function.[5,6] Such adverse changes induced by alterations in myocardial cellular and molecular composition can finally culminate in HF.[5,6] This prompted various attempts to modulate the behavior of myeloid cells to prevent the development of HF. With macrophages being the most abundant cells observed in the infarcted hearts,[7] previous attempts were focused on targeting these cells using broad immunosuppressive strategies.[8] Such strategies failed due to an apparent lack of understanding of macrophage diversity and their associated functions.[8] Here, we provide an update to the current understanding on macrophages and enumerate their role in MI and HF linked with it. We will also discuss the potential relevance of this new knowledge to the development of personalized therapies.

Cardiac macrophages

In the healthy heart, macrophages proliferate locally and self-renew independently of circulating monocytes.[9] These resident cardiac macrophages (RCMs) comprise 7%–8% of non-cardiomyocytes in the heart.[9] They are located throughout the entire heart, and this includes the interstitial space between myocytes, fibroblasts, and endothelial cells, and are closely associated with vessels, and in the conducting system.[10,11] The long-standing perception that all tissue macrophages originate from adult mononuclear cells in the peripheral circulation[12] was deceived with recent studies showing that macrophages in general (including RCMs) are already established during embryonic stages.[13,14] Thus, developed RCMs persist till adulthood but with limited self-renewal capacity as the heart ages. Studies performed using genetic mapping and lineage tracing techniques confirmed that the majority of RCMs originate from the yolk sac or fetal liver

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(MHC-II$^{hi/lo}$ CCR2^{-}) during embryonic development,[13] with an additional population derived from bone-marrow-derived monocytes that begin to infiltrate the heart at 14 days postpartum (postnatal development).[14] Postnatal macrophages (MHC-II^{hi} CCR2^{+}) become an additional major population that is primarily supplemented by continuous colonization of peripheral blood monocytes during later stages.[14] The presence of major histocompatibility complex class II (MHC class II) makes the RCMs more efficient in antigen presentation, T cell activation, and in immune surveillance and adaptive immunity, whereas its absence makes them efficient phagocytes, thereby playing a predominant role in tissue homeostasis and innate immunity.[15] Further, RCMs that are both negative and positive for C-C chemokine receptor type 2 (CCR2) expression show distinct physiological roles in cardiac development. Primitive CCR2^{-} embryonic macrophages play a crucial role in coronary development through their contribution in vascular remodeling of the primitive coronary plexus.[16] On the other hand, owing to their participation in facilitation of action potential propagation, both MHC-II^{hi}CCR2^{+} and MHC-II^{hi}CCR2^{-} RCMs have been shown to be directly connected to cardiomyocytes via connexin 43-containing gap junctions that enable electrical charge exchange and promote efficient repolarization in both murine and human hearts.[10] Difference in cardiac macrophage functions based on CCR2 expression (i.e., CCR2^{+} vs. CCR2^{-}) was also demonstrated in adult human hearts in a study involving sex-mismatched heart transplant recipients.[17] Indeed, macrophages in uninjured human hearts in this study were primarily HLA-DR^{hi}CCR2^{-}, with HLA-DR being a homologue of MHC class II.[17] Additionally, T cell immunoglobulin and mucin-domain-containing protein 4 (TIMD4) is another key marker that distinguishes embryonic (TIMD4^{+}) derived from monocyte (TIMD4^{-})-derived macrophages.[18] Moreover, RCMs expressing MerTK, CD206, and other receptors are widely distributed around cardiomyocytes and show their potential role in clearing extracellular vesicles, including damaged mitochondria and other organelles that are released through cell exocytosis.[19,20]

CARDIAC REMODELING POST-MI

Cardiac repair post-MI can be broadly divided into three distinct phases: the inflammatory, proliferative, and maturation phases.[21,22] The initial inflammatory phase peaks at ~3 days post-injury, whereas the proliferative phase lasts to ~10 days, and the maturation phase extends over months.[23] The proliferative phase is characterized by the upregulation of anti-inflammatory and resolving signaling molecules as well as activation of mesenchymal reparative cells that are predominantly myofibroblasts and vascular cells. Reparative cells, especially myofibroblasts, are composed of cross-linked collagen that helps in scar formation, and apoptosis of these cells marks the end of the proliferative phase.[24] The final maturation marks the remodeling of extracellular matrix, resulting in optimal cardiac repair or maladaptive remodeling leading to the development of HF.[6]

Inflammatory phase

It is well known that acute MI occurs as a resultant of rupture of coronary atherosclerotic plaques, obstructing blood circulation in the heart due to thrombus formation. This obstruction for long period leads to cardiac hypoxia, activating cell death programs in cardiomyocytes and parenchymal cells. Hypoxia also impairs vascular integrity to augment vessel permeability and facilitate leukocyte infiltration.[25] Danger-associated molecular patterns (DAMPs) released from necrotic and stressed cells binds to pattern recognition receptors on surviving parenchymal cells and infiltrating leukocytes.[26–32] Inflammasome activation due to this binding in turn stimulates caspase activity and interleukin-1β (IL-1β) release, thereby causing continued cell death due to activation of a range of inflammatory mediators that include inflammatory cytokines, chemokines, and cells adhesion molecules.[27,28,30,33–35] Several DAMPs that are released during MI include S100 proteins, high-mobility group box-1, ATP, uric acid, fibronectin extra domain A, IL-1α, heat shock proteins, low-molecular-weight hyaluronic acid, complement, mitochondrial DNA, double-stranded RNA (dsRNA), and single-stranded RNA (ssRNA).[25,27,30,32,35] Prominent targets for the DAMPs to bind include Toll-like receptor 4 (TLR4), IL-1 receptor, receptor for advanced glycation end products and activation of the cytosolic nucleotide-binding oligomerization domain (NOD)-like receptors.[32,34–37] These bindings activate signaling pathways that finally converge to stimulate mitogen-activated protein kinases (MAPKs) and nuclear factor κB (NF-κB) pathways. Such pathways drive the expression of inflammatory genes coding for cytokines such as tumor necrosis factor (TNF); IL-1β, IL-6, and IL-18; and chemokines of CXC that act as neutrophil chemoattractants and CC that attract monocytes and T lymphocytes and cell adhesion molecules.[35,38,39] Leukocyte recruitment further amplifies the inflammatory response and promotes the clearance of dying cells by efferocytosis and tissue digestion by release of various proteases.[24,40] It should also be noted that some of these intracellular components are likely presented as antigens to engage the adaptive arm of immunity, which can further impact the outcome following ischemic events, but a robust discussion on this topic is beyond the scope of this review.

Monocytes and macrophages in inflammation

Infiltration of monocytes and macrophages follows influx of neutrophil into the infarct, and it occurs in two sequential phases[41,42]: a primary inflammatory phase and a secondary anti-inflammatory phase. These phases signify a phenotypic switch of both cellular types and signaling molecules. Although inflammatory monocytes are Ly6C high (Ly6C^{hi}) and appear between days 1 and 4 post-MI, anti-inflammatory monocytes are Ly6C low (Ly6C^{lo}) appearing between days 4 and 7. CCR2^{+} RCMs are responsible for initial recruitment of monocytes to the injured area through a myeloid differentiation primary response 88 (MYD88)-dependent pathway.[43] Activation of this pathway resulted in release of monocyte chemoattractant proteins and monocyte mobilization.[43] On the contrary, the absence of CCR2 (CCR2^{-}) in RCMs inhibits the recruitment process.[43] Multiple models of cardiomyocyte death demonstrated Ly6C^{hi}CCR2^{+} monocytes and CCR2^{+}MHC-II^{hi} macrophages in the heart, indicating Ly6C^{hi} monocyte recruitment and MHC-II^{hi} macrophage accumulation as common stereotyped responses to cardiomyocyte death.[43] These inflammatory macrophages are highly phagocytic, secrete proteases, and produce ROS and inflammatory cytokines like

TNF-α.[44] This initial inflammatory phase is crucial for debris clearance and infarct healing. Depletion of inflammatory monocytes and macrophages[14,44–46] or preventing monocyte recruitment[47] resulted in impaired infarct healing, thereby causing increased accumulation of necrotic debris, persistent cardiac inflammation, reduced ejection fraction, and ultimately HF. On the contrary, overrecruitment of these myeloid cells during the inflammation phase results in impaired wound healing. Monocytosis (as indicated by drastic increase in Ly6C^{hi} monocytes at the injury site) in mice and patients impaired cardiac function,[48–50] whereas dampening, but not ablating, this process was found to be beneficial.[51–55] The determinantal outcome to oversupply of Ly6C^{hi} monocytes after MI was shown in the *Apoe*$^{-/-}$ mice, where chronically elevated monocyte counts caused a higher recruitment of Ly6C^{hi} monocytes, resulting in accelerated progression toward HF.[49] Furthermore, a study in 2017 showed that MI provokes activation of interferon regulatory transcription factor 3-interferon (IRF3-interferon) axis in a distinct population of interferon-inducible cells (IFNICs), which were classified as cardiac macrophages.[56] These cells recognized the DNA that is released from dying cardiomyocytes, which ultimately fueled to a maladaptive IRF3-dependent innate immune (type 1 interferon) response.[56] Single-cell RNA-seq (scRNA-seq) analysis was utilized to examine the gene expression of the cell-surface markers for mononuclear cells linked to ontological and phenotypic subsets of leukocytes. IFNICs were identified as cells expressing *Adgre1* (encoding F4/80), *H2.Aa* that encodes for MHCII, and *Ccr2*, but not expressing *Ly6c2* that encodes Ly6C.[56] Such expression profile led to classifying IFNICs as monocyte-derived cardiac macrophages,[14] and this cell population was not identifiable in scRNA-seq data derived from leukocytes of *Irf3*$^{-/-}$ mice or wild-type (WT) sham controls. Deletion of crucial components of this pathway or administration of interferon alpha receptor neutralizing antibody following coronary artery ligation (CAL) improved ventricular size, contractile function, and mouse survival.[56] These studies indicate that therapeutic approaches aimed at blocking or lowering recruitment of monocytes (e.g., anti-CD20 aimed to deplete B cells needed for monocyte recruitment[57]) or inhibiting IRF3 signaling demonstrate beneficial effects.[56] All the abovementioned discussion also summarizes the importance of optimal levels of inflammatory monocytes and macrophages for efficient participation in inflammatory phase.

Reservoirs for monocyte replenishment

MI leads to production of inflammatory molecules like IL-1β,[32] TLR ligands, and chemokines that can act on hematopoietic stem cell (HSC) receptors to bias them toward a myeloid lineage.[58–60] Accumulation of monocytes and macrophages in the myocardium is accompanied by an increase in circulating monocyte levels.[44,61] Accounting for ~40% of monocytes recruited to the myocardium after MI,[61] spleen acts as the immediate major reservoir for these circulating monocytes via two ways: (1) its ability to mobilize a reservoir of locally stored undifferentiated monocytes in response to injury-associated triggers and (2) replenishment by local expansion of myeloid progenitor cells supplied from the bone marrow, a process termed as extramedullary emergency monocytopoiesis.[61,62] Myeloid cell recruitment to the injury site is also induced by other sources such as chemokine (C-C motif) ligand 7 produced by B cells[57] and granulocyte/macrophage colony stimulating factor (GM-CSF) produced by fibroblasts in the infarct site.[63] GM-CSF has been shown to act locally and distally to generate and recruit inflammatory and proteolytic cells.[63] Interestingly, in the study involving GM-CSF, splenectomy on day 2 had no impact on the leukocyte counts in the infarcted heart on day 3, indicating that the GM-CSF-induced cell accumulation was spleen independent. In fact, GM-CSF was shown to stimulate a distinct myeloid-based progenitor subset in the bone marrow.[63] Similarly, increased progenitor cell presence was also observed in liver of splenectomized mice 4 days after MI.[50] Moreover, monocytopoiesis after MI is also regulated by sympathetic nervous system (SNS) signaling.[50,64] Enhanced SNS signaling after MI results in downregulation of several quiescence and retention factors (like CXCL12) in the bone marrow (BM), leading to increased mobilization and activation of HSCs.[50] Activation of SNS also resulted in release of progenitors from BM niches and their hosting in the spleen because of increased stem cell factor production, leading to amplified extra-medullary hematopoiesis.[50] Experiments to understand if there exists a difference in the gene expression of Ly6C^{hi} monocytes isolated from spleen and bone marrow on day 4 post-MI showed markedly different mRNA levels for 11 of 32 genes assessed. For e.g., there was a 60- and 6-fold higher mRNA expression of IL-1β and cathepsin B expression in inflammatory monocytes isolated from spleen than in bone marrow, and these findings were consistently observed in those isolated from atherosclerotic plaques after MI.[50] All the abovementioned findings put forth the importance of studying different progenitor subset populations that may be unknown in different reservoirs and determine their role in extramedullary myelopoiesis post-MI. Further, these studies will also help in clearly defining the impact of these reservoirs (spleen vs. bone marrow) with respect to their primacy, as current findings doubt the prominence of spleen as the major reservoir for monocyte mobilization in inflammatory phase of MI.

Proliferative phase

This phase is dominated by Ly6C^{lo} monocytes/macrophages, which promote healing via myofibroblast accumulation, angiogenesis, and collagen deposition.[44] Ly6C^{lo} monocytes derived from Ly6C^{hi} monocytes peaks around day 7 post-MI.[44,65,66] The switch from Ly6C^{hi} monocytes to Ly6C^{lo} monocytes is crucial for myocardial tissue repair,[67,68] and this includes the production of anti-inflammatory cytokines like IL-10 and transforming growth factor β (TGF-β).[69,70] Ly6C^{lo} macrophages produce vascular endothelial growth factor (VEGF), which induces angiogenesis as well as matrix metalloproteinase (MMP), and tissue inhibitors of metalloproteinases, which regulate extracellular matrix deposition. Orphan nuclear hormone receptor nuclear receptor subfamily 4, group a, member 1 (Nr4a1) has been identified as a key molecular switch that controls many cellular functions involved in inflammation, apoptosis, and proliferation.[66] Its significance in the context of MI primarily comes from its necessity for the survival of Ly6C^{lo} monocytes.[71] Indeed, *Nr4a1* was demonstrated to transduce signals from upstream signals like cluster differentiation (CD)36, with the latter been identified to be necessary for early clearance of dead cardiomyocytes.[72] Activation of *Nr4a1* results in its binding-based induction of the downstream myeloid-epithelial-reproductive tyrosine kinase (MerTK) expression, signifying the importance of *cd36-Nr4a1-Mertk* axis in phagocytosis post-MI.[72] This process of clearance of apoptotic cells by MerTK marks the

transition from the inflammatory phase to inflammation resolution and wound healing.[73] Deficient efferocytosis (clearance of apoptotic cells) results in larger infarct size, less angiogenesis, and greater fibrotic area due to lack of VEGFA.[74] The change from the inflammatory to a resolving milieu also promotes myofibroblast proliferation and migration to the infarct. Recent studies also indicated that *Nr4a1* is dispensable to the differentiation of Ly6C^{lo} macrophages.[75] In fact, inflammatory Nr4a1^{lo} Ly6C^{hi} monocytes from the bone marrow and spleen were found to infiltrate to infarcted myocardium in response to a brief CCR2 burst and later differentiate to reparative Nr4a1^{hi} F4/80^{hi} Ly6C^{lo} macrophages that proliferate locally.[66] Furthermore, *Nr4a1*$^{-/-}$ *mice* displayed an absence of Ly6C^{lo} monocytes in circulation, resulting in abnormally inflamed macrophages, poor healing, increased fibrosis, and finally accelerated HF.[66] A similar pattern of monocyte recruitment is also observed in the remote myocardium[41]; however, Ly6C^{lo} cell numbers peak at day 5, and Ly6C^{hi} monocytes and macrophages persist in the non-ischemic myocardium.[64] Monocyte and macrophage populations at the infarct site return to baseline by 2 weeks post-MI but may persist for months after MI in the remote, remodeling myocardium.[64] Absolute numbers of macrophages in the remote myocardium expand and persist following MI via increased monocyte recruitment and differentiation.[18,64] Overall studies show that both monocytes and macrophages are crucial to balance the initial inflammatory phase and the subsequent reparative phase.[44,66] With inflammation and resolution phases governing the infarct size and cardiac structural changes, respectively, therapeutic interventions aimed at the reparative phase would be cardioprotective,[76] whereas inhibition of excessive inflammation may promote infarct healing.[77]

Maturation phase

This is the final phase in the cardiac repair process, which is characterized by cellular (mainly fibroblasts and leukocytes) clearance from the areas of tissue damage in injured myocardium and cessation of angiogenesis as well as collagen cross-linking. All the changes that underpin the maturation phase lead to extracellular matrix remodeling and formation of an acellular fibrous scar.[78] With this process continuing for months, it is not surprising to expect that it may also be accompanied by low-level inflammation that is required for cardiomyocyte apoptosis and suppression of cardiac contractility along with additional extracellular matrix remodeling. Insights into the role of macrophages in the maturation phase have been delineated in some murine studies. Such studies indicate that continued infiltration of monocytes contribute to inflammatory macrophage populations in the myocardium, which can further lead to long-term fibrosis and adverse remodeling.[64,79] Nevertheless, studies looking into these changes are very few; therefore, the molecular mechanisms behind regulation of macrophages remain poorly understood. Further studies both at the clinical and pre-clinical levels are warranted for better understanding of the underlying processes. In a nutshell, cellular changes in these three phases signify the requirement of a perfect balance in the effects caused due to inflammatory and anti-inflammatory mediators. This is because sustained inflammation (caused by either excessive inflammatory mediators or lack of anti-inflammatory ones) can lead to structural and functional changes in the left ventricle, a process that is termed adverse remodeling and may ultimately result in HF.[80]

HEART FAILURE POST-MI

Adverse left ventricular (LV) remodeling (caused due to excessive inflammation), chronic neurohumoral activation (a resultant of cardiomyocyte death and scar formation), and impaired healing of cardiac tissue underpins the development of HF after MI hospitalization.[81] Upregulated renin-angiotensin-aldosterone and SNS activities form the basis behind the neurohumoral activation as observed in those hospitalized after MI.[82] Abnormal ventricular remodeling has shown to change ventricular geometry that includes wall thinning, ischemic mitral regurgitation, and a continued cardiomyocyte loss.[82] With a diagnosis percentages approximating to 13% of patients up to 30 days and 20%–30% at 1 year post-discharge,[83,84] it is obvious to predict that MI-induced HF is more prevalent in patients discharged from hospital following the attack. Its incidence after discharge is highest in the first months, which then drops and later remains stable at a rate of 1.3%–2.2% per year afterward.[84] While the total mortality risk increases 3-fold and cardiovascular mortality to 4-fold in patients developing HF post-MI, regardless of the type of HF.[85] To understand the specific contribution of monocyte and macrophage subsets on remodeling processes post-MI, studies have focused majorly on manipulating these populations from myocardial and non-myocardial sources.

Myocardial monocytes and macrophages

Both activity and counts of embryo-derived RCMs decrease as a part of normal cardiac aging process. This also includes their declined self-renewal even in the absence of inflammation and progressive replacement with fibrotic monocyte-derived macrophages in the heart.[86] Even a 2-month-old normal mouse heart shows about 10-fold decrease in resident macrophage proliferation capacity over newborn pups upon diversification into subpopulations.[86] It is also appreciated that low-grade inflammation caused by mechanical stress would lead to their depletion and replacement from circulating monocytes with MHC-II^{hi} expression.[86] This replacement also correlates with diminished cardiac regeneration capacity and increased risk for HF. Apart from these RCMs, the role of monocyte-derived macrophages in the infarcted myocardium is significant and needs attention. As discussed in section 2.2., Nr4a1 is essential to the development of Ly6C^{lo} monocytes, but it has also been found that Nr4a1 is dispensable to the differentiation of Ly6C^{lo} macrophages.[75] In the absence of Nr4a1 (*Nr4a1*$^{-/-}$), mice with Ly6C^{hi} monocytes showed an increased expression of CCR2 on their surface, indicating their avid infiltration into the myocardium.[66] These monocytes differentiate to abnormally inflammatory macrophages with an inflammatory M1-phenotype-based gene expression profiling, which finally leads to compromised heart function through severe LV dysfunction (as assessed at day 21 post-coronary artery ligation).[66] Surprisingly, *Nr4a1*$^{-/-}$ mice also showed significantly higher Ly6C^{lo} macrophages in the myocardium on day 7. Having said that, the role of different

RCMs (MHC-II$^{hi/lo}$ CCR2$^{+/-}$ TIMD$^{+/-}$) with respect to their structural and functional changes during HF development needs extensive investigations.

Pericardial macrophages

The pericardial cavity also acts as an alternative pool for macrophages, although much has not been unveiled in respect to their role. Immunophenotyping of cells from pericardial lavage under steady-state conditions has identified Gata6^{+} (MHCII^{-}CD102^{+}) pericardial macrophages or GPCMs as the most abundant cell type (34.2%).[87] Following experimental MI, these macrophages were found to invade the epicardium and perform anti-fibrotic functions despite losing the Gata6 expression.[87] And their presence in the human pericardial fluid supports the notion that this reparative function is relevant in human disease. The very idea that GPCMs demonstrate anti-fibrotic activity in an infarcted heart has been challenged in a subsequent study that involved genetic lineage tracing techniques.[88] Post MI, GPCMs were identified to be recruited to the heart surface, restricting into the thickened epicardial layer with only a minimal invasion to the underlying myocardium.[88] Surprisingly, there was no significant change in cardiac fibrosis or function of injured hearts as observed in cell ablation or Gata6 knockout in GPCMs experiments.[88] Despite the diversified results observed in these studies, it is still reasonable to anticipate that the field of pericardial myeloid cells is worth exploring, given the fact that pericardial cavity disruption resulted in accelerated maladaptive post-MI remodeling.[87] Furthermore, the role of pericardial adipose tissue in MI should not be ignored, given the ability of this depot to host inflammatory monocyte-derived macrophages.[89] Indeed, a lead study in this line reported its importance in regulating granulopoiesis, fibrosis, and cardiac function in infarcted hearts.[89] We propose the concept that a pericardial-myocardial axis involving several cells and signaling process serves to protect the injured heart following MI or other cardiac challenges.

Monocytes from non-myocardial sources

A variety of approaches has been utilized to understand the impact of monocytes that are resulted from different reservoirs of mischief. These approaches include inhibition of monocytopoiesis (e.g., beta-adrenergic blockade), depletion of circulating monocytes (e.g., clodronate-loaded liposomes), depletion or inhibition of extra medullary reservoirs (e.g., splenectomy, Agt1ar antagonism), inhibition of monocyte release from the bone marrow (e.g., Ccr2$^{-/-}$), or entry into the myocardium (e.g., Ccr2$^{-/-}$).

Monocytopoiesis and monocytosis

Post-MI, the production of monocytes is sustained, resulting in monocytosis in the circulation, driven by various mechanisms. In particular, enhanced SNS signaling during MI promotes hematopoietic stem and progenitor cells mobilization and extra-medullary myelopoiesis, thereby increasing the level of circulating monocytes.[50,90–94] Blockade of β-adrenergic following MI may have protective effect via suppression of monocytosis.[64] IL-1β is a cytokine that promotes monocytopoiesis; therefore, targeting IL-1β following MI also reduces circulating monocyte levels and mitigates deterioration of LV function at 3 weeks.[95] Empirical studies also found a correlation between monocytosis and the degree of LV dysfunction after MI. For example, the Apoe$^{-/-}$ mice with chronically elevated levels of circulating Ly6C^{hi} monocytes show elevated infiltration of Ly6C^{hi} monocytes in the myocardium on day 5 and reduced left ventricular ejection fraction (LVEF) at 3 weeks, compared to wild-type mice.[49] Similarly, in patients following ST-segment elevation MI (STEMI), peripheral monocytosis is associated with LV dysfunction and LV aneurysm.[96] In another study, peak levels of CD14^{+}CD16^{-} monocytes (that corresponds to Ly6C^{hi} type in mice) following STEMI negatively correlated with the extent of myocardial salvage and the recovery of left ventricular function after MI.[97] CCR2 is necessary for mobilization of Ly6C^{hi} monocytes from the bone marrow, seeding of the spleen by monocytes, and in attraction of Ly6C^{hi} monocytes to the myocardium.[98] Performing CAL in *Ccr2*$^{-/-}$ mice or in wild-type mice subjected to siRNA-mediated knockdown of CCR2 has demonstrated reduced myocardial infiltration of Ly6C^{hi} cells and improved LVEF and left ventricular end-diastolic volume.[44,99] Release of monocytes from the spleen occurs in a CCR2-independent manner, and splenic monocytes also contribute to maladaptive ventricular remodeling.[61] Splenectomy in mice 8 weeks post-CAL improves ventricular function,[100] and adoptive transfer of splenic monocytes isolated from mice with ventricular dysfunction cause maladaptive ventricular remodeling in the recipients. Adoptive transfer of LPS-stimulated splenic monocytes does not induce ventricular remodeling, suggesting that it is not an inflammatory monocyte phenotype rather a spleen-specific modification that is responsible for HF induction. Release of splenic monocytes following MI is also regulated by angiotensin II type 1a (Atgr1a) receptor, and this was based on two findings: (1) the inability of the spleen to expel monocytes efficiently in *Atgr1a*$^{-/-}$ mice and (2) sustained exogenous administration of Ang II in wild-type mice reproduced monocyte egress.[61] The beneficial effect of RAS inhibition on inflammation-associated acute cardiac rupture post-MI mediated by blockade of splenic monocytes release was pharmacologically proven preclinically in mice using drugs like losartan or perindopril,[101] whereas translating these findings at the clinical side needs extensive investigations in the future.

KNOWLEDGE FROM POLARIZED (I.E., M1/M2) MACROPHAGES

Over the years, approaches aimed at protecting against impaired cardiac remodeling and HF post-MI were majorly focused toward altering macrophage phenotypes. It was widely accepted that macrophage polarization occurs during the disease pathogenesis following MI. It is also widely appreciated that there is an abundance of macrophage subsets, all ascribed with various functions. However, in the context of inflammation and resolution of inflammation, the classical M1/M2 polarization and characterization studies have provided us with useful knowledge on the role of macrophages and the inflammatory or reparative status of the tissue. Thus, in the context of MI both broad and targeted

strategies have been explored in maladaptive ventricular remodeling. Upregulation of protective M2 type genes using IL-4 or IL-10 treatment or gamma aminobutyric acid A receptor agonism post-CAL demonstrated significant protection[102–104]; nevertheless, such approaches involved manipulation of other cell types including T-reg cells. This led to the exploration for development of more targeted approaches aimed at manipulating specific phenotypes (M2 or M1). Tribbles homolog 1 (TRIB1) is an adaptor protein and a member of Ca^{2+}/calmodulin-dependent protein kinase Ser/Thr protein kinase family with its roles exhibited in protein degradation and lipid metabolism.[102,105] Studies also implicated that it is critical for the differentiation of $F4/80^{+}$ MR^{+} tissue-resident macrophages; these share characteristics with M2 macrophages and so termed as M2-like macrophages.[105] *Trib1*$^{-/-}$ mice exhibited a selective depletion of M2-like macrophages in multiple organs[105] along with functional deficits that include frequent cardiac rupture due to impaired fibroblast activation and collagen fibril formation in the infarct area.[102] Interestingly, this compromised tissue repair was entirely rescued by an external supply of M2-like macrophages.[102] Similarly, MMP28 or epilysin, a member of MMP family proteinases has been shown to promote polarization of macrophages toward M2 phenotype.[106] Genetic ablation of MMP28 exhibited an increase in mortality by day 7 after CAL due to significant cardiac rupture as well as severe LV dysfunction with a worse LV remodeling index and increased lung edema.[107] As expected, these mice showed an increase in canonical M1 and a decrease in M2 markers. Moreover, efferocytosis through MerTK shifts macrophages to a reparative phenotype, and knockout of this receptor leads to increased expression of M1 genes and LV dysfunction after CAL.[73]

Conversely, suppression of inflammation by blocking pathways needed for M1 macrophage polarization have also been explored. Interferon regulatory factor 5 (Irf5) serves as a master transcription factor of macrophage polarization. It translates danger signals, including TLR ligands, into inflammatory gene expression, giving rise to M1 macrophages. siRNA-mediated targeting of Irf5 in mice subjected to CAL resulted in reduced expression of M1 markers TNF- α and IL-1β, but no major changes were observed in M2 marker expressions (by day 4). These mice showed similar infarct size but LV dilation was reduced at 3 weeks.[77] These observations suggest a scenario where strategies directed toward activation of M2 macrophage phenotype would be protective against adverse remodeling after MI. Targeting through this strategy may not be straightforward as M2 macrophages were shown to develop four distinct subtypes (M2a, M2b, M2c, and M2d), when encountered with diverse stimuli.[108] This can also be substantiated by the fact that macrophages isolated from the infarct area of mice (on days 1, 3, and 7 post-CAL) did not fully align with either M1 or M2 phenotype.[109] Surprisingly, inflammatory macrophages on day 1 showed overexpression of M2 marker Arg1, and on Day 3, phagocytic macrophages also showed gene expression related to mitochondrial function and oxidative phosphorylation.[109] Moreover, the outcomes from a study that involves mice with myeloid specific deficiency of GATA-binding factor 3 (Gata3) challenge the current understanding that the presence of excessive proinflammatory $Ly6C^{hi}$ populations in the initial inflammatory phase is deleterious. Gata3 is a transcription factor required for differentiation of M2 macrophages.[110] Selective deletion of Gata3 in myeloid cells depletes only $Ly6C^{lo}$ macrophages following CAL. Surprisingly, these mice demonstrated higher levels of $Ly6C^{hi}$ macrophages despite improved LV size and function at 2 months post-MI.[110] Conflicting observations in these studies put forth an opinion to reconcile at the canonical M1-M2 macrophage classification. The growing body of evidence that macrophages exhibit phenotypic spectrum and the existence of non-monocyte-derived macrophage subsets in the disease pathogenesis indeed provides strong empirical evidence for a more comprehensive classification (Figure 1). May be different approaches used above to selectively manipulate macrophage subtypes end up depleting limited subtypes belonging to M1 or M2 but not as totality of one phenotype.[110] For example, in the study involving *Gata3*$^{-/-}$ mice, only partial depletion of $Ly6C^{lo}$ macrophages was observed, indicating merely a proportion of these cells are Gata3 dependent.[110]

MACROPHAGE HETEROGENEITY IN THE OMICS ERA

The idea that there exist multiple clusters of cell populations for macrophages owing to diversified activation under different stimuli opened a new research arena. The advent of advanced technologies that can demonstrate superior sensitivities with an ability to detect and report multiple outcomes with high rigor and robustness made this field truly exciting. The capability to use "OMICS" approaches at different levels to identify the comprehensive cellular signatures is growing (Figure 1). Single-cell RNA sequencing (scRNA-seq) has made it possible to identify the sub-populations that are not been limited by the choice of few cell-surface markers and are bound to be game changers in future.[111,112] One classic example is the identification of macrophages enriched for oxidized low-density lipoprotein receptor 1 (*Olr1*) and glycoprotein non-metastatic melanoma protein b (*Gpnmb*)-positive macrophage clusters.[113] While *Olr1* enriched cluster is of inflammatory nature, *Gpnmb*-positive subset exhibited higher phagocytosis and fatty acid oxidation preference.[113] Utilizing scRNA-seq for analyzing murine aortic macrophages defined three distinct macrophage or clusters: (1) inflammatory, (2) resident-like, and (3) a distinct population named triggered receptor expressed on myeloid cells 2 ($TREM2^{hi}$) macrophages.[114] Later, these seemingly non-inflammatory lipid-associated *Trem2*^{hi} macrophages were also reported to exist in the ischemic area of the infarcted hearts as two sequentially populating subpopulations, *Trem2*^{hi}*Spp1*^{hi} monocyte-to-macrophage intermediates and fully differentiated *Trem2*^{hi}*Gdf15*^{hi} macrophages.[115] Combining a unique cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) technique with fate-mapping analysis and targeted deletions, the same study also demonstrated that macrophage landscape of the infarcted heart was dominated by monocyte-derived populations comprising of two inflammatory populations, viz., interferon-stimulated gene 15 (*Isg15*^{hi}) and $MCHII^{+}$*Il1b*$^{+}$ cells.[115] Another notable phenotype reported here is the ischemic injury shift of circulating $Ly6C^{hi}$ monocytes toward a *Chil3*^{hi} state with granulocyte-like features.[115] A similar upregulation in *Trem2*^{hi} macrophages was also observed in another independent MI study that involved spatial transcriptome sequencing (ST-seq) and scRNA-seq.[7] Although these techniques provide additional insights into the biology following MI, it is still important to validate these putative findings (e.g., interactomes) with functional biology.

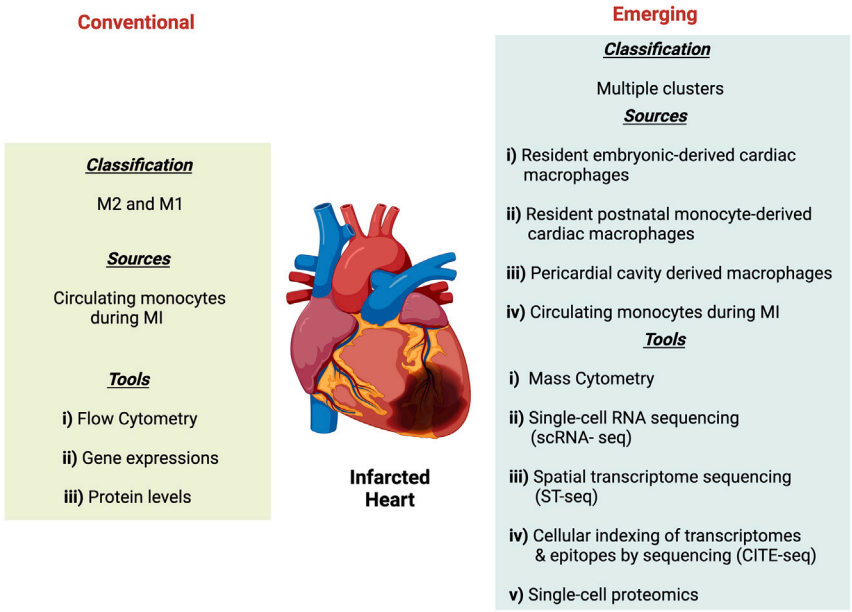

Figure 1. Emerging dimensions in the understanding of the macrophage heterogeneity

In the past decade, the area of macrophage heterogeneity was widely explored using different advancing technologies, which resulted in disseminating knowledge from different directions. The outcomes are comprehensive but undelineated, thus prompting future investigations. Created with BioRender.com.

The ever-growing complexity of macrophage signatures

We present here, in brief, a few case studies that have further embraced the field of heterogeneity for two key reasons: (1) the ever-growing complexity of the macrophage signatures based on the findings that utilize different techniques and (2) studies involving diverse stimuli aimed to determine how differences in the activation signals lead to variations in the pattern of macrophage signatures. Dick et al. investigated cardiac macrophage heterogeneity in steady-state through the combined use of genetic fate mapping, long-term parabiosis, and scRNA-seq and observed four distinct populations in a healthy adult murine myocardium, including a subset that can self-renew with negligible monocyte input (TIMD4^{+}LYVE1^{+}MHC-II^{lo}CCR2^{-}), a subset that is partially replaced by monocytes (TIMD4^{-}LYVE1^{-}MHC-II^{hi}CCR2^{-}), and two CCR2^{+}MHC-II^{hi} subsets that are fully replaced by monocytes.[18] Post-MI, TIMD and CCR emerged as mutually exclusive markers for resident and recruited macrophages. This is because MI reduced TIMD^{+} and TIMD^{-} resident macrophage abundance, whereas CCR2^{+} monocyte-derived macrophages adopted multiple cell fates within infarcted tissue, including those nearly distinguishable from resident macrophages. Absence of TIMD4 expression in recruited macrophages highlights its ability to track a subset of resident macrophages in the absence of fate mapping. Despite this similarity, inducible depletion of resident macrophages using a Cx3cr1-based system led to impaired cardiac function and promoted adverse remodeling in the peri-infarct zone, revealing a nonredundant cardioprotective role of resident cardiac macrophages.[18] Out of 13 different myeloid clusters shown in mouse heart following MI, there exist 6 unique macrophage populations within infarct area that are distinct from baseline populations. Studies involving cardiac macrophages from human subjects also identified subsets that share similar gene signatures of some murine macrophage clusters. Sorting CD45^{+}CD64^{+}CD14^{+} human cardiac macrophages based on expression of MHC class II (HLA-DR) and CCR2 identified three subsets that are similar to mouse cardiac macrophages (i.e., CCR2^{-}MHC-II^{hi}, CCR2^{+}MHC-II^{hi}, and CCR2^{+}MHC-II^{lo}).[18] The human CCR2^{-}MHC-II^{hi} macrophages expressed many genes that are observed in the murine *Timd4* cluster (including *TIMD4*, *LYVE1*, *CD163*, *FOLR2*, *IGF1* and *MAF*), whereas human CCR2^{+}MHC-II^{hi} macrophages and CCR2^{+}MHC-II^{lo} monocytes are associated with murine gene signatures belonging to *Isg* and *Ccr2* clusters and monocytes.[18] Although human subjects used in the study show end-stage cardiomyopathy, the outcomes highlight the complexity and plasticity that underlies behind recruited and resident cardiac macrophages at steady state and particularly within infarcted hearts.[18] In an independent investigation, Epelman and colleagues also showed four subsets of steady-state resident macrophages in the adult murine heart but with a distinctive classification. The first and second subsets are Ly6C^{-} MHC-II^{hi} CX3CR1^{hi} CD206^{int} MerTK^{+} CD11c^{hi} CCR2^{-} CD64^{+} macrophages and Ly6C^{-} MHC-II^{low} CX3CR1^{int} CD206^{hi} MerTK^{+} CD11c^{low} CCR2^{-} CD64^{+} macrophages, respectively, whereas the third population retained Ly6C expression and the fourth one could be separated from Ly6C^{+} having third population by the lack of MerTK and CD206 expressions. Striking differences were observed between the classifications proposed by the Dick and Epelman groups, which needs further studies determining their significance in MI-induced cardiac repair including adverse remodeling leading to HF.[14] An overview of different studies showing unique macrophage or macrophage-like clusters was presented in Table 1.

With CCR2 expression (CCR2^{+} and CCR2^{-}) been demonstrated even in the subsets belonging to healthy myocardium and given their role in adverse remodeling and injury resolution, respectively, it is not surprising to expect that such differences lead to variations in the resident macrophage populations during MI. Selective depletion of CCR2^{+} macrophages results in reduced monocyte recruitment to the infarcted

Table 1. Overview of different studies showing unique macrophage or macrophage-like clusters

S. No.	Subset or cluster/expression pattern	Activity	Species	Origin	Reference
1	IFNICs interferon-inducible	Activation of IRF3-interferon axis	Mouse	Heart	King et al., 2017[56]
2	(i) MAC_Olr1 and (ii) MAC_Gpnmb	(i) Proinflammatory (ii) Phagocytosis and FA oxidation	Mouse	Heart	Zhuang et al., 2022[113]
3	(i) Isg15^{hi} and (ii) MHCII^{+}Il1b^{+} (iii) Trem2^{hi}Spp1^{hi} and (iv) Trem2^{hi}Gdf15^{hi}	(i & ii) Proinflammatory (iii & iv) Non-inflammatory; lipid-like macrophage	Mouse	Heart	Rizzo et al., 2022[115]
4	Multiple clusters	Determination of RCMs signature and macrophage heterogeneity	Mouse	Heart	Dick et al., 2019[18]
5	Multiple clusters	Determination of RCMs signature and macrophage heterogeneity	Mouse	Heart	Epelman et al., 2014[14]
6	F4/80^{+} MR^{+}	M2 like-macrophages	Mouse	Spleen	Satoh et al., 2013[105]
7	Gata6^{+} (MHCII^{-}CD102^{+})	Reparative immune response	Mouse & human	Pericardial cavity	Deniset et al., 2019[87]

myocardium with improved LV systolic function, smaller LV chamber dimensions, and reduced akinetic myocardium compared with controls 28 days after ischemia reperfusion (IR) injury.[43] Whereas depletion of CCR2^{-} macrophages resulted in the opposite. Unlike similar infarct sizes on day 2 (CCR2^{+} vs. CCR2^{-}), depletion of CCR2^{-} macrophages resulted in larger sized infarcts at day 28 when compared to CCR2^{+} counterparts,[43] and this indicates that tissue-resident macrophages affect post-infarction LV remodeling rather than the initial infarct size. Considering the technical advancements that motivated toward the understanding of complex molecular and cellular signatures globally, one can anticipate that investigations performed on isolated macrophages may yield multiple clusters. The existence of such distinct macrophage clusters even in the transcriptomes of single cardiac nuclei commends the importance of resident macrophages and their function.[116] Large-scale transcriptome sequencing of 287,269 single cardiac nuclei revealed 9 major cell types and 20 subclusters of cell types within the human heart, of which 2 distinct groups are resident macrophages with a few key markers overlapping with TIMD4^{+} or CCR2^{+} macrophages.[116] Furthermore, the impact of diverse stimuli in the overwhelming world of heterogeneity is groundbreaking. Stimulation of human macrophages using diverse activation signals resulted in a dataset having 299 macrophage transcriptomes.[117] A total of 28 different stimuli including pattern recognition receptor ligands, cytokines, and metabolic cues were utilized, and combining all these data resulted in identification of 9,498 genes that were expressed in at least one condition. Further analysis revealed a spectrum of activation states in macrophages, which essentially extends the M1 versus M2 polarization model. Network analyses identified key transcriptional regulators associated with all macrophage activation, which is complemented by regulators related to stimulus-specific programs.[117] Comparison of different genes in resting human and mouse macrophages using different *in vitro* and *ex vivo* models in another study defined a set of highly expressed molecules, representing a consistent 87-gene combined molecular signature that appears specific to macrophages.[118] Although transcriptomics and genomics-based outcomes appear to help in revealing the comprehensive macrophage signature, it poses challenges that exist in accurately translating the changes at the transcriptional and metabolic level to cell function. Also, the inherent limitations that prevailing antibody and mass spectrometry technologies possess resulted in the emergence of single-cell proteomics, and this enabled high-throughput analysis of protein content in single cells at an affordable cost through use of automated micro-sample preparation techniques. Successful quantification of over 3,042 proteins in 1,490 single nuclei/macrophage in a 10-day time frame even in the absence of polarizing factors is a testament for the substantial heterogeneity demonstrated by macrophages.[119]

Identification of multiple monocyte populations

The concept that there exists a heterogeneity in the cardiac macrophages also becomes more appealing with studies indicating that their precursors, monocytes, too exhibit heterogeneous populations.[120] In humans, monocytes display considerable heterogeneity, which is reflected through a nomenclature based on the expression levels of cluster of differentiation CD14 and CD16. Traditionally three clusters were identified in human monocytes, viz., classical (CD14^{++}CD16^{-}), intermediate (CD14^{++}CD16^{+}), and non-classical (CD14^{+}CD16^{++}) monocytes.[121] Whereas only two major monocyte subsets have been described in mice, namely Ly6C^{hi} and Ly6C^{lo}, that resemble classical and non-classical human monocytes, respectively.[122] Over the years, it was realized that the CD14/CD16-based definition might not be sufficient for unambiguous identification.[123] Factors to include here are (1) the nature of CD14/CD16 expression, i.e., their activation status and disease context and (2) similarity in the expression profile between natural killer (NK) cells and classical monocytes, with both cells being CD14^{++}CD16^{-}.[123–125] To improve monocyte subset identification, Villani et al. performed scRNA-seq analysis on human monocytes that were already fluorescence-activated cell sorter (FACS) sorted using CD14 and CD16 gating system, and this revealed four clusters, of which two were located within the intermediate monocyte subset.[126] Further, human intermediate monocyte subset was also found to cluster with both classical and non-classical monocytes, and newly identified Ly6C^{Int} monocytes in mice were found to show transcriptional as well as phenotypic overlap to both Ly6C^{hi} and Ly6C^{lo} monocytes.[127,128] These and other studies have finally suggested the possibility of a monocyte separation that is independent of CD14/CD16 gating system.[123,129] With application of high-parameter cytometry and scRNA-seq technology combined with high-dimensional analyses advanced the identification of new subsets within the three existing monocyte

populations.[123,126,128,130,131] For example, Hammers et al. identified eight monocyte subsets using CyTOF, of which three subsets fall within non-classical and four within classical monocyte categories,[130] and using 17-color FACS, Merah-Mourah et al. identified a set each of large and small monocytes, which could be further divided into a total of six monocyte subsets based on the expression of CD16.[131] In the context of MI, different studies reported that patients demonstrate accumulation of almost all three types of traditionally discussed monocytes, with some predicting that persistent elevation of intermediate type is associated with risk for major cardiovascular events.[97,132–136]

Future directions

Studies from Epelman, Dick, and Bajpai groups along with others clearly signify the presence of distinct macrophage subsets in human and mouse heart.[14,18,43] These distinct subsets have been shown to possess different origins, and their recruitment in steady-state and in infarcted heart involves a higher degree of complexity and plasticity. Although these studies help in expanding our current knowledge of RCMs to a larger extent, they also signify the need for greater understanding of the underlying dynamic processes required to delineate the probable differential contribution of these individual clusters to tissue repair post-MI injury. With the fact that molecular changes do occur in the infarcted heart at three different regions, viz., infarct, peri-infarct, and remote zones, future studies that involve comprehensive clustering approaches should also consider the importance of mapping spatiotemporal variations throughout the heart (Figure 2). Moreover, multiple findings affirm the notion that the field of monocyte heterogeneity is ever-growing. With the fact that monocyte replenishment plays a critical role in pathogenesis of acute MI through emergency hematopoiesis, there is a high possibility that multiple but distinct monocyte populations resulted from bone marrow and/or spleen (which are the possible sources for monocyte heterogeneity) may enter the heart, where they differentiate into multiple macrophage types based on the local milieu (Figure 2). It would be ideal to trace the different monocyte populations and their corresponding macrophage clusters while understanding their impact on infarcted heart, and any such successful effort would lead to a game-changer while developing targeted therapeutics. Additionally, future experiments should also be aimed at determining the impact of diverse stimuli on the newly identified monocyte/macrophage clusters. The idea, therefore, is to have a comprehensive cellular characterization followed by functional validation of the individual clusters for their importance in steady-state and infarcted hearts.

THERAPEUTIC IMPLICATIONS

The pathogenesis of MI involves aberrations in multiple biological processes through the participation of different cell types via diverse signal transduction mechanisms. Nevertheless, keeping in mind the translational potential based on the patient-specific needs in the clinical setup, promising therapies could be classified into four categories: (1) anti-inflammation, (2) anti-fibrosis, (3) cardioprotection and cardiac regeneration, and (4) pro-angiogenesis.[137] It is rational to predict that the selection of a single therapy or multiple therapies would be based on the stage of the MI complication (e.g., acute MI with inflammation or a recurrent MI with HF associated with it). The choice of key pathways for selecting appropriate therapy(ies) is based on their involvement in the disease pathogenesis, and this includes NLRP3/caspase-1 and TLR4/MyD88/NF-kB in inflammatory response; TGF-β/SMADs and Wnt/β-catenin in fibrosis; Notch, Hippo/YAP, RhoA/ROCK, Nrf2/HO-1, and Sonic hedgehog in cardiac protection (the crucial mediators in oxidative stress and apoptosis); and PI3K/Akt, MAPK, JAK/STAT, and Sonic hedgehog in angiogenesis.[137] Accordingly, multiple therapeutic strategies are emerging such as drug, protein, gene, cell, extracellular vesicle (e.g., exosome), carrier-based (e.g., nanoparticles), etc.[137] While providing an overview about anti-inflammatory and immunomodulatory agents with a focus on how they failed as therapeutic approaches against MI, here we delve into the basis behind macrophage as a promising therapeutic target by providing some lead findings in this line.

Targeting inflammation and immunomodulation

Broad spectrum anti-inflammatory agents

As discussed earlier, remodeling myocardium post-MI is associated with several mechanisms that can activate inflammation. This critical role of inflammation in multiple aspects of myocardial response post-injury draws attention, suggesting targeting it may hold promising beneficial effects. Such beneficial effects may include and not limited to preventing leukocyte-mediated cardiomyocyte injury in surviving cardiomyocytes, protecting cardiomyocytes from chronic apoptosis, increasing the tensile strength of the healing scar by restraining protease activation, suppressing inflammation-driven fibrogenic signaling, and selective activation of chemokine-dependent recruitment of progenitor cells to promote angiogenesis in the infarct zone.[8] Early attempts to inhibit inflammation were focused on broad anti-inflammatory strategies like use of glucocorticoids (GCs) and nonsteroidal anti-inflammatory drugs (NSAIDs).[8] These approaches were often associated with adverse consequences.[8] Though different studies suggested that GCs protect the infarcted myocardium by reducing cardiomyocyte necrosis and apoptosis,[138,139] higher-dose corticosteroid therapy has been shown to impair clearance of dead cells from the infarct.[140] This may disrupt fibroblast formation and ultimately lead to formation of thinner scars.[140] Moreover, clinical studies in patients with MI have produced conflicting results with the use of corticosteroids.[8,141,142] Because GC receptors are ubiquitously expressed in all nucleated cells, GCs exhibit a wide range of effects on several molecular cascades belonging to different cell types,[143] causing a range of adverse effects and therefore become unattractive options for treating MI patients.[8] Alternatively, NSAIDs, especially selective COX-2 inhibitors, were introduced in late 1990s as another broad anti-inflammatory strategy.[144] Although some initial studies in animal models suggested both non-selective non-aspirin NSAIDs and selective COX-2 inhibitors may have protective effects against MI injury,[145,146] others have shown detrimental effects on infarct healing causing a scar thinning and an accentuated systolic dysfunction.[147–150] On the clinical

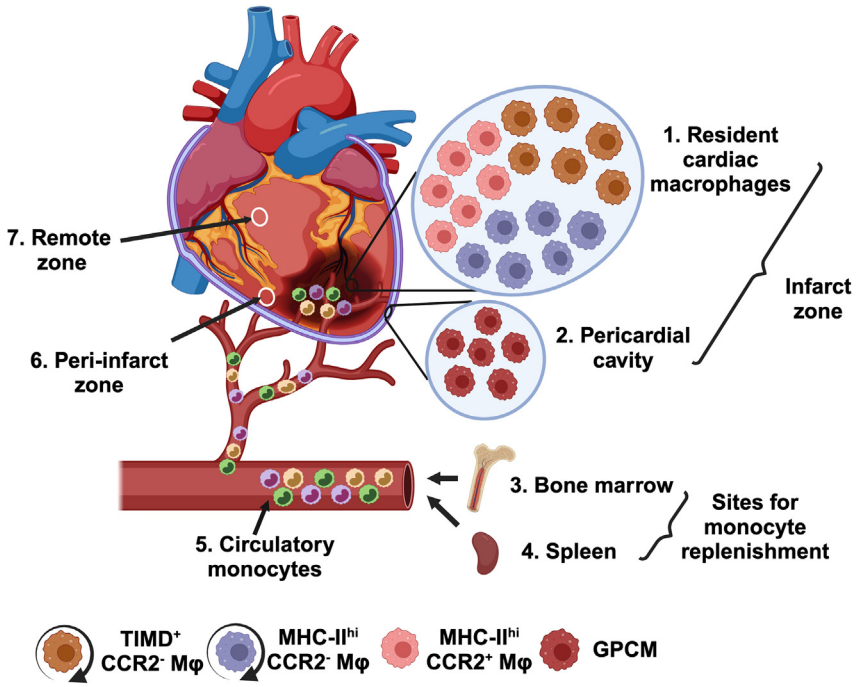

Figure 2. Different sites for sources of macrophage heterogeneity in an infarcted heart

An infarcted myocardium shows three functionally distinct regions, viz., infarct zone, per-infarct zone, and remote zone. Bone marrow and spleen act as the reservoirs to replenish the circulatory monocyte populations during MI, and multiple colors for depicting these cells indicate the possibility of multiple but distinctive monocyte populations entering the circulation. RCMs were shown with three different types of cells, which act as pillars upon further clustering of subsets that took place based on the presence or absence of other markers. Circulatory arrows around $TIMD^{+}$ $CCR2^{-}$ Mφ and $MHC\text{-}II^{hi}$ $CCR2^{-}$ Mφ indicate their ability to self-proliferate and renew locally in the heart. Apart from being the sources of global macrophage heterogeneity, all these sites may also act as the points of determination for spatiotemporal variations in macrophages. Created with BioRender.com.

side, these drugs increased the risk of death and reinfarction and an increased risk of hospitalization due to HF in post-MI patients.[151,152] One possible reason behind the failure of broad-inflammation-based therapies lies in the global participation of inflammatory cascades during MI pathogenesis.[153] For example, inflammatory pathways have been demonstrated to play crucial role during cardiac injury and repair phases post-MI.[153] Additionally, inflammatory mediators have also been shown to regulate cardiac angiogenesis by recruiting angiogenic progenitor cells to the infarct site.[154] Another major contributor that further complicates therapeutic implementation of promising targets is the notable heterogeneity in the underlying pathogenesis in human patients.[8] All these bring in the need to take into account important temporal and spatial considerations based on patient-specific needs while designing therapeutic strategies targeting inflammation.[8]

Targeted anti-inflammatory agents and immunomodulators

With significant advancement in our understanding of crucial molecular signals that are involved in the process of inflammation mediation following MI and in the light of the failure of the broad anti-inflammatory agents,[8] therapeutic interventions that are aimed at targeted inhibition of specific inflammatory signals were explored later, and the outcomes in the animal models suggest that such targeted inhibitions protect the infarcted hearts from acute injury and prevent adverse remodeling. Despite encouraging outcomes in preclinical studies, therapeutic implementation of inflammatory targets in patients with MI has been challenging.[8] Additionally, limited studies have tested the effects of non-specific immunomodulation and immunosuppression strategies as therapeutic modalities to treat MI. Early administration of low-dose immunosuppressive agents has been reported to exhibit beneficial effects in animal models.[155–157] Like broad-spectrum anti-inflammatory agents, these immunosuppressive agents have failed to demonstrate favorable outcomes in clinical settings. In a clinical trial involving limited STEMI patients, methotrexate administration did not attenuate the infarct size but worsened systolic dysfunction 3 months after the acute MI.[158] Similar results of failure to reduce infarct size and attenuate adverse remodeling in STEMI patients were also observed with intravenous injection of immunoglobulin.[159] Additionally, cyclosporine (another potent immunosuppressant) has attracted attention as a therapeutic agent, because of its effects as a cyclophilin B inhibitor. Unfortunately, a large randomized double-blind controlled trial showed no beneficial effects of its bolus dose in STEMI patients undergoing percutaneous coronary intervention.[160] All these findings draw our attention to aim for the therapeutic strategies regulating other aspects of the cardiac remodeling.

Macrophage as a promising therapeutic target

The idea that macrophages could become a druggable therapeutic target stems from their substantial impact on different aspects of the cardiac remodeling post-MI. Cardiac remodeling involves participation of multiple cardiac cells, and studies have revealed that macrophages interact with different cell types, causing divergent effects. Cardiomyocytes occupy about 70% of the heart volume,[161] whereas endothelial cells constitute majority of the non-cardiomyocyte cells in the heart.[162] Post-MI, damaged cardiomyocytes interact with RCMs and monocyte-derived macrophages to initiate additional recruitment of immune cells to drive the inflammatory response further. Interaction of endothelial cells with cardiac macrophages, on the other hand, has been shown to be essential for revascularization and angiogenesis. Following MI, formation of new blood vessels is essential for controlling compensatory hypertrophy and cardiac remodeling. Production of pro-angiogenic factors such as VEGFA,[66,102,163] platelet-derived growth factor α/β,[163] and insulin growth factor-1[163] by activated macrophages acts as a testament to their participation in angiogenesis post-injury.[164,165] STAT3 has been acknowledged as a critical transcriptional activator for this process,[166] and JAK/STAT signaling pathway has been indicated in promoting angiogenesis and myocardial functional reconstruction through induction of M2 macrophage polarization.[167] Moreover, environmental eustress-induced BDNF-mediated ERK1/2 and AKT pathways contributed to increased survival of $Ly6C^{lo}$ macrophages (and their CCR2-$MHCII^{lo}$ subsets), thereby improving cardiac function and ameliorated adverse ventricular remodeling after MI in mice.[168] Activation of ERK1/2 pathway has also been implicated in the prevention of polarization and chemotaxis of M1 macrophages in a study involving C-X-X chemokine receptor type 7 inhibition.[169] Further, VEGFC secreted by cardiac macrophages promoted myocardial lymphangiogenesis and ameliorated cardiac injury post-MI.[170] In heart, cardiac fibroblasts act as primary producers of extracellular matrix and have been critical in ventricular remodeling after MI. Post-MI, activated macrophages released pro-fibrotic factors like TGF-β[66] and fibronectin[102] that help to mechanically strengthen the heart. Moreover, coculturing of macrophages and cardiac fibroblasts resulted in increased production of fibroblast-derived IL-6, which finally led to increased SMAD3 phosphorylation due to augmented production of TGF-β in both cell types.[171] In the era of macrophage heterogeneity where multiple subsets or clusters were observed in both normal and MI hearts, it becomes critical to determine if there exist significant differences in the functional aspects of the newly discovered subsets (e.g., phagocytotic/efferocytotic/chemotactic). In other words, it becomes imperative to determine the differences, if exist, among multiple subsets in regulating cardiac processes governing overall cardiac remodeling post-MI and their possible therapeutic implications (Figure 3). Such studies may open new avenues in the selection of an appropriate therapy(ies) and the right strategy(ies) for their deployment.

Different treatment approaches exploiting macrophage phenotype

Studies aimed at exogenous cardioprotection using stem cells or stromal cells, which were intended to modulate macrophage polarization, have been explored as one of the new therapeutic approaches, and they demonstrated positive outcomes. Injection of fractionated bone marrow mononuclear cells or cardiac progenitor cells improved heart function through an acute sterile immune response characterized by temporal and regional induction of $CCR2^{+}$ and $CX3CR1^{+}$ macrophages.[172] This selective macrophage response has been shown to improve cardiac fibroblast activity, thereby enhancing the mechanical properties in the ischemic area. Additionally, injection of cells that were resultant of coculture of bone-marrow-derived macrophages with bone-marrow-derived mesenchymal stem cells in a rat model of MI resulted in a better and improved overall cardiac remodeling than the group that had injection of bone-marrow-derived mesenchymal stem cells alone.[173] Better cardiac remodeling in the mixed cell group was attributed to higher abundance of M2 phenotype macrophages, and these findings highlight the importance of priming as a complementary therapy for cardiac repair.[173] Extracellular vesicles (EVs), which have received increasing attention as cell-free therapeutics for regenerative medicine because of their lipid bilayer structure and cargos,[174] were also utilized in a study where CDC-derived EVs polarized macrophages to a special phenotype that is highly phagocytic and anti-inflammatory to display cardioprotection.[175] CDCs are cardiosphere-derived cells that are essentially cardiac stromal cells and represent a promising stem cell source for repairing damaged heart tissue.[176] Further, siRNAs that can inhibit the surface expression of markers on inflammatory monocytes were studied. Owing to their phagocytic abilities on particles ranging between nanometer and micrometer range, selected siRNA nanomolecules that can specifically silence CCR2 mRNA in the inflammatory monocytes were synthesized; these molecules were promptly taken up by monocytes, causing CCR2 mRNA degradation in the targeted monocytes and finally reducing infarct size.[52] Studies that involve different treatment approaches are highly encouraging and require exploiting macrophage-specific subsets in the evolving framework of heterogeneity (Figure 3).

Heterogeneity and the need for development of small molecule precision therapeutics

Alternatively, newly discovered macrophage clusters can also be utilized for development of precision medicine strategies through identification of unique small molecules that can target a specific subset. scRNA-seq analysis of human macrophages that are activated by IFNɣ (M(IFNɣ)) identified two major clusters, viz., inflammatory $(M(IFN\gamma)^{i})$ and phagocytotic $(M(IFN\gamma)^{p})$.[177] Library of integrated-network-based cellular signatures (LINCS) is a drug-gene interaction database that comprises >1 million gene expression profiles of chemically perturbed human cell lines. Using LINCS, BI-2536, a PLK (Polo-like kinase) inhibitor was identified to shift the macrophage phenotype, modulated inflammation, and decreased atherosclerosis and calcification.[177] To summarize, the emerging framework of macrophage heterogeneity looks promising for designing different therapeutic approaches that can demonstrate unique benefits for patient-specific needs (Figure 3).

Conclusion

Given the phenotypic variation exhibited by macrophages owing to their spectrum of activation states in response to a diversity of stimuli, understanding their role in MI may require looking beyond the M1-M2 paradigm. This realization dovetail's rather nicely with the maturation

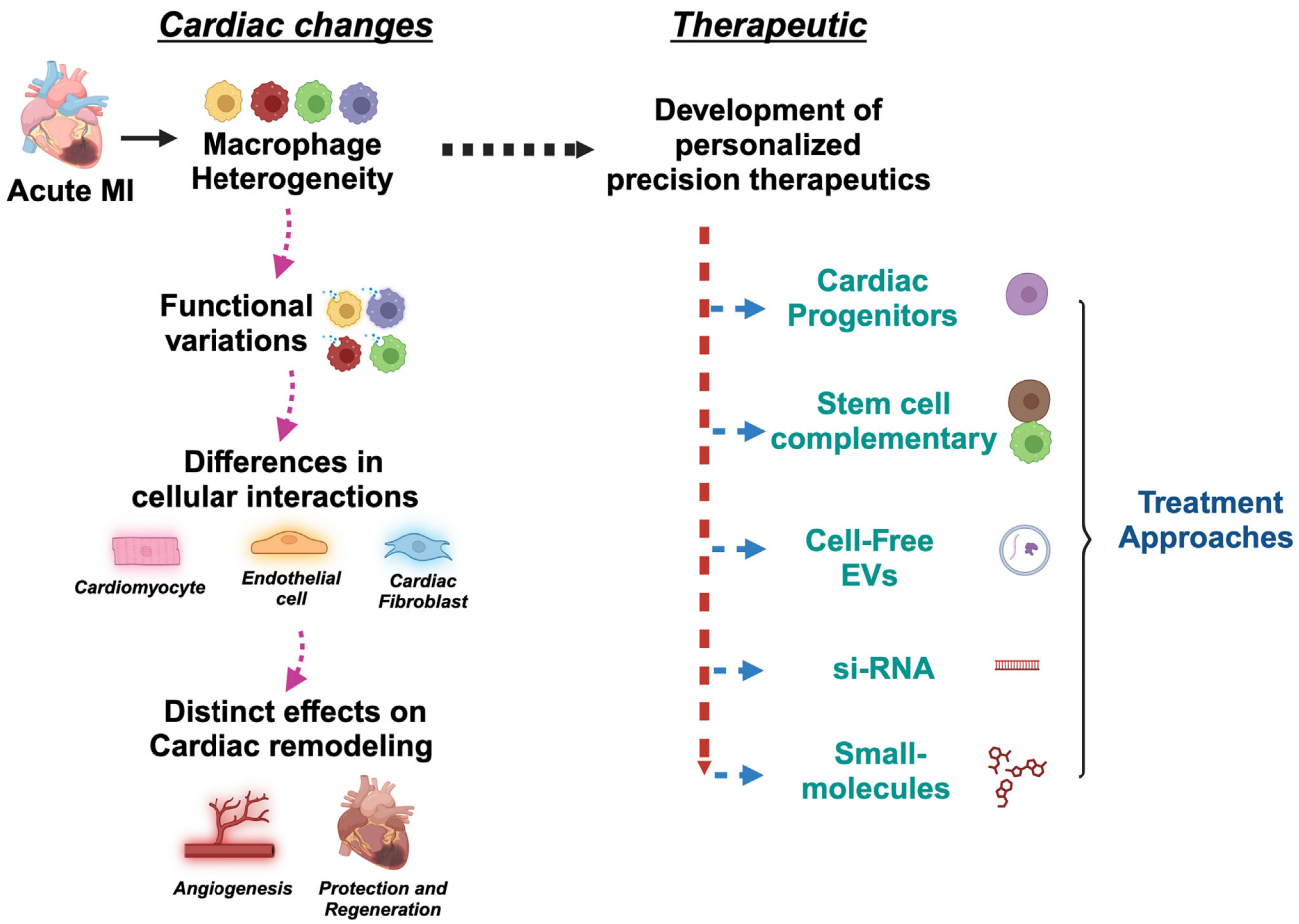

Figure 3. Therapeutic implications of macrophage heterogeneity in an infarcted heart

An infarcted heart consists of heterogeneous macrophage populations resulted from resident and monocyte-derived macrophage sources. Both temporal and spatial considerations determine the nature and type of heterogeneity involved, and such variations would form the basis behind their distinct functional effects, if exist. And this indeed acts as a rationale for developing personalized precision therapeutics based on patient-specific needs. Cardiac progenitors as primary or complementary therapies, cell-free therapeutic approaches such as extracellular vesicles (EVs), and design and development of targeted RNA-based silencing as well as small molecule inhibitors, all these approaches could be utilized for exploiting macrophage phenotype in the evolving framework of heterogeneity. Dotted lines indicate the need for further studies in this area of research. Created with BioRender.com.

of high-throughput single-cell technology, which allows us to move beyond a known set of cell-surface markers and use unbiased clustering approaches to identify macrophage subsets. These unique clustering approaches may further shed light on previously unknown macrophage populations influencing repair and maladaptive remodeling in the heart after MI. However, functional validation is critical in dissecting the biology of these new approaches. Nonetheless, generating new knowledge for the better understanding of such macrophage subsets as well as their precursors (i.e., different monocyte populations) and the pathways involved in their development, function, and interaction with other cells would allow us to design rationalized druggable targets. This ultimately leads to development of therapeutics that could explicitly work on those specific therapeutic targets by excluding any unwanted off-target effects.

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AUTHOR CONTRIBUTIONS

B.K.: conceptualization, writing—original drafting, writing—review & editing, and visualization. G.S.: writing—original drafting and editing. P.B.: writing—review and visualization. A.M.: writing—review and editing, visualization, and valuable suggestions. P.R.N.: supervision, reviewed, and editing. All authors have reviewed and approved the final manuscript for publication.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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