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REVIEW ARTICLE

Mitochondria-derived damage-associated molecular patterns and inflammation in the ischemic-reperfused heart

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Abstract

Cardiac cell death after myocardial infarction release endogenous structures termed damage-associated molecular patterns (DAMPs) that trigger the innate immune system and initiate a sterile inflammation in the myocardium. Cardiomyocytes are energy demanding cells and 30% of their volume are mitochondria. Mitochondria are evolutionary endosymbionts originating from bacteria containing molecular patterns similar to bacteria, termed mitochondrial DAMPs (mDAMPs). Consequently, mitochondrial debris may be particularly immunogenic and damaging. However, the role of mDAMPs in myocardial infarction is not clarified. Identifying the most harmful mDAMPs and inhibiting their early inflammatory signaling may reduce infarct size and the risk of developing post-infarct heart failure. The focus of this review is the role of mDAMPs in the immediate pro-inflammatory phase after myocardial infarction before arrival of immune cells in the myocardium. We discuss different mDAMPs, their role in physiology and present knowledge regarding their role in the inflammatory response of acute myocardial infarction.

KEYWORDS

cardiac cells, heart, ischemia–reperfusion, mitochondrial DAMPs, sterile inflammation

1 | INTRODUCTION

Acute myocardial infarction is the major cause of death worldwide and the most frequent cause of heart failure due to post-infarct remodeling.[1,2] Myocardial ischemia, in particular in combination with reperfusion, triggers an inflammatory response. Cell injury and death in the myocardium release endogenous structures from dying cells that initiate local acute sterile inflammation.[3] A systemic response is also initiated, but that is beyond the scope of this review. The endogenous structures are termed damage-associated molecular patterns (DAMPs) and are defined by their ability to signal to the innate immune system about danger and tissue injury.[4] An important role of the innate immune system is clearance and healing of tissue injury. Activation of the innate immune system by extracellular DAMPs triggers a sterile inflammatory response including production of pro-inflammatory cytokines. This defines the initial *inflammation phase*, including the pro-inflammation phase, before infiltration of immune cells (Figure 1).[5]

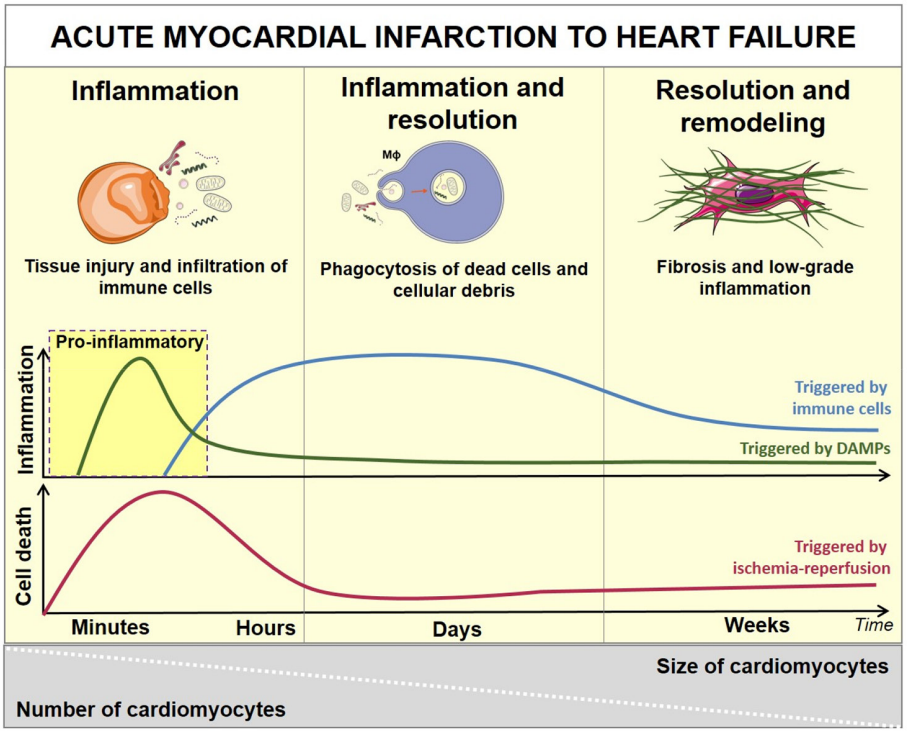

FIGURE 1 Schematic overview of the transition from myocardial infarction to heart failure. Myocardial infarction results in myocardial necrosis, release of damage-associated molecular patterns (DAMPs), and production of pro-inflammatory mediators. These mediators recruit immune cells that infiltrate the infarct and remove cellular fragments. Due to cell death triggered by ischemia–reperfusion, the heart compensate and initiate remodeling and fibrosis. Inflammation is reduced by anti-inflammatory cytokines in the resolution phase, but a low-grade chronic inflammation continues. The number of cardiomyocytes are decreasing due to myocardial necrosis, but their size increase due to increased mechanical work and compensatory hypertrophy. Some elements in the figure are modified from Servier Medical Art, http://smart.servier.com/

The innate immune system is classically activated when myeloid-originated leukocytes come across mediators of either pathologic origin, termed pathogen associated molecular patterns (PAMPs), or DAMPs released from damaged tissue. PAMPs and DAMPs bind and activate specific pattern recognition receptors (PRRs) on the immune cells.[6] However, it is widely accepted that non-immune cells, such as cardiomyocytes, cardiac fibroblasts, and cardiac endothelial cells express innate immune receptors.[7,8] PRRs are highly conserved between mammalian species and are not just important in cardiac tissue injury, but also in recognizing PAMPs in bacterial or viral triggered myocarditis.[9–11] The different immune receptors are classified into groups: formyl peptide receptors (FPRs), Toll-like receptors (TLRs), NOD-like receptors (NLRs), C-type lectin receptors (CLRs), RIG-like receptors (RLRs), and AIM-like receptors (ALRs).[12] Consequently, the released DAMPs are able to trigger and activate the cardiomyocytes and cardiac fibroblast to produce and release of pro-inflammatory cytokines. The consequence of this pro-inflammatory phase is attraction of immune cells.

Recruitment of immune cells to the infarcted area causes a strong inflammation, but it also starts the *inflammation and resolution phase* by clearing the infarct of dead cells and cellular debris (Figure 1).[13,14] The heart has few tissue-resident immune cells; however, immune cells are recruited and infiltrate the infarcted area 6–8 h after injury demonstrated in human tissue samples.[15,16] DAMPs, cytokines, and components of the complement system are responsible for immune cell extravasation, mainly neutrophils and monocytes.[17,18] Leukocytes infiltrating the infarct area initiate the resolution phase by phagocytosing necrotic cells and cellular debris. Moreover, DAMPs per se can have fatal consequences for cardiomyocytes and directly trigger cell death.[19] The healing process is essential to avoid cardiac rupture and eventually produce anti-inflammatory cytokines that slow down the inflammatory response.[20,21]

Severe cardiac tissue injury causes improperly regulated and unresolved inflammation, which contributes to excessive or prolonged low-grade chronic inflammation that contributes to structural changes of the heart, that is, the *resolution and remodeling phase* (Figure 1).[22–24] It should be emphasized that the therapeutic approaches of these three phases of the inflammatory response are very different. This review will focus on the pro-inflammatory

phase, as the size of the infarcted myocardium and loss of contractile cardiomyocytes are significant determinants that increase the mechanical work of the surviving myocardium and eventually increase the risk of developing heart failure.[25]

2 | MITOCHONDRIA-DERIVED DAMPS IN CARDIAC INFLAMMATORY SIGNALING

The heart is a highly energy demanding organ and >30% of the cardiomyocyte volume comprises of mitochondria.[26] Mitochondria are evolutionary endosymbionts originating from bacteria containing immunogenic and harmful molecular patterns, some of which are similar to bacteria.[19] Hence, necrotic cardiomyocytes release numerous mitochondrial DAMPs (mDAMPs) that are able to trigger the immune system. Controlled inflammatory responses after myocardial infarction are essential, as excessive inflammation gives collateral damage to the heart whereas inefficient early immune responses can cause fatal cardiac rupture.[27–29] It is therefore important to understand which endogenous structures that trigger inflammatory responses in the pro-inflammatory phase and how their down-stream actions are executed. The ancient endosymbiosis of the α-Proteobacteria allowed the eukaryotic cell to exploit oxygen in order to produce energy in the form of ATP.[30] Although energy production is highly beneficial for the host, certain bacterial structures are preserved in the mitochondria. An intuitive hypothesis is therefore that mitochondrial debris is particularly immunogenic. The list of identified mDAMPs include mitochondrial DNA (mtDNA), *N*-formyl peptides, cardiolipin, mitochondrial transcription factor A (TFAM), ATP, reactive oxygen species (ROS), succinate, and cytochrome c.[31–34] Intriguingly, physiological concentrations of non-specific mitochondrial debris have proven to be immunostimulatory in several studies.[32,35,36] mDAMPs are either released from damaged or stressed mitochondria into the cytosol, or from necrotic cells into the extracellular space. The different mDAMPs therefore exert different roles depending on their newly exposed milieu.

2.1 | Mitochondrial DNA

mtDNA is a circular double-stranded DNA molecule of 16.569 base pair coding for 13 proteins involved in the respiratory chain, transfer RNAs, and ribosomal RNAs.[37,38] Similar to bacterial DNA, mtDNA contains clusters of unmethylated CpG motifs, but the degree of methylation is not clear and varies among species.[39,40] mtDNA is organized in nucleoids with TFAM proteins in order to keep integrity in the mitochondrial matrix.[41] mtDNA is a highly potent trigger of the innate immune system in general (reviewed in[42,43]) but the focus of this chapter is inflammation caused by mtDNA in cardiac cells.

Cardiac cells can be exposed to mtDNA either intracellularly or extracellularly. It has been proposed that mtDNA is released intracellularly through permeabilization of both the inner and outer mitochondrial membrane in damaged or stressed mitochondria.[44] mtDNA can then become oxidized in contact with ROS and thereby trigger different inflammatory pathways, including: (1) the interferon pathway through the cytosolic DNA sensing pathway, cyclic GMP-AMP synthase (cGAS)/stimulator of interferon genes protein (STING), (2) NLRP3 inflammasome activation, and (3) TLR9 activation.[45–48] This has recently been reviewed.[49] Ischemia-injured cardiomyocytes show increased expression of retinoic acid early transcript 1 (RAE-1) in the early phase after ischemia, which is thought to be activated through the STING pathway. It is possible that mtDNA indirectly activates RAE-1 expression that is involved in cardiac fibrosis and remodeling in the remodeling phase.[50,51]

Normally, extracellular mtDNA originates from various sources, including in the formation of neutrophil extracellular traps or from platelets.[52,53] However, in the pro-inflammatory phase after ischemia–reperfusion injury, most extracellular mtDNA originates from necrotic cardiomyocytes and elevated levels of mtDNA has been found in circulation of myocardial infarction patients.[54] Whatever the source, the common denominator is that mtDNA avoids degradation by DNases. Consequently, mtDNA is able to trigger inflammatory signaling pathways in neighboring, surviving cardiomyocytes and endothelial cells.[19,55,56] It was demonstrated by Yang et al.[57] that mtDNA increased infarct size in isolated rat hearts exposed to ischemia–reperfusion. Moreover, both blood-perfused and buffer-perfused isolated hearts treated with DNases during reperfusion after global ischemia had reduced infarct size.[57]

Extracellular mtDNA is suggested to bind to TLR9, which is associated with endosomal membranes inside the cell. We have recently proposed a mechanism of internalization of extracellular DNA, including mtDNA, via membrane-bound nucleolin in cardiomyocytes.[58] However, there is no direct evidence of a co-localization of membrane-bound nucleolin and mtDNA in cardiomyocytes, thus other proteins may be involved. mtDNA activates the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway in cardiomyocytes in order to express and release pro-inflammatory cytokines, which participate in the local and systemic inflammatory response.[19,58]

mtDNA is highly cytotoxic to cardiomyocytes, where cardiomyocyte viability is dose-dependently reduced by mtDNA exposure.[19] Although the majority of dead cardiomyocytes is believed to be due to necrotic cell death, other cell death pathways need further investigation. As mentioned above, intracellular oxidized mtDNA is thought to activate the NLRP3 inflammasome leading to cleavage of pro-interleukin (IL)-1β, pro-IL-18, and gasdermin D. Gasdermin D proteins form a pore-structure in the plasma membrane that allow release of cytokines. However, the pore is unspecific and holds a size of 10–20 nm in diameter, which allows unspecific release of cytoplasmic proteins and additional DAMP release, including mtDNA.[59,60] This might eventually destabilize the membrane integrity and cause pyroptotic cell death.[45] Intriguingly, there is at present no evidence of NLRP3 expression or inflammasome assembly in primary, adult cardiomyocytes. Whether this is an evolutionary defense mechanism in cardiomyocytes preventing irreversible pyroptotic cell death remains to be elucidated. Cardiac fibroblasts, conversely, form the NLRP3 inflammasome and release IL-1β and IL-18.[61] To our knowledge, there is no evidence supporting that mtDNA activates pyroptosis in adult cardiac fibroblasts or that fibroblasts undergo pyroptotic cell death. However, it has been shown that caspase-1 play a major role in reperfusion and it cleaves gasdermin D in ischemia-reperfused hearts and in neonatal cardiac fibroblasts.[62,63]

Similar to NLRP3, absent in melanoma 2 (AIM2) form inflammasome complexes triggered by cytosolic double-stranded DNA.[64] Recent discoveries showed that mtDNA trigger AIM2 inflammasome-dependent caspase-1 activation and IL-1β release in macrophages and contributes to chronic inflammation in heart failure.[64,65] The expression of AIM2 and the formation of AIM2 inflammasomes in adult cardiac cells remains unclear.

2.2 | Mitochondrial *N*-formyl peptides

In bacteria, formylation of a methionine and its association with transfer RNA (tRNA) is a requirement for initiation of protein synthesis. A formylated methionine is found at the N-terminus of bacterial proteins. This formylation, a well-described PAMP, is detected by the innate immune system as part of the host defense mechanism against invading bacterial pathogens.[66,67] The mtDNA-encoded proteins of the electron transport chain are transcribed and translated in the mitochondria.[68] Thus, the proteins contain a formylation of their N-terminal and are referred to as mitochondrial *N*-formyl peptides.[69,70] Extracellular mitochondrial *N*-formyl peptides activate the FPRs, which are plasma membrane bound G-protein coupled receptors. However, little is reported about the exact expression level of the FPRs in the heart. We have studied the absolute mRNA transcripts in mouse adult cardiomyocytes and cardiac fibroblasts and found no expression of the three described FPRs; FPR1, FPR2, and FPR3 (Figure 2; see Figures S2, S3). Mitochondrial *N*-formyl peptides are intuitively still released in massive amounts during ischemia–reperfusion injury. Locally, mitochondrial *N*-formyl peptides are thought to guide cytokine-recruited leukocytes to the accurate site in the injured myocardium.[71] Systemically, mitochondrial *N*-formyl peptides may bind FPRs on circulating leukocytes and activate these cells directly.[31,72,73] In summary, evidence indicates that mitochondrial *N*-formyl peptides may be more involved in the general inflammation phase rather than the pro-inflammatory phase (Figure 2).[31,74,75]

2.3 | Cardiolipin

Cardiolipin was first observed in heart tissue; contradictory to cardiolipin's given nomenclature, the effect of cardiolipin as a mDAMP in the heart is not yet clear. Cardiolipin constitute 20% of the phospholipid composition of the inner mitochondrial membrane.[77–79] It is important in mitochondrial signaling and stabilizing mitochondrial respiratory supercomplexes.[80,81] The resemblance of the cardiolipin-rich inner mitochondrial membrane and the bacterial plasma membrane makes cardiolipin a potentially potent inflammatory mDAMP in ischemia–reperfusion injury. Cytosolic cardiolipin activates the NLRP3 inflammasome in monocytes, but its extracellular role in the pro-inflammatory phase in the heart remains unknown.[82,83]

2.4 | Extracellular cytochrome c

Cytochrome c is involved in two mechanistic pathways, mitochondrial oxidative phosphorylation and activation of apoptotic cell death. Cytochrome c is normally located in the mitochondrial intermembrane space working as an electron carrier in oxidative phosphorylation.[84] In ischemia–reperfusion, mitochondria experience a rapid increase in pH and concentrations of calcium, which cause opening of the mitochondrial permeability transition pore.[85] This pore allows cytochrome c to leak into the cell interior and activate the intrinsic pathway of apoptosis.[86] In contrast, very little is known about its extracellular effect in ischemia–reperfusion injury and the pro-inflammatory phase. It has been reported that necrotic cells release cytochrome c, and it has been used as a marker for mitochondrial injury in patients of resuscitation after cardiac arrest.[87–89] Moreover, it has been shown

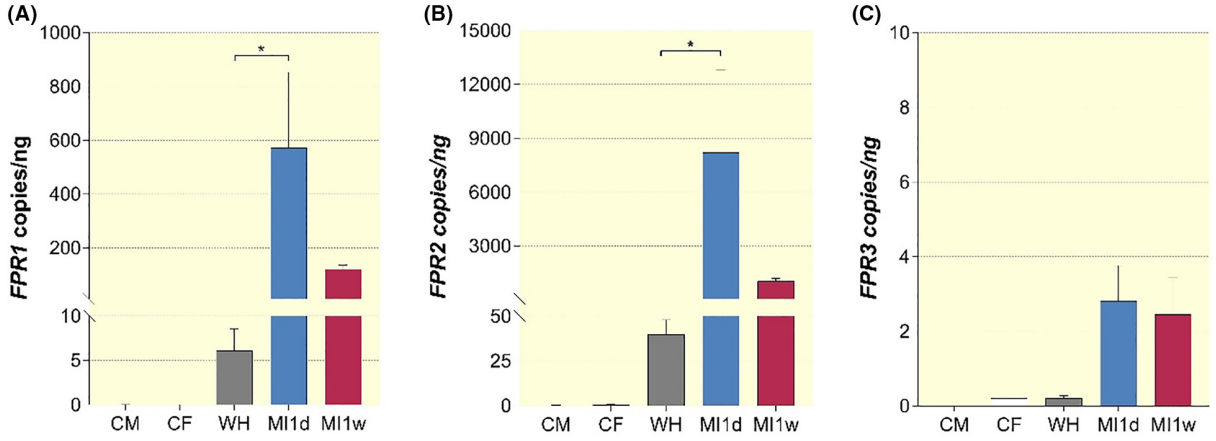

FIGURE 2 Absolute quantification of the FPRs. Absolute number of mRNA copies per ng of the three formyl peptide receptors (A) FPR1, (B) FPR2, and (C) FPR3 in CM, CF, WH, and MI1d or MI1w. Quantification of FPRs indicated very low expression of all three receptors in cardiomyocytes and cardiac fibroblasts. In the perfused whole heart tissue, both FPR1 and FPR2 were expressed, indicating expression in other cardiac cells. Previous studies have shown expression of FPRs in both cardiac smooth muscle cells and tissue-resident macrophages.[76] All the FPRs were highly expressed in cardiac tissue 1 day after coronary artery ligation and slightly lower 1 week after ligation. This is a strong indicator of infiltration of FPR-expressing neutrophils and monocytes secondary to tissue injury. Data are presented as mean ± SEM and statistical differences were tested with one-way ANOVA and Dunnett's multiple comparison test ($n = 6$). * indicates $p \leq 0.05$. Summary of materials and methods can be found in the supplementary data.[172] FPR, Formyl peptide receptor; CM, cardiomyocytes; CF, cardiac fibroblasts; WH, whole heart tissue; MI1d, one day after myocardial infarction; MI1w, one week after myocardial infarction

that extracellular cytochrome c is a trigger of inflammation in neutrophils and monocytes.[90] Unfortunately, the level of endotoxins in the cytochrome c ligands used has not been reported in these studies. We have to this date not found a proper, commercially available cytochrome c ligand with endotoxin levels below FDAs recommendations. The exact role of extracellular cytochrome c in cardiomyocytes is unknown.

2.5 | Other known mDAMPs

ROS play a major role in cardiac reperfusion injury and are highly cytotoxic to the fragile cardiomyocytes. However, this has been reviewed elsewhere and is beyond the scope of this review.[91,92] Furthermore, ROS originate from multiple sources and it is difficult to differentiate a particular role of mitochondria-derived ROS. An alternative role for ROS is their ability to activate inflammation. Mitochondria-originating cytosolic ROS or extracellular ROS activate the NLRP3 inflammasome, which cleaves and releases IL-1β and IL-18.[61,93]

Similar roles are observed for extracellular ATP, which binds to P2Y receptors on the plasma membrane and activates the pre-assembled NLRP3 inflammasome.[94,95] Extracellular ATP also induce chemotaxis and direct phagocytosing cells to sites of apoptotic bodies.[96] Mouse cardiomyocytes obtain a round, unhealthy morphology and die when exposed to physiologically relevant doses of ATP (unpublished observations).

TFAM is nuclear encoded and belongs to the family of high-mobility group proteins. Its function is similar to the nuclear-encoded high-mobility group box 1 (HMGB1), which activates inflammatory pathways, but TFAM and HMGB1 are not structurally similar.[97,98] Nonetheless, it has been shown that TFAM triggers an inflammatory response, in particular in combination with mtDNA in dendritic cells.[99] However, the role of TFAM and its inflammatory signaling pathways in the heart remains unknown.

Succinate, a metabolite formed in the Krebs cycle and a substrate for the electron transport chain complex II, has proven to be a highly pro-inflammatory mDAMP. During ischemia, massive amounts of succinate accumulate as the electron transport chain enters a reverse mode.[100] Besides facilitating ROS production in reperfusion, succinate is released extracellularly from dying cells and activates GPR91, a G-protein coupled receptor. GPR91 triggers hypertrophic growth of cardiomyocytes and inflammatory processes in innate immune cells.[34,101] If succinate trigger inflammation in adult cardiac cells remains to be answered.

3 | INFLAMMATION IN CARDIAC CELLS

Endogenous DAMPs are normally hidden from immune cell recognition (Figure 3a), but ischemia and reperfusion causes loss of plasma membrane integrity and necrotic

cell death (Figure 3b).[102,103] Although loss of mitochondrial membrane potential is a large contributor to necrotic cell death, release of mitochondrial cytochrome c into the cytosol of surviving cells can also initiate the apoptotic machinery.[104,105] If phagocytosis is delayed or fails to remove the apoptotic bodies, secondary necrosis occurs as the apoptotic bodies loose membrane integrity. In the border zone of the infarct, surviving cardiomyocytes are exposed to a storm of cellular debris that are either immunostimulatory, cytotoxic, or both (Figure 3b).[4,19,106] It has been extensively documented that ischemia-induced necrosis is a process that develops over time in reperfusion and is termed the *wavefront phenomenon*.[107–109] It is believed that the release of DAMPs from necrotic cells increase the expression of pro-inflammatory markers within the first 3 h of reperfusion, leaving the window for therapeutic intervention within the first hours of the onset of reperfusion.[110]

Cardiac cells are very different in both morphology and function, and inflammation caused by mDAMPs may therefore have different roles on the cells during the pro-inflammatory phase before the arrival of immune cells.

3.1 | Cardiomyocytes

The adult heart comprises a fixed amount of terminally differentiated cardiomyocytes that is sustained throughout

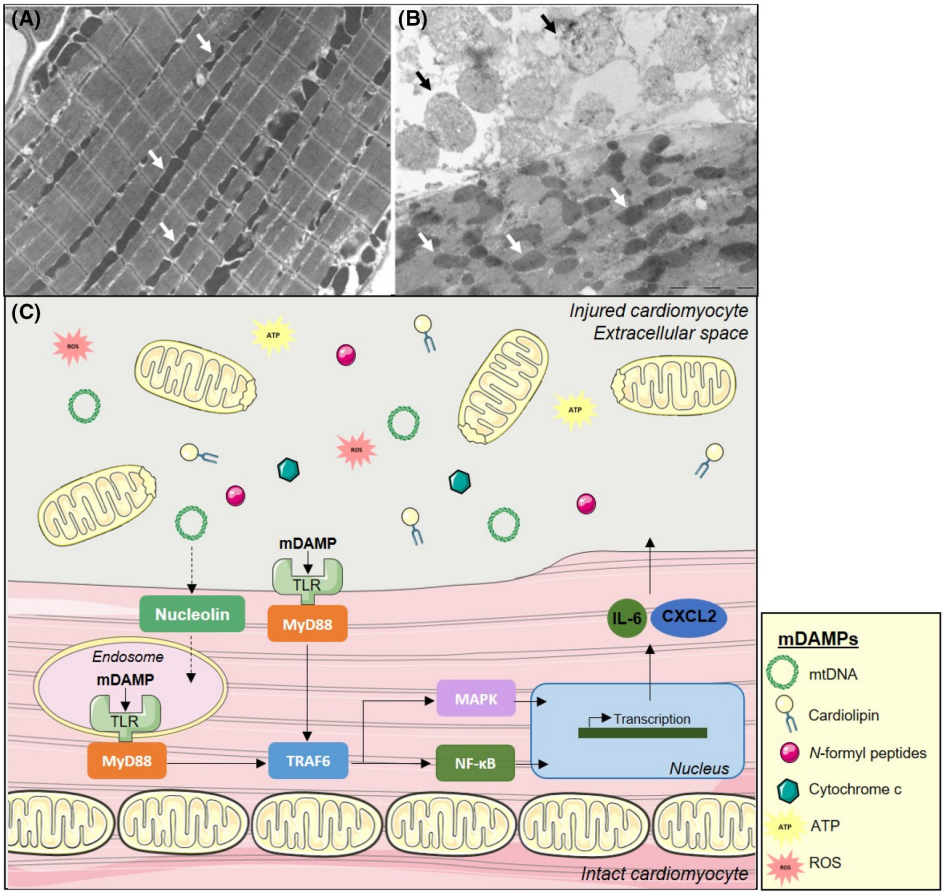

FIGURE 3 Electron micrographs of healthy and necrotic myocardium. (A) Healthy intact myocardium. Mitochondria (white arrows) are neatly arranged between the contractile filaments in the heart. (B) Necrotic cell (top part of the image) releasing its content onto a neighboring intact cardiomyocyte (bottom part of the image). The mitochondria from the necrotic cell are swelled and perhaps at the boarder to burst (black arrows). (C) mDAMPs released from an injured cardiomyocyte onto an intact cardiomyocyte. Pathogen recognition receptors with identified expression in primary adult cardiomyocytes includes the TLR, which are triggered by mDAMPs either on the cell surface or in endosomes. mtDNA is suggested to be imported to the endosomes via nucleolin.[58] The TLRs signal through, *inter alia*, MyD88, TRAF6, the MAPK and/or NF-κB pathway leading to the expression of pro-inflammatory cytokines. The cytokines are released into the extracellular milieu and trigger local cytokine receptors or enter the blood stream to recruit immune cells to the site of injury. Summary of materials and methods can be found in the supplementary data.[173] mDAMPs, mitochondrial damage associated molecular patterns; TLR, toll-like receptors; mtDNA, mitochondrial DNA; MyD88, myeloid differentiation primary response 88; TRAF6, TNF receptor associated factor 6; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells. Elements in (C) are modified from Servier Medical Art, http://smart.servier.com/

life.[111–114] As cardiomyocytes are unable to proliferate, they undergo compensatory hypertrophy in response to excessive wall stress.[115] Cardiomyocytes are large (100–200 μm in length) occupying approximately 70–80% of the heart volume, although, counting for only 25–35% of the total number of cells in the heart.[116–118] Ninety-five per cent of the energy required by cardiomyocytes originate from oxidative phosphorylation.[119] During ischemia, cells are not able to sustain ATP production through oxidative phosphorylation and ATP is rapidly consumed. Hence, oxygen-deprivation of cardiomyocytes is rapidly fatal and causes irreversible loss of cardiomyocytes. Cardiomyocytes occupy most of the cardiac volume and their cellular content are believed to be the main stimulus of the inflammatory response. It has been shown by us and others that cardiomyocytes produce cytokines in response to mDAMPs.[58,120] A large production of cytokines takes place that may have detrimental consequences for the cardiac cells. IL-6 is highly expressed by cardiomyocytes in the viable border zone of the infarct.[121] IL-6 was the most robust marker of inflammation in hypoxia–reoxygenation studies with cultured primary mouse cardiomyocytes, indicating that IL-6 is relevant in the local pro-inflammatory phase.[58] Furthermore, IL-6 contributes to cardiomyocyte hypertrophy in co-culture with cardiac fibroblasts and reduce cardiac fibroblast differentiation.[122]

3.2 | Cardiac fibroblasts

Non-cardiomyocytes counts for 65–70% of the total number of cells in the heart. Of these, cardiac fibroblasts occupy the third largest population of cells after endothelial cells, accounting for approximately 20% of the total number of cells, although this varies among species.[123] Cardiac fibroblasts, with their membrane protruding morphology, are quiescent cells, situated between the cardiomyocytes in the myocardium. In the healthy myocardium, their pivotal roles are maintaining extracellular network and signaling.[124,125] Cardiac fibroblasts have high inflammatory potential and are rapidly triggered by released DAMPs.[126] They are able to form and activate the NLRP3 inflammasome after myocardial infarction leading to cleavage and release the pro-inflammatory cytokines, IL-1β and IL-18.[61] Moreover, in dysmetabolic models, such as high levels of saturated fatty acids, have been associated with increased NLRP3 inflammasome activity in cardiac fibroblasts.[127,128]

Activation and assembly of the NLRP3 inflammasome in cardiovascular diseases has been reviewed elsewhere.[129,130] NLRP3 activity has been associated with mitochondrial dysfunction and inhibition of NLRP3 with the selective inhibitor MCC950 has shown promising effect on the ischemia-reperfused heart.[130,131] Cardiac fibroblasts are large contributors to IL-1β release in the heart. Administration of caspase-1 inhibitors at reperfusion in patients with myocardial infarction preserved ventricular function and reduced IL-1β release.[132–134] Moreover, IL-1β causes downregulation of genes involved in sarcoplasmic reticulum calcium handling in cardiomyocytes. This causes dysregulation of excitation-contraction coupling.[132,133] Identifying targets that reduce release of pro-inflammatory cytokines may be valuable in treating ischemia–reperfusion injury.

3.3 | Cardiac endothelial cells

There are different population of endothelial cells in the heart with different function such as *endocardial* endothelial cells and *vascular* endothelial cells, and they account for around 60% of the non-cardiomyocytes in the heart.[123,135] Little is known about the role of the endocardium in myocardial infarction. The capillary density is approximately 3.000–4.000/mm^2. The capillary endothelial cells communicate with adjacent cardiomyocytes in regulating metabolism, growth, contractility and rhythmicity.[136,137] Exposure of mDAMPs, including mtDNA, ATP and mitochondrial *N*-formyl peptides, increase the permeability across the epithelial layer preparing for transmigration of arriving immune cells heading for the injured myocardium.[138–140] The endothelial cells are also important for activation of the NLRP3 inflammasome and IL-1β release, which recruit pro-inflammatory monocytes and neutrophils. These cells are the first immune cells that arrive to the infarcted myocardium in the resolution phase and they initiate phagocytosis of dead cells and cellular debris.[13,141] Cytokines, such as IL-1β and tumor necrosis factor (TNF), originating from cardiac cells, facilitate adherence and migration of leukocytes due to increased expression of adhesive proteins on the endothelial cell surface.[142,143]

4 | STERILE MYOCARDIAL INFLAMMATION FROM BENCH TO BEDSIDE

Publications describing post-infarct inflammation started in 1956, when proteins of the complement system and C-reactive proteins were found to be increased in the serum of patients with myocardial infarction.[144] Immunohistochemistry revealed the presence of complement proteins in the myocardium in the 1970s.[145] In the 1980s, it was shown that myocardial infarction per se triggered activation of the complement system and infiltration of granulocytes into the myocardium.[146] However,

production of ROS in ischemia–reperfusion injury dominated the literature in the mid-80s.[147,148]

The first publication, in which reperfusion injury was called an inflammatory response, was in 1989.[149] However, it was not until 1990 that researchers understood that the innate immune system could have a direct pathogenic role in myocardial infarction.[150,151] Rapidly increasing mRNA expression of intercellular adhesion molecule 1 (ICAM-1) was shown in the border zone of myocardial infarction.[152] Furthermore, the discovery that the immune system could respond to endogenous intracellular alarmins was described in the danger theory by Polly Matzinger in 1994.[4] We now know a lot more about the molecular basis of inflammation after myocardial infarction. However, clinical trials have been disappointing and inconclusive so far.[153,154] Studies with non-selective immunosuppressive drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids, have shown catastrophic consequences on post-myocardial infarction remodeling, with subsequent increased risk of recurrent myocardial infarction, stroke, and vascular death due to improper healing processes.[155–158] Recently, a more targeted trial named CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study) trial, where canakinumab, an anti-IL-1β monoclonal antibody, was administered in patients with previous history of myocardial infarction. The drug reduced serum levels of C-reactive proteins and reduced the rate of recurrent cardiovascular events. However, the CANTOS trial showed no difference in mortality.[159] The COLCOT (Colchicine Cardiovascular Outcomes Trial) study introduced the anti-inflammatory drug colchicine, which targets the NLRP3 inflammasome and subsequently IL-1β secretion, to patients that suffered myocardial infarction. Early treatment with the low-cost drug colchicine improved the primary endpoints.[160,161] The ASSAIL-MI (ASSessing the effect of Anti-IL-6 treatment in Myocardial Infarction) trial was more successful. In this trial, a single dose of an IL-6 receptor antagonist (tocilizumab) was given to NSTEMI patients 2 days after symptoms of myocardial infarction. Treated patients showed reduced C-reactive protein levels and reduced myocardial tissue damage.[162] The two latter clinical trials focus on important cytokines in the inflammatory process, however, the underlying molecular mechanisms of sterile inflammation in the pro-inflammatory phase and specifically the role of mDAMPs in myocardial infarction are still not sufficiently understood.

Infarct size correlates with increasing levels of mDAMP release and cytokine production.[163] As long as necrosis is ongoing, the inflammatory and cytotoxicity cascades are prolonged and the myocardial fate is significantly worsened. Excessive early inflammation amplifies degradation of the extracellular matrix and increases risk of cardiac rupture.[164] Additionally, disproportionate production of pro-inflammatory cytokines activates apoptotic signaling in cardiomyocytes. High concentrations of circulating cytokines in patients are associated with increased infarct size and adverse outcomes.[21,165,166] Patients with myocardial infarction, atrial fibrillation, and heart failure have increased levels of mDAMPs, in particular mtDNA, in the circulation.[54,167,168] Unfortunately, little has been done with regard to mDAMPs in patients with myocardial infarction. Increased plasma levels of mtDNA has been shown in patients undergoing open heart surgery with cardiopulmonary bypass.[169] Increased mtDNA appeared both to be free in the plasma as well as in microvesicles.[170] Patients with chronic heart failure, mainly caused by myocardial infarction, have higher levels of circulating mtDNA compared to healthy individuals.[171] Moreover, the study shows that high levels of mtDNA in patients with chronic heart failure give better survival compared to low levels; the low levels correlate with increased mortality. These results are slightly inconclusive and the authors have no explanation for these results.[171] At the time being, there are no therapeutic interventions protecting cardiac cells against injurious mDAMPs. More detailed knowledge can potentially provide better and more targeted treatment.

5 | CONCLUSIONS

Due to their bacterial origin, mitochondria may potentially be more immunogenic than other cellular components. Significant levels of mDAMPs are released upon injury and necrosis of cardiac cells, causing innate immune responses and exacerbated myocardial damage. We still need more knowledge about mDAMPs in myocardial infarction, and an efficient strategy could be to identify the most harmful mDAMPs and find ways to inhibit their early inflammatory signaling.

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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