Immune cells as targets for cardioprotection: new players and novel therapeutic opportunities

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Abstract

New therapies are required to reduce myocardial infarct (MI) size and prevent the onset of heart failure in patients presenting with acute myocardial infarction (AMI), one of the leading causes of death and disability globally. In this regard, the immune cell response to AMI, which comprises an initial pro-inflammatory reaction followed by an anti-inflammatory phase, contributes to final MI size and post-AMI remodelling [changes in left ventricular (LV) size and function]. The transition between these two phases is critical in this regard, with a persistent and severe pro-inflammatory reaction leading to adverse LV remodelling and increased propensity for developing heart failure. In this review article, we provide an overview of the immune cells involved in orchestrating the complex and dynamic inflammatory response to AMI—these include neutrophils, monocytes/macrophages, and emerging players such as dendritic cells, lymphocytes, pericardial lymphoid cells, endothelial cells, and cardiac fibroblasts. We discuss potential reasons for past failures of anti-inflammatory cardioprotective therapies, and highlight new treatment targets for modulating the immune cell response to AMI, as a potential therapeutic strategy to improve clinical outcomes in AMI patients. This article is part of a Cardiovascular Research Spotlight Issue entitled ‘Cardioprotection Beyond the Cardiomyocyte’, and emerged as part of the discussions of the European Union (EU)-CARDIOPROTECTION Cooperation in Science and Technology (COST) Action, CA16225.

Keywords

Inflammation • Myocardial ischaemia/reperfusion injury • Acute myocardial infarction • Monocytes • Macrophages • Lymphocytes • Dendritic cells • Fibroblasts

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1. Introduction

Acute myocardial infarction (AMI) and the heart failure which often follows are among the leading causes of death and disability worldwide. Although short-term mortality (in-hospital and 30 days) following AMI is on the decline,¹ long-term mortality (1 year and beyond) post-AMI remains significant, and this is, in part, due to the increase in the number of patients who survive their AMI, and go onto to develop heart failure.^{2,3} As such, new treatments are required to reduce myocardial infarct (MI) size, in order to preserve left ventricular (LV) function and prevent the onset of heart failure in AMI patients. In this regard, the acute inflammatory response to AMI is a key determinant of final MI size and subsequent adverse post-AMI LV remodelling, making inflammation an important target for cardioprotection.^{4,5} The initial acute inflammatory response to AMI is triggered by the innate immune response to cell necrosis, which includes the release of fragments of mitochondrial DNA into the tissue that can act as danger-associated molecular patterns (DAMPs),⁶ complement activation, formation of the inflammasome, and so on, and is the focus of another article in this Cardiovascular Research Spotlight Issue.⁷ The innate immune response activates and triggers the accumulation of immune cells into the ischaemic myocardium, and orchestrates the initial pro-inflammatory reaction, the main purpose of which is to clear necrotic cell debris from the MI zone. The immune cell response to AMI is characterized by early infiltration of neutrophils into the MI zone from 6 to 24 h post-AMI, followed by the accumulation of pro-inflammatory monocytes and macrophages over the next 48 to 72 h, both of which contribute to the cardiomyocyte death and myocardial injury which occurs in response to myocardial ischaemia/reperfusion injury (IRI).⁵ This is followed by an anti-inflammatory reparative phase (days 4–7) which is mainly driven by anti-inflammatory monocytes/macrophages and is mediated by suppression, resolution, and containment of the initial pro-inflammatory response, and which permits wound healing and scar formation to occur. Because the inflammatory response to AMI evolves over several hours to days following reperfusion, anti-inflammatory cardioprotective strategies discussed in this article are unlikely to reduce *acute* MI size (defined in this article as ≤ 6 h of AMI) arising from the early reperfusion injury that occurs in the first few minutes of reperfusion. As such, MI size limitation using an anti-inflammatory cardioprotective strategy would be expected to only manifest at least 6–24 h after reperfusion, and therefore mainly arises from a reduction in *early* MI size (defined in this article as ≤ 24 h of AMI), and reduction in *late* MI size (defined in this article as ≥ 24 h of AMI) from beneficial effects on infarct-remodelling. Disturbances in both the balance and transition between the initial pro-inflammatory reaction and the subsequent anti-inflammatory reparative phase can augment myocardial IRI, and worsen post-MI adverse LV remodelling thereby increasing the propensity for the development of heart failure following AMI.

In this review article, we provide an overview of the immune cells involved in orchestrating the complex and dynamic inflammatory response to AMI, which include neutrophils, monocytes/macrophages, and emerging players such as dendritic cells (DCs), lymphocytes, pericardial lymphoid cells, endothelial cells, and cardiac fibroblasts. We review ways in which the immune cell response to AMI is modulated by endogenous cardioprotective strategies such as ischaemic preconditioning (IPC), ischaemic postconditioning (IPost), and remote ischaemic conditioning (RIC). Finally, we discuss potential reasons for past failures of anti-inflammatory cardioprotective therapies, and highlight emerging targets for modulating the inflammatory response to AMI, as potential novel therapeutic strategies to improve clinical outcomes following AMI.

2. Neutrophils as targets for cardioprotection

Neutrophils are the first immune cells recruited into the ischaemic heart following AMI. Increased circulating number⁸ or volume of neutrophils⁹ in patients suffering an AMI positively correlates with MI size, subsequent LV function and clinical outcomes. Once recruited in the ischaemic myocardium, neutrophils maintain the initial acute pro-inflammatory response to IRI. Their rapid degradation and degranulation propagates the acute inflammatory response to neighbouring areas of the myocardium (so-called 'neutrophil-induced injury')¹⁰ and triggers monocyte infiltration into the ischaemic tissue.¹¹ Interestingly, the recruitment of neutrophils into the heart after AMI demonstrates a circadian pattern, which can impact on late MI size and LV function.¹² It has been demonstrated that neutrophils can polarize macrophages towards a reparative phenotype, and thus contribute to the healing phase following AMI, highlighting a potential protective role for neutrophils.¹³ Therefore, therapeutic strategies targeted to neutrophils should take into consideration the potential beneficial effects of neutrophils in post-AMI healing.

Neutrophil function following AMI can also be modulated by endogenous cardioprotective phenomena such as IPost,¹⁴ in which brief cycles of non-lethal ischaemia and reperfusion applied at the onset of reperfusion reduced neutrophil accumulation into the MI zone.¹⁵ However, whether the reduction in myocardial accumulation of neutrophils observed with IPost is an epiphenomenon of improved myocardial salvage or is actually required for cardioprotection is not clear. Furthermore, a clinical study demonstrated that RIC (brief cycles of non-lethal ischaemia and reperfusion applied to the upper arm) down-regulated the expression of kinin B1 and B2 receptors in neutrophils of patients undergoing cardiac surgery.¹⁶

In summary, neutrophils are recruited into the ischaemic heart and their rapid degradation and degranulation results in an acute pro-inflammatory response which triggers monocyte infiltration in the first few hours. Novel approaches to regulate the neutrophils are RIC or IPost which modulate the expression of kinin B1 and B2 receptors in neutrophils (Figure 1).

2.1 Therapeutic targeting of neutrophils for cardioprotection

A number of treatment strategies which target neutrophils have been shown to reduce early and late MI size following AMI. These include those which reduce the accumulation of neutrophils into the MI zone, and include those directed to interleukin (IL)-1 inhibition,¹⁷ anti-nuclear factor kappa-B ligand [RANKL],¹⁸ transient receptor potential melastatin 2 ablation,¹⁹ plasminogen activator inhibitor-1 ablation,²⁰ proteasome-mediated I kappa-B alpha inhibition,²¹ complement C5a,²² and glucagon-like peptide-1²³ (see Table 1 for details). Another group of therapies have been shown to be beneficial following AMI by inhibiting neutrophil activity, such as lipoxygenase-cyclooxygenase,²⁴ Urge-8,²⁵ CI-959,²⁶ Lidocaine,²⁷ Tetrandrine,²⁸ myeloperoxidase (MPO) inhibition,²⁹ and so on (see Table 1 for details). There have also been neutral experimental studies targeting inflammation induced by neutrophils.^{30–32} Therapeutic targeting of neutrophils to reduce early MI size in the clinical setting following AMI has proven to be very challenging. For example, clinical studies targeting CD11/CD18 subunits of the $\beta 2$ integrin adhesion receptors to prevent neutrophil adhesion did not report any cardioprotective effect on early MI size following AMI^{33,34} (see Table 1 for details).

3. Monocytes and macrophages as targets for cardioprotection

tumour necrosis factor- α (TNF- α) and IL-1 β , produce proteolytic enzymes, and secrete matrix metalloproteinases (MMPs), which degrades the extracellular matrix.³⁶ The Ly6C^{high} monocytes differentiate into activated pro-inflammatory macrophages (M1 or CCR2+) that express IL-1 β and TNF- α .³⁷ In the later stages of AMI, the Ly-6C^{low} monocytes and M2 (or CCR2-) phenotypes become the predominant subtype, promote the healing response of AMI, and contribute to angiogenesis and collagen deposition to form scar tissue to replace the lost cardiomyocytes in the MI zone.^{36,37} We must clarify herein that Ly6C monocyte subsets refer to those encountered in mice. Therefore, balancing the dynamic roles of monocytes/macrophages is prerequisite for optimal cardiac healing following AMI. In the clinical setting, it has been shown in AMI patients that higher levels of circulating pro-inflammatory monocytes and monocyte-platelet complexes were associated with increased myocardial injury and impaired recovery of LV function.^{38,39}

Following AMI, a number of factors mediate the recruitment of Ly6C^{high} monocyte migration into the MI zone including: monocyte chemoattractant protein-1 (MCP-1) production, which binds to C-C

Table 1 Major studies investigating anti-inflammatory cardioprotective strategies targeting the immune cell response to reduce MI size and preventing adverse LV remodelling

Study	Experimental acute IRI model	Anti-inflammatory therapeutic strategy	Cardioprotective effect	Proposed mechanism(s) and translational potential
Neutrophils				
Burke et al. ²⁶	<i>In vivo</i> dogs 90 min ischaemia and 6 h reperfusion	CI-959 Before and during reperfusion	Reduction of acute MI size	Inhibiting the formation of toxic oxygen radicals by inflammatory cells
Tanaka et al. ¹⁴⁷	<i>In vivo</i> dogs 90 min ischaemia and 3 h reperfusion	Anti-CD18 Prior to ischaemia	Reduction of acute MI size	Prevents adhesion and accumulation of neutrophils. Clinical studies using this approach have been neutral (LIMIT-AMI and HALT-AMI) ^{33,34}
Amsterdam et al. ²⁴	<i>In vivo</i> pigs 50 min ischaemia and 3 h reperfusion	BW755C Prior to ischaemia	Reduction of acute MI size	Selective inhibition of neutrophil cytotoxic activity by inhibiting dual cyclooxygenase-lipoxygenase blocking agent without affecting neutrophil migration into injured myocardium
Vitola et al. ²⁷	<i>In vivo</i> rabbits 30 min ischaemia and 48 h reperfusion	Lidocaine First 10 min of ischaemia	Reduction of late MI size	Sodium channel blocker which inhibits several neutrophil functions
Shen et al. ²⁸	<i>In vivo</i> rats 30 min ischaemia and 1 h reperfusion	Tetrandrine Prior to ischaemia	Reduction of acute MI size	Inhibition of neutrophil priming, adhesion, and activation, and abolishment of subsequent infiltration and ROS production
Bao et al. ²¹	<i>In vivo</i> rats 30 min ischaemia and 24 h reperfusion	PR-39 At reperfusion	Reduced late MI size	Inhibitor of proteasome-mediated I kappa-B alpha degradation and its truncated form PR-11 resulting in less neutrophil infiltration, MPO activity, and expression of ICAM-1 and VCAM-1
Kohtani et al. ²⁵	<i>In vivo</i> rats 60 min ischaemia and 24 h reperfusion	Urge-8 Prior to ischaemia and at reperfusion	Reduction of early MI size	Anti-neutrophil monoclonal antibody which inhibits chemotactic activity, superoxide production, phagocytic function, and neutrophil degranulation
Granfeldt et al. ¹⁵	<i>In vivo</i> rats 30 min ischaemia and 3 h reperfusion	IPost At reperfusion	Reduction of acute MI size	Reduced accumulation of neutrophils in MI zone. No further reduction in MI size with IPost in neutrophil-depleted rats
Wu et al. ¹⁴⁸	<i>In vivo</i> mice 30 min ischaemia and 24 h reperfusion	Recombinant human IL-37 At reperfusion	Reduction of late MI size	IL-37 protected cardiomyocytes from apoptosis and suppressed the migration ability of neutrophils towards the chemokine LIX
Carbone et al. ¹⁸	Mice 30 min ischaemia and 24 h reperfusion	Anti-RANKL IgG During ischaemia	Reduced late MI size and preserved LV function	Antibody to neutralization of receptor activator of nuclear factor kappa-B ligand (RANKL) reduced neutrophil infiltration, ROS and MMP-9 release.
Ali et al. ²⁹	Mice 30 min ischaemia and 48 h reperfusion	PF-1355 Post-reperfusion for 1 week	Less post-AMI LV remodelling	Pharmacological MPO inhibitor started 1 h post-AMI reduced leucocyte accumulation and prevented adverse LV remodelling.

Continued

Table 1 Continued

Study	Experimental acute IRI model	Anti-inflammatory therapeutic strategy	Cardioprotective effect	Proposed mechanism(s) and translational potential
Monocytes/macrophages				
Nakano <i>et al.</i> ⁵⁶	<i>In vivo</i> mice 30 min ischaemia and 12 and 24 h reperfusion	lbesartan At reperfusion	Reduction of early and late MI size	PPAR- γ -dependent anti-inflammatory mechanisms. Antagonizing monocyte-mediated inflammation
Zhou <i>et al.</i> ⁵²	<i>In vivo</i> rat 45 min ischaemia and 30 days reperfusion	Phenylethanol delivered in liposomes to monocytes and macrophages at day 2 and 4 post-AMI	Reduction of late MI size and less LV fibrosis	Non-selective VGSC inhibitor inhibited pro-inflammatory monocytes and promoted differentiation of M1 to M2 macrophages
Wang <i>et al.</i> ⁵⁵	<i>In vivo</i> murine Permanent ischaemia	Topiramate	Reduction of late MI size and less adverse LV remodelling	GABA-A receptor agonist inhibited pro-inflammatory monocytes and promoted differentiation of M1 to M2 macrophages
Tian <i>et al.</i> ⁵⁴	<i>In vivo</i> mice 40 min ischaemia and 1 h reperfusion	BAY 60-6583 Prior to ischaemia	Reduction of acute MI size	Adenosine 2B Receptor agonist, BAY 60-6583, has anti-inflammatory effects, probably by modulating macrophages phenotype switching via a PI3K/Akt pathway
Lymphocytes				
Yang <i>et al.</i> ⁶⁴	<i>In vivo</i> mice 45 min ischaemia and 24 h reperfusion	ATL146e At reperfusion	Reduction of late MI size	A2AR agonist which inhibits CD4 ⁺ T cell accumulation and activation in the reperfused heart. CD4 ⁺ but not CD8 ⁺ T lymphocytes contribute to acute myocardial IRI
Zouggar <i>et al.</i> ⁴²	<i>In vivo</i> murine Permanent ischaemia	Rituximab One hour after infarction	Less adverse LV fibrosis and remodelling	Depletion of B cells by rituximab (CD20 specific antibody) inhibited pro-inflammatory monocyte infiltration
Mast cells				
Parikh <i>et al.</i> ⁷⁹	<i>In vitro</i> rat 30 min ischaemia and 30 min reperfusion	Disodium cromoglycate During ischaemia and reperfusion	Reduction of acute MI size	DSCG stabilized mast cells and reduced MPO activation DSCG blocked cardioprotective effects of IPC
Parikh <i>et al.</i> ⁸⁷	<i>In vitro</i> rat 30 min ischaemia and 30 min reperfusion	NE Prior to ischaemia	Reduction of acute MI size	NE stabilized mast cells and reduced MPO activation
Jaggi <i>et al.</i> ⁷⁸	<i>In vitro</i> rat 30 min ischaemia and 120 min reperfusion	Ketotifen and carvedilol Compound 48/80 prior to ischaemia	Reduction of acute MI size	Ketotifen and carvedilol inhibited mast cell degranulation during ischaemia/reperfusion Compound 48/80, a mast cell granulator, was cardioprotective when administered prior to ischaemia, to degranulate mast cells prior to AMI
Cardiac fibroblasts				
Abrial <i>et al.</i> ⁹⁵	<i>In vivo</i> rats 60 min and 24 h of reperfusion	To investigate the role of CF	Late MI size reduction was observed in CF secretome treated mice compared with control	CFs participate in cardioprotection during the acute phase of ischaemia-reperfusion via a paracrine pathway involving TIMP-1

CF, cardiac fibroblast; ROS, reactive oxygen species.

chemokine receptor type 2 (CCR2) expressed on the surface of monocytes^{40,41} expression of chemokine (C-C motif) ligand 2 (CCL2) and chemokine (C-C motif) ligand 7 (CCL7), which also mediate CCR2-dependent monocyte migration.⁴² Very recently it has been demonstrated that the human myocardium also contains distinct subsets of CCR2- and CCR2+ macrophages which have distinct functional properties, analogous to reparative CCR2- and inflammatory CCR2+ macrophages in the mouse heart.⁴³ Angiotensin II (ANG II), the concentration of which is increased in the circulation following AMI, recruit monocytes from the splenic reservoir⁴⁴ the sympathetic nervous system that stimulates the entry of haematopoietic stem cells from the bone marrow through β 3-adrenergic receptor signalling, and β 2-adrenergic receptors, which play a crucial role in the migration of monocytes/macrophages to MI zone.⁴⁵ The orphan nuclear hormone receptor, nuclear receptor subfamily 4, group a, member 1 (Nr4a1) is critical for limiting the migration of pro-inflammatory monocytes to the site of infarction and for increasing the differentiation of reparative macrophages, and eventually for preventing adverse cardiac remodelling.⁴⁶

Macrophage migration inhibitory factor (MIF) plays dual roles in the setting of myocardial IRI. MIF released by cardiomyocytes in a model of IRI was shown to exert cardioprotective effects via the CD74/5' AMP-activated protein kinase/c-Jun N-terminal kinase axis (CD74/AMPK/JNK),^{47,48} and the cardioprotective effect was enhanced by S-nitrosylation of MIF.⁴⁹ In contrast, another study reported that MIF deficiency protected the heart from IRI by suppressing the pro-inflammatory response, suggesting a detrimental role for MIF following AMI.⁵⁰ The compartmentalized and opposing effects of MIF after myocardial IRI are largely mediated by C-X-C motif chemokine receptor 2 (CXCR2). MIF confers protective effects improving myocardial healing and function through CXCR2 in resident cells, however, it exerts detrimental effects on CXCR2-bearing inflammatory cells, increasing monocyte infiltration and impairing heart function.³⁰ During reperfusion in mice, macrophage myeloid-epithelial-reproductive tyrosine kinase (MerTK) deficiency led to decreased cardiac wound debridement, increased MI size, and depressed cardiac function, whereas blockade of CCR2-dependent monocyte infiltration into the heart reduced soluble MER (myeloid-epithelial-reproductive tyrosine kinase; i.e. soluble MER) levels post-AMI, implicating monocyte-induced MerTK cleavage as a novel contributor and therapeutic target for preventing myocardial IRI.⁵¹ Signals and receptors involved in monocyte/macrophage recruitment/internalization are illustrated in Figures 1–3.

A number of therapies have been shown to protect the heart post-AMI by promoting the monocyte/macrophage phenotypic switch from pro-inflammatory to anti-inflammatory phenotypes including phenytoin, a non-selective voltage-gated sodium channel (VGSC) inhibitor,⁵² BAY 60-6583, an adenosine A2B receptor ($A_{2B}R$) agonist,^{53,54} and γ -aminobutyric acid (GABA) receptor antagonists such as topiramate or bicuculline⁵⁵ (see Table 1 for details).

3.1 Therapeutic targeting of monocytes/macrophages for cardioprotection

Emerging evidence shows that anti-inflammatory strategies targeting the pro-inflammatory monocyte/macrophage subset can reduce excessive inflammation and improve cardiovascular outcomes. Angiotensin-converting enzyme (ACE) inhibitors have been shown to reduce monocyte migration from the spleen.⁴⁴ These data could explain the benefits of ACE inhibitors in patients with an early stage of AMI as demonstrated by the ability of these drugs to reduce inflammation and lower mortality.⁴⁴

Insufficient local drug concentration in the ischaemic heart may be one factor for prior failed clinical cardioprotection studies, and nanoparticles may be used to improve delivery of cardioprotective therapies to the ischaemic heart following AMI. In this regard, it has been shown that irbesartan, an ANG II type 1 receptor blocker with a peroxisome proliferator activated receptor (PPAR- γ) agonistic effect, delivered at reperfusion using nanoparticles inhibited the recruitment of pro-inflammatory monocytes to the infarcted myocardium, and ameliorated LV remodelling at 21 days in a murine model of myocardial IRI.⁵⁶

In summary, the monocyte/macrophage response to AMI is biphasic and therefore anti-inflammatory therapies targeting these immune cells need to take this into consideration.

4. Lymphocytes as emerging targets for cardioprotection

A role for lymphocytes in the inflammatory response to AMI has recently emerged. T lymphocytes are activated within a few days after AMI in lymph nodes draining the myocardium.⁵⁷ Analysis of transcoronary gradients from AMI patients has demonstrated early recruitment of T and B lymphocytes into myocardium within 90 min of reperfusion.⁵⁸ Animals deficient in helper T cells develop impaired healing after AMI, and this is associated with an increase in myocardial monocytes/macrophages. Additionally, very recently it has been shown that T-bet (a transcription factor that regulates the differentiation and function of immune cells) deficiency attenuates pressure overload-induced cardiac remodelling in rats, indicating that targeting T-bet in T cells may be of great importance for the treatment of heart failure.⁵⁹ Regulatory T cells (T_{reg}) are known to down-regulate the innate immune response. Indeed it has been shown that a lack of T_{reg} mirrors the phenotype of T-helper cell deficient animals after AMI. In contrast, activation of T_{reg} by superagonistic anti-CD28 monoclonal antibody administered 2 days after AMI improved healing and survival.⁶⁰ This is most likely due to differentiation effects on monocytes/macrophages that developed a 'pro-healing' phenotype after T_{reg} activation in a transforming growth factor beta 1 (TGF- β 1)-dependent manner.⁶⁰ This concept is supported by another study where depletion of T_{reg} after AMI worsened LV remodelling. Moreover, T_{reg} changed the phenotype of cardiac fibroblasts with a reduced contraction of fibroblast-populated collagen pads.⁶¹ Injection of tolerogenic DCs can modulate T_{reg} . Indeed, such DCs primed with lysate from infarcts induced a shift towards reparative macrophages via activation of T_{reg} and better healing after AMI.⁶² It has been shown that some cardioprotective effects of IPC appear to depend on adequate T_{reg} function— T_{reg} depletion was associated with increased late MI size and pronounced infiltration of inflammatory cells in a rat model of IPC.⁶³

Recombination activating gene 1 knockout (RAG1 KO) mice, which are deficient in lymphocytes, had significant smaller MI size when compared with wild-type animals. Adoptive transfer of CD4⁺ T cells in RAG1-KO mice blunted the protective effect.⁶⁴ Accordingly, CD4⁺ T cell deficient animals had smaller late MI size after AMI.⁶⁵ This effect could be reversed by an adenosine receptor agonist. Adenosine can be synthesized by CD39 and 73 from nucleotides released by tissue injury. CD39 and CD73 are expressed on lymphocytes and cardiomyocytes.⁶⁶

Finally, B lymphocytes are also recruited to the injured myocardium after AMI. B lymphocytes produced CCL7 mediates the recruitment of Ly6C^{high} monocytes. Depletion of B cells by rituximab (CD20 specific antibody) had beneficial effects after AMI.⁴²

Figure 2 Immune cell changes hours to days following AMI. B and T lymphocytes are recruited early in the injured myocardium. B lymphocytes produce CCL7 which mediates the recruitment of Ly6C^{high} monocytes. The spleen provides a steady source of monocytes and they migrate to the site of myocardial injury under the regulation of IL-1 β , ANG II and the binding of the chemokine ligand 2 (CCL2) to the chemokine receptor 2 (CCR2). MCP-1 and CCL7 also mediate CCR2-dependent monocyte migration. Moreover, β 2-adrenergic receptors play a crucial role in the migration of monocytes/macrophages to the site of infarction following AMI. The first populations of monocytes that migrate to the site of infarction are the pro-inflammatory Ly6C^{high} monocytes which differentiate into activated pro-inflammatory macrophages (M1) that express IL-1 β and TNF- α . Gradually, the Ly6C^{low} monocytes become the predominant subtype and promote the healing response to AMI and the nuclear receptor subfamily 4, group a, member 1 (Nr4a1) is essential for Ly6C^{low} monocyte production. IL-13, the CXCL12/CXCR4 axis, GABA-A receptor activity and VGSCs represent targets for the monocyte/macrophage phenotypic switch from pro-inflammatory to anti-inflammatory. The PPAR- γ -dependent anti-inflammatory mechanisms and monocyte-induced myeloid-epithelial-reproductive tyrosine kinase (MerTK) cleavage are considered as novel contributors and therapeutic targets for preventing IRI.

Overall, these data suggest a differential role of T cells after AMI with some T cells contributing to myocardial IRI, and others contributing to healing after AMI. The latter process seems to be antigen dependent. B cells seem to aggravate the remodelling process after AMI. The complex roles of lymphocytes to the inflammatory response to AMI, may make clinical translation difficult. A successful therapy will highly be dependent on the kind of injury and the individual time points in which lymphocytes are modified. Nevertheless, some drugs to modify a B- or T-cell response are under development and some are already in clinical use. Since lymphocytes influence other cells, their effects are multiplied moreover, lymphocyte responses are context dependent and might therefore be an ideal tool to support healing after AMI.

4.1 Pericardial lymphoid clusters as emerging targets for cardioprotection

Pericardial adipose tissue contains a high density of lymphoid clusters which serve as secondary lymphoid organs in which different types of cells such as B cells, DCs, and T cells enlarge in response to AMI.⁶⁶ In a very recent study,⁶⁷ it was demonstrated that surgical removal of murine pericardial adipose tissue, B cell depletion, and granulocyte-macrophage colony-stimulating factor blockade, reduced LV fibrosis, and preserved cardiac function following AMI in mice. The above findings highlight the major role of pericardial adipose tissue and B cells in the modulation of post-AMI outcome and indicate that activation of pericardial lymphoid clusters in response to AMI might also affect cardiac healing.

Figure 3 Immune cell changes in the healing period following AMI. Regulatory T cells (T_{reg}) undergo DC regulation and down-regulate the innate immune response. The activation of T_{reg} is favourably associated with improved healing and survival. T_{reg} cells also determine the phenotype of cardiac fibroblasts, which affects the wound healing process. DCs might mediate the activation of CD4⁺ T cells in the infarcted area, improving cardiac function post-AMI. There is increasing evidence that miRNAs (miRs) regulate the immune system by altering the function of DCs. DCs also produce miRs and exosomes regulating the inflammatory response after AMI. Pericardial lymphoid clusters can modulate the post-AMI outcome, indicating that activation of these clusters might affect as well cardiac healing. Cardiac fibroblasts are subjected to regulation by neurohumoral signals (ANG II and aldosterone), growth factors, such as TGF-β1 and secreted mediators that bind to fibroblast cell surface receptors (cytokine and growth factor receptors, integrins, syndecans, and CD44). These signals trigger the activation of fibrogenic programmes while TGF-β1 plays a critical role in conversion of cardiac fibroblasts into an organized scar with well-aligned myofibroblasts. However, TGF-β1 stimulation profoundly alters the electrophysiological phenotype of cardiac myofibroblasts, enhancing gap junctional coupling between myofibroblasts and cardiomyocytes by increasing connexin 43, thus contributing to arrhythmogenesis in the fibrotic heart.

5. DCs as emerging targets for cardioprotection

DCs are antigen-presenting cells that contribute to innate immunity and provide a link to the adaptive immune system.⁶⁸ In the heart, DCs are important both for promoting an immune response to specific pathogens, and for maintaining self-tolerance.⁶⁹ Myocardial IRI following AMI induces an inflammatory response which includes the migration and accumulation of DCs to the ischaemic region.^{70,71} Mice depleted of DCs had worse post-MI remodelling than control mice suggesting that DCs are protective in the early pro-inflammatory response to AMI.⁷¹ The infarcted myocardium of DC-depleted mice also displayed increased pro-inflammatory monocyte and

macrophage recruitment and pro-inflammatory cytokines.⁷¹ Interestingly, a correlation between decreased levels of DCs, increased levels of macrophages, and impaired reparative fibrosis followed by cardiac rupture, has been shown in histological samples of infarcted myocardium from patients deceased shortly after AMI.⁷² This suggests a protective role of DCs against cardiac rupture.

DCs have been shown to activate cardiac-specific autoreactive CD4⁺ T cells in a murine experimental AMI model.⁷³ Another study reported that exosomes secreted by DCs might mediate the activation of CD4⁺ T cells in the infarcted area, improving cardiac function in mice post-AMI.⁷⁴ However, these findings are discordant with the data from another study showing detrimental effects of CD4⁺ T cells in the setting of myocardial IRI.⁶⁵ It has recently been shown that administration of heart-

specific tolerogenic DCs in post-AMI mice was beneficial to cardiac remodelling, function, and survival of infarcted mice by inducing regulatory T_{regs} that promoted a macrophage-specific repair programme from inflammatory to reparative.⁶² Thus, targeting DCs could be an alternative therapeutic strategy to stimulate the beneficial action of T_{regs} and improve cardiac remodelling in post-AMI patients.

6. Resident cardiac mast cells as targets for cardioprotection

The existence of a resident population of mast cells in heart tissue has been demonstrated,⁷⁵ where they are mainly located in the adventitial lining of the coronary vasculature and within the cardiac interstitium.⁷⁶ Activation of cardiac mast cells has been shown to play an important role in AMI,⁷⁷ and may therefore present an important target for cardioprotection.

Mast cell activation and/or degranulation are noted to release pro-inflammatory mediators, and their release can be prevented by a variety of pharmacological interventions including low-dose ketotifen and carvedilol, and compound 48/80, a specific mast cell degranulating agent.⁷⁸ Similarly, disodium cromoglycate (DSCG), has been shown to stabilize mast cells and reduce myocardial injury, attenuate MPO release, and prevent post-IRI arrhythmias following AMI.⁷⁹ It should also be mentioned that dual targeting of neutrophil- and mast cell-derived proteases may represent a novel therapeutic strategy to reduce post-IRI inflammation and improve cardiac remodelling.⁸⁰

The release of mast cell toxic aldehydes formed by lipid peroxidation, and neuropeptides such as substance P, released by juxtaposed cardiac sensory nerves, promotes mast cell degranulation and release of mast cell-derived renin.⁸¹ Consequently, mast cell-derived renin induces reperfusion arrhythmias following myocardial IRI through the activation of the cardiac renin-angiotensin system (RAS) and locally formed ANG II that exacerbates release of norepinephrine (NE) from isolated sympathetic nerve terminals.^{82–84} Activation of Gi-coupled receptors, such as histamine-H₄, adenosine-A₃, and sphingosinephosphate-1 receptors, all expressed at the mast cell surface, significantly reduced RAS-system activation and induced cardioprotective anti-RAS effects.

Similarly, IPC has been shown to mimic the cardioprotective effects of anti-RAS signalling. In both situations, anti-RAS cardioprotection was induced by the activation of protein kinase C epsilon (PKC-ε), and mitochondrial aldehyde dehydrogenase-2 (ALDH2), regulating aldehyde-induced mast cell renin release.^{82–84} Furthermore, IPC also produced cardioprotection and decreased release of mast cell MPO following myocardial IRI, by activating the Na⁺/H⁺ exchanger and consequent degranulation of resident cardiac mast cells.⁸⁵ In addition, it has recently been proposed that ATP-induced renin release from mast cells exacerbates myocardial IRI, through the regulation of ecto-nucleoside triphosphate diphosphohydrolase 1/CD39 (CD39) and ATP availability at the mast cell surface. Thus, it has been demonstrated that overexpression of CD39 prevents ATP-induced renin release and exerts a cardioprotective effect.⁸⁶ Controversially, pharmacological preconditioning with NE produces a cardioprotective and anti-arrhythmic effect similar to IPC through degranulation of resident cardiac mast cells, reduction of lactate dehydrogenase, and MPO activation,⁸⁷ following myocardial IRI.

In summary, resident cardiac mast cells are an important detector system for initiating the pro-inflammatory response to AMI, and they are therefore potential targets for cardioprotection.

7. Cardiac fibroblasts as targets for cardioprotection

Cardiac homeostasis is supported by a network of direct and indirect interactions between cardiomyocytes and other resident myocardial cell types, such as endothelial cells, fibroblasts, pericytes and vascular smooth muscle cells, macrophages, mast cells, and epicardial adipocytes.^{88,89} Because the adult mammalian myocardium has negligible regenerative capacity, healing of the injured heart following AMI is dependent on a superbly orchestrated cellular response that is driven by an inflammatory cascade, and ultimately leads to formation of a collagen-based scar.⁹⁰ As the infarct micro-environment changes, cardiac fibroblasts undergo dramatic phenotypic alterations that critically regulate repair and remodelling of the infarcted heart.⁹¹ During the inflammatory phase of infarct healing, cardiac fibroblasts respond to DAMPs released by dying cells, and activate pro-inflammatory and matrix-degrading programmes.^{92,93} Considering that several other cell types, including endothelial cells, immune cells, and ischaemic cardiomyocytes can also secrete pro-inflammatory mediators, the relative contribution of fibroblasts remains unclear. A recent study suggested that fibroblast-derived granulocyte-macrophage colony-stimulating factor (GM-CSF) release may play an important role in chemotactic attraction of neutrophils and monocytes in the infarcted myocardium.⁹⁴ It has also been suggested that during the early post-ischaemic period, fibroblasts may modulate survival pathways in cardiomyocytes, affecting their susceptibility to ischaemic death.^{95,96} These effects may be mediated through secretion of soluble pro- or anti-apoptotic mediators by fibroblasts,⁹⁵ via release of exosomes containing miRNAs,⁹⁷ or through modulation of the extracellular matrix by fibroblast-derived MMPs.⁹⁸

As professional phagocytes clear the infarct from dead cells and matrix debris, mediators repressing inflammation are released, and anti-inflammatory mononuclear cell subsets predominate.^{36,99} Suppression of the inflammatory response is associated with expansion and activation of a reparative programme in cardiac fibroblasts. In the healing infarct, resident fibroblast populations are recruited to the area of necrosis, proliferate and aggregate, mediating formation of a scar.¹⁰⁰ Moreover, local activation of neurohumoral pathways (such as ANG II and aldosterone), and generation of growth factors, such as TGF-β1 play a critical role in conversion of cardiac fibroblasts into myofibroblasts, activated matrix-synthetic cells that incorporate contractile proteins (such as α-smooth muscle actin) in stress fibers.¹⁰¹ Secreted mediators bind to fibroblast cell surface receptors (such as cytokine and growth factor receptors, integrins, syndecans, and CD44), triggering downstream signalling cascades that activate a fibrogenic programme.^{102,103}

TGF-β1 has a wide range of effects on cardiac fibroblasts, mediated through activation of a series of intracellular effectors, the Smads.^{104,105} Smad3-mediated induction of an integrin-mediated programme in cardiac fibroblasts has been demonstrated to play a critical role in repair of the infarcted heart, promoting formation of an organized scar with well-aligned myofibroblasts, and preserving the structural integrity of the ventricle.¹⁰⁵ Some of the fibrogenic effects of TGF-β1 may be mediated through activation of other fibrogenic mediators, such as IL-11.¹⁰⁶ Additionally to TGF-β1, *in vivo* infusion of IL-10 post-MI in mice increased fibroblast activation, and improved the LV micro-environment to diminish inflammation and facilitate cardiac wound healing.¹⁰⁷

Scar maturation is associated with reduction in the population of infarct fibroblasts,¹⁰⁸ loss of myofibroblast phenotype, apoptosis of many activated fibroblasts,¹⁰⁹ and expression of specialized matrix proteins by

surviving fibroblast subsets.¹¹⁰ In contrast, in non-infarcted remodelling segments, resident fibroblasts may exhibit chronic activation in response to pressure and volume loads, contributing to adverse post-infarction remodelling.

Considering their crucial involvement in both injurious and reparative processes, targeting the fibroblasts following AMI poses major challenges. Characterization of fibroblast subpopulations with distinct functional profiles, and identification of specific fibroblast-derived mediators involved in injury, repair and adverse remodelling are needed in order to develop safe and effective therapeutic approaches.

8. Targeting long ncRNA and miRNA as a cardioprotective strategy

Non-coding RNAs (ncRNAs) are recognized as important regulators of several pathophysiological processes, including cardiovascular and inflammatory diseases, and have been investigated for their diagnostic and therapeutic potential, including in cardioprotection (reviewed in Refs.^{111,112}). The many types of ncRNAs are broadly classified in small, <200 nucleotides, and long ncRNA (lncRNA), >200 nucleotides. Although there is much more information about the role of small miRNAs in the regulation of inflammatory processes after AMI, the importance of lncRNAs has been increasingly demonstrated,¹¹³ including one particular type of lncRNA, the circular RNAs (circRNAs). MiRNAs control the metabolism of immune cells such as macrophages, T cells, B cells, and DCs,^{114,115} and lncRNAs have been implicated in regulating inflammation, inflammatory factors, and macrophage activation.^{116–119}

The lncRNAs ANRIL and KCNQ1OT1, were shown to be positively associated with LV dysfunction after AMI, supporting a deleterious role for these lncRNAs.¹²⁰ On the other hand, the lncRNA, autophagy-promoting factor has been suggested to be cardioprotective, because it can inhibit autophagy by sequestering one of its inducers, miR-188-3p.¹²¹

The circRNA, MICRA, was identified as a potential cardioprotective RNA in IRI.¹²² However, these are mere associations between blood inflammatory cell levels and IRI and LV function following AMI. Mechanistic studies are therefore required to address the cardioprotective and therapeutic potential of these RNA molecules. This will likely be the aim of future research, considering that therapeutic tools based on RNA modulation are applicable in the clinic, and are the topic of active investigation.

There is increasing evidence of miRNA regulation of the immune system, including the development and function of DCs and the inflammatory response after AMI.^{123,124} For instance, miR-155 is required for DCs to activate CD4⁺ T cells.^{125,126} It has been demonstrated *in vitro* that while down-regulation of miR-let-7i impaired DCs' ability to activate T cells, it improved their capability to induce T_{reg} expansion.¹²⁷ Functional experiments in mice showed that overexpression of miR-148a, miR-148b, and miR-152 inhibited DC secretion of pro-inflammatory cytokines and attenuated the expansion of CD4⁺ T cells.¹²⁸ The up- or down-regulation of miR-23b, miR-30b, miR-99a, and miR-125a in tolerogenic DCs affected their capacity to produce ILs.^{129,130} MiR-181a reduced the DC-mediated inflammatory response by oxidized low-density lipoproteins in atherosclerosis.¹³¹ Notably, miR-181a and miR-150 play an important role in the regulation of apoptosis and the DC-mediated inflammatory response to MI.^{132,133} *In vitro* overexpression of miR-181a or miR-150 suppressed cardiomyocyte apoptosis under hypoxia by attenuating the DC inflammatory response.¹³² Interestingly, the

cardioprotective role of miR-150 in AMI was suggested due to its regulation of monocyte migration and pro-inflammatory cytokine production in mice and human.¹³⁴ In addition, low-circulating levels of miR-150 in blood cells following an AMI were associated with inflammation, LV remodelling and poor outcome,¹³⁵ thus suggesting that this miRNA, mostly expressed in monocytes and with paracrine roles in angiogenesis,¹²² could act as a cardioprotective agent in IRI. Therefore, targeting miRNAs regulating DCs may constitute another cardioprotective approach.

9. Future perspectives

A number of anti-inflammatory cardioprotective strategies, which have been shown in the experimental setting to reduce early and late MI size and prevent adverse LV remodelling, have failed in the clinical setting to show any benefit in AMI patients. The reasons for this are complex and multi-factorial, and have been extensively reviewed elsewhere^{136–139} and only an overview is provided in this section.

9.1 Addressing the complex immune cell response to AMI

As highlighted in this review, the inflammatory response to AMI is hugely complex and involves a number of different immune cell types, many of which play dynamic and divergent roles by sustaining inflammation in the early phase, and/or resolving inflammation during the reparative healing phase. The natural history of the inflammatory/fibrotic response post-AMI has been illustrated in pigs indicating that there is a timely co-ordinated cellular response to myocardial IRI, and understanding the mechanisms involved in tissue repair will provide valuable information in the development of novel cardioprotective strategies.¹⁴⁰ The interaction of the immune cells with each other and with non-immune cells such as cardiomyocytes, endothelial cells, and fibroblasts further complicates the inflammatory response to AMI. Therefore, this needs to be taken into consideration when targeting immune cells following AMI, and requires a better understanding of the immune cell response to myocardial IRI. This may be achieved by timing the administration of anti-inflammatory cardioprotective therapies to specifically target either the initial pro-inflammatory or subsequent anti-inflammatory reparative phase, either separately or in combination. The contribution of immune cells to myocardial damage and repair in the setting of AMI at different time points, during ischaemia, early after reperfusion and during scar formation and wound healing is illustrated in Figures 1–3.

9.2 Multi-target approach to cardioprotection

The complex inflammatory response to AMI has many players, including the interplay between the innate immune response and subsequent immune cell infiltration into the MI zone. However, the majority of anti-inflammatory cardioprotective strategies which have failed in the clinical setting has been directed to a single component of the inflammatory response, such as a particular cytokine or a specific immune cell type, and this may have contributed to the failure of these studies. Therefore, a multi-target anti-inflammatory strategy aimed at different components of the inflammatory response to AMI may be more effective at preventing myocardial IRI. For example, one could combine the early administration of an agent which inhibits the pro-inflammatory response to AMI (e.g. pro-inflammatory monocytes) with the subsequent administration of agent which activates the anti-inflammatory reparative response to AMI (e.g. anti-inflammatory monocytes).

However, the situation is further complicated, when one takes into consideration the other non-inflammatory components of myocardial IRI, such as the cardiomyocytes (mitochondrial dysfunction, calcium overload, and oxidative stress), coronary endothelial dysfunction and microvascular obstruction, platelet activation, and so on. As such, multi-target cardioprotective therapies should also address these different components of myocardial IRI.¹⁴¹

9.3 Preclinical rigour and reproducibility and more clinically relevant animal AMI models

The preclinical animal AMI models that have been used to test anti-inflammatory cardioprotective strategies do not represent the typical AMI patient. In most experimental studies, AMI is induced in juvenile healthy animals by occluding a healthy coronary artery, whereas in the clinical setting AMI typically occurs in middle-aged patients and is due to rupture of a coronary atherosclerotic plaque and superimposed thrombosis and inflammation.¹³⁷ In addition, more clinically relevant animal AMI models need to be used, which take into consideration co-morbidities (such as age, diabetes, hypertension, hyperlipidaemia, and so on), and co-medications (such as statins, P2Y₁₂ inhibitors, beta-blocker), which are known to interfere with cardioprotection (reviewed in Refs.^{137,142}).

Another important reason for the failed translation of cardioprotection from the bench-side to the bed-side has been the lack of rigour and reproducibility used in the preclinical testing of novel cardioprotective therapies.¹⁴³ This can be addressed by setting up robust animal AMI protocols and setting up of a network of research centres for undertaking preclinical multicentre randomized placebo-controlled small and large animal studies. This network can then be used to test carefully selected cardioprotective therapies, using standardized myocardial IRI protocols and centralized data analysis. This approach was utilized by the US-based Consortium for preclinical assessment of cardioprotective therapies (CAESAR),^{144,145} and has been proposed by our EU-CARDIOPROTECTION CA16225 COST Action.¹⁴⁶

In conclusion, further studies are required to determine whether targeting the immune response to myocardial IRI can improve clinical outcomes following AMI. Although a number of anti-inflammatory cardioprotective therapies have failed to reduce early and late MI size and improve clinical outcomes following AMI, the majority has been single-targeted and directed to immune cells such as neutrophils and monocytes/macrophages. Experimental studies have reported beneficial effects from targeting other components of the immune cell response to AMI, including lymphocytes, DCs, lymphocytes, and mast cells, and these have the potential to improve clinical outcomes following AMI.

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