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 (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 new players such as dendritic cells, lymphocytes, pericardial lymphoid cells, and their interaction with cardiomyocytes, endothelial cells, and cardiac fibroblasts. We discuss potential reasons for past failures of anti-inflammatory cardioprotective therapies, and highlight emerging 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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Abbreviations

$A_{2B}R$, adenosine A2B receptor; ACE, angiotensin-converting-enzyme; ALDH2, mitochondrial aldehyde dehydrogenase-type 2; AMI, Acute myocardial infarction; AMPK, AMP-activated protein kinase; ANG II, angiotensin II; APF, autophagy-promoting factor; CCL2, chemokine (C-C motif) ligand 2; CCL7, chemokine (C-C motif) ligand 7; CCR2, C-C chemokine receptor type 2; circRNAs, circular RNAs; CXCR2, C-X-C motif chemokine receptor 2; DAMPs, danger-associated molecular patterns; DCs, dendritic cells; DSCG, disodium cromoglycate; GABA, γ-aminobutyric acid; GM-CSF, fibroblast-derived granulocyte-macrophage colony stimulating factor; lncRNA, long ncRNAs; JNK, c-Jun N-terminal kinase; IL-1β, interleukin-1β. IPC, ischaemic preconditioning; IPost, ischaemic postconditioning; IRI, ischaemia/reperfusion injury; LV, left ventricular; MCP-1, monocyte chemoattractant protein-1; MerTK, macrophage myeloid-epithelial-reproductive tyrosine kinase; MI, myocardial infarct; MIF, macrophage migration inhibitory factor; MMPs, metalloproteinases; MPO, myeloperoxidase; ncRNAs, non-coding RNAs; NE, norepinephrine; Nr4a1, nuclear receptor subfamily 4, group a, member 1; PKC-ε, protein kinase C epsilon type; PPAR-γ, peroxisome proliferator activated receptor; RAG1, recombination activating gene 1; RANKL, anti-nuclear factor kappa-B ligand; RAS, renin-angiotensin system; RIC, remote ischaemic conditioning; TNF-α, tumor necrosis factor; TGF-β1, transforming growth factor beta 1; T_{reg}. regulatory T-cells; VGSC, voltage-gated sodium channel.

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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 decline1, long-term mortality (one 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 characterised by early infiltration of neutrophils into the MI zone from 6 to 24 hours post-AMI, followed by the accumulation of pro-inflammatory monocytes and macrophages over the next 48 to 72 hours, 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 the acute MI size (defined in this article as ≤6 hours 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 hours after reperfusion, and therefore mainly arises from a reduction in early MI size (defined in this article as <24 hours of AMI), and reduction in late MI size (defined in this article as ≥24 hours 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 new players such as dendritic cells, lymphocytes, pericardial lymphoid cells, and their interaction with cardiomyocytes, 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 treatment targets for modulating the inflammatory response to AMI, as potential novel therapeutic strategies to improve clinical outcomes following AMI.

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2. Neutrophils as targets for cardioprotection

Neutrophils are the first immune cells recruited into the ischaemic heart following AMI. Increased circulating number[8] or volume of neutrophils[9] 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].

Briefly we can summarise that neutrophils are recruited into the ischaemic heart and their rapid degradation and degranulation results in an acute pro-

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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).

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-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-1[23] (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[30,31,32]. 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 β2 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).

In summary, although, it is well-established that neutrophils are an early contributor to the pro-inflammatory response following AMI, anti-inflammatory therapies directed towards neutrophils have failed in the clinical setting. The reasons for this failure to translate cardioprotection into the clinical setting are multiple and relate to the timing of therapy, the complexity of the inflammatory response to AMI, and the multiple players involved (see section 9).

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3. Monocytes and macrophages as targets for cardioprotection

Monocytes produced in the bone marrow and spleen, enter the bloodstream, and are recruited to the MI zone in the first few hours following AMI, with the spleen providing a steady source of monocytes once their reserves are depleted in the bone marrow[35]. The first population of monocytes that migrate to the site of infarction (peaking at day 3 after AMI) are the pro-inflammatory (Ly6C^{high}) monocytes that express tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β_, 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-1β 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 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+

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macrophages which have distinct functional properties, analogous to reparative CCR2− and inflammatory CCR2+ macrophages in the mouse heart[43]. Angiotensin II, the concentration of which is increased in the circulation following AMI, recruit monocytes from the splenic reservoir[44]; the sympathetic nervous system that stimulates the entry of haematopoietic stem cells from the bone marrow through β3-adrenergic receptor signaling, and β2-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 compartmentalised 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 levels post-AMI, implicating monocyte-induced MerTK

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cleavage as a novel contributor and therapeutic target for preventing myocardial IRI[51]. Signals and receptors involved in monocyte/macrophage recruitment/internalisation are illustrated in Figures 1, 2 and 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 bicuculline[55] (see Table 1 for details).

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 angiotensin 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].

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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 downregulate 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-β1)-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 dendritic cells (DCs) can modulate T_{reg}. Indeed, such DCs primed with lysate from infarcts induced a shift toward reparative macrophages via activation of T_{reg} and

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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 to 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 synthesised 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].

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.

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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, dendritic cells 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.

5. Dendritic cells as emerging targets for cardioprotection

Dendritic cells (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.

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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 program 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 stabilise mast cells and reduce myocardial injury, attenuated MPO release, and prevented 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 remodeling[80].

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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 angiotensin II (ANG II) that exacerbates release of norepinephrine (NE) from isolated sympathetic nerve terminals[82,83,84]. Activation of Gi-coupled receptors, such as histamine-H4, adenosine-A3 and sphingosine-1-phosphate-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 type (PKC-ε), and mitochondrial aldehyde dehydrogenase-type 2 (ALDH2), regulating the 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 Na^+/H^+ exchange 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.

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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 microenvironment 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 programs[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

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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 program 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 angiotensin 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 program[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 program in cardiac fibroblasts has been demonstrated to play a critical role in repair of the infarcted heart, promoting formation of an organised 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 interleukin (IL)-11[106]. Additionally to TGF-β1, *in vivo* infusion of IL-10 post-MI in mice increased fibroblast activation, and improved the LV microenvironment 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

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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. Characterisation 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 lncRNA and miRNA as a cardioprotective strategy

Non-coding RNAs (ncRNAs) are recognised 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 [111,112]]. The many types of ncRNAs are broadly classified in small, <200 nucleotides, and long (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 (APF) has

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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 downregulation 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 interleukins[129,130]. MiR-181a reduced the DC-mediated inflammatory response by oxidised 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

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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.

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 coordinated 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-

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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.

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 have 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].

Preclinical rigour and reproducibility and more clinically relevant animal AMI models

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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 [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 pre-clinical 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 pre-clinical multicentre randomised placebo-controlled small and large animal studies. This network can then be used to test carefully selected cardioprotective therapies, using standardised myocardial IRI protocols and centralised data analysis. This approach was utilised by the U.S. 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 have been single-targeted and directed to immune cells such as neutrophils and monocytes/macrophages. Experimental studies have reported beneficial effects from

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targeting other components of the immune cell response to AMI, including lymphocytes, dendritic cells, lymphocytes, and mast cells, and these have the potential to improve clinical outcomes following AMI.

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References

1. Schmidt M, Jacobsen JB, Lash TL, Bøtker HE, Sørensen HT. 25 year trends in first time hospitalisation for acute myocardial infarction, subsequent short and long term mortality, and the prognostic impact of sex and comorbidity: a Danish nationwide cohort study. *BMJ* 2012; **344**:e356.
2. Szummer K, Wallentin L, Lindhagen L, Alfredsson J, Erlinge D, Held C, James S, Kellerth T, Lindahl B, Ravn-Fischer A, Rydberg E, Yndigegn T, Jernberg T. Improved outcomes in patients with ST-elevation myocardial infarction during the last 20 years are related to implementation of evidence-based treatments: experiences from the SWEDEHEART registry 1995-2014. *Eur Heart J* 2017; **38**:3056-3065.
3. Chen J, Hsieh AF, Dharmarajan K, Masoudi FA, Krumholz HM. National trends in heart failure hospitalization after acute myocardial infarction for Medicare beneficiaries: 1998-2010. *Circulation* 2013; **128**:2577-2584.
4. Liu J, Wang H, Li J. Inflammation and inflammatory cells in myocardial infarction and reperfusion injury: a double-edged sword. *Clin Med Insights Cardiol* 2016;**10**:CMC.S33164.
5. Ong S-B, Hernández-Reséndiz S, Crespo-Avilan GE, Mukhametshina RT, Kwek X-Y, Cabrera-Fuentes HA, Hausenloy DJ. Inflammation following acute myocardial infarction: Multiple players, dynamic roles, and novel therapeutic opportunities. *Pharmacol Ther* 2018;**186**:73–87.
6. Yang XM, Cui L, White J, Kuck J, Ruchko MV, Wilson GL, Alexeyev M, Gillespie MN, Downey JM, Cohen MV. Mitochondrially targeted

Endonuclease III has a powerful anti-infarct effect in an in vivo rat model of myocardial ischemia/reperfusion. *Basic Res Cardiol* 2015; **110**(2):3.
7. Zuurbier C, Abbate A, Cabrera-Fuentes H, De Kleijn D, Downey J, Pagliaro P, Preissner K, Takahashi M, Yndestad A, Davidson S.
8. Chia S, Nagurney JT, Brown DFM, Raffel OC, Bamberg F, Senatore F, Wackers FJT, Jang I-K. Association of leukocyte and neutrophil counts with infarct size, left ventricular function and outcomes after percutaneous coronary intervention for ST-elevation myocardial infarction. *Am J Cardiol* 2009;**103**:333–337.
9. van Hout GP, van Solinge WW, Gijsberts CM, Teuben MP, Leliefeld PH, Heeres M, Nijhoff F, de Jong S, Bosch L, de Jager SC, Huisman A, Stella PR, Pasterkamp G, Koenderman LJ, Hoefer IE. Elevated mean neutrophil volume represents altered neutrophil composition and reflects damage after myocardial infarction. *Basic Res Cardiol* 2015;**110**.
10. Frangogiannis NG. Interleukin-1 in cardiac injury, repair, and remodeling: pathophysiologic and translational concepts. *Discoveries* 2015;**3**:e41.
11. Soehnlein O, Zernecke A, Eriksson EE, Rothfuchs AG, Pham CT, Herwald H, Bidzhekov K, Rottenberg ME, Weber C, Lindbom L. Neutrophil secretion products pave the way for inflammatory monocytes. *Blood* 2008;**112**:1461–1471.
12. Schloss MJ, Horckmans M, Nitz K, Duchene J, Drechsler M, Bidzhekov K, Scheiermann C, Weber C, Soehnlein O, Steffens S. The time-of-day of myocardial infarction onset affects healing through oscillations in cardiac neutrophil recruitment. *EMBO Mol Med* 2016;**8**:937–948.
13. Horckmans M, Ring L, Duchene J, Santovito D, Schloss MJ, Drechsler M, Weber C, Soehnlein O, Steffens S. Neutrophils orchestrate post-myocardial infarction healing by polarizing macrophages towards a reparative phenotype. *Eur Heart J* 2016;ehw002.
14. Zhao Z-Q, Corvera JS, Halkos ME, Kerendi F, Wang N-P, Guyton RA, Vinten-Johansen J. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol - Heart Circ Physiol* 2003;**285**:H579–H588.
15. Granfeldt A, Jiang R, Wang NP, Mykytenko J, Eldaif S, Deneve J, Zhao ZQ, Guyton RA, Tønnesen E, Vinten-Johansen J. Neutrophil inhibition contributes to cardioprotection by postconditioning. *Acta Anaesthesiol Scand* 2011;**56**:48–56.
16. Saxena P, Aggarwal S, Misso NL, Passage J, Newman MAJ, Thompson PJ, Udekem Y d', Praporski S, Konstantinov IE. Remote ischaemic preconditioning down-regulates kinin receptor expression in neutrophils of patients undergoing heart surgery. *Interact Cardiovasc Thorac Surg* 2013;**17**:653–658.
17. Bujak M, Frangogiannis NG. The role of IL-1 in the pathogenesis of heart disease. *Arch Immunol Ther Exp (Warsz)* 2009;**57**:165–176.
18. Carbone F, Crowe LA, Roth A, Burger F, Lenglet S, Braunersreuther V, Brandt KJ, Quercioli A, Mach F, Vallée J-P, Montecucco F. Treatment with anti-RANKL antibody reduces infarct size and attenuates dysfunction impacting on neutrophil-mediated injury. *J Mol Cell Cardiol* 2016;**94**:82–94.
19. Hiroi T, Wajima T, Negoro T, Ishii M, Nakano Y, Kiuchi Y, Mori Y, Shimizu S. Neutrophil TRPM2 channels are implicated in the exacerbation of myocardial ischaemia/reperfusion injury. *Cardiovasc Res* 2012;**97**:271–281.
20. Praetner M, Zuchtriegel G, Holzer M, Uhl B, Schaubächer J, Mittmann L, Fabritius M, Fürst R, Zahler S, Funken D, Lerchenberger M, Khandoga A, Kanse S, Lauber K, Krombach F, Reichel CA. Plasminogen activator inhibitor-

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1 promotes neutrophil infiltration and tissue injury on ischemia–reperfusion. *Arterioscler Thromb Vasc Biol* 2018;**38**:829–842.
21. Bao J, Sato K, Li M, Gao Y, Abid R, Aird W, Simons M, Post MJ. PR-39 and PR-11 peptides inhibit ischemia-reperfusion injury by blocking proteasome-mediated I kappa B alpha degradation. *Am J Physiol-Heart Circ Physiol* 2001;**281**:H2612–H2618.
22. van der Pals J, Koul S, Andersson P, Götberg M, Ubachs JF, Kanski M, Arheden H akan, Olivecrona GK, Larsson B, Erlinge D. Treatment with the C5a receptor antagonist ADC-1004 reduces myocardial infarction in a porcine ischemia-reperfusion model. *BMC Cardiovasc Disord* 2010;**10**.
23. Dokken BB, Bonte LRL, Davis-Gorman G, Teachey MK, Seaver N, McDonagh PF. Glucagon-like peptide-1 (GLP-1), immediately prior to reperfusion, decreases neutrophil activation and reduces myocardial infarct size in rodents. *Horm Metab Res* 2011;**43**:300–305.
24. Amsterdam EA, Pan H-L, Rendig SV, Symons JD, Fletcher MP, Longhurst JC. Limitation of myocardial infarct size in pigs with a dual lipoxygenase-cyclooxygenase blocking agent by inhibition of neutrophil activity without reduction of neutrophil migration. *J Am Coll Cardiol* 1993;**22**:1738–1744.
25. Kohtani T, Abe Y, Sato M, Miyauchi K, Kawachi K. Protective effects of anti-neutrophil antibody against myocardial ischemia/reperfusion injury in rats. *Eur Surg Res* 2002;**34**:313–320.
26. Burke SE, Wright CD, Potoczak RE, Boucher DM, Dodd GD, Taylor DG, Kaplan HR. Reduction of canine myocardial infarct size by CI-959, an inhibitor of inflammatory cell activation. *J Cardiovasc Pharmacol* 1992;**20**:619–629.
27. Vitola JV, Forman MB, Holsinger JP, Atkinson JB, Murray JJ. Reduction of myocardial infarct size in rabbits and inhibition of activation of rabbit and human neutrophils by lidocaine. *Am Heart J* 1997;**133**:315–322.
28. Shen Y-C, Chen C-F, Sung Y-J. Tetrandrine ameliorates ischaemia-reperfusion injury of rat myocardium through inhibition of neutrophil priming and activation. *Br J Pharmacol* 1999;**128**:1593–1601.
29. Ali M, Pulli B, Courties G, Tricot B, Sebas M, Iwamoto Y, Hilgendorf I, Schob S, Dong A, Zheng W, Skoura A, Kalgukar A, Cortes C, Ruggeri R, Swirski FK, Nahrendorf M, Buckbinder L, Chen JW. Myeloperoxidase inhibition improves ventricular function and remodeling after experimental myocardial infarction. *JACC Basic Transl Sci* 2016;**1**:633–643.
30. Liehn EA, Kanzler I, Konschalla S, Kroh A, Simsekyilmaz S, Sonmez TT, Bucala R, Bernhagen J, Weber C. Compartmentalized protective and detrimental effects of endogenous macrophage migration-inhibitory factor mediated by CXCR2 in a mouse model of myocardial ischemia/reperfusion. *Arterioscler Thromb Vasc Biol* 2013;**33**:2180–2186.
31. Oral H, Kanzler I, Tuchscheerer N, Curaj A, Simsekyilmaz S, Sönmez TT, Radu E, Postea O, Weber C, Schuh A, Liehn EA. CXC chemokine KC fails to induce neutrophil infiltration and neoangiogenesis in a mouse model of myocardial infarction. *J Mol Cell Cardiol* 2013;**60**:1–7.
32. Curaj A, Staudt M, Fatu R, Kraaijeveld AO, Jankowski J, Biessen EAL, Liehn EA. Blockade of CCR3 retains the neutrophils, preserving their survival during healing after myocardial infarction. *Discoveries* 2015;**3**:e45.
33. Baran KW, Nguyen M, McKendall GR, Lambrew CT, Dykstra G, Palmeri ST, Gibbons RJ, Borzak S, Sobel BE, Gourlay SG, Rundle AC, Gibson CM, Barron HV. Double-blind, randomized trial of an anti-CD18 antibody in conjunction with recombinant tissue plasminogen activator for acute myocardial infarction: Limitation of myocardial infarction following thrombolysis in acute myocardial infarction (LIMIT AMI) Study. *Circulation* 2001;**104**:2778–2783.

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34. Faxon DP, Gibbons RJ, Chronos NAF, Gurbel PA, Sheehan F. The effect of blockade of the CD11/CD18 integrin receptor on infarct size in patients with acute myocardial infarction treated with direct angioplasty: the results of the HALT-MI study. *J Am Coll Cardiol* 2002;**40**:1199–1204.
35. Leuschner F, Rauch PJ, Ueno T, Gorbatov R, Marinelli B, Lee WW, Dutta P, Wei Y, Robbins C, Iwamoto Y, Sena B, Chudnovskiy A, Panizzi P, Keliher E, Higgins JM, Libby P, Moskowitz MA, Pittet MJ, Swirski FK, Weissleder R, Nahrendorf M. Rapid monocyte kinetics in acute myocardial infarction are sustained by extramedullary monocytopoiesis. *J Exp Med* 2012;**209**:123–137.
36. Nahrendorf M, Swirski FK, Aikawa E, Stangenberg L, Wurdinger T, Figueiredo J-L, Libby P, Weissleder R, Pittet MJ. The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. *J Exp Med* 2007;**204**:3037–3047.
37. Nahrendorf M, Swirski FK. Monocyte and macrophage heterogeneity in the heart. *Circ Res* 2013;**112**:1624–1633.
38. van der Laan AM, Hirsch A, Robbers LF, Nijveldt R, Lommerse I, Delewi R, van der Vleuten PA, Biemond BJ, Zwaginga JJ, van der Giessen WJ, Zijlstra F, van Rossum AC, Voermans C, van der Schoot CE, Piek JJ. A proinflammatory monocyte response is associated with myocardial injury and impaired functional outcome in patients with ST-segment elevation myocardial infarction. *Am Heart J* 2012;**163**:57–65.e2.
39. Tsujioka H, Imanishi T, Ikejima H, Kuroi A, Takarada S, Tanimoto T, Kitabata H, Okochi K, Arita Y, Ishibashi K, Komukai K, Kataiwa H, Nakamura N, Hirata K, Tanaka A, Akasaka T. Impact of heterogeneity of human peripheral blood monocyte subsets on myocardial salvage in patients with primary acute myocardial infarction. *J Am Coll Cardiol* 2009;**54**:130–138.
40. Kakio T, Matsumori A, Ono K, Ito H, Matsushima K, Sasayama S. Roles and relationship of macrophages and monocyte chemotactic and activating factor/monocyte chemoattractant protein-1 in the ischemic and reperfused rat heart. *Lab Invest* 2000;**80**:1127–1136.
41. Martinovic I, Abegunewardene N, Seul M, Vosseler M, Horstick G, Buerke M, Darius H, Lindemann S. Elevated monocyte chemoattractant protein-1 serum levels in patients at risk for coronary artery disease. *Circ J* 2005;**69**:1484–1489.
42. Zouggari Y, Ait-Oufella H, Bonnin P, Simon T, Sage AP, Guérin C, Vilar J, Caligiuri G, Tsiantoulas D, Laurans L, Dumeau E, Kotti S, Bruneval P, Charo IF, Binder CJ, Danchin N, Tedgui A, Tedder TF, Silvestre J-S, Mallat Z. B lymphocytes trigger monocyte mobilization and impair heart function after acute myocardial infarction. *Nat Med* 2013;**19**:1273–1280.
43. Bajpai G, Schneider C, Wong N, Bredemeyer A, Hulsmans M, Nahrendorf M, Epelman S, Kreisel D, Liu Y, Itoh A, Shankar TS, Selzman CH, Drakos SG, Lavine KJ. The human heart contains distinct macrophage subsets with divergent origins and functions. *Nat Med* 2018; **24**:1234-1245.
44. Leuschner F, Panizzi P, Chico-Calero I, Lee WW, Ueno T, Cortez-Retamozo V, Waterman P, Gorbatov R, Marinelli B, Iwamoto Y, Chudnovskiy A, Figueiredo J-L, Sosnovik DE, Pittet MJ, Swirski FK, Weissleder R, Nahrendorf M. Angiotensin-converting enzyme inhibition prevents the release of monocytes from their splenic reservoir in mice with myocardial infarction. *Circ Res* 2010;**107**:1364–1373.
45. Grisanti LA, Gumpert AM, Traynham CJ, Gorsky JE, Repas AA, Gao E, Carter RL, Yu D, Calvert JW, García AP, Ibáñez B, Rabinowitz JE, Koch WJ, Tilley DG. Leukocyte-expressed β2-adrenergic receptors are essential for survival after acute myocardial injury. *Circulation* 2016;**134**:153–167.

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46. Hilgendorf I, Gerhardt LMS, Tan TC, Winter C, Holderried TAW, Chousterman BG, Iwamoto Y, Liao R, Zirlik A, Scherer-Crosbie M, Hedrick CC, Libby P, Nahrendorf M, Weissleder R, Swirski FK. Ly-6Chigh monocytes depend on Nr4a1 to balance both inflammatory and reparative phases in the infarcted myocardium. *Circ Res* 2014;**114**:1611–1622.
47. Miller EJ, Li J, Leng L, McDonald C, Atsumi T, Bucala R, Young LH. Macrophage migration inhibitory factor stimulates AMP-activated protein kinase in the ischaemic heart. *Nature* 2008;**451**:578–582.
48. Qi D, Hu X, Wu X, Merk M, Leng L, Bucala R, Young LH. Cardiac macrophage migration inhibitory factor inhibits JNK pathway activation and injury during ischemia/reperfusion. *J Clin Invest* 2009;**119**:3807–3816.
49. Luedike P, Hendgen-Cotta UB, Sobierajski J, Totzeck M, Reeh M, Dewor M, Lue H, Krisp C, Wolters D, Kelm M, Bernhagen J, Rassaf T. Cardioprotection through S-nitros(yl)ation of macrophage migration inhibitory factor. *Circulation* 2012;**125**:1880–1889.
50. Gao X-M, Liu Y, White D, Su Y, Drew BG, Bruce CR, Kiriazis H, Xu Q, Jennings N, Bobik A, Febbraio MA, Kingwell BA, Bucala R, Fingerle-Rowson G, Dart AM, Morand EF, Du X-J. Deletion of macrophage migration inhibitory factor protects the heart from severe ischemia–reperfusion injury: A predominant role of anti-inflammation. *J Mol Cell Cardiol* 2011;**50**:991–999.
51. DeBerge M, Yeap XY, Dehn S, Zhang S, Grigoryeva L, Misener S, Procissi D, Zhou X, Lee DC, Muller WA, Luo X, Rothlin C, Tabas I, Thorp EB. MerTK cleavage on resident cardiac macrophages compromises repair after myocardial ischemia reperfusion injury. *Circ Res* 2017;**121**:930–940.
52. Zhou X, Luo Y-C, Ji W-J, Zhang L, Dong Y, Ge L, Lu R-Y, Sun H-Y, Guo Z-Z, Yang G-H, Jiang T-M, Li Y-M. Modulation of mononuclear phagocyte inflammatory response by liposome-encapsulated voltage gated sodium channel inhibitor ameliorates myocardial ischemia/reperfusion injury in rats. *PLoS ONE* 2013;**8**:e74390.
53. Koeppen M, Harter PN, Bonney S, Bonney M, Reithel S, Zachskorn C, Mittelbronn M, Eckle T. Adora2b signaling on bone marrow derived cells dampens myocardial ischemia-reperfusion injury. *Anesthesiology* 2012;**116**:1245–1257.
54. Tian Y, Piras BA, Kron IL, French BA, Yang Z. Adenosine 2B receptor activation reduces myocardial reperfusion injury by promoting aAnti-inflammatory macrophages differentiation via PI3K/Akt pathway. *Oxid Med Cell Longev* 2015;**2015**:1–8.
55. Wang Z, Huang S, Sheng Y, Peng X, Liu H, Jin N, Cai J, Shu Y, Li T, Li P, Fan C, Hu X, Zhang W, Long R, You Y, Huang C, Song Y, Xiang C, Wang J, Yang Y, Liu K. Topiramate modulates post-infarction inflammation primarily by targeting monocytes or macrophages. *Cardiovasc Res* 2017;**113**:475–487.
56. Nakano Y, Matoba T, Tokutome M, Funamoto D, Katsuki S, Ikeda G, Nagaoka K, Ishikita A, Nakano K, Koga J, Sunagawa K, Egashira K. Nanoparticle-mediated delivery of irbesartan induces cardioprotection from myocardial ischemia-reperfusion injury by antagonizing monocyte-mediated inflammation. *Sci Rep* 2016;**6**.
57. Hofmann U, Beyersdorf N, Weirather J, Podolskaya A, Bauersachs J, Ertl G, Kerkau T, Frantz S. Activation of CD4+ T lymphocytes improves wound healing and survival after experimental myocardial infarction in mice. *Circulation* 2012;**125**:1652–1663.
58. Boag SE, Das R, Shmeleva EV, Bagnall A, Egred M, Howard N, Bennaceur K, Zaman A, Keavney B, Spyridopoulos I. T lymphocytes and fractalkine contribute to myocardial ischemia/reperfusion injury in patients. *J Clin Invest* 2015;**125**:3063–3076.

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59. Ma ZG, Dai J, Yuan YP, Bian ZY, Xu SC, Jin YG, Zhang X, Tang QZ. T-bet deficiency attenuates cardiac remodelling in rats. *Basic Res Cardiol* 2018; **113**:19.
60. Weirather J, Hofmann UDW, Beyersdorf N, Ramos GC, Vogel B, Frey A, Ertl G, Kerkau T, Frantz S. Foxp3+ CD4+ T cells improve healing after myocardial infarction by modulating monocyte/macrophage differentiation. *Circ Res* 2014;**115**:55–67.
61. Saxena A, Dobaczewski M, Rai V, Haque Z, Chen W, Li N, Frangogiannis NG. Regulatory T cells are recruited in the infarcted mouse myocardium and may modulate fibroblast phenotype and function. *Am J Physiol-Heart Circ Physiol* 2014;**307**:H1233–H1242.
62. Choo EH, Lee J-H, Park E-H, Park HE, Jung N-C, Kim T-H, Koh Y-S, Kim E, Seung K-B, Park C, Hong K-S, Kang K, Song J-Y, Seo HG, Lim D-S, Chang K. Infarcted myocardium-primed dendritic cells improve remodeling and cardiac function after myocardial infarction by modulating the regulatory T cell and macrophage polarization. *Circulation* 2017;**135**:1444–1457.
63. Ke D, Fang J, Fan L, Chen L. Targeted deletion of regulatory T cells attenuates the protective effects of myocardial ischemic preconditioning in rats. *Scand Cardiovasc J* 2015;**49**:64-71.
64. Yang Z, Day Y-J, Toufektsian M-C, Xu Y, Ramos SI, Marshall MA, French BA, Linden J. Myocardial infarct-sparing effect of adenosine A2A receptor activation is due to its action on CD4+ T lymphocytes. *Circulation* 2006;**114**:2056–2064.
65. Mathes D, Weirather J, Nordbeck P, Arias-Loza AP, Burkard M, Pachel C, Kerkau T, Beyersdorf N, Frantz S and Hofmann U. CD4+ Foxp3+ T-cells contribute to myocardial ischemia-reperfusion injury. *J Mol Cell Cardiol* 2016;**101**:99-105.
66. Borg N, Alter C, Görldt N, Jacoby C, Ding Z, Steckel B, Quast C, Bönner F, Friebe D, Temme S, Flögel U, Schrader J. CD73 on T cells orchestrates cardiac wound healing after myocardial infarction by purinergic metabolic reprogramming. *Circulation* 2017;**136**:297–313.
67. Horckmans M, Bianchini M, Santovito D, Megens RTA, Springael J-Y, Negri I, Vacca M, Eusanio MD, Moschetta A, Weber C, Duchene J, Steffens S. Pericardial adipose tissue regulates granulopoiesis, fibrosis, and cardiac function after myocardial infarction. *Circulation* 2017;**137**:948–960.
68. Mildner A, Jung S. Development and function of dendritic cell subsets. *Immunity* 2014;**40**:642–656.
69. Hart DN. Demonstration and characterization of Ia-positive dendritic cells in the interstitial connective tissues of rat heart and other tissues, but not brain. *J Exp Med* 1981;**154**:347–361.
70. Zhang J, Yu ZX, Fujita S, Yamaguchi ML, Ferrans VJ. Interstitial dendritic cells of the rat heart. Quantitative and ultrastructural changes in experimental myocardial infarction. *Circulation* 1993;**87**:909–920.
71. Anzai A, Anzai T, Nagai S, Maekawa Y, Naito K, Kaneko H, Sugano Y, Takahashi T, Abe H, Mochizuki S, Sano M, Yoshikawa T, Okada Y, Koyasu S, Ogawa S, Fukuda K. Regulatory role of dendritic cells in postinfarction healing and left ventricular remodeling. *Circulation* 2012;**125**:1234–1245.
72. Nagai T, Honda S, Sugano Y, Matsuyama T, Ohta-Ogo K, Asaumi Y, Ikeda Y, Kusano K, Ishihara M, Yasuda S, Ogawa H, Ishibashi-Ueda H, Anzai T. Decreased myocardial dendritic cells is associated with impaired reparative fibrosis and development of cardiac rupture after myocardial infarction in humans. *J Am Heart Assoc* 2014;**3**:e000839–e000839.
73. Van der Borght K, Scott CL, Nindl V, Bouché A, Martens L, Sichien D, Van Moorleghem J, Vanheerswynghels M, De Prijck S, Saeys Y, Ludewig B,

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Gillebert T, Guilliams M, Carmeliet P, Lambrecht BN. Myocardial infarction primes autoreactive T cells through activation of dendritic cells. *Cell Rep* 2017;**18**:3005–3017.
74. Liu H, Gao W, Yuan J, Wu C, Yao K, Zhang L, Ma L, Zhu J, Zou Y, Ge J. Exosomes derived from dendritic cells improve cardiac function via activation of CD4(+) T lymphocytes after myocardial infarction. *J Mol Cell Cardiol* 2016;**91**:123–133.
75. Singh M, Saini HK. Resident cardiac mast cells and ischemia-reperfusion injury. *J Cardiovasc Pharmacol Ther* 2003;**8**:135–148.
76. Rydzyński K, Dalen H, Saetersdal T, Engedal H. Morphologic and morphometric analysis of the mast cells from human heart biopsies. *Agents Actions* 1987;**20**:288–290.
77. Frangogiannis NG, Lindsey ML, Michael LH, Youker KA, Bressler RB, Mendoza LH, Spengler RN, Smith CW, Entman ML. Resident cardiac mast cells degranulate and release preformed TNF-alpha, initiating the cytokine cascade in experimental canine myocardial ischemia/reperfusion. *Circulation* 1998;**98**:699–710.
78. Jaggi AS, Singh M, Sharma A, Singh D, Singh N. Cardioprotective effects of mast cell modulators in ischemia-reperfusion-induced injury in rats. *Methods Find Exp Clin Pharmacol* 2007;**29**:593–600.
79. Parikh V, Singh M. Cardiac mast cell stabilization and cardioprotective effect of ischemic preconditioning in isolated rat heart. *J Cardiovasc Pharmacol* 1998;**31**:779–785.
80. Hooshdaran B, Kolpakov MA, Guo X, Miller SA, Wang T, Tilley DG, Rafiq K, Sabri A. Dual inhibition of cathepsin G and chymase reduces myocyte death and improves cardiac remodeling after myocardial ischemia reperfusion injury. *Basic Res Cardiol* 2017; **112**:62.
81. Mackins CJ, Kano S, Seyedi N, Schäfer U, Reid AC, Machida T, Silver RB, Levi R. Cardiac mast cell-derived renin promotes local angiotensin formation, norepinephrine release, and arrhythmias in ischemia/reperfusion. *J Clin Invest* 2006;**116**:1063–1070.
82. Morrey C, Brazin J, Seyedi N, Corti F, Silver RB, Levi R. Interaction between sensory C-fibers and cardiac mast cells in ischemia/reperfusion: activation of a local renin-angiotensin system culminating in severe arrhythmic dysfunction. *J Pharmacol Exp Ther* 2010;**335**:76–84.
83. Marino A, Sakamoto T, Robador PA, Tomita K, Levi R. S1P receptor 1-mediated anti-renin-angiotensin system cardioprotection: pivotal role of mast cell aldehyde dehydrogenase type 2. *J Pharmacol Exp Ther* 2017;**362**:230–242.
84. Koda K, Salazar-Rodriguez M, Corti F, Chan NY-K, Estephan R, Silver RB, Mochly-Rosen D, Levi R. Aldehyde dehydrogenase activation prevents reperfusion arrhythmias by inhibiting local renin release from cardiac mast cells. *Circulation* 2010;**122**:771–781.
85. Kaur H, Parikh V, Sharma A, Singh M. Effect of amiloride A Na+/H+ exchange inhibitor on cardioprotective effect of ischaemic preconditioning: possible involvement of resident cardiac mast cells. *Pharmacol Res* 1997;**36**:95–102.
86. Aldi S, Marino A, Tomita K, Corti F, Anand R, Olson KE, Marcus AJ, Levi R. E-NTPDase1/CD39 modulates renin release from heart mast cells during ischemia/reperfusion: a novel cardioprotective role. *FASEB J* 2015;**29**:61–69.
87. Parikh V, Singh M. Possible role of cardiac mast cells in norepinephrine-induced myocardial preconditioning. *Methods Find Exp Clin Pharmacol* 1999;**21**:269–274.

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88. Hirsch E, Hilfiker-Kleiner D, Balligand J-L, Tarone G, Windt LD, Bauersachs J, Ferdinandy P, Davidson S, Hausenloy DJ, Schulz R. Interaction of the heart and its close and distant neighbours: report of the Meeting of the ESC Working Groups Myocardial Function and Cellular Biology. *Cardiovasc Res* 2013;**99**:595–599.
89. Rohrbach S, Troidl C, Hamm C, Schulz R. Ischemia and reperfusion related myocardial inflammation: A network of cells and mediators targeting the cardiomyocyte. *IUBMB Life* 2015;**67**:110–119.
90. Prabhu SD, Frangogiannis NG. The biological basis for cardiac repair after myocardial infarction. *Circ Res* 2016;**119**:91–112.
91. Shinde AV, Frangogiannis NG. Fibroblasts in myocardial infarction: A role in inflammation and repair. *J Mol Cell Cardiol* 2014;**70**:74–82.
92. Saxena A, Chen W, Su Y, Rai V, Uche OU, Li N, Frangogiannis NG. IL-1 induces proinflammatory leukocyte infiltration and regulates fibroblast phenotype in the infarcted myocardium. *J Immunol* 2013;**191**:4838–4848.
93. Turner NA. Inflammatory and fibrotic responses of cardiac fibroblasts to myocardial damage associated molecular patterns (DAMPs). *J Mol Cell Cardiol* 2016;**94**:189–200.
94. Anzai A, Choi JL, He S, Fenn AM, Nairz M, Rattik S, McAlpine CS, Mindur JE, Chan CT, Iwamoto Y, Tricot B, Wojtkiewicz GR, Weissleder R, Libby P, Nahrendorf M, Stone JR, Becher B, Swirski FK. The infarcted myocardium solicits GM-CSF for the detrimental oversupply of inflammatory leukocytes. *J Exp Med* 2017;jem.20170689.
95. Abrial M, Silva CCD, Pillot B, Augeul L, Ivanes F, Teixeira G, Cartier R, Angoulvant D, Ovize M, Ferrera R. Cardiac fibroblasts protect cardiomyocytes against lethal ischemia–reperfusion injury. *J Mol Cell Cardiol* 2014;**68**:56–65.
96. Frangogiannis NG. The functional pluralism of fibroblasts in the infarcted myocardium. *Circ Res* 2016;**119**:1049–1051.
97. Tian C, Gao L, Zimmerman MC, Zucker IH. Myocardial infarction-induced microRNA-enriched exosomes contribute to cardiac Nrf2 dysregulation in chronic heart failure. *Am J Physiol-Heart Circ Physiol* 2018;**314**:H928–H939.
98. Piccoli M-T, Gupta SK, Viereck J, Foinquinos A, Samolovac S, Kramer FL, Garg A, Remke J, Zimmer K, Batkai S, Thum T. Inhibition of the cardiac fibroblast–enriched lncRNA Meg3 prevents cardiac fibrosis and diastolic dysfunction. *Circ Res* 2017;**121**:575–583.
99. Shiraishi M, Shintani Y, Shintani Y, Ishida H, Saba R, Yamaguchi A, Adachi H, Yashiro K, Suzuki K. Alternatively activated macrophages determine repair of the infarcted adult murine heart. *J Clin Invest* 2016;**126**:2151–2166.
100. Shinde AV, Frangogiannis NG. Mechanisms of fibroblast activation in the remodeling myocardium. *Curr Pathobiol Rep* 2017;**5**:145–152.
101. Shinde AV, Humeres C, Frangogiannis NG. **T**he role of α-smooth muscle actin in fibroblast-mediated matrix contraction and remodeling. *Biochim Biophys Acta BBA - Mol Basis Dis* 2017;**1863**:298–309.
102. Travers JG, Kamal FA, Valiente-Alandi I, Nieman ML, Sargent MA, Lorenz JN, Molkentin JD, Blaxall BC. Pharmacological and activated fibroblast targeting of Gβγ-GRK2 after myocardial ischemia attenuates heart failure progression. *J Am Coll Cardiol* 2017;**70**:958–971.
103. Frangogiannis NG. Cardiac fibrosis: Cell biological mechanisms, molecular pathways and therapeutic opportunities. *Mol Aspects Med* 2018.
104. Dobaczewski M, Bujak M, Li N, Gonzalez-Quesada C, Mendoza LH, Wang X-F, Frangogiannis NG. Smad3 signaling critically regulates fibroblast phenotype and function in healing myocardial infarction. *Circ Res* 2010;**107**:418–428.

Downloaded from https://academic.oup.com/cardiovascres/advance-article-abstract/doi/10.1093/cvr/cvz050/5368489 by UCL (University College London) user on 11 March 2019

105. Kong P, Shinde AV, Su Y, Russo I, Chen B, Saxena A, Conway SJ, Graff JM, Frangogiannis NG. Opposing actions of fibroblast and cardiomyocyte Smad3 signaling in the infarcted myocardium. *Circulation* 2017;**137**:707–724.
106. Schafer S, Viswanathan S, Widjaja AA, Lim W-W, Moreno-Moral A, DeLaughter DM, Ng B, Patone G, Chow K, Khin E, Tan J, Chothani SP, Ye L, Rackham OJL, Ko NSJ, Sahib NE, Pua CJ, Zhen NTG, Xie C, Wang M, Maatz H, Lim S, Saar K, Blachut S, Petretto E, Schmidt S, Putoczki T, Guimarães-Camboa N, Wakimoto H, van Heesch S, Sigmundsson K, Lim SL, Soon JL, Chao VTT, Chua YL, Tan TE, Evans SM, Loh YJ, Jamal MH, Ong KK, Chua KC, Ong BH, Chakaramakkil MJ, Seidman JG, Seidman CE, Hubner N, Sin KYK, Cook SA. IL11 is a crucial determinant of cardiovascular fibrosis. *Nature* 2017; **552**:110-115.
107. Jung M, Ma Y, Iyer RP, DeLeon-Pennell KY, Yabluchanskiy A, Garrett MR, Lindsey ML. IL-10 improves cardiac remodeling after myocardial infarction by stimulating M2 macrophage polarization and fibroblast activation. *Basic Res Cardiol* 2017;**112**:33.
108. Frangogiannis N. Myofibroblasts in reperfused myocardial infarcts express the embryonic form of smooth muscle myosin heavy chain (SMemb). *Cardiovasc Res* 2000;**48**:89–100.
109. Takemura G, Ohno M, Hayakawa Y, Misao J, Kanoh M, Ohno A, Uno Y, Minatoguchi S, Fujiwara T, Fujiwara H. Role of apoptosis in the disappearance of infiltrated and proliferated interstitial cells after myocardial infarction. *Circ Res* 1998;**82**:1130–1138.
110. Fu X, Khalil H, Kanisicak O, Boyer JG, Vagnozzi RJ, Maliken BD, Sargent MA, Prasad V, Valiente-Alandi I, Blaxall BC, Molkentin JD. Specialized fibroblast differentiated states underlie scar formation in the infarcted mouse heart. *J Clin Invest* 2018;**128**:2127–2143.
111. Ong S-B, Katwadi K, Kwek X-Y, Ismail NI, Chinda K, Ong S-G, Hausenloy DJ. Non-coding RNAs as therapeutic targets for preventing myocardial ischemia-reperfusion injury. *Expert Opin Ther Targets* 2018;**22**:247–261.
112. Gomes CPC, Spencer H, Ford KL, Michel LYM, Baker AH, Emanueli C, Balligand J-L, Devaux Y. The function and therapeutic potential of long non-coding RNAs in cardiovascular development and disease. *Mol Ther - Nucleic Acids* 2017;**8**:494–507.
113. Wang S, Yu W, Chen J, Yao T, Deng F. LncRNA MALAT1 sponges miR-203 to promote inflammation in myocardial ischemia-reperfusion injury. *Int J Cardiol* 2018;**268**:245.
114. Ouimet M, Ediriweera HN, Gundra UM, Sheedy FJ, Ramkhelawon B, Hutchison SB, Rinehold K, Solingen C van, Fullerton MD, Cecchini K, Rayner KJ, Steinberg GR, Zamore PD, Fisher EA, Loke P, Moore KJ. MicroRNA-33–dependent regulation of macrophage metabolism directs immune cell polarization in atherosclerosis. *J Clin Invest* 2015;**125**:4334–4348.
115. Jiang S, Yan W, Wang SE, Baltimore D. Let-7 suppresses B cell activation through restricting the availability of necessary nutrients. *Cell Metabolism* 2018; **27**:393-403.
116. Du M, Yuan L, Tan X, Huang D, Wang X, Zheng Z, Mao X, Li X, Yang L, Huang K, Zhang F, Wang Y, Luo X, Huang D, Huang K. The LPS-inducible lncRNA Mirt2 is a negative regulator of inflammation. *Nat Commun* 2017;**8**.
117. Li X, Dai Y, Yan S, Shi Y, Han B, Li J, Cha L, Mu J. Down-regulation of lncRNA KCNQ1OT1 protects against myocardial ischemia/reperfusion injury following acute myocardial infarction. *Biochem Biophys Res Commun* 2017;**491**:1026–1033.

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118. Xu Y, Shao B. Circulating lncRNA IFNG-AS1 expression correlates with increased disease risk, higher disease severity and elevated inflammation in patients with coronary artery disease. *J Clin Lab Anal* 2018;e22452.
119. Zhou Z, Jiang R, Yang X, Guo H, Fang S, Zhang Y, Cheng Y, Wang J, Yao H, Chao J. circRNA mediates silica-induced macrophage activation via HECTD1/ZC3H12A-dependent ubiquitination. *Theranostics* 2018;**8**:575–592.
120. Vausort M, Wagner DR, Devaux Y. Long noncoding RNAs in patients with acute myocardial infarction. *Circ Res* 2014;**115**:668–677.
121. Wang K, Liu C-Y, Zhou L-Y, Wang J-X, Wang M, Zhao B, Zhao W-K, Xu S-J, Fan L-H, Zhang X-J, Feng C, Wang C-Q, Zhao Y-F, Li P-F. APF lncRNA regulates autophagy and myocardial infarction by targeting miR-188-3p. *Nat Commun* 2015;**6**.
122. Salgado-Somoza A, Zhang L, Vausort M, Devaux Y. The circular RNA MICRA for risk stratification after myocardial infarction. *IJC Heart & Vasculature* 2017; 33–36.
123. O Connell RM, Rao DS, Chaudhuri AA, Baltimore D. Physiological and pathological roles for microRNAs in the immune system. *Nat Rev Immunol* 2010;**10**:111–122.
124. Smyth LA, Boardman DA, Tung SL, Lechler R, Lombardi G. MicroRNAs affect dendritic cell function and phenotype. *Immunology* 2015;**144**:197–205.
125. Dunand-Sauthier I, Santiago-Raber M-L, Capponi L, Vejnar CE, Schaad O, Irla M, Seguin-Estevez Q, Descombes P, Zdobnov EM, Acha-Orbea H, Reith W. Silencing of c-Fos expression by microRNA-155 is critical for dendritic cell maturation and function. *Blood* 2011;**117**:4490–4500.
126. Rodriguez A, Vigorito E, Clare S, Warren MV, Couttet P, Soond DR, Dongen S van, Grocock RJ, Das PP, Miska EA, Vetrie D, Okkenhaug K, Enright AJ, Dougan G, Turner M, Bradley A. Requirement of bic/microRNA-155 for normal immune function. *Science* 2007;**316**:608–611.
127. Zhang M, Liu F, Jia H, Zhang Q, Yin L, Liu W, Li H, Yu B, Wu J. Inhibition of MicroRNA let-7i depresses maturation and functional state of dendritic cells in response to lipopolysaccharide stimulation via targeting suppressor of cytokine signaling 1. *J Immunol* 2011;**187**:1674–1683.
128. Liu X, Zhan Z, Xu L, Ma F, Li D, Guo Z, Li N, Cao X. MicroRNA-148/152 Impair innate response and antigen presentation of TLR-triggered dendritic cells by targeting CaMKII. *J Immunol* 2010;**185**:7244–7251.
129. Zheng J, Jiang H-Y, Li J, Tang H-C, Zhang X-M, Wang X-R, Du J-T, Li H-B, Xu G. MicroRNA-23b promotes tolerogenic properties of dendritic cells in vitro through inhibiting Notch1/NF-κB signalling pathways. *Allergy* 2012;**67**:362–370.
130. Su X, Qian C, Zhang Q, Hou J, Gu Y, Han Y, Chen Y, Jiang M, Cao X. miRNomes of haematopoietic stem cells and dendritic cells identify miR-30b as a regulator of Notch1. *Nat Commun* 2013;**4**.
131. Wu C, Gong Y, Yuan J, Zhang W, Zhao G, Li H, Sun A, KaiHu, Zou Y, Ge J. microRNA-181a represses ox-LDL-stimulated inflammatory response in dendritic cell by targeting c-Fos. *J Lipid Res* 2012;**53**:2355–2363.
132. Zhu J, Yao K, Guo J, Shi H, Ma L, Wang Q, Liu H, Gao W, Sun A, Zou Y, Ge J. miR-181a and miR-150 regulate dendritic cell immune inflammatory responses and cardiomyocyte apoptosis via targeting JAK1-STAT1/c-Fos pathway. *J Cell Mol Med* 2017;**21**:2884–2895.
133. Zhao H, Zhang X, Zheng Y, Li Y, Wang X, Hu N, Zhou P, Wang K. Propofol protects rat cardiomyocytes from anthracycline-induced apoptosis by

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regulating microRNA-181a in vitro and in vivo. *Oxid Med Cell Longev* 2018;**2018**:1–11.
134. Liu Z, Ye P, Wang S, Wu J, Sun Y, Zhang A, Ren L, Cheng C, Huang X, Wang K, Deng P, Wu C, Yue Z, Xia J. MicroRNA-150 protects the heart from injury by inhibiting monocyte accumulation in a mouse model of acute myocardial infarction. *Circ Cardiovasc Genet* 2015; **8**:11-20.
135. Devaux Y, Vausort M, McCann GP, Zangrando J, Kelly D, Razvi N, Zhang L, Ng LL, Wagner DR, Squire IB. MicroRNA-150: A novel marker of left ventricular remodeling after acute myocardial infarction. *Circ Cardiovasc Genet* 2013;**6**:290–298.
136. Hausenloy DJ, Garcia-Dorado D, Bøtker HE, Davidson SM, Downey J, Engel FB, Jennings R, Lecour S, Leor J, Madonna R, Ovize M, Perrino C, Prunier F, Schulz R, Sluijter JPG, Laake V, W L, Vinten-Johansen J, Yellon DM, Ytrehus K, Heusch G, Ferdinandy P. Novel targets and future strategies for acute cardioprotection: Position Paper of the European Society of Cardiology Working Group on Cellular Biology of the Heart. *Cardiovasc Res* 2017;**113**:564–585.
137. Lecour S, Bøtker HE, Condorelli G, Davidson SM, Garcia-Dorado D, Engel FB, Ferdinandy P, Heusch G, Madonna R, Ovize M, Ruiz-Meana M, Schulz R, Sluijter JPG, Van Laake LW, Yellon DM, Hausenloy DJ. ESC working group cellular biology of the heart: position paper: improving the preclinical assessment of novel cardioprotective therapies. *Cardiovasc Res* 2014;**104**:399–411.
138. Hausenloy DJ, Bøtker HE, Condorelli G, Ferdinandy P, Garcia-Dorado D, Heusch G, Lecour S, Laake LW van, Madonna R, Ruiz-Meana M, Schulz R, Sluijter JPG, Yellon DM, Ovize M. Translating cardioprotection for patient benefit: position paper from the Working Group of Cellular Biology of the Heart of the European Society of Cardiology. *Cardiovasc Res* 2013;**98**:7–27.
139. Hausenloy DJ, Botker HE, Engstrom T, Erlinge D, Heusch G, Ibanez B, Kloner RA, Ovize M, Yellon DM, Garcia-Dorado D. Targeting reperfusion injury in patients with ST-segment elevation myocardial infarction: trials and tribulations. *Eur Heart J* 2017;**38**:935–941.
140. Vilahur G, Juan-Babot O, Peña E, Oñate B, Casaní L, Badimon L. Molecular and cellular mechanisms involved in cardiac remodeling after acute myocardial infarction. *J Mol Cell Cardiol* 2011; **50**:522-533.
141. Davidson SM, Ferdinandy P, Andreadou DJ I, Bøtker HE, Heusch G, Ibáñez B, Ovize M, Schulz R, Yellon DM, Hausenloy MD, Garcia-Dorado D. Multi-target strategies to reduce myocardial ischemia/reperfusion injury. *J Am Coll Cardiol*, 2018.
142. Ferdinandy P, Hausenloy DJ, Heusch G, Baxter GF, Schulz R. Interaction of risk factors, comorbidities, and comedications with ischemia/reperfusion injury and cardioprotection by preconditioning, postconditioning, and remote Conditioning. *Pharmacol Rev* 2014;**66**:1142–1174.
143. Bøtker HE, Hausenloy D, Andreadou I, Antonucci S, Boengler K, Davidson SM, Deshwal S, Devaux Y, Di Lisa F, Di Sante M, Efentakis P, Femminò S, García-Dorado D, Giricz Z, Ibanez B, Iliodromitis E, Kaludercic N, Kleinbongard P, Neuhäuser M, Ovize M, Pagliaro P, Rahbek-Schmidt M, Ruiz-Meana M, Schlüter K-D, Schulz R, Skyschally A, Wilder C, Yellon DM, Ferdinandy P, Heusch G. Practical guidelines for rigor and reproducibility in preclinical and clinical studies on cardioprotection. *Basic Res Cardiol* 2018;**113**:39.
144. Lefer DJ, Bolli R. Development of an NIH consortium for preclinicAl AssESsment of CARdioprotective therapies (CAESAR): a paradigm shift in

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studies of infarct size limitation. *J Cardiovasc Pharmacol Ther* 2011;**16**:332–339.

145. Jones SP, Tang X-L, Guo Y, Steenbergen C, Lefer DJ, Kukreja RC, Kong M, Li Q, Bhushan S, Zhu X, Du J, Nong Y, Stowers HL, Kondo K, Hunt GN, Goodchild TT, Orr A, Chang CC, Ockaili R, Salloum FN, Bolli R. The NHLBI-sponsored Consortium for preclinicAl assESsment of cARdioprotective therapies (CAESAR): a new paradigm for rigorous, accurate, and reproducible evaluation of putative infarct-sparing interventions in mice, rabbits, and pigs. *Circ Res* 2015;**116**:572–586.

146. Andreadou I, Adamovski P, Bartekova M, Bertrand L, Biedermann D, Borutaite V, Bøtker HE, Chlopicki S, Dambrova M, Davidson S, Devaux Y, Di Lisa S, Djuric D, Erlinge D, Falcao-Pires I, Galatou E, García-Dorado D, Garcia-Sosa AT, Girão H, Giricz Z, Gyongyosi M, Healy D, Heusch G, Jakovljevic V, Jovanic J, Kolar F, Kwak B, Leszek P, Liepins E, Longnus S, et al. Realizing the therapeutic potential of novel cardioprotective therapies: The EU-CARDIOPROTECTION COST Action – CA16225. *Cond Med* 2018;**1**:116–123.

147. Tanaka M, Brooks SE, Richard VJ, FitzHarris GP, Stoler RC, Jennings RB, Arfors KE, Reimer KA. Effect of anti-cd18 antibody on myocardial neutrophil accumulation and infarct size after ischemia and reperfusion in dogs. *Circulation* 1993;**87**:526-535.

148. Wu B, Meng K, Ji Q, Cheng M, Yu K, Zhao X, Tony H, Liu Y, Zhou Y, Chang C, Zhong Y, Zhu Z, Zhang W, Mao X, Zeng Q. Interleukin-37 ameliorates myocardial ischaemia/reperfusion injury in mice. *Clinical Exper Immunol* 2014;**176**:438-451.

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Table 1: Major studies investigating anti-inflammatory cardioprotective strategies targeting the immune cell response to reduce myocardial infarct size and preventing adverse left ventricular remodelling

Study	*Experimental acute IRI model*	*Anti-inflammatory therapeutic strategy*	*Cardioprotective effect*	*Proposed mechanism(s) and translational potential*
Neutrophils				
Burke SE, et al, 1992[26]	*In vivo* 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 M, et al, 1993[147]	*In vivo* 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, 1993[24]	*In vivo* 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 JV, et al, 1997[27]	*In vivo* rabbits 30 min ischaemia and 48 h reperfusion	Lidocaine First 10 min of ischaemia	Reduction of late MI size	Sodium channel blocker which inhibits of several neutrophil functions
Shen YC, et al, 1999[28]	*In vivo* 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 J, et al, 2001[21]	*In vivo* 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, myeloperoxidase activity, and expression of ICAM-1 and VCAM-1.
Kohtani T, et al, 2002[25]	*In vivo* 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 A, et al, 2012[15]	*In vivo* rats 30 min ischaemia and 3h reperfusion	IPost At reperfusion	Reduced acute MI size and less myocardial	Reduced accumulation of neutrophils in MI zone. No further reduction in MI size with IPost in neutrophil-depleted rats
Wu B, et al, 2014[148]	*In vivo* mice 30 min ischaemia and 24 and 72 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 F, et al, 2016[18]	Mice 30 min ischaemia and 24h 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, reactive oxygen species (ROS) and MMP-9 release.
Ali et al 2016[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 hour post-AMI reduced leucocyte accumulation and prevented adverse LV remodelling.

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Monocytes/macrophages				
Nakano, et al, 2016[56]	*In vivo* mice (30 min ischaemia and 12 and 24 h of reperfusion	Ibesartan At reperfusion	Reduction of early and late MI size	PPARγ-dependent anti-inflammatory mechanisms. Antagonizing monocyte-mediated inflammation
Zhou et al 2013 [52]	*In vivo* rat 45 min ischaemia and 30 days of reperfusion	Phenytoin 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 et al 2017[55]	*In vivo* murine Permanent ischaemia	Topiramate After ischaemia	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, et al, 2015[54]	*In vivo* 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, et al, 2006[64]	*In vivo* mice 45 min ischaemia and 24 h of 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.
Zouggari,et al, 2013[42]	*In vivo* 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 et al 1998[79]	*In vitro* rat 30 min ischaemia and 30 min reperfusion	Disodium cromoglycate During ischaemia and reperfusion	Reduced acute MI size	DSCG stabilised mast cells and reduced MPO activation. DSCG blocked cardioprotective effects of IPC
Parikh et al 1999[87]	*In vitro* rat 30 min ischaemia and 30 min reperfusion	Norepinephrine Prior to ischaemia	Reduced early MI size	Norepinephrine stabilised mast cells and reduced MPO activation.
Jaggi et al 2007 [78]	*In vitro* rat 30 min ischaemia and 120 min reperfusion	Ketotifen and carvedilol Compound 48/80 prior to ischaemia	Reduced early 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 M, et al, 2014[95]	*In vivo* rats (60 min and 24 h of reperfusion)	To investigate the role of cardiac fibroblasts (CF)	Late MI size reduction was observed in CF secretome treated mice compared to control	CFs participate in cardioprotection during the acute phase of ischaemia-reperfusion via a paracrine pathway involving TIMP-1

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Figure 1: Immune cell changes several minutes to hours following AMI
Following AMI, neutrophils are recruited into the ischaemic heart. Their rapid degradation and degranulation result in an acute pro-inflammatory response and trigger 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. Migration inhibitory factor (MIF) is released by cardiomyocytes in response to reactive oxygen species (ROS) and hypoxia. MIF exerts compartmentalised and opposing effects after myocardial IRI mediated by CXCR2. At early reperfusion, fibroblast-derived granulocyte-macrophage colony stimulating factor (GM-CSF) release plays an important role in chemotactic attraction of neutrophils and monocytes into the infarcted myocardium. Mast cell activation and/or degranulation leads to the release of pro-inflammatory mediators while mast cell–derived renin induces reperfusion arrhythmias through the activation of RAS. IPC activates Gi-coupled receptors and stabilizes mast cells. The activation of Na^+/H^+ exchange attenuates MPO release and prevents post-IRI arrhythmias. Anti-RAS cardioprotection is also induced by the activation of PKC-ε and mitochondrial ALDH2.

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-1β, angiotensin II and the binding of the chemokine ligand 2 (CCL2) to the chemokine receptor 2 (CCR2). Monocyte chemoattractant protein-1 (MCP-1) and CCL7 also mediate CCR2-dependent monocyte migration. Moreover, β2-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-1β and TNF-α. Gradually, the $Ly\text{-}6C^{low}$ 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 $Ly\text{-}6C^{low}$ monocyte production. Interleukin-13 (IL-13), the CXCL12/CXCR4 axis, GABAA receptor activity and VGSCs represent targets for the monocyte/macrophage phenotypic switch from pro-inflammatory to anti-inflammatory. The PPARγ 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.

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Figure 3: Immune cell changes in the healing period following AMI
Regulatory T-cells (T_{reg}) undergo dendritic cell (DC) regulation and downregulate the innate immune response. The activation of T_{reg} through CD28 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 (angiotensin 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 programs 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.

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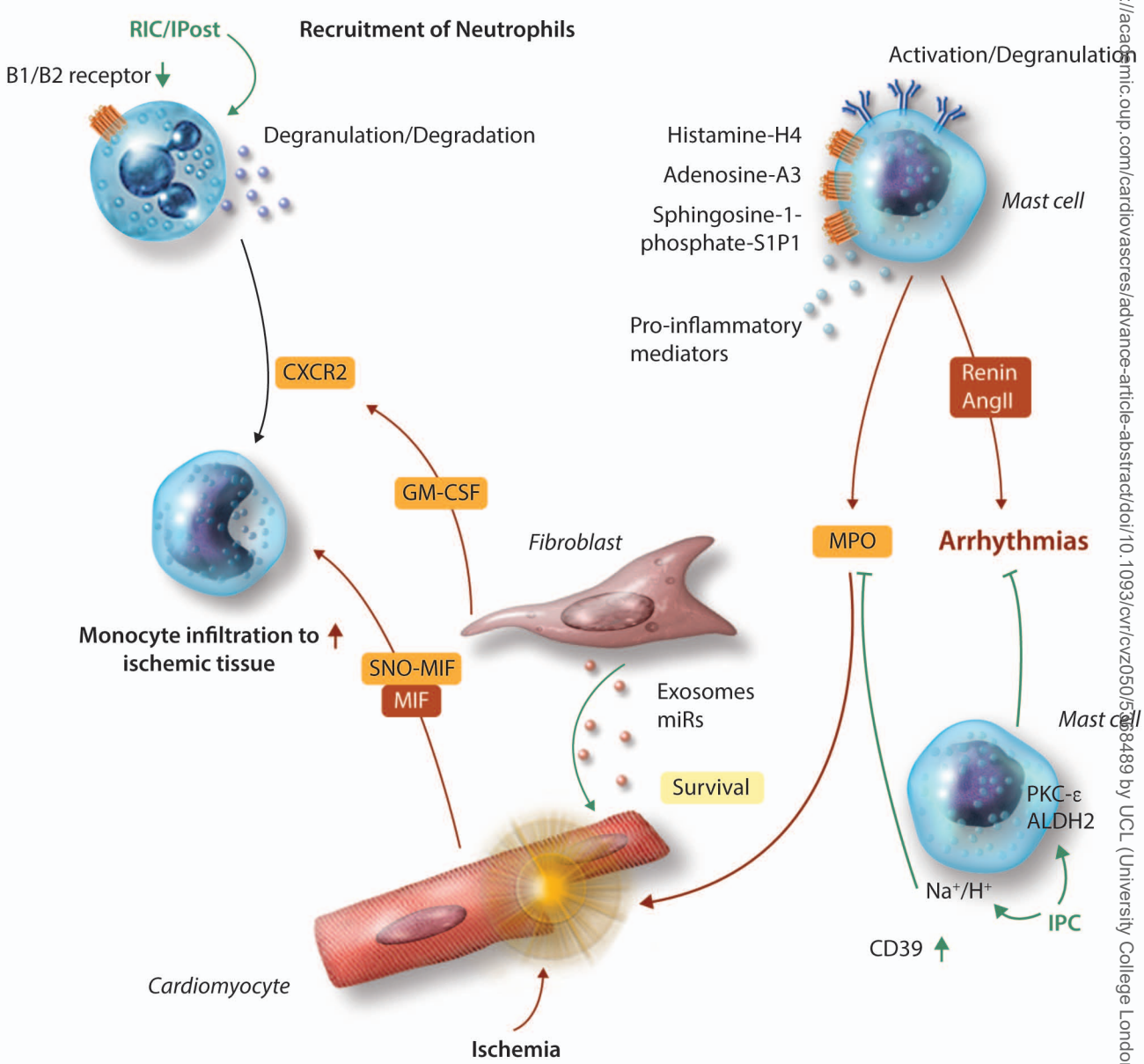

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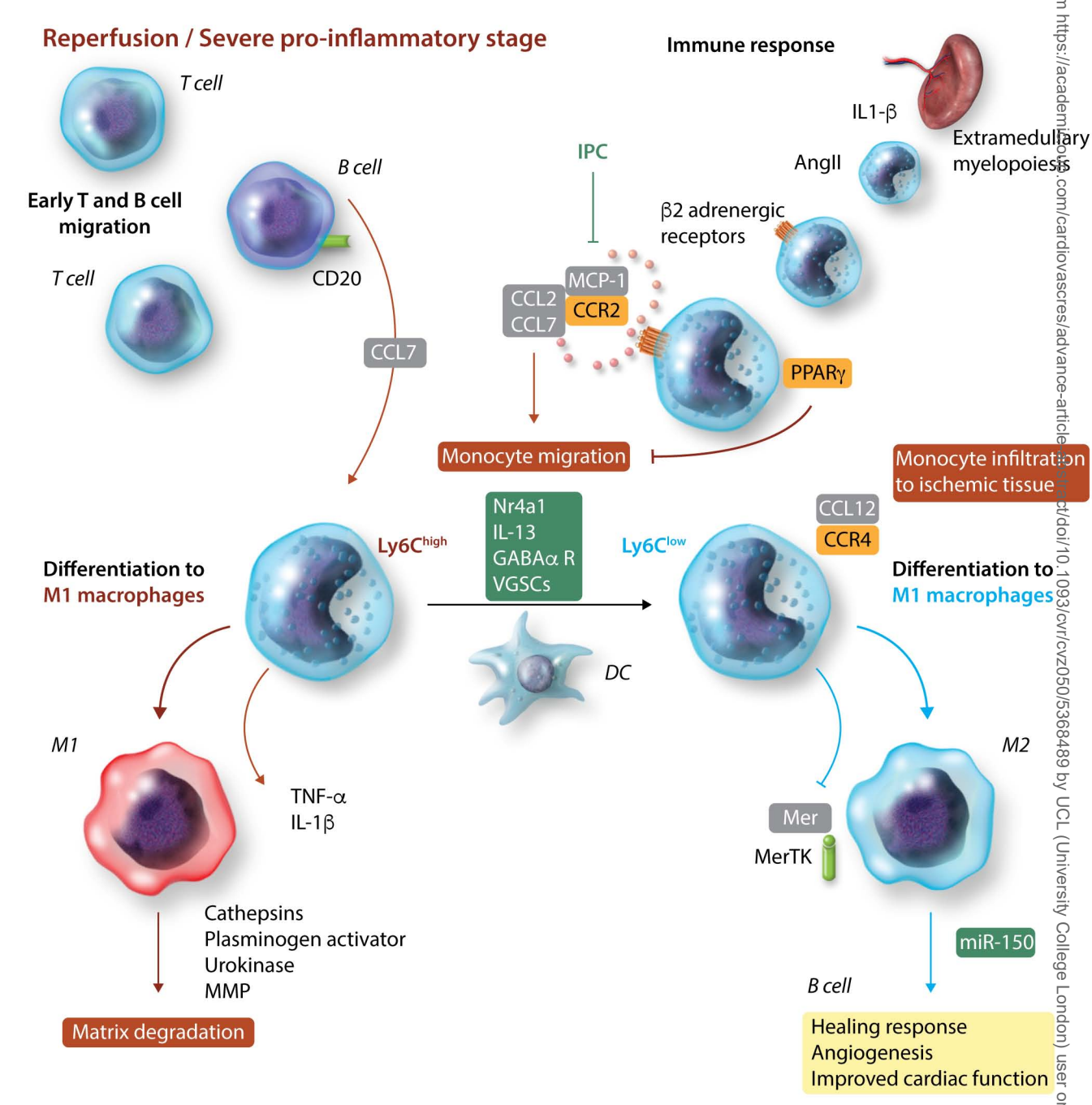

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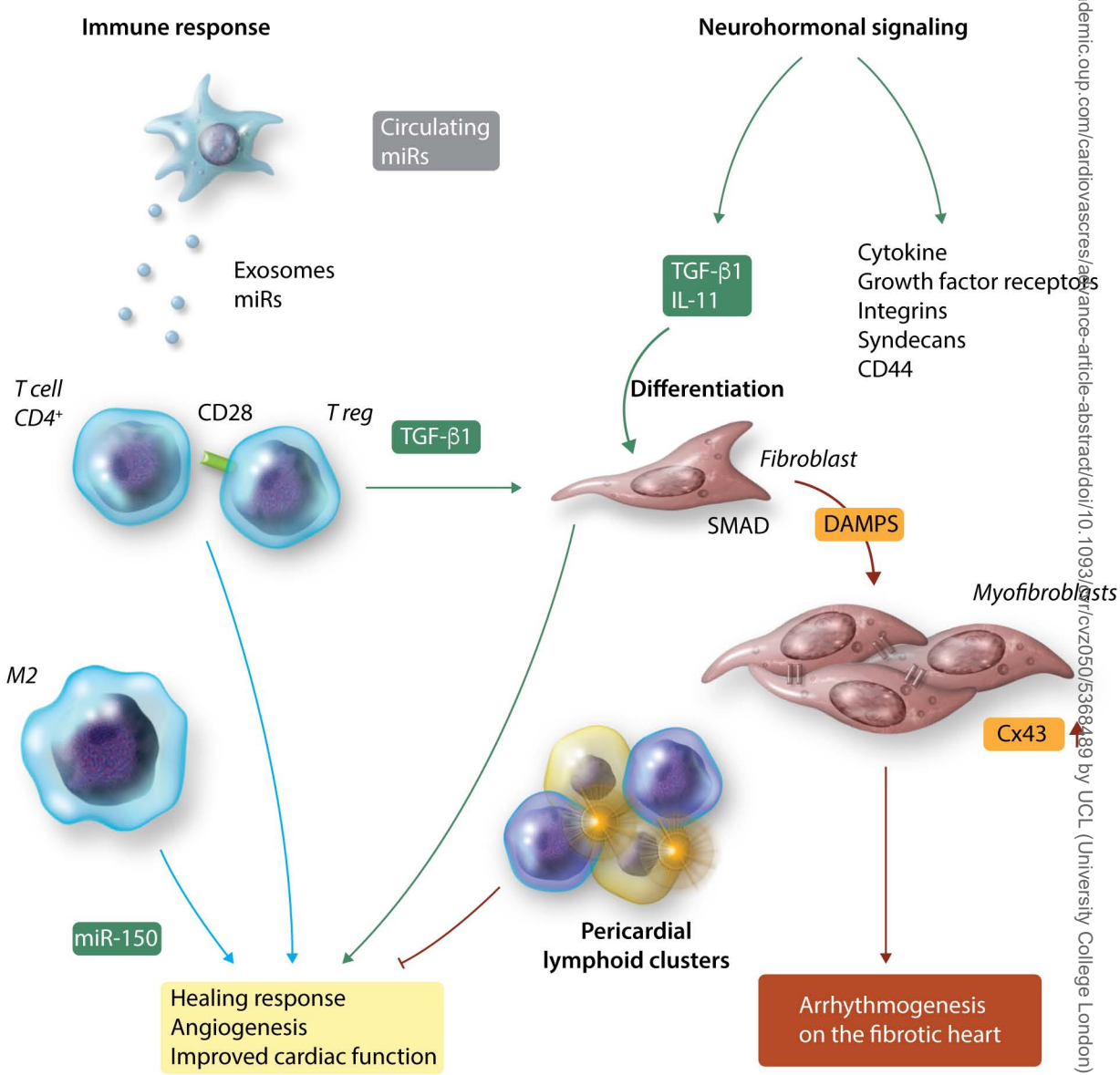

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