Myocyte signalling in leucocyte recruitment to the heart

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Myocardial damage, by different noxious causes, triggers an inflammatory reaction driving post-injury repair mechanisms and chronic remodelling processes that are largely detrimental to cardiac function. Cardiomyocytes have recently emerged as key players in orchestrating this inflammatory response. Injured cardiomyocytes release damage-associated molecular pattern molecules, such as high-mobility group box 1 (HMGB1), DNA fragments, heat shock proteins, and matricellular proteins, which instruct surrounding healthy cardiomyocytes to produce inflammatory mediators. These mediators, mainly interleukin (IL)-1β, IL-6, macrophage chemoattractant protein (MCP)-1, and tumour necrosis factor α (TNF-α), in turn activate versatile signalling networks within surviving cardiomyocytes and trigger leucocyte activation and recruitment. In this review, we will focus on recently characterized signalling pathways activated in cardiomyocytes that mediate inflammatory responses during myocardial infarction, hypertensive heart disease, and myocarditis.

Keywords  Cardiac inflammation  •  Leucocyte recruitment  •  Signalling pathways  •  Heart disease  •  Cytokines

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1. Introduction

The myocardium responds to aetiology-different pathological injuries through a common multistep process involving highly co-ordinated interactions between cardiac and immune cells. Three temporally distinct events characterize the process of myocardial injury and subsequent inflammation: (i) production of inflammatory mediators (mainly cytokines and chemokines) by stressed/damaged myocardial cells; (ii) transmigration of inflammatory cells to the damaged tissue leading to secondary cytokine amplification, wound-healing processes, and tissue remodelling, and (iii) release of anti-inflammatory signals to restrain leucocyte invasion and terminate inflammation and wound healing.

Among cardiac cells, fibroblasts are the principal source of inflammatory signals in ischaemic hearts, while endothelial cells primarily release inflammatory molecules in response to pressure overload. In diverse pathological conditions, cardiomyocytes further contribute to the establishment of a pro-inflammatory environment in the myocardium by producing different cytokines and chemokines. Stress exerted by different noxae (e.g. hypoxia, pathological mechanical stretch, and infection) stimulates cardiomyocytes to mobilize inflammatory mediators and signalling pathways that are silent or minimally active in the healthy adult myocardium. This can occur via direct engagement by mechanical stimuli of mechanosensors, such as integrins, cytoskeleton, and sarcosomal proteins. Alternatively, stressed/damaged cardiomyocytes can mobilize intra- or extracellular ‘danger signals’, also known as damage-associated molecular pattern (DAMP) molecules, that are recognized by pattern recognition receptors, such as Toll-like receptors (TLRs), localized either within the signalling cell or in neighbouring cardiomyocytes. In turn, receptor activation (either by myocardial stress or DAMPs) triggers intracellular crosstalk signal transduction routes, such as mitogen-activated protein kinase (MAPK), Janus kinase (JAK)—signal transducer and activator of transcription (STAT), and calcineurin-dependent pathways. These cascades converge downstream to activate nuclear transcription factors [mainly nuclear factor kappa B (NF-κB) and activator protein 1 (AP-1)], which are required for the induction of most cytokine and chemokine genes, including the pro-inflammatory cytokines tumour necrosis factor α (TNF-α), IL-1β, and IL-6, and chemokines like macrophage chemoattractant protein (MCP)-1/CCL2.

Several cardiomyocyte-derived molecules function as ‘leucocyte mobilization factors’ that promote immune cell extravasation and subsequent recruitment to sites of myocardial damage. These effects mostly stem from the ability of cytokines and chemokines to up-regulate adhesion molecules on endothelial cells, such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule (VCAM), and to promote leucocyte directional migration via direct binding to leucocyte cell surface receptors, such as the C-X-C motif chemokine receptor type 4 (CXCR4). After recruitment, immune cells (especially phagocytes) contribute to the removal of necrotic, apoptotic, and infected cardiomyocytes, and clear cellular debris. Of note, infiltrating...
Table 1  Signalling pathways underlying cardiomyocyte–leucocyte communication in ischaemic heart disease

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immune cells also constitute an additional source of cytokine production, which amplify the initial local inflammatory reaction and contribute to the establishment of a more sustained inflammatory response. In chronic inflammation, cytokines critically affect the entity and pace of cardiomyocyte apoptosis and hypertrophy, extracellular matrix (ECM) deposition, as well as cardiomyocyte and vascular regeneration. These events constitute a complex pathophysiological response called pathological myocardial remodelling.

Recruitment of inflammatory cells must be tightly controlled to guarantee tissue healing while avoiding both quantitatively and temporally excessive post-injury inflammatory responses that lead to maladaptive remodelling and contractile dysfunction. Cardiac cells limit immune cell infiltration and terminate chronic inflammation by secreting a broad range of anti-inflammatory signals, such as the macrophage-inhibiting cytokine 1 (growth differentiation factor 15, GDF-15).

A large cohort of signalling pathways is sequentially activated in cardiomyocytes and in leucocytes to ensure proper spatio-temporal orchestration of post-injury myocardial remodelling. In this review, we will focus on signal transduction routes that are specifically activated in cardiomyocytes to attract immune cells to the diseased myocardium. In particular, we will discuss the more recently characterized signalling pathways acting in the context of key pathological cardiac diseases, such as myocardial infarction (MI; Table 1 and Figure 1), hypertensive heart disease (Table 2 and Figure 2), and myocarditis (Table 3 and Figure 3).

1.1 Cardiomyocyte signals recruiting leucocytes to the infarcted myocardium

MI is a condition of irreversible myocardial necrosis that results from a prolonged ischaemic insult. Owing to the limited regenerative potential of the myocardium, cardiomyocyte death elicits a reparative response that ultimately results in the formation of a scar, leading to focal contractile impairment and ventricular remodelling. Damaged cardiomyocytes in the infarcted area initiate the reparative response by releasing specific DAMPs, which are recognized by TLRs expressed on leucocytes, parenchymal cells, and also on healthy neighbouring cardiomyocytes. The high-mobility group box 1 (HMGB1) is a prototypical ‘danger signal’ released under hypoxic stress conditions. HMGB1 is a ubiquitously expressed and highly conserved nuclear protein mobilized by necrotic and severely stressed cells in different damaged tissues. Indeed, cardiomyocytes have been shown to passively release HMGB1 both in vitro in response to peroxynitrite-induced oxidative stress and necrosis, and in vivo after myocardial ischaemia–reperfusion injury.

Once secreted, DAMPs such as HMGB1 exert their chemoattractive effects by diffusing to the peri-infarct zone, where they engage TLRs on healthy cardiomyocytes. While their highest expression levels are found in cells of the myeloid lineage, TLRs are also expressed in tissues devoid of a specialised immune function, including the myocardium. Among cardiac TLRs, TLR-4 is the major sensor of ischaemia-induced DAMPs. Engagement of cardiomyocyte TLR-4 by these danger signals, in turn, promotes the up-regulation of CXC-type chemokines and of adhesion molecules such as ICAM-1 and VCAM, thereby driving monocyte homing to the infarct. Upon recruitment, leucocytes actively produce a whole battery of cytokines including TNF-α, IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-17, interferon γ (IFN-γ), and granulocyte monocyte colony-stimulating factor (GMCSF), affecting ECM protein function and the ensuing remodelling process.

Evidence has shown that TLR-4 activation in ischaemic hearts is maladaptive, since engagement of TLR-4 is associated with the release of massive amounts of inflammatory mediators and attracts a substantial number of leucocytes. Accordingly, cytokine production and phagocyte homing to the infarct and peri-infarct zones have been dampened in whole-body TLR-4 knock-out mice, leading to a significantly smaller infarct size than in wild-type controls. Similarly, TLR-4 inhibition, via intravenous administration of the TLR-4 antagonist eritoran, reduces infarct size in a myocardial ischaemia–reperfusion injury model. However, the results of these studies are confounded by the use of whole-body knock-out models, where cardiomyocyte- and leucocyte-dependent TLR-4 signalling events can hardly be distinguished. Only the availability of tissue-specific knock-out systems may help to elucidate the specific contribution of TLR-4 activation within cardiomyocytes.

Besides engagement of canonical TLR pathways, DAMPs such as HMGB1 may promote immune cell recruitment to sites of injury via alternative mechanisms that do not involve receptor activation. For instance, HMGB1 released by injured muscles can bind directly to the endothelial chemokine C-X-C motif chemokine 12 (CXCL12), which ensures trafficking of leucocytes that express the corresponding receptor, CXCR4. Within HMGB1–CXCL12 heterocomplexes, HMGB1 induces key conformational changes in residues 3–12 of the receptor, CXCR4. Within HMGB1–CXCL12 heterocomplexes, HMGB1 induces key conformational changes in residues 3–12 of the receptor, CXCR4, and in turn favours...
Figure 1 Cardiomyocyte signalling pathways controlling cardiomyocyte–leucocyte communication in MI. Necrotic heart muscle cells attract leucocytes to the myocardium by releasing danger signals (HMGB1) and cytokines (IL-6) that diffuse to the peri-infarct zone and trigger Toll-like (TLRs) as well as cytokine (gp130) receptors expressed by neighbouring healthy cardiomyocytes. Engagement of the corresponding intracellular signalling cascades ultimately results in the up-regulation of cytokines and complement elements that, by binding to cognate leucocyte receptors, either activate or inhibit immune cell migration. Most cardiomyocyte-derived cytokines, such as IL-6, can initiate positive feedback circuits thereby promoting cytokine amplification and the establishment of a chronic inflammatory state.

Table 2 Signalling pathways underlying cardiomyocyte–leucocyte communication in hypertensive heart disease

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<tr>
<td>mito-DNA/TLR-9/MyD88/ NF-κB</td>
<td>IL-1β, IL-6</td>
<td>Mø recruitment</td>
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<td>TLR-4/mTOR/NF-κB</td>
<td>TNF-α, IL-1β, IL-6</td>
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<td>AngII/HSP70/TLR-4</td>
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<td>β-AR + IL-1βR/cAMP/PKA</td>
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<td>TLR-4/p38MAPK/ERK/NF-κB</td>
<td>TNF-α</td>
<td>Mast cell recruitment</td>
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<td>TNF-α/Nox2-4</td>
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<td>Mø activation</td>
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<td>RANKL/RANK/TRA2-6/PLC/PKC/NF-κB</td>
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<td>IL-18/MyD88/IRAK4/TRA6/JNK/Sp-1</td>
<td>EMMPRIN</td>
<td>MMP2 secretion by Mø</td>
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early recruitment of monocytes after injury in in vivo models of air pouches and injured muscles. Although conclusive experimental evidence is still pending, it is plausible that cardiomyocyte HMGB1 promotes immune cell infiltration into the infarcted myocardium through similar mechanisms. A further layer of complexity in HMGB1-mediated signalling is due to the high versatility of this molecule. Although it has well documented chemoattractant activities, HMGB1 appears to either favour or inhibit immune cell recruitment depending on the
microenvironment, the type of cells/organs, and the duration/degree of extracellular HMGB1 up-regulation. Accordingly, administration of exogenous HMGB1 to mice with coronary artery ligation inhibits instead of triggering dendritic cell recruitment to the peri-infarct area, eventually improving contractile function. However, the mechanism whereby HMGB1 affects dendritic cell recruitment is still controversial and requires further investigation. Altogether, these findings demonstrate that necrotic cardiac cell release of HMGB1 stimulates immune cell recruitment to the infarcted myocardium both directly, via interaction with CXCL12/CXCR4, and indirectly, by triggering TLR signalling in viable neighbouring cardiomyocytes.

Signalling transduction events downstream of DAMPs-activated TLRs rely on the activity of myeloid differentiation primary response gene 88 (MyD88), an adaptor protein without known enzymatic activity, but that is critical in the activation of NF-κB transcription factors. In cardiomyocytes, TLR-4-mediated engagement of MyD88 controls reactive oxygen species production, likely via NADPH oxidase (Nox), and promotes oxidation-dependent activation of calmodulin-dependent protein kinase

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**Table 3** Signalling pathways underlying cardiomyocyte–leucocyte communication in viral myocarditis

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<td>CAR/adherens junctions</td>
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<td>MΦ, T-cell, and NK-cell recruitment</td>
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**Figure 2** Cardiomyocyte signalling pathways controlling cardiomyocyte–leucocyte communication in hypertensive heart disease. Cardiomyocyte injury by pressure overload promotes the release of DAMPs, both intracellularly (mitochondrial DNA) and extracellularly (HSP70). These DAMPs are sensed by membrane (TLR-4) and endosomal TLRs (TLR-9), both converging at NF-κB-mediated induction of cytokine genes. Alternatively, heart cells secrete matricellular proteins (EMMPRIN) and cytokines (RANKL, IL-1) that are recognized by integrins and cytokine receptors on surrounding cardiomyocytes, wherein specific intracellular cascades are initiated. Many molecular crosstalks, such as that between IL-1β and β-AR signalling, further contribute to transcriptional up-regulation of leucocyte recruiting factors.
II δ (CaMKII δ). In turn, oxidation-activated CaMKII δ up-regulates a variety of NF-κB target genes, including MCP-1, TNF-α, and the complement factor B, which cooperate to stimulate leucocyte recruitment to the site of injury. 13,14 Accordingly, evidence has shown that whole-body MyD88 knock-out mice have attenuated neutrophil infiltration in the infarct area and improved survival, stemming from a defective production of pro-inflammatory mediators by MyD88-deficient cardiomyocytes upon TLR-4 activation. 13 Similarly, whole-body genetic deletion of CaMKII δ dramatically reduces monocyte infiltration and protects the heart against ischaemia/reperfusion damage. Notably, cardiomyocyte-restricted inactivation of CaMKII δ phenocopies global CaMKII δ disruption, 14 indicating that cardiomyocyte TLR/MyD88/CaMKII/NF-κB signalling is a key route for the recruitment of leucocytes to the infarcted heart.

Besides the release of DAMPs, cardiomyocytes can actively recruit immune cells to the infarct zone via secretion of the cytokine IL-6. Hypoxic stress and post-ischaemic lymph directly trigger IL-6 production by cardiomyocytes in vitro and in the viable border zone of a myocardial infarct. 47,48 IL-6 binds to the common receptor of IL-6 cytokines on neighbouring cardiomyocytes, the glycoprotein 130 (gp130), which is coupled to the JAK/STAT3 transduction machinery. In ischaemic hearts, where high levels of IL-6 are actively produced by stressed cardiomyocytes, the gp130/STAT3 signalling cascade is engaged and promotes a massive release of cytokines, chemokines, and factors associated with the complement system, ultimately instructing leucocytes to reach the injured area. In keeping with these findings, mice carrying a point mutation in cardiomyocyte gp130 (Y757F) exhibited the unrestrained gp130 signalling occurring in MI with enhanced macrophage homing to non-ischaemic and border infarct zones. This results in increased mortality, associated with enhanced left ventricular rupture rate. Intriguingly, monoallelical STAT3 deletion in Y757F cardiomyocytes, but not in non-myocytes, has been shown to prevent complement activation and sustained inflammation, as well as lower left ventricular rupture rate, heart failure, and mortality after subacute MI. 49 Therefore, cardiomyocyte IL-6/gp130/JAK/STAT3 signalling constitutes a key pathway whereby heart muscle cells communicate with and attract leucocytes to the ischaemic heart. Interestingly, IL-6 itself is among the variety of inflammatory molecules produced by cardiomyocytes upon IL-6-mediated engagement of gp130/STAT3 signalling. 50 This results in positive feedback activation of the IL-6/gp130/JAK/STAT3 cascade, representing a key paracrine circuit that promotes cytokine amplification and the establishment of a chronic inflammatory state within the infarcted myocardium.

Chemokines, together with cytokines, are additional key communication factors between injured cardiomyocytes and immune cells. Major chemokines released by heart muscle cells in response to ischaemic insults include CXCL16, the macrophage-inhibitory factor (MIF) and CXCL12, also known as stromal cell-derived factor-1 (SDF-1). CXCL16 is a classical chemoattractant for various leucocyte subsets and is dramatically up-regulated early after ischaemia, both in cardiomyocytes and in non-cardiomyocytes in infarcted and non-infarcted areas. CXCL16 has been shown to be significantly induced in isolated
cardiomyocytes by different heart failure-related stimuli, including TNF-α, IFN-γ, TLR-2, and TLR-4 agonists, with a prominent effect of IL-1β.51 Similarly, MIF is weakly expressed in normal heart cells, but specifically up-regulated and released by surviving cardiomyocytes in infarcted vs. non-infarcted regions, and promotes early macrophage infiltration after damage. In isolated cardiomyocytes, MIF is produced and actively secreted via Protein kinase C (PKC)-dependent pathways in response to oxidative stress, but not to other stimuli relevant to heart failure, such as angiotensin II (Ang II), endothelin-1, IL-1β, and TNF-α.17,18 Cardiomyocyte-derived MIF, in turn, activates either cardiomyocyte or leucocyte CXCR2 and, depending on the target cell compartment, exerts either beneficial or detrimental functions. While engagement of myocyte CXCR2 is cardioprotective and limits infarct size, MIF-mediated recruitment of CXCR2-positive monocytes to the myocardium is maladaptive and eventually hampers infarct healing. Notably, MIF-dependent mobilization is a multistep process, whereby MIF first triggers monocyte arrest through the CXCR axis and then promotes monocyte transmigration through the intermediate production of MCP-1.19

A prominent role in post-infarction immune cell recruitment is also played by the CXCL12/CXCR4 axis. CXCL12 is induced by anoxia and reoxygenation stress in cultured cardiomyocytes and in vivo in the myocardium following ischaemia/reperfusion injury, through a cGMP/endothelial nitric oxide synthase-mediated signal transduction cascade.20 CXCL12 primarily exerts its chemoattractant properties by direct engagement of leucocyte CXCR4. Early after ischaemia, neutrophil infiltration of the infarcted area is severely impaired in whole-body CXCR4 heterozygous mice (Cxcr4+/−) and correlates with reduced infarct size. Notably, macrophages (MΦ) that infiltrate Cxcr4+/− hearts belong to immune-modulating rather than pro-inflammatory subsets, and are likely the cause of more efficient repair processes. Intriguingly, Cxcr4+/− mice transplanted with wild-type bone marrow emulate the inflammatory pattern of Cxcr4+/− mice, thereby suggesting that the CXCR4 receptor may exert an additional role other than leucocyte recruitment.21

Timely repression of leucocyte infiltration to the infarct area is necessary to ensure infarct healing and avoid cardiac rupture. Indeed, cardiomyocytes also produce relevant signals that limit immune cell invasion of the diseased myocardium. Among these negative regulators of monocyte infiltration into the infarct, the CXCL12/CXCR4 axis is physiologically eliminated via autophagic mechanisms known as mitophagy, during pressure overload injured mitochondria accumulate, escape usual mitophagy, and release their DNA content.22 This in turn is recognized intracellularly by a specialized TLR isoform, TLR-9,54 that is primarily located on endosomes. Upon recognition of exogenous DNA, TLR-9 signals via the common TLR adaptor MyD88, leading to the production of leucocyte recruiting factors, such as IL-6.22 Similar to DAMP-mediated engagement of TLR-4 in ischaemic hearts, pressure-overload activation of TLR-9 by mitochondrial DNA is harmful. Whole-body genetic inactivation of TLR-9 fully prevents pressure overload-induced macrophage infiltration and cardiac decompensation after aortic constriction. Similarly, evidence has shown that administration of TLR-9 inhibitory oligodeoxynucleotides limits pressure overload-mediated inflammatory responses and improves survival of wild-type mice challenged by a high severity pressure overload.22 Intriguingly, TLR-9 receptor priming with synthetic oligonucleotides also blocks TLR-9 signalling, limiting macrophage recruitment and activation in overloaded hearts to delay cardiac failure.55 However, conditional deletion studies are required to corroborate the involvement of cardiomyocyte rather than leucocyte TLR-9 in these mechanisms.

In addition to intracellular DAMPs, stressed cardiomyocytes can release heat shock proteins in the extracellular environment to serve as ligands for TLRs expressed on the membrane of surrounding myocytes.24,56 The heat shock protein HSP70 is abundantly secreted in the extracellular space by stressed cardiomyocytes, likely in response to pressure overload-induced increase of Ang II, a vasoactive peptide actively produced in hypertensive patients. Extracellular HSP70 accumulates on the membrane of cardiomyocytes and initiates pro-inflammatory pathways by binding cardiomyocyte TLR-4. Blockade
of HSP70 release or binding to its receptor prevents MCP1-mediated recruitment of Mφ and consequent fibrosis, thus limiting pressure overload-induced myocardial remodelling.24

A key intracellular effector, downstream of TLR-4 activation in pressure-overloaded hearts, is mTOR. mTOR functions as a negative regulator of TLR-4 signalling when overexpressed, as it inhibits TLR-4-dependent secretion of TNF-α, IL-1-β, and IL-6 in cultured cardiomyocytes.23 Similarly, cardiomyocyte-specific mTOR overexpression in mice limits myocyte-dependent production of IL-1β and IL-6 after banding, thus reducing cardiac recruitment of immune cells and ameliorating maladaptive remodelling. These findings suggest that cardiomyocyte-restricted up-regulation of mTOR in human patients with heart failure is a physiological response to limit TLR-4-dependent inflammation.23 Conversely, TLR-4 signalling is positively controlled by the γ isomorph of phosphoinositide 3 kinase (PI3K). Genetic or pharmacological inactivation of this enzyme has been shown to impair TLR-4-mediated secretion of the pro-inflammatory molecule HMGB1 and to be beneficial in lipopolysaccharide (LPS)-induced heart failure.25

A large body of evidence points to a major role of PI3Kγ downstream of another cardiomyocyte surface receptor subtype, the β-adrenergic receptor (β-AR).57,58 In pressure-overloaded hearts, constitutive engagement of β-ARs by high levels of circulating catecholamines eventually results in β-AR desensitization, a key determinant of contractile dysfunction in failing hearts.59 In addition, β-AR signalling may contribute to maladaptive cardiac remodelling by promoting cardiomyocyte-mediated release of pro-inflammatory cytokines and the ensuing immune cell recruitment and activation. This stems from a crosstalk between β-AR and IL-1β cascades via cyclic AMP (cAMP)/protein kinase A (PKA) signalling hubs, which ultimately activate IL-6 secretion in cultured cardiomyocytes.26 Given the role of PI3Kγ downstream of β-ARs and TLR-4, it is likely that PI3Kγ activity influences cytokine release by pressure-overloaded cardiomyocytes. Accordingly, whole-body inactivation of PI3Kγ [PI3Kγ kinase-dead (PI3Kγ KD)] leads to reduced infiltration of leucocytes in the myocardium after aortic banding.60 However, bone marrow transplantation experiments demonstrate that the defective recruitment of PI3Kγ KD leucocytes is due to an impaired immune cell response to chemotactic stimuli, and not to defective secretion of inflammatory mediators by cardiac myocytes. In keeping with this finding, inflammatory chemokines and cytokines, to an impaired immune cell response to chemotactic stimuli, and not demonstrate that the defective recruitment of PI3Kγ KD leucocytes is due to an impaired immune cell response to chemotactic stimuli, and not to defective secretion of inflammatory mediators by cardiac myocytes. In keeping with this finding, inflammatory chemokines and cytokines, to an impaired immune cell response to chemotactic stimuli, and not demonstrate that the defective recruitment of PI3Kγ KD leucocytes is due to an impaired immune cell response to chemotactic stimuli, and not to defective secretion of inflammatory mediators by cardiac myocytes. In keeping with this finding, inflammatory chemokines and cytokines, to an impaired immune cell response to chemotactic stimuli, and not demonstrate that the defective recruitment of PI3Kγ KD leucocytes is due to an impaired immune cell response to chemotactic stimuli, and not demonstrate that the defective recruitment of PI3Kγ KD leucocytes is due to an impaired immune cell response to chemotactic stimuli, and not demonstrate that the defective recruitment of PI3Kγ KD leucocytes is due to an impaired immune cell response to chemotactic stimuli, and not demonstrative of treating protocols are used.

A small study (ATTACH) even reported a significant increase in death and hospitalization in patients receiving a chimeric anti-TNF-α antibody (infliximab).71 Possible explanations of these results are that selective disruption of TNF-α signalling is insufficient to significantly modify the complex inflammatory milieu occurring in chronic heart failure, or that TNF-α blockade may even hamper the establishment of protective signals.72 Nonetheless, it is still possible that TNF-α inhibition may provide beneficial effects in other patient groups or if different treatment protocols are used.

Similar to TNF-α, another member of the TNF superfamily, the receptor activator of NF-κB ligand (RANKL), is released by cardiomyocytes in response to Ang II and is rapidly induced after aortic banding.34 Upon release, RANKL is recognized by the cardiomyocyte surface receptor, receptor activator of NF-κB (RANK), which is coupled to a signalling cascade involving TNF receptor associated factor 2 (TRAF2)/TRAF6, PLC, and PKC. This eventually leads to NF-κB nuclear translocation and the ensuing transcriptional up-regulation of TNFα, IL-1α, and IL-1β.73 Besides release of DAMPs and TNF cytokines, pressure-overloaded cardiomyocytes can signal to leucocytes by up-regulating the extracellular matrix metalloproteinase inducer (EMMPRIN). High levels of catecholamines and reactive oxygen species detected in pressure-overloaded hearts are major stimuli for EMMPRIN induction. Accordingly, in isolated cardiomyocytes, β-AR stimulation and oxidative stress increase EMMPRIN expression via ROS-dependent activation of the JNK

MAPK phosphatase 1,66 and glycogen synthase kinase (GSK)3.67 A major mediator of LPS-induced heart failure in vivo is TLR-4 that and, as a surface receptor, is also engaged by pressure overload-related DAMPs.68,69 Therefore, it is plausible that the same signalling routes govern TNF-α up-regulation in contexts of hypertensive heart disease. Cardiomyocyte-derived TNF-α can, in turn, be sensed by the same myocyte, thereby initiating autocrine circuits that ensure sustained secretion of inflammatory mediators. In vitro stimulation of cardiomyocytes with TNF-α consistently initiates an intracellular cascade involving NADPH oxidases, Nox2 and Nox4, and culminates in the production of pro-inflammatory cytokines, such as IL-1β and IL-6.33 These molecules instruct leucocytes to reach the myocardium and to produce further inflammatory mediators, establishing a chronic inflammatory response. In keeping with these findings, TNF-α whole-body knock-out mice have attenuated inflammation after aortic banding that results in reduced reparative fibrosis and improved cardiac function.30 Notably, TNF-α can control ECM remodelling both indirectly, by attracting leucocytes to the injured heart, and directly, by promoting myocyte apoptosis and activation of matrix metalloproteinases (MMPs).34 Experiments with conditional knock-out models conclusively demonstrate a central role of infiltrating immune cells in TNF-α-mediated matrix secretion. Hence, cardiomyocyte-restricted overexpression is sufficient to increase the number of mast cells in the heart and, consequently, to promote TGF-β-dependent matrix remodelling and heart failure.32 Therefore, cardiomyocyte TNF-α critically controls the maladaptive transition to heart failure by exacerbating immune responses and the ensuing ECM reorganization.

Circulating levels of TNF-α are elevated in human patients with heart failure.69 Nonetheless, while the detrimental effect of pathological TNF-α signalling in the myocardium has been extensively documented in vitro and in animal models, the RECOVER, RENAISSANCE, and RENEWAL randomized placebo-controlled trials failed to demonstrate any beneficial effect of TNF-α inhibition with etanercept in patients with chronic heart failure.70 Another smaller study (ATTACH) even reported a significant increase in death and hospitalization in patients receiving a chimeric anti-TNF-α antibody (infliximab).71 Possible explanations of these results are that selective disruption of TNF-α signalling is insufficient to significantly modify the complex inflammatory milieu occurring in chronic heart failure, or that TNF-α blockade may even hamper the establishment of protective signals.72 Nonetheless, it is still possible that TNF-α inhibition may provide beneficial effects in other patient groups or if different treatment protocols are used.
pathway. Mechanically, extracellular EMMPRIN serves as a ligand for integrins expressed on the membrane of surrounding cardiomyocytes. In turn, activated integrins trigger Ras-related C3 botulinum toxin substrate 1 (Rac1)-dependent PI3K/Akt/IKK/NF-κB and MAPK kinase (MKK)/JNK/AP-1 signalling to promote NF-κB and AP-1 transcription factors and the subsequent induction of pro-inflammatory cytokines, mainly IL-18. Intriguingly, IL-18 can stimulate EMMPRIN transcriptional up-regulation within cardiomyocytes via MyD88/IRAK4/TRAF6/JNK-dependent Sp1 activation, thus initiating a positive feedback loop of IL-18 production. IL-18-induced EMMPRIN expression, in turn, favours MMP expression in both cardiomyocytes and monocytes, eventually driving maladaptive remodelling of the pressure-overloaded myocardium.

Termination of pressure overload-dependent inflammation is guaranteed by IL-10 that, by engaging cardiomyocyte IL-10R, signals through p38 MAPK and JAK–STAT3 pathways to antagonize NF-κB activation and TNF-α induction. Accordingly, administration of recombinant IL-10 to pressure-overloaded animals inhibits leucocyte recruitment, reduces fibrosis, and ultimately limits the transition to heart failure. Intriguingly, IL-10 treatment not only limits the progression, but also reverses aortic constriction-induced adverse cardiac remodelling once hypertrophy has been established. However, although some evidence demonstrates that septic stimuli of isolated cardiomyocytes can produce IL-10 in vitro, the most accepted view is that mononuclear cells are the main source of IL-10 in models of heart disease.

### 1.3 Cardiomyocyte signals recruiting leucocytes to the infected myocardium

Myocarditis is defined by primary activation of pathological immune processes within the myocardium. Immune cell infiltration of the myocardium can be elicited by infections (viral, bacterial, and protozoan), and also by non-infectious causes, such as hypersensitivity, autoimmunity, and cardiotoxicity. Besides initial myocyte damage, activation of the immune system is recognized as the most relevant pathological stress contributing to disease development. Cytotoxic T-lymphocytes and natural killer (NK) cells directly destroy infected cardiomyocytes, while innate immune cells, such as Nδ, Mδ, and mast cells, contribute to cardiac damage through uncontrolled ROS production. This in turn results in chamber stiffening and contractile impairment, culminating in the development of dilated cardiomyopathy (DCM).

Viruses, including entroviral coxsackievirus B3 (CVB3), adenoviruses, parvovirus B19, and human herpes virus 6, are the most common causes of myocarditis in western countries. Early after infection, cardiomyocytes expose on their plasma membrane the viral antigens in complex with Class I major histocompatibility complex (MHC) molecules. MHC-antigen complexes are then recognized by immune cell T cell receptors (TCRs) that in turn trigger a signalling cascade modulating leucocyte activation and subsequent aggression of infected cells. This process is accompanied by massive release of soluble factors by cardiomyocytes and immune cells, which further contribute to attract leucocytes to the infected myocardium.

Several pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, and IFN-γ, as well as anti-inflammatory agents, such as IL-10, are found in the myocardium early after viral infection. Detection of these molecules in the heart, before visible immune cell infiltration, supports the notion of cardiac cells as early and relevant producers of inflammatory mediators. For example, myocyte release of TNF-α is a potent initiator of systemic inflammatory responses. Cardiomyocyte-restricted overexpression of TNF-α is sufficient to trigger cardiac inflammation and a severe form of myocarditis and DCM. Notably, TNF-α is the hub of a complex cytokine network that ultimately fine-tunes the expression levels of TNF-α itself during viral myocarditis. For example, systemic IFN-γ overexpression enhances TNF-α levels in healthy hearts and induces myocarditis. IL-1β is another positive regulator of TNF-α, while IL-10 antagonizes the expression of both TNF-α and its positive modulator IFN-γ.

Another regulator of TNF-α levels in the infected myocardium is IL-6. Despite a clear pro-inflammatory function of this cytokine in viral myocarditis, in the acute phase after CVB3 infection, IL-6 operates as a negative regulator of TNF-α. In this phase, IL-6 also decreases cardiac production of the chemoattractant MCP-1 and the immunomodulatory cytokine IL-10, thus limiting immune cell recruitment and anti-inflammatory signals. The opposing effects exerted by IL-6 ultimately favour inflammation resolution, as demonstrated by increased myocarditis severity in CVB3-infected IL-6-deficient mice. It is possible that, by enhancing selected pro-inflammatory signals (hepatic antiviral response and complement activation) and limiting others (cardiac TNF-α and MCP-1 release), IL-6 ensures rapid viral clearance and better regulation of the early inflammatory responses that in turn control the severity of the subsequent chronic disease pathology. IL-6-dependent fine-tuning of immune responses to viruses is partly orchestrated by cardiomyocytes. Targeted deletion of the IL-6 transducer STAT3 in cardiomyocytes results in enhanced chronic inflammation after CVB3 infection and impaired myocarditis resolution. Therefore, despite the success of blocking the IL-6 pathway in models of MI and autoimmune myocarditis, depletion of IL-6-signalling to treat viral infections of the heart may be detrimental.

Cytokines released by infected cardiomyocytes exert their pro-inflammatory effects through mechanisms similar to those occurring in ischaemia and pressure overload, i.e. by inducing key chemotactic factors named chemokines. While CVB3 directly stimulates cardiomyocytes to secrete MCP-1, expression of the chemoattractant CXCL10/IFN-γ-induced protein 10 (IP10) cannot be induced in vitro by viral infection per se, but is triggered by IFN-γ stimulation. In CVB3-infected hearts, IFN-γ up-regulation precedes CXCL10 expression. Both factors are secreted before the peak of leucocyte infiltration, thus suggesting that resident cardiac cells are the main source of CXCL10. Cardiomyocyte-restricted overexpression of CXCL10 favours the mounting of an early immune response to CVB3 infection and limits viral replication. Interestingly, in non-infected hearts, overexpression of CXCL10 within myocytes is sufficient to recruit Mδ, T-cells, and NK cells and to promote a positive feedback loop of cardiac up-regulation of IFN-γ, but not to elicit pathological myocardial remodelling. On the contrary, cardiomyocyte-restricted overexpression of another chemokine typically up-regulated by CVB3 infection, named osteopontin (OPN), leads to chronic myocarditis culminating in DCM and premature death. Cardiomyocyte OPN primarily promotes cytotoxic T lymphocyte expansion and activation against infected cardiac cells. Accordingly, inhibition of OPN in MHC-OPN mice with DCM reverses the inflammatory process, thus suggesting a potential therapeutic benefit of targeting this chemokine in chronic myocarditis.

Another signalling pathway activated within cardiomyocytes to attract immune cells to the infected myocardium is the coxsackievirus and adenovirus receptor (CAR) pathway. CAR is a member of the immunoglobulin family and is exposed on cardiomyocyte membranes, where it mediates the entry of viral particles. In keeping with this finding,
CAR-deficient mice are resistant to both viral infection and myocarditis development. Intriguingly, cardiomyocyte-restricted CAR overexpression is able to trigger cardiac inflammation even in the absence of viral infection. In these mice, spontaneous inflammatory cardiomyopathy is characterized by cardiac up-regulation of several inflammatory mediators (MCP-1, TNF-α, IL-1β, IL-6, IL-12, and IFN-γ) and by the ensuing infiltration of M₆b, T-cells, and NK cells. CAR-induced inflammation can arise from either CAR-mediated disruption of cardiomyocyte adherence junctions or CAR-mediated activation of β-catenin hypertrophic signalling. However, the most accepted view is that the MAPK signalling cascade links CAR activation to cytokine up-regulation, since both stress-activated JNK and mitogen activated protein kinase (p38MAPK) are significantly activated in CARD-overexpressing hearts prior to onset of inflammation. Therefore, CAR-induced stress-activated MAPK signalling may contribute to the development of cardiac inflammation unrelated to viral infection. In keeping with these findings, the CAR transcript was found to be up-regulated in patients with DCM that did not show signs of viral infection. Transcriptional induction of CAR occurs in the late chronic phase of the disease and can thus be a key factor in the maintenance of the chronic inflammation underlying DCM progression.

2. Conclusion
Several studies have begun to uncover the molecular pathways underlying the highly relevant crosstalk between cardiomyocytes and leukocytes during and after myocardial injury. These pathways tend to be more disease-specific in the initial phases of myocardial damage, but they become progressively more stereotyped in the later phases of myocardial remodelling, culminating in ventricular fibrotic remodelling and failure. Dissection of these signalling pathways and their crosstalk has and will provide a large number of novel potential candidates for drug targeting and/or molecular treatments, and also promises the identification of the best timing for the administration of pathway-specific or more broad-spectrum therapies. In particular, inhibition or modulation of leukocyte recruitment to, and activation in, the injured myocardium in MI and myocarditis) via TLR4/phosphatidylinositol migration inhibitory factor mediated by CXCR2 in a mouse model of myocardial ischemia/ reperfusion.

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