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CONTEMPORARY REVIEW

Impact of Reperfusion on Temporal Immune Cell Dynamics After Myocardial Infarction

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ABSTRACT: Excessive inflammation and impaired healing of cardiac tissue following a myocardial infarction (MI) can drive the development of heart failure. Cardiac repair begins immediately after the onset of MI and continues for months. The repair process can be divided into the following 3 overlapping phases, each having distinct functions and sequelae: the inflammatory phase, the proliferative phase, and the maturation phase. Macrophages, neutrophils, and lymphocytes are present in the myocardium throughout the repair process and govern the duration and function of each of these phases. However, changes in the functions of these cell types across each phase are poorly characterized. Numerous immunomodulatory therapies that specifically target inflammation have been developed for promoting cardiac repair and preventing heart failure after MI. However, these treatments have been largely unsuccessful in large-scale clinical randomized controlled trials. A potential explanation for this failure is the lack of a thorough understanding of the time-dependent evolution of the functions of immune cells after a major cardiovascular event. Failure to account for this temporal plasticity in cell function may reduce the efficacy of immunomodulatory approaches that target cardiac repair. This review is concerned with how the functions of different immune cells change with time following an MI. Improved understanding of the temporal changes in immune cell function is important for the future development of effective and targeted treatments for preventing heart failure after MI.

Key Words: cardiac remodeling ■ heart failure ■ inflammation ■ myocardial infarction ■ reperfusion injury

Acute myocardial infarction (MI) and its sequelae comprise the single highest cause of mortality and disease burden globally.[1] MI is most typically a consequence of atherothrombosis, wherein blood flow through a coronary artery is obstructed by the formation of an occlusive thrombus over a ruptured or eroded atherosclerotic plaque. Despite advancements in therapy, cardiac function remains suboptimal in most patients after an MI, and the risk of progression to ischemic heart failure (HF) remains high. Adverse left ventricular (LV) remodeling underpins the development of HF after an MI. This is largely determined by the initial size of the infarction and effectiveness of healing after MI. Large infarcts[2] and excessive inflammation[3] both compromise post-MI cardiac repair and increase the risk of developing HF. Although timely reperfusion of the occluded coronary artery by primary percutaneous coronary intervention (PCI) is the gold standard treatment for reducing infarct size, there are few effective therapies that successfully mitigate the aberrant inflammatory response and improve healing and recovery after MI.[1]

As immune cells play a pivotal role in mediating inflammation and promoting tissue repair after MI, they are a promising target for the development of cardioprotective therapies and HF prevention. Although the major types of immune cells have distinct functions that vary with time after MI, how these functions evolve over time is poorly characterized. Improved understanding of these immune responses could enable the identification of potential treatment strategies for curbing adverse LV remodeling and the development of HF.

Immune cell dynamics after MI differ depending on whether reperfusion of the myocardium is achieved. Reperfusion causes inflammatory leukocytes to infiltrate more rapidly into the previously ischemic area.[4] This

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Nonstandard Abbreviations and Acronyms

IRI	ischemia/reperfusion injury
NETs	neutrophil extracellular traps
ROS	reactive oxygen species
Tregs	regulatory T lymphocytes

review describes how inflammatory cell infiltration and the actions of the different immune cells change with time following MI and reperfusion. This knowledge is significant in the clinical context, where most patients with MI undergo PCI or thrombolytic therapy to achieve myocardial reperfusion. Novel mechanistic insights from studies of nonreperfused MI are also described.

PHASES OF CARDIAC REPAIR AFTER MI

Before outlining the dynamic roles of inflammatory leukocytes and immune cells after MI and reperfusion, the different stages of healing after MI are summarized. Cardiac repair begins immediately after the onset of ischemia. It has the following 3 distinct phases[5]: the inflammatory phase, the proliferative phase, and the maturation phase.

Inflammatory Phase

Immediately following MI, the immune system mounts a sterile inflammatory response to initiate the healing of damaged cardiac tissue. The initial ischemic insult induces massive cardiomyocyte necrosis and cell death in the ischemic myocardium. Cell components released from dying cardiomyocytes act as danger signals, or damage-associated molecular patterns, resulting in inflammasome activation. This in turn stimulates caspase activity and the release of IL (interleukin)-1β, causing continued cell death and the production of proinflammatory cytokines and chemokines that recruit leukocytes to the infarct zone (Figure 1).[5] This initial inflammatory response clears necrotic cells from areas of damage in preparation for scar formation. The inflammatory phase lasts up to 4 days from the onset of MI in humans and up to 3 days in mice.[5]

Reperfusion therapy exerts a number of effects on cardiac healing during the inflammatory phase. PCI and thrombolysis restore blood flow and reoxygenate the myocardium after MI. Although timely reperfusion is necessary to limit the ischemic insult and prevent further myocardial necrosis, it can also exacerbate tissue injury and inflammation. The rapid restoration of oxygen and the newly recruited immune cells create a pro-oxidant and proinflammatory environment that can initiate apoptosis and cause death of otherwise salvageable cardiomyocytes for up to 3 days after successful reperfusion.[1,6] The paradoxical myocardial injury that accompanies reperfusion is termed *ischemia/reperfusion injury* (IRI) and accounts for up to 50% of the final infarct size.[7]

Proliferative Phase

Resolution of the post-MI inflammatory phase marks the onset of the proliferative phase and is characterized by the development of early granulation tissue. Inflammatory leukocytes are either cleared from areas of tissue damage or transformed into reparative leukocytes that release anti-inflammatory, reparative cytokines.[5] Granulation tissue is formed during the proliferative phase in a process driven by angiogenesis and the deposition of collagen by myofibroblasts that leads to the development of scar tissue. In humans, this phase primarily occurs from day 5 to day 14 after MI and from days 4 to 7 in mice.[5]

Maturation Phase

The maturation phase is the final stage of cardiac repair after MI. This phase is characterized by collagen cross-linking and the cessation of angiogenesis as well as clearance of fibroblasts and leukocytes from areas of tissue damage. The extracellular matrix remodeling that underpins the maturation phase of post-MI cardiac repair results in the formation of a final fibrous, acellular scar.[5] The maturation phase can continue for months after resolution of the proliferative phase and may be accompanied by low-level inflammation that drives cardiomyocyte apoptosis, suppression of cardiac contractility, and additional extracellular matrix remodeling. Inappropriate inflammation across these 3 phases mediates structural and functional changes to the left ventricle that diminish cardiac output, a process that is termed *adverse remodeling* and may ultimately result in HF.[1]

IMMUNE CELL DYNAMICS AFTER MI AND REPERFUSION

Immune cells are indispensable for effective healing and rapidly infiltrate into the myocardium after MI to coordinate cardiac tissue repair. Figure 2 outlines the kinetics of immune cell infiltration into damaged myocardium after MI and reperfusion in mice. The specific functions of each cell type vary according to the phase of healing.

Monocytes and Macrophages

Inflammatory Phase

Following the onset of MI, activated circulating monocytes adopt a proinflammatory phenotype and rapidly

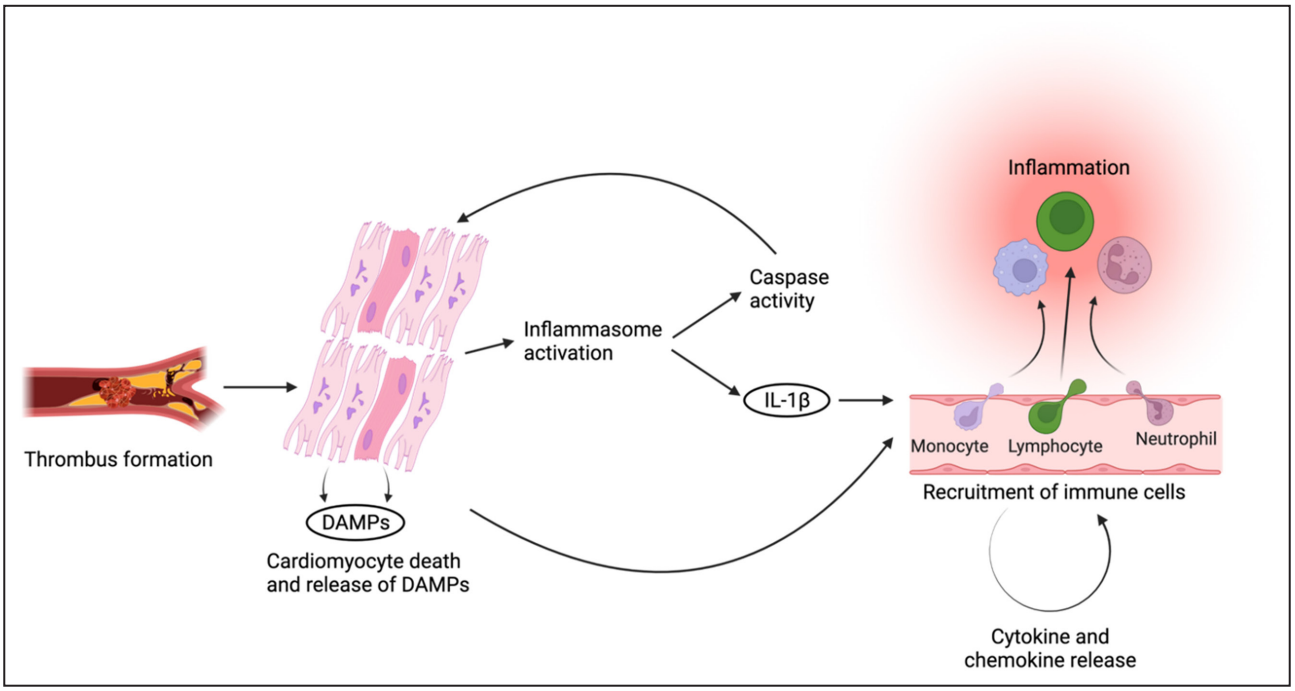

Figure 1. The cascade of cellular events resulting in the initiation of the inflammatory phase of healing after myocardial infarction.

During myocardial infarction, the formation of an occlusive thrombus in the coronary artery causes massive cardiomyocyte necrosis in the myocardium. The dying cardiomyocytes release DAMPs, which activate inflammasomes and initiate the recruitment of immune cells. Activation of inflammasomes in turn activates caspases within cardiac cells, triggering apoptosis and further cell death. The activated inflammasomes also induce the release of IL-1β, which additionally stimulates immune cell recruitment. As immune cells infiltrate into the infarcted myocardium, they release proinflammatory cytokines and chemokines that further enhance immune cell recruitment, initiating a sterile inflammatory response that signals the onset of the inflammatory phase of healing. DAMPs indicates damage-associated molecular patterns; and IL-1β, interleukin-1β. Created with BioRender.

infiltrate into the myocardium, where they differentiate into macrophages.[8]

When assessed 2 days after reperfusion by PCI, proinflammatory cluster of differentiation (CD) $14^{++}CD16^{-}$ monocyte levels in patients with MI increase, and anti-inflammatory $CD14^{dim}CD16^{+}$ monocyte levels decrease.[9] Moreover, as early as 90 minutes following reperfusion, anti-inflammatory monocytes are preferentially depleted in the circulation of patients with ST-segment–elevation MI, and the rapid fall in anti-inflammatory monocytes correlates with larger infarct size and lower LV ejection fraction (LVEF).[10] Taken together, these findings suggest that the systemic increase in proinflammatory monocyte levels has the capacity to exacerbate IRI in humans. It is, however, important to note that the definitions of proinflammatory and anti-inflammatory monocyte and macrophage markers are controversial.

Few studies have examined the precise role of macrophages in the late inflammatory phase after MI in humans. Human autopsy studies have shown that the macrophages that accumulate in the myocardium during the first 5 days after MI are mostly proinflammatory and monocytic in origin.[11,12] However, these studies did not differentiate between patients who had undergone successful reperfusion therapy from patients who had not. Mobilization of splenic monocytes and increased proinflammatory blood monocyte levels ensure sustained influx of monocytes into the damaged myocardium and characterize the end of the inflammatory phase.[9,12,13]

Preclinical studies have provided important mechanistic insights into the contributions of monocytes and macrophages to the inflammatory phase. A recently identified subset of self-renewing, tissue-resident macrophages that are derived from embryonic precursors have been found to play an important role in cardiac repair following MI in mice.[14] Although most tissue-resident macrophages in the myocardium are lost during the initial ischemic insult, those that do remain eventually contribute to the anti-inflammatory and reparative actions of macrophages in the proliferative phase of healing.[15–18] In mice, monocytes are also released from the spleen into the circulation at the onset of ischemia and rapidly infiltrate into the myocardium.[4,11,18] Reperfusion further promotes rapid monocyte infiltration during this stage.[10] After recruitment to areas of myocardial damage, monocytes differentiate

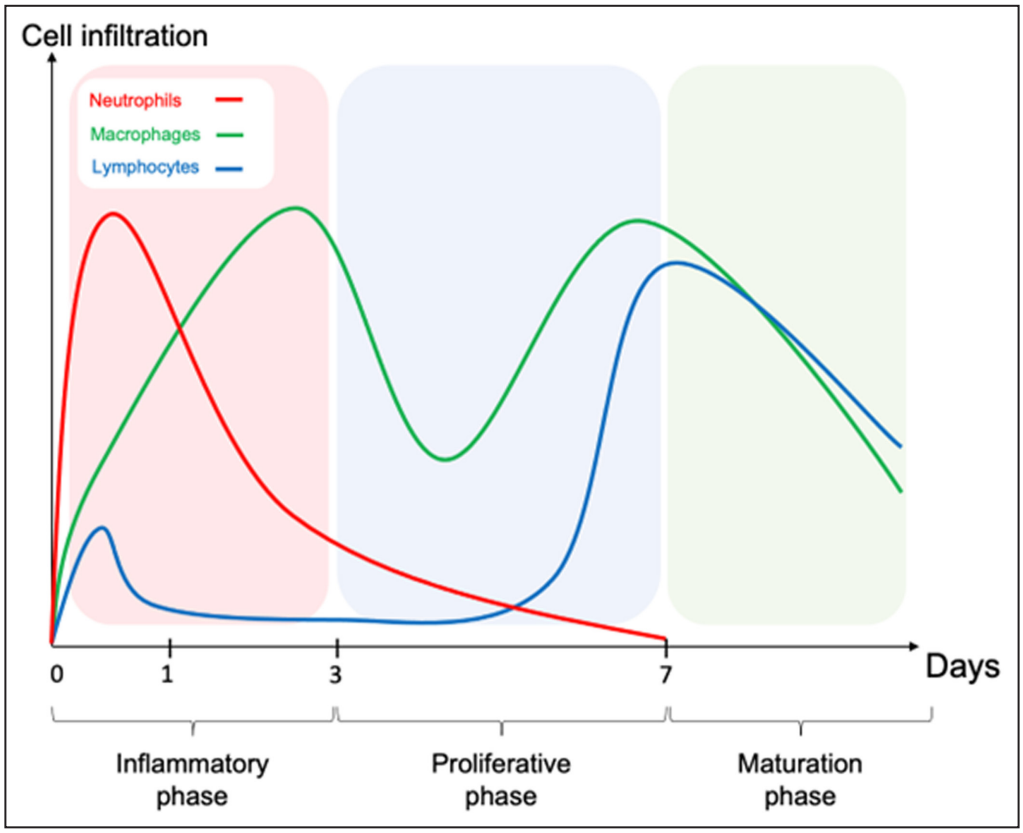

Figure 2. Neutrophil, macrophage, and lymphocyte cellular infiltration kinetics after myocardial infarction in mice.
The inflammatory, proliferative, and maturation phases of healing are each characterized by unique patterns of immune cell infiltration. These leukocyte infiltration kinetics govern the length and function of each of the 3 phases of cardiac repair. Compiled from Yan et al[4] and Rusinkevich et al.[19]

into proinflammatory macrophages that clear necrotic cells and break down the extracellular matrix in the infarct zone. Myocardial macrophage numbers peak at around 3 days after reperfusion (Figure 2).[4,19] The presence of proinflammatory macrophages in the myocardium after an MI is crucial for effective healing, as impaired clearance of necrotic and apoptotic cells from areas of tissue damage prolongs the inflammatory phase, resulting in poor scar formation and an increased risk of complications such as ventricular rupture.[20,21] Inhibition of early monocyte recruitment by microvascular obstruction, wherein occlusion of the microvasculature by leukocytes and platelet thrombi prevents successful reperfusion of the myocardium, results in the loss of these important macrophage functions, leading to poorer long-term healing.[22]

Proinflammatory macrophages may also exacerbate IRI through the release of reactive oxygen species (ROS), proteolytic enzymes, and inflammatory cytokines, all of which induce cardiomyocyte apoptosis to cause further tissue damage (Figure 3A).[23] These macrophages additionally exacerbate tissue damage by recruiting interferon-γ–producing T lymphocytes, which in turn amplify the local inflammatory immune response in a process that is inhibited by the lectin receptor, Dectin-2.[24] Studies in which this excessive proinflammatory macrophage activity is supressed by deletion of the cell surface receptors Dectin-2 or Lgr4 (leucine-rich repeat-containing G protein-coupled receptor 4) have successfully ameliorated infarct size and reduced long-term adverse remodeling.[24,25] Other studies have shown that manipulation of macrophage polarization toward an anti-inflammatory phenotype after induction of ischemia and reperfusion alleviates IRI, decreases infarct size and myocardial damage, and improves ventricular function.[26–29] These studies further demonstrate that anti-inflammatory macrophages can be induced as early as 1 day after reperfusion, thus identifying macrophage polarization as a promising therapeutic target.

Proliferative Phase

Resolution of the initial post-MI inflammation marks the onset of reparative cell proliferation and the formation of scar tissue. At this point, the macrophages in the myocardium, most of which have an anti-inflammatory

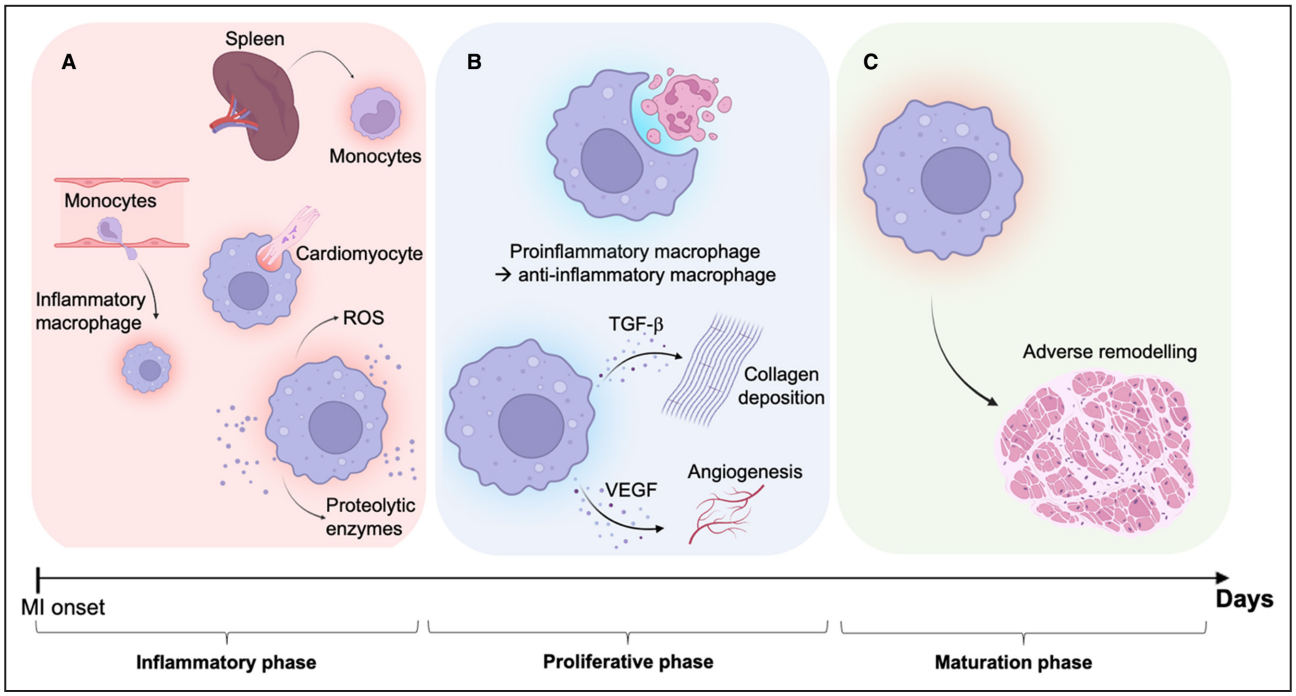

Figure 3. Temporal dynamics of macrophage infiltration and function after MI and reperfusion.
A, During the initial period of ischemia/reperfusion injury, macrophages in the myocardium are derived largely from circulating proinflammatory monocytes. Splenic monocyte release sustains the large influx of monocytes into the infarct zone. After entering the myocardial tissue, proinflammatory monocytes differentiate into macrophages that phagocytose dying cardiomyocytes and release ROS and proteolytic enzymes. **B**, At the onset of the proliferative phase, macrophages polarize from the proinflammatory to the anti-inflammatory phenotype. Throughout this phase, macrophages coordinate the formation of early granulation tissue by inducing deposition of collagen and angiogenesis through the release of TGF-β and VEGF, respectively. **C**, Months after MI, proinflammatory macrophages may persist in the myocardium and contribute to adverse remodeling of the left ventricle. MI indicates myocardial infarction; ROS, reactive oxygen species; TGF-β, transforming growth factor-β; and VEGF, vascular endothelial growth factor. Created with BioRender.

phenotype, begin to coordinate the formation of granulation tissue. Although autopsy studies of patients with MI indicate that this process begins in the subacute phase of healing, from 5 to 20 days,[11,12] the kinetics of the transition between the inflammatory and proliferative phases in humans remain poorly understood.

A key process driving the transition from the inflammatory to the proliferative phase is efferocytosis, wherein macrophages phagocytose apoptotic cells. Efferocytosis of apoptotic neutrophils and cardiomyocytes polarizes macrophages toward the anti-inflammatory phenotype approximately 3 days after reperfusion in mice (Figure 3B).[21,30] Impaired efferocytosis prolongs the inflammatory phase and delays healing after MI and reperfusion.[31] Once polarized toward an anti-inflammatory, reparative phenotype, macrophages secrete anti-inflammatory, angiogenic, and profibrotic cytokines that inhibit further proinflammatory leukocyte influx, such as TGF-β (transforming growth factor–β) and VEGF (vascular endothelial growth factor), and promote angiogenesis and collagen deposition in a process that initiates scar tissue formation (Figure 3B).[32] The polarization of macrophages toward an anti-inflammatory phenotype is associated with a second peak in macrophage numbers in the myocardium after reperfusion (Figure 2). This further drives tissue repair during the proliferative phase of healing.[4,19] Of translational relevance is the fact that polarization of macrophages to an anti-inflammatory phenotype reduces long-term adverse remodeling in mice, even if the polarizing therapy is not initiated until 3 days after reperfusion, at the start of the proliferative phase.[33]

Maturation Phase

Few studies have delineated the role of macrophages in the maturation phase following reperfusion after MI. In humans, myocardial biopsies of patients with HF taken at the time of LV assist device insertion showed the presence of both tissue-resident and monocyte-derived macrophages.[34] In these samples, a greater abundance of proinflammatory monocyte-derived macrophages was associated with increased remodeling and LV dysfunction. Other studies have shown that lower levels of circulating anti-inflammatory monocytes are associated with an increased risk of mural thrombi formation months after MI.[20] Together, these observations provide evidence for an ongoing detrimental role

of proinflammatory monocytes and macrophages during the maturation phase of healing (Figure 3C).

To date, insights into the role of macrophages in the maturation phase have been limited to preclinical studies in nonreperfused murine MI models. These studies show that continued infiltration of monocytes contributes to inflammatory macrophage populations in the nonreperfused myocardium, where they can worsen long-term fibrosis and adverse remodeling.[35,36] However, the pro- and anti-inflammatory activities of macrophages and the regulation of macrophage levels in areas of damage remain poorly understood in the months after MI and reperfusion. Further clinical and preclinical studies are warranted to better understand these longer term processes.

Neutrophils

Inflammatory Phase

Similar to monocytes and macrophages, neutrophils have also been implicated in the pathogenesis of IRI. Clinical studies have consistently shown that high levels of circulating neutrophils or a high neutrophil-to-lymphocyte ratio around the time of reperfusion therapy are both associated with increased mortality, poorer LVEF, and an increased risk of developing HF after MI.[37] Neutrophils are systemically and maximally activated in humans within 4 hours after the onset of MI, leading to the release of ROS and proinflammatory enzymes, such as myeloperoxidase, neutrophil elastase, and matrix metalloproteinases.[38,39]

Neutrophils are also the primary source of the proinflammatory alarmins, S100A8 and S100A9, after MI.[40] The release of S100A8 and S100A9 in the myocardium stimulates additional leukocyte recruitment and increases the secretion of proinflammatory cytokines. In addition, high serum S100A8 and S100A9 levels after PCI are associated with poor LVEF and an increased incidence of HF.[40,41] Another mechanism by which neutrophils exacerbate IRI is via formation of neutrophil extracellular traps (NETs), which are large networks of decondensed chromatin and cytoplasmic proteins that contribute to inflammation after MI (Figure 4A). Approximately a quarter of thrombi retrieved from patients with MI contain NETs, and higher levels of NETs in the thrombus[42,43] and serum[44–46] after PCI are associated with increased infarct size and poorer LVEF. The NETs in a culprit coronary artery can also induce

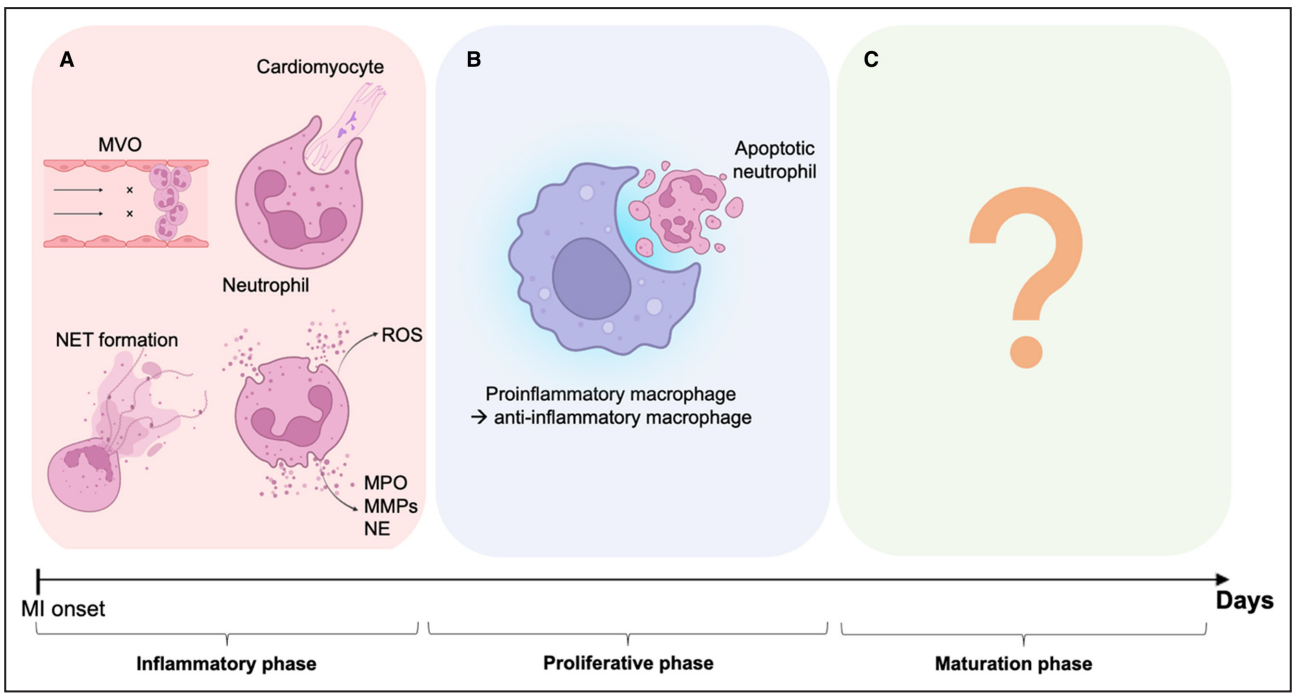

Figure 4. Temporal dynamics of neutrophil infiltration and function after MI and reperfusion.
A, In the immediate inflammatory response to MI, neutrophils accumulate in the microvasculature and impair reperfusion by blocking further blood flow, causing MVO. The formation of NETs can also exacerbate ischemia/reperfusion injury. Once in the myocardial tissue, neutrophils can phagocytose dying cardiomyocytes, produce ROS, and release inflammatory enzymes, including myeloperoxidase, matrix metalloproteinases, and neutrophil elastase, all of which exacerbate inflammation and cause additional myocardial injury. **B**, In the proliferative phase, neutrophils reach the end of their natural lifespan, undergo apoptosis, and are taken up by macrophages through efferocytosis. This polarizes the macrophages to an anti-inflammatory phenotype, leading to the resolution of inflammation and the initiation of the proliferative phase. **C**, The long-term actions of neutrophils in the maturation phase remain poorly understood. MI indicates myocardial infarction; MMPs, matrix metalloproteinases; MPO, myeloperoxidase; MVO, microvascular obstruction; NE, neutrophil elastase; NET, neutrophil extracellular trap; and ROS, reactive oxygen species. Created with BioRender.

monocyte chemoattractant protein-1 and increase adhesion molecule expression in endothelial cells, both of which increase inflammatory cell recruitment and infiltration.[47] Of particular clinical significance is that metoprolol, the only cardioprotective drug that has been shown to decrease infarct size in patients after IRI, exerts its cardioprotective effects by inhibiting neutrophil infiltration and activation in mice and patients with MI.[48–50]

Studies of ischemia and reperfusion in mice are mostly consistent with human studies and have confirmed the detrimental impact of neutrophils on IRI. Neutrophil infiltration into the myocardium peaks within 24 hours after reperfusion (Figure 2).[4,19] Increased neutrophil infiltration is associated with proinflammatory macrophage polarization and poor outcomes, whereas decreased neutrophil influx is cardioprotective.[27] Neutrophils exacerbate IRI primarily by contributing to oxidative stress and aggravating microvascular obstruction (Figure 4A). It has long been known that the pro-oxidant enzyme, myeloperoxidase, which is secreted from neutrophils during reperfusion, produces ROS, causes cellular damage, and induces cardiomyocyte apoptosis.[51] Neutralizing ROS in the myocardium with antioxidants such as α-tocopherol can reduce neutrophil infiltration and decrease infarct size.[52] Studies in mice have further established that neutrophil-derived S100A8 and S100A9 exacerbate IRI and cardiomyocyte death by impairing cardiomyocyte mitochondrial complex I activity,[40] acting as chemoattractants for monocytes,[53] and amplifying granulopoiesis, which increases IL-1β secretion.[54]

Beyond the initial period of IRI, neutrophils continue to act as effectors of myocardial damage during the remainder of the inflammatory phase of healing. Inhibiting neutrophil infiltration in mice decreased infarct size and improved LVEF 1 day after MI and reperfusion.[55] However, this study failed to show further improvement despite continued inhibition of neutrophil infiltration for a further 6 weeks after reperfusion. Given that neutrophil infiltration into the myocardium peaks at <1 day after reperfusion,[4,19] these findings collectively indicate that the deleterious short-term effects of neutrophils are mostly restricted to the early inflammatory phase, where they secrete proinflammatory cytokines and pro-oxidant enzymes that exacerbate cardiomyocyte death, leading to poor recovery and loss of ventricular function (Figure 4A).[56,57] Accordingly, some studies have shown that early, short-term reduction of neutrophil infiltration by knocking down or pharmacologically inhibiting 1 of the surface markers expressed on neutrophils, TREM-1 (triggering receptor expressed on myeloid cells–1), during the acute inflammatory post-MI period reduces cardiac fibrosis and improves long-term ventricular function.[58,59] However, emerging evidence indicates that neutrophils may not only cause myocardial damage after reperfusion but also may exert some cardioprotective effects. For example, healing is impaired and mortality increases in the absence of NET formation[60] and the neutrophil-derived peptide, netrin-1.[61] Further investigation into the mechanisms by which neutrophils promote healing after MI and reperfusion may enable the development of future neutrophil-targeted treatments.

Proliferative Phase

Neutrophils indirectly drive the onset of the proliferative phase of cardiac repair by secreting neutrophil gelatinase-associated lipocalin, which induces anti-inflammatory macrophage polarization (Figure 4B).[30] However, whether neutrophils have an ongoing role in the proliferative phase after MI is poorly understood. Neutrophil numbers have been reported to return to baseline by the onset of the proliferative phase following reperfusion after ST-elevation MI in humans, suggesting that they no longer promote myocardial tissue damage.[62] This is consistent with what has been reported in mice, where the few neutrophils that do remain during the proliferative phase of the repair process adopt a noninflammatory phenotype.[63,64]

Maturation Phase

During the final maturation phase following reperfusion, neutrophils contribute to the persistent, low-level inflammatory state that predisposes patients with MI to developing HF via mechanisms that are poorly understood (Figure 4C). Many patients with HF have increased circulating neutrophil levels or an increased neutrophil-to-lymphocyte ratio as well as elevated neutrophil gelatinase-associated lipocalin levels.[65,66] Neutrophils isolated from patients with HF also release the proinflammatory cytokines IL-6 and IL-8 and have impaired secretion of the proangiogenic cytokine VEGF and the anti-inflammatory cytokine IL-1RA.[67] Furthermore, although BNP (B-type natriuretic peptide) from healthy patients can suppress neutrophil superoxide generation, this effect is lost in patients with HF, indicating that neutrophils from these patients are resistant to BNP-mediated ROS suppression and are thus likely to experience increased oxidative stress.[68] These findings suggest that neutrophils likely play a proinflammatory role in the maturation phase and may exacerbate progression to HF in humans. Unfortunately, few preclinical studies have focused on the role of neutrophils in cardiac repair weeks to months after MI, indicating an area in need of further research.

Lymphocytes

Inflammatory Phase

The contribution of lymphocytes to IRI varies widely. Several subsets of lymphocytes are involved in cardiac

repair, including B lymphocytes, CD4+ T lymphocytes, CD8+ T lymphocytes, and regulatory T lymphocytes (Tregs), a subset of CD4+ lymphocytes with immunosuppressive properties. The importance of lymphocytes in cardiac repair in humans is well established. Lymphocytes have prognostic value, with their sequestration from the circulation into the myocardium being associated with worse clinical outcomes.[37,69] Indeed, the reduction in circulating lymphocytes immediately after reperfusion correlates with infarct size and the exacerbation of IRI.[69] Lymphocytes also contribute to microvascular obstruction, impaired reperfusion, and exacerbation of IRI in humans (Figure 5A).[70] Patients with high circulating levels of activated CD8+ T lymphocytes during this phase tend to have increased cardiac injury and poorer long-term ventricular function.[71] CD8+ T lymphocytes from these patients have enhanced cytotoxic properties that can cause further injury to the infarcted myocardium.[72] Activated CD4+ T lymphocytes also secrete proinflammatory cytokines, which amplify the cytotoxic effects of CD8+ T lymphocytes and exacerbate cardiac injury in humans after MI (Figure 5A).[73] Attempts at reducing this response with the immunosuppressant cyclosporine have reduced postreperfusion lymphopenia, but failed to improve clinical outcomes, suggesting that the deleterious lymphocyte actions are not inhibited by cyclosporine.[74] Better understanding of these effects is needed for targeted development of treatments that reduce IRI. B lymphocytes additionally aggravate inflammatory myocardial injury by releasing CCL7 (chemokine ligand 7), which increases monocyte infiltration.[75]

Preclinical studies have similarly implicated lymphocytes as culprits in IRI. Although lymphocytes typically require activation by a specific antigen to perform their immune functions, early involvement of lymphocytes in IRI is likely not antigen specific.[76] Although dendritic cells activate cardiac antigen-specific autoreactive T lymphocytes during the initial ischemic insult, adaptive immune responses take weeks to develop before they influence healing after MI and reperfusion.[77,78] The immediate onset of these lymphocyte actions is therefore most likely triggered directly by the inflammatory environment and the release of damage-associated molecular patterns.[79] Lymphocytes infiltrate into the myocardium within minutes after reperfusion in mice, peaking between 1 and 3 days after reperfusion (Figure 2) and exacerbate IRI by accumulating in the

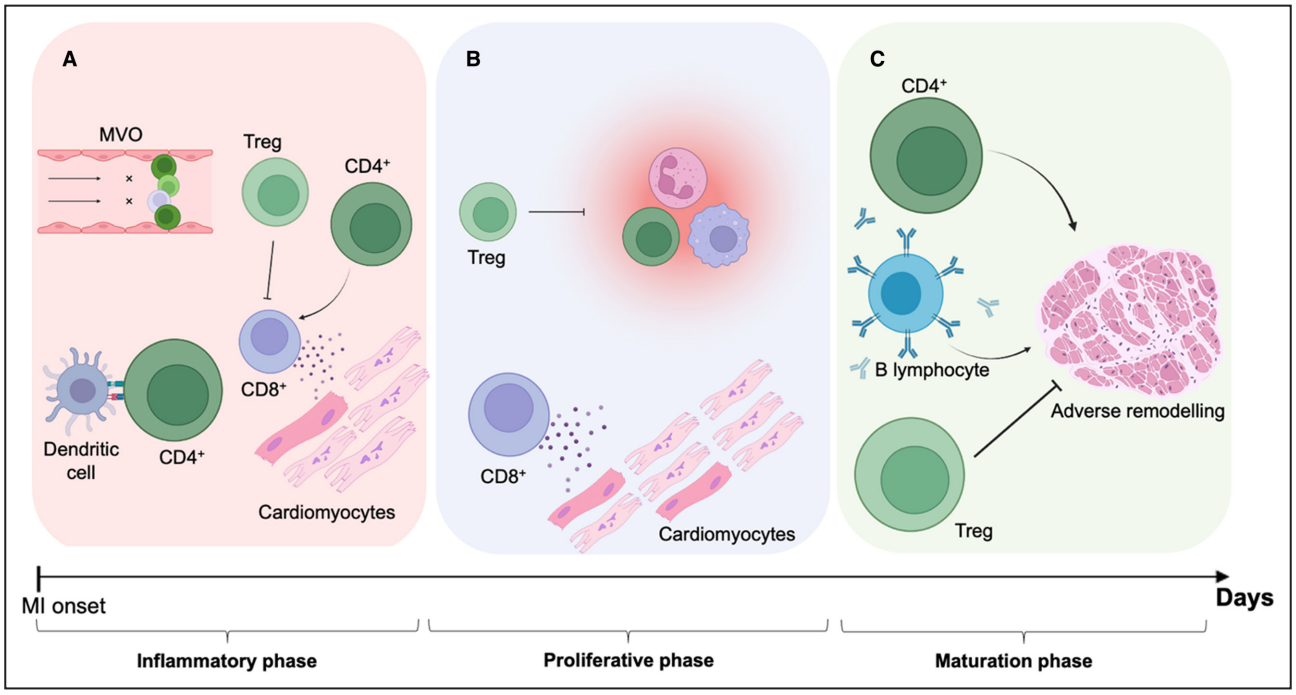

Figure 5. Temporal dynamics of lymphocyte infiltration and function after MI and reperfusion.
A, Immediately following reperfusion, lymphocytes aggregate in the microvasculature and cause MVO, aggravating ischemia/reperfusion injury. Concurrently, dendritic cells also prime the adaptive, antigen-specific immune response by presenting cardiac autoantigens to CD4+ T lymphocytes. In the later stages of the inflammatory phase, CD8+ T lymphocytes impair healing by releasing cytotoxic enzymes, an effect that may be amplified by CD4+ T lymphocytes. However, Tregs can inhibit these detrimental actions and limit cardiac damage. **B**, Throughout the proliferative phase, CD8+ T lymphocytes secrete cytotoxic enzymes while Tregs promote the resolution of inflammation. **C**, In the late maturation phase, the adaptive immune response mounts an autoantigen-specific response involving T and B lymphocytes causing inflammation that contributes to adverse left ventricular remodeling. This detrimental action is opposed by Tregs. CD indicates cluster of differentiation; MI, myocardial infarction; MVO, microvascular obstruction; and Treg, regulatory T lymphocyte. Created with BioRender.

microvasculature, where they obstruct blood flow and exacerbate myocardial injury (Figure 5A).[4,19,76,80,81] CD4+ T lymphocytes also secrete proinflammatory cytokines, such as interferon-γ, which accelerate inflammation by increasing leukocyte infiltration and further increase infarct size.[82]

However, some lymphocyte subsets, such as Tregs, play cardioprotective roles after ischemia and reperfusion (Figure 5A). Tregs infiltrate into the myocardium and alleviate IRI by secreting anti-inflammatory cytokines and mediators, such as IL-10, TGF-β, and adenosine.[76,83] Tregs can also reduce neutrophil infiltration,[76] modulate anti-inflammatory macrophage polarization,[80] and limit collagen deposition,[81] leading to the formation of early scar tissue and improved ventricular function post-MI in mice. Therapies that enhance Treg activity after IRI may thus provide potential novel therapeutic avenues for enhancing cardiac healing early after MI.

Proliferative Phase

Lymphocytes continue to have beneficial as well as adverse functions during the proliferative phase of myocardial healing in humans. Anti-inflammatory Tregs are the predominant lymphocyte population in this phase.[84] Increased Treg activation is associated with improved ventricular function and clinical outcomes.[85] However, CD8+ T lymphocytes and granzyme B, a cytotoxic enzyme released by CD8+ T lymphocytes, are also present in the myocardium of patients with MI during this phase, and continue to cause inflammation and tissue damage (Figure 5B).[86] Unfortunately, preclinical studies of lymphocyte function in mice after MI typically do not report on the effects of lymphocytes during the proliferative phase. The role of these immune cells during this stage of healing clearly warrants additional exploration.

Maturation Phase

Lymphocyte dysfunction in the maturation phase contributes to long-term adverse remodeling and the development of HF in patients with MI. In humans, both T and B lymphocytes are present in the myocardium months after MI onset, where they exert antigen-specific responses that produce anticardiac autoantibodies (Figure 5C).[84] Circulating B lymphocytes can also trigger inflammatory responses that further impair ventricular function up to a month after MI.[87] In patients with established HF, anticardiac autoantibodies are present in the circulation and the myocardium and are associated with disease progression.[88]

Further evidence for an ongoing detrimental role of lymphocytes in the maturation phase has emerged from studies in mice, where lymphocytes remain elevated in the myocardium 7 days after reperfusion, contributing to autoantibody formation and decreasing survival and ventricular function (Figure 2).[78] Unfortunately, further insights into the function of lymphocytes during the maturation phase of healing come only from studies conducted on mice with nonreperfused MI. These studies have shown that CD4+ T lymphocytes, CD8+ T lymphocytes, and dysfunctional Tregs continue to exert chronic proinflammatory effects that can last for months and ultimately induce HF.[89,90] Additional studies that explore the cellular dynamics of lymphocytes in the maturation phase following reperfused MI are clearly needed for these events to be better understood.

Dendritic Cells

Dendritic cells are of lympho-myeloid origin and coordinate the innate and adaptive immune responses. In humans, dendritic cells are rapidly recruited from the circulation to the myocardium, where they coordinate the immune response after MI.[91] Autopsy studies have revealed that decreased dendritic cell numbers within the myocardium after MI correlate strongly with increased macrophage infiltration and elevated risk of cardiac rupture.[92] A similar trend exists in patients with established ischemic HF, where lower levels of circulating and myocardial dendritic cells are associated with decreased LVEF.[93] These studies suggest that dendritic cells play an important and beneficial regulatory role in short- and long-term cardiac healing after MI in humans.

Little is known about the role of dendritic cells in cardiac healing after MI in animal models. Most studies have been performed in mice with nonreperfused MI and reveal additional complexities that have not been reported in clinical studies. A subset of tolerogenic dendritic cells can induce Treg activity and anti-inflammatory macrophage polarization to improve MI outcomes.[77,94] However, other subsets of conventional and monocyte-derived dendritic cells worsen outcomes by activating autoreactive inflammatory CD4+ and CD8+ T lymphocytes.[78,79] Further research is required to better understand whether dendritic cells exert their beneficial and detrimental effects in a time-specific manner after MI and reperfusion and whether it is possible to develop strategies that regulate these effects.

Other Immune Cells

Other immune cells, such as basophils, eosinophils, and mast cells, also participate in cardiac repair. Peripheral eosinophils and basophils are activated and migrate into cardiac tissue in patients with MI.[95] Reduced peripheral eosinophil or basophil counts at the time of admission or after reperfusion are associated with poor

30-day mortality.[96] Studies in mice with nonreperfused MI have identified a potentially cardioprotective role for eosinophils and basophils in post-MI hearts, although how these cells exert their effects following reperfusion is not understood.[97,98] Mast cell degranulation may also contribute to the inflammatory response following IRI, with inhibition of degranulation improving outcome after reperfusion.[99] Additional investigation is required in this area to better elucidate the temporal dynamics of these immune cell types after reperfusion following MI.

POTENTIAL AVENUES FOR THERAPEUTICS AND FUTURE DIRECTIONS

Given the significant role of inflammation in the development of adverse remodeling and HF after MI, it follows that anti-inflammatory and immunomodulatory drugs have the potential to enhance post-MI healing and reduce long-term complications. Unfortunately, no drugs have yet been approved that specifically target inflammation after MI and prevent HF. The most noteworthy anti-inflammatory therapy trialed to date is canakinumab, a monoclonal antibody that abrogates inflammation by targeting the proinflammatory cytokine IL-1β.[100,101] To date, canakinumab remains the only drug to successfully prevent HF and HF-related mortality after MI in a large randomized controlled trial.[102] Colchicine is another anti-inflammatory drug that inhibits systemic inflammation in patients with MI and HF.[103] Colchicine inhibits tubulin polymerization, disrupts neutrophil function, and prevents neutrophil influx to sites of tissue injury, hence reducing inflammation. Although colchicine does not reduce infarct size in patients, it does decrease the risk of recurrent cardiovascular events and may also prevent HF.[104,105] Further large-scale randomized controlled trials are required to establish whether either of these drugs enhance cardiac repair after MI. However, administration of canakinumab or colchicine has also been demonstrated in large randomized controlled trials to increase the risk of severe infection after MI, which also indicates a continued need for the development of more selective anti-inflammatory therapies that target pathogenic inflammatory cardiac events while maintaining physiologic immune responses against infection.[100,105]

Some immunomodulatory strategies have had early small-scale clinical success at improving cardiac function in patients with MI.[106] Cyclosporine, a calcineurin inhibitor that inhibits T lymphocyte activation, has been studied extensively as a strategy for protecting against lymphocyte-mediated injury after MI. Unfortunately, treatment with cyclosporine after MI has failed to improve clinical outcomes in large randomized controlled trials.[74] Rituximab, another therapy that targets lymphocytes, is under investigation in the context of HF treatment. A monoclonal antibody to CD20 that specifically depletes B lymphocytes from the circulation, rituximab is currently in use for the treatment of hematological malignancies and inflammatory conditions such as rheumatoid arthritis. Preclinical studies have shown B lymphocyte depletion to be cardioprotective, and early clinical trials demonstrate a favorable safety profile after MI.[75,107] Studies on the efficacy of rituximab and its impact on outcomes after MI in humans are underway.[108]

Overall, however, despite the many potential cardioprotective immunomodulatory therapies that have been investigated, or are currently under investigation, for reducing cardiac damage patients with MI, clinical trials have demonstrated little benefit. This is largely because immune cells have pleiotropic effects, and targeting or inhibiting the activation of a particular cell type may cause unintended adverse effects that negate their cardioprotective benefit. Moreover, immune cells are functionally dynamic after MI, and prolonged immunomodulation is likely to have different effects at each stage of healing, which may hinder tissue repair and worsen outcomes during the proliferative phase. Development of effective cardioprotection after MI may therefore require the use of therapies that target the temporal dynamics of immune cells during specific phases of the healing process.

Future studies may also benefit from addressing the limitations of preclinical models of MI. For example, innate immunological differences exist between mice and humans. Whereas most humans presenting with MI tend to be older and have several comorbidities, such as hypertension, diabetes, or dyslipidemia, mouse models of MI and reperfusion are mostly conducted by surgically ligating the left anterior descending coronary artery in young, healthy mice.[1] As aging, hyperlipidemia, hyperglycemia, hypertension, and atherosclerosis prime innate immunity effectors and are associated with low-level systemic inflammation, these comorbidities are likely to impair endogenous repair mechanisms in humans, which may explain, at least in part, why cardioprotection in animal models has failed to translate into benefit in the clinic. Similarly, most patients presenting with acute MI have several comedications and receive additional medications such as antiplatelet agents, anticoagulants, opioids, and β-blockers at the time of presentation. All of these can potentially interact with the ensuing inflammatory response. This represents another factor that animal models have largely been unable to account for.[1] To address this issue, a position paper put forward by the European Society of Cardiology in 2014 made a number of recommendations specifically supporting the use of in vivo models that employ transient occlusion of the main left coronary followed by coronary reflow to induce IRI and recommending that any such small animal models be complemented by large animal

models that also emulate both ischemia and reperfusion injury.[109] Because this review focuses on novel cardioprotective therapies for preventing IRI, we have not reviewed experimental models of permanent coronary artery ligation, which are most often used to investigate post-MI HF. Another methodological limitation lies in the fact that male mice are used in most preclinical studies despite significant sex-specific differences in outcomes after MI.[110] These studies are thus unlikely to provide insights into cardiovascular disease in women. Moreover, most preclinical studies only examine immune cell function at a single timepoint after MI and thus provide no information about how immune cell function evolves with time after reperfusion. Further studies that investigate immune cell function during each phase of healing after IRI are clearly warranted. In addition, development and widespread implementation of in vivo methods that accurately quantify real-time inflammatory and immune cell infiltration and activity also remain pressing needs as strategies for facilitating understanding of immune cell dynamics after MI. Several such methods have been proposed, although none have been routinely implemented in studies of cardiac repair following MI.[111,112] Addressing these limitations will undoubtedly generate new insights into immune cell dynamics that are amenable to translation into the human situation and assist with the development of novel therapies for preventing and reversing HF in patients with MI.

CONCLUSIONS

There is an undisputed need for interventions that improve cardiac repair after MI. Reducing inflammation and autoimmunity are critical for healing cardiac tissue damage after MI and are undoubtedly targets for reducing adverse LV remodeling and HF. However, cardiac repair following MI is complex, and current therapies are just beginning to address these intricacies. The functional heterogeneity of immune cell types that develops with increasing time after MI has important therapeutic implications. Development of treatments that target specific immune cell subsets during different phases of repair processes is the first step toward successful cardioprotective therapies.

ARTICLE INFORMATION

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