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Role of innate immunity in cardiac inflammation after myocardial infarction

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1. ABSTRACT

Over the past two decades, inflammation has emerged as a key pathophysiological process during myocardial infarction. It develops consecutively to the activation of innate immune defense mechanisms, in response to the release of endogenous molecules by necrotic cells and the extracellular matrix. These danger signals are sensed by cellular receptors normally involved in antimicrobial defenses, including toll-like receptors and a subset of NOD-like receptors, which promote intracellular signaling dependent on nuclear factor kappaB and on the formation of the inflammasome. These mechanisms stimulate the expression of multiple inflammatory mediators and growth factors, sequentially inducing the recruitment of inflammatory cells, the clearance of injured tissue, angiogenesis, and the proliferation of fibroblasts, eventually resulting in scar formation and infarct healing. Dysregulation of these responses may result in continued cardiomyocyte loss, fibrosis beyond the limits of the infarcted area, reactive hypertrophy and chamber dilatation, a process termed adverse cardiac remodeling, leading to functional compromise and heart failure. This review presents the current state of knowledge on the process of immune activation within the infarcted myocardium and its consequences.

2. INTRODUCTION

Myocardial infarction (MI) is the major cause of chronic heart failure in developed countries. In the past two decades, significant progresses have been made in the understanding of the cellular and molecular mechanisms implicated in the complex pathophysiology of MI. Acute coronary occlusion induces myocardial ischemia, resulting in the cessation of aerobic metabolism and high-energy phosphate generation. In the absence of restoration of blood flow, irreversible cardiomyocyte injury develops already after 30 minutes, characterized mainly by necrotic cell death. Rapid reperfusion of the ischemic myocardium is therefore mandatory to prevent such irreversible damage (1). However, reperfusion by itself may paradoxically promote myocardial damage, a concept termed myocardial ischemia-reperfusion injury (2), which is related to mutliple identified mechanisms, including intracellular calcium overload, alterations of intracellular pH, dissipation of mitochondrial membrane potential with opening of the mitochondrial permeability transition pore and the generation of toxic free radicals and oxidant species (1, 2).

Following these hyper acute derangements, an early inflammatory response develops within the infarcted myocardium, triggered by the activation of endothelial cells, cardiomyocytes and leukocytes, and which is

characterized by the expression of adhesion molecules, chemokines and inflammatory cytokines, complement activation, and the recruitment of inflammatory cells (3, 4). This response is essential for the successful adaptation of the heart to the acute injury, as it sets in motion a series of homeostatic mechanisms aimed at the healing and the preservation of function of the injured myocardium (5). However, when this response is dysregulated, excessive and/or sustained, maladaptive consequences develop, characterized by continued cardiomyocyte loss, fibrosis beyond the limits of the infarcted area together with reactive hypertrophy and ventricular chamber dilatation (5, 6). This process, termed adverse cardiac remodeling eventually results in functional compromise and clinical heart failure (7). Understanding the detailed molecular mechanism of these inflammatory changes is therefore crucial for the future development of innovative therapies to prevent or treat such adverse cardiac remodeling.

3. INFLAMMATORY GENE EXPRESSION IN THE INFARCTED MYOCARDIUM

3.1. Inflammatory cytokines

Key inflammatory cytokines are rapidly expressed within the infarcted myocardium, including TNF alpha, IL-1 beta, IL-18 and IL-6, producing pleiotropic and often contradictory effects on the heart, as summarized in Table 1. TNF alpha mediates inflammatory injury (8) and promotes the activity of matrix metalloproteinases (MMPs) in cardiac fibroblasts (9) after MI. It reduces myocardial contractility by dysregulating calcium homeostasis in cardiac myocytes and by triggering the expression of additional cardiodepressant molecules, such as IL-1 beta, and nitric oxide (10). Alternatively, TNF alpha also triggers cytoprotective signals preventing cardiomyocyte apoptosis and adverse remodeling, as identified in studies in TNF alpha receptor KO mice (11), and as suggested by the lack of benefit of anti-TNF alpha strategies in humans with chronic heart failure (12, 13). The contrasted roles of TNF alpha could be related to opposite signals triggered by the two classes of TNF receptors, p55 (TNFR1) and p75 (TNFR2), as recently proposed by Kishore and co-workers. Using KO mice from either receptor, these authors found that post-MI survival and left ventricular functional recovery were much improved in p55KO, whereas they were markedly reduced in p75 KO. Thus, signaling via TNFR1/p55appears responsible for the deleterious effects of TNF alpha, whereas TNFR2/p75 signaling seems to convey mainly beneficial effects from TNF alpha (14).

IL-1 beta is markedly upregulated in the infarcted heart, playing a major role in myocardial inflammation, hypertrophy and adverse remodeling (15, 16), as shown by studies using mice lacking the IL-1 beta receptor (17) or overexpressing IL-1 beta in the myocardium (18). Also, it was recently found that deficiency in IRAK-4, a key downstream intermediate in IL-1 beta signaling, had favorable effects on left ventricular remodeling by blunting the detrimental mobilization of bone marrow dendritic cells in the myocardium (19). Further supporting such a detrimental role, a recent small clinical study indicated that post-infarction left ventricular remodeling was improved in patients treated with anakinra, a recombinant form of the natural IL-1beta receptor antagonist IL-1Ra (20). Another member of the IL-1 family, IL-18, has been also shown to be upregulated after MI (21). Like IL-1 beta, Il-18 is present primarily as an inactive precursor (pro-IL-18), which is processed in stimulated cells by the action of caspase-1, also known as IL-1 beta-converting enzyme (ICE). Mature IL-18 is a potent pro-inflammatory cytokine whose precise role in post-MI inflammation and repair remains unknown, although a link between the levels of serum IL-18 concentrations and adverse clinical events after MI has been established (21, 22).

IL-6 and related cytokines such as IL-11, IL-27 or leukemia-inhibitory factor-1, are additional inflammatory cytokines overexpressed in the injured myocardium (23, 24). IL-6 cytokines act through binding to the IL-6Ralpha/gp130 signal transduction complex, triggering the activation of several signal transduction pathways, including the Janus-activated kinase-signal transducer and activator of transcription (JAK-STAT) pathway (24). More specifically, IL-6 and its related congeners act via STAT3, a transcription factor regulating a myriad of genes involved in the regulation of cell growth, apoptosis, differentiation, and survival, and which also acts as a mitochondrial protein involved in energy production, as extensively reviewed in (25). Most significantly, IL-6-STAT3-dependent signaling regulates crucial cell-cell interactions which are essential for the maintenance of normal heart architecture, as shown by enhanced myocyte apoptosis, cardiac fibrosis and ventricular chamber dilatation observed in IL-6 null mice (23). With respect to MI, controversial results have been obtained regarding the role of IL-6. Fuchs *et al.* reported that the lack of IL-6 did not affect long-term MI size or left ventricle function, remodeling, and survival in mice, probably because compensatory mechanisms were set in motion to maintain JAK-STAT3 signaling (26). In marked contrast, a recent study by Kobara *et al.* demonstrated that an anti-IL-6 receptor antibody, administered to mice after the induction of MI, markedly reduced myocardial infiltration by neutrophils and macrophages, suppressed MMP-2 activity in the infarct region and reduced both cardiomyocyte hypertrophy and interstitial fibrosis in the non-infarct region. Overall, the inhibition of IL-6 signaling resulted in a reduction of left ventricle dilatation and contractile dysfunction, together with an increased survival rate (27). In line with these findings, Hilfiker-Kleiner *et al.* recently reported that mice with a cardiomyocyte-restricted mutant form of gp-130, promoting continuous activation of gp130-STAT3 signaling, displayed higher mortality associated with increased left ventricular rupture rate, sustained cardiac inflammation, and heart failure after MI (28).

3.2. Chemokines, adhesion molecules and leukocyte trafficking

Chemoattractant cytokines, or chemokines, are further key mediators of post-MI inflammatory responses, which orchestrate the accumulation of leukocyte within the infarcted myocardium (reviewed in (29)). Leukocyte trafficking also depends on the upregulated expression of selectins, integrins and adhesion molecules such as ICAM-

Table 1. Innate immune mechanisms in myocardial infarction: contribution of studies using transgenic mice

Transgenic strain	Model of MI	Main findings	Ref.
TNF alpha pathway			
TNF alpha KO	I/R (30'-120')	Reduced infarct size and contractile dysfunction. Reduced NF-kappaB activation, chemokine expression, PMN infiltration.	(8)
TNF alpha KO	P.O. (up to 7 D)	Improved cardiac function. Reduced myocardial ICAM-1 expression.	(157)
TNFR1-R2 KO	P.O. (24 h)	Increased infarct size, increased apoptosis of cardiac myocytes.	(11)
TNFR1 (p55) KO	P.O. 7-28 D	Reduced mortality, improved functional recovery.	(14)
TNFR2 (p75) KO	P.O. 7-28 D	Increased mortality, increased LV dysfunction.	(14)
IL-1 beta pathway			
IL-1R KO	I/R (1 h/6 h to 7 D)	Reduced chemokine, cytokine and MMPs expression. Reduced PMN infiltration, LV dilatation and LV fibrosis.	(17)
IL-6 pathway			
gp-130 mutant	P.O. (up to 2 W)	Persistent myocardial STAT3 activation, increased myocardial inflammation, LV dysfunction and LV rupture.	(28)
IL-10 pathway			
IL-10 KO	I/R (30'-2-24 h)	Increased PMN infiltration, ICAM-1/TNF expression and infarct size.	(158, 159)
IL-10 KO	I/R (1 h/6 h to 7 D)	Increased expression of TNF and CCL2. No effect on LV dysfunction and remodeling.	(160)
TGF beta pathway			
Smad3 KO	I/R (1 h/6 h to 7 D)	Reduced PMN infiltration and chemokine expression. Reduced dilatative LV remodeling and diastolic dysfunction.	(45)
TSP-1 KO	I/R (1 h/3 h to 7 D)	Increased and prolonged expression of IL-1, IL-6 and TGF-beta. Enhanced LV adverse remodeling with increased fibrosis.	(161)
Chemokines, adhesion molecules			
CXCR4 KO	P.O. (3 W)	No influence on LV systolic dysfunction and adverse remodeling	(37)
IP-10 KO	I/R (1 h/6 h to 7 D)	Expansion of fibrosis, increased remodeling and systolic dysfunction Increased PMN (after 24 and 72 h) and macrophages (after 3 to 7 D).	(34)
MCP-1 KO	I/R (1 h/6 h to 7 D)	Delayed macrophage recruitment and myofibroblast accumulation. Reduced cytokine expression. Reduced LV remodeling.	(38)
CCR2 KO	P.O. (up to 28 D)	Reduced macrophage infiltration, MMP expression and collagen deposition. Reduced LV systolic dysfunction and LV remodeling.	(39)
CCR1 KO	P.O. (up to 21 D)	Reduced PMN infiltration, decreased apoptosis, increased proliferation of myofibroblasts in the infracted tissue. Reduced LV remodeling.	(42)
ICAM-1 KO	I/R (30'/2 h-3 W)	Reduced reperfusion injury at 2 h.	(162, 163)
ICAM-1/P-sel KO	I/R (30'-1 h/3-24 h)	Reduced PMN infiltration at 24 h, but no change in infarct size.	(164)
CD-18 KO	I/R (30'/2 h)	Reduced PMN infiltration and smaller infarct size (at 2 h reperfusion)	(163)
NF-kappaB pathway			
Cardiac restricted mutated IkappaB	P.O. (4 W)	Diminished NF-kB p65 activation and cytokine expression. Reduced LV remodeling, fibrosis and systolic function.	(64)
Cardiac-restricted mutated IkappaB	P.O. (24 h)	Increased infarct size and apoptosis. Reduced expression of anti-apoptotic proteins (Bcl-2 and c-IAP1).	(68)
p50 KO	I/R (30'/24 h)	Reduced PMN infiltration, decreased infarct size	(165)
p50 KO	P.O. (up to 8 W)	Reduced mortality, LV remodeling and systolic dysfunction	(62, 63)
p50 KO	P.O. (4 W)	Increased expression of cytokines, chemokines and MMPs. Enhanced remodeling, fibrosis, hypertrophy and systolic dysfunction.	(69)
TLR pathway			
TLR2 KO	I/R (30'/60') (isolated hearts)	Improved post-ischemic functional recovery. Blunted myocardial expression of TNF alpha and IL-1 beta.	(85)
TLR2 KO	P.O. (1 to 4 W)	Reduced TGF beta expression and fibrosis. Reduced LV remodeling and systolic dysfunction.	(86)
TLR2 KO	I/R (30'/60')	Reduced myocardial PMN accumulation, ROS formation and IL-1 beta expression. Suppressed coronary endothelial dysfunction.	(84)
TLR4 deficient (C3H/HeJ mice)	P.O. (up to 4 W)	Reduced remodeling, preserved systolic function. Reduced fibrosis and expression of cytokines.	(81)
TLR4 KO	P.O. (1-4 W)	Reduced systolic dysfunction and remodeling. Decreased TGF beta expression. Reduced lymphocyte infiltration and apoptosis.	(82)
TLR4 KO	I/R (1 h/24 h)	Reduced infarct size, myocardial PMN infiltration and oxidative stress.	(79)
MyD88 KO	I/R (1 h/up to 7 D)	Reduced infarct size, reduced LV dysfunction, reduced myocardial PMN infiltration, MCP-1 and ICAM-1 expression.	(92)
RAGE pathway			
RAGE KO	I/R (30'/48 h)	Reduced infarct size, myocardial inflammation and dysfunction.	(94, 166)
Inflammasome pathway			
Caspase-1 KO	I/R (30'/48 h)	Reduced infarct size (48 h reperfusion).	(115)
Caspase-1 KO	P.O. (up to 9 D)	Reduced LV remodeling, MMP-3 and IL-18 expression and apoptosis.	(114)
ASC KO	I/R (30'/up to 2 W)	Reduced infarct size at 48 h. Reduced LV fibrosis, remodeling and systolic dysfunction. Reduced myocardial cytokine expression.	(115)

Abbreviations: ASC: apoptosis-associated speck-like protein containing a caspase recruitment domain; Bcl-2: B-cell CLL/lymphoma 2;c-IAP-1: inhibitor of apoptosis protein-1; CCL: chemokine (C-C motif) ligand ; CCR: chemokine (C-C motif) receptor; CXCR: chemokine (C-X-C motif) receptor; D: Day; H: hour;ICAM-1: inter-cellular adhesion moelcule-1; IkappaB: inhibitor of KappaB; IL: interleukin; IL-1R: IL-1 beta receptor; IP-10: Interferon-inducing protein 10; I/R: Ischemia/Reperfusion; KO: Knockout; LV: left ventricle; MCP-1: monocyte chemoattractant protein-1; MMP: matrix metalloproteinase; MyD88: myeloid differentiation primary response gene 88; NF-kappaB: nuclear factor kappaB; PMN: ploymorphonuclear cell; P.O.: permanent occlusion; P-sel: P-selectin; RAGE: receptor for advanced glycation end-products; ROS: reactive oxygen species; SDF-1: stromal cell-derived factor-1; STAT: signal transducer and activator of transcription; TGF beta: transforming growth factor beta; TLR: toll-like receptor; TNF alpha: tumor necrosis factor alpha; TNFR1: TNF receptor 1; TNFR2: TNF receptor 2; TSP-1: Thrombospondin-1; W: week

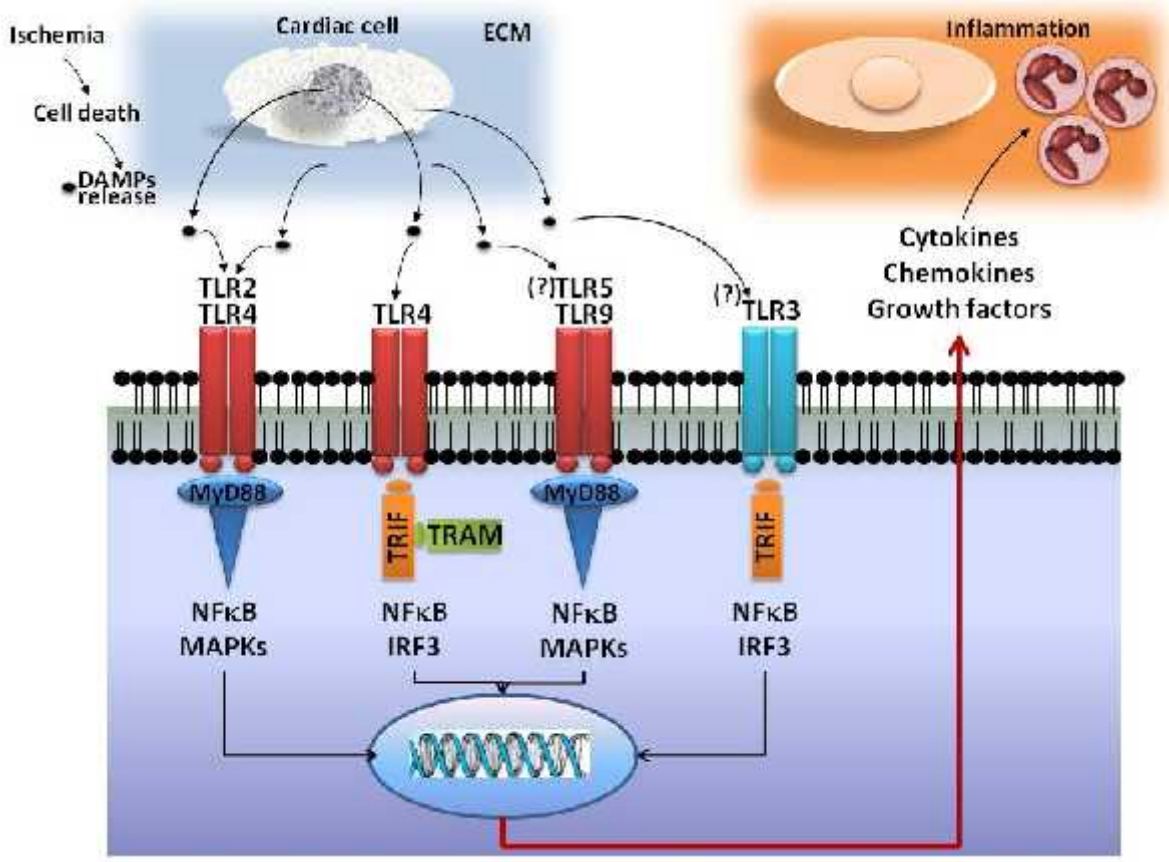

Figure 1. Innate immune mechanisms trigger post-ischemic myocardial inflammation. Myocardial ischemia promotes necrosis of cardiac cells (mainly cardiomyocytes) and damages to the extracellular matrix (ECM). In turn, dead cells and damaged ECM release various danger associated molecular patterns (DAMPs), including high mobility group box-1 (HMGB1), heat shock proteins (HSPs), myosin, hyaluronic acid, fibrinogen, fibronectin EDA and nucleic acids. Released DAMPs are sensed by toll-like receptors (TLRs), especially TLR2 and TLR4. The contribution of other TLRs, such as TLR3, TLR5 and TLR9 remains speculative in the context of myocardial ischemia. Activated TLRs trigger intracellular signaling which mainly depends on the recruitment of the adapter protein MyD88 (myeloid differentiation primary response gene 88), responsible for the downstream activation of the transcription factor nuclear factor kappa B and of the Mitogen Activated Protein Kinase (MAPKs) family of signaling proteins. A MyD88-independent pathway is also used by TLR3, which depends on the activation of TRIF (TIR domain-containing adaptor inducing interferon-beta), and by TLR4, which can also recruit TRIF using the adapter TRAM (TRIF-related adapter molecule), resulting in the downstream activation of NF kappa B and IRF3 (Interferon Regulating Factor 3). The activation of NF kappaB, TRIF and IRF-3 induces the transcription of multiple genes encoding inflammatory proteins such as cytokines and chemokines, as well as growth factors, whose release promotes the recruitment of activated leukocytes and a robust inflammatory response in the myocardium.

and VCAM-1 (30), which promote rolling, adhesion and endothelial transmigration of polymorphonuclear cells (PMNs). By generating free radicals, proteases and cytokines, PMNs may amplify cardiomyocyte injury and enhance the degradation of the extracellular matrix, and may thereby participate to the process of adverse remodeling and ventricular dilatation (30). Alternatively, PMNs also contribute to the healing process through their apoptotic death, which is followed by their clearance by macrophages, an event that promotes the release of TGF beta and the resolution of inflammation (see below) (3).

With respect to mononuclear cells, two distinct subsets are sequentially recruited within the infarcted myocardium. Ly-6C hi monocytes, which express the MCP-1 (monocyte chemoattractant protein-1) receptor CCR2, dominate the early phase, with potent phagocytic and pro-inflammatory actions, whereas Ly-6C lo cells, expressing the fractalkine receptor CX3CR, are recruited later and promote healing via profibrotic and angiogenic properties (31). About half of the monocytes acutely recruited within the infarct originate from a splenic reservoir (32), in a process which is highly dependent on angiotensin II (33). Accordingly, this may explain, at least in part, the beneficial effects of angiotensin-converting enzyme inhibitors against adverse post-MI remodeling. Indeed, administration of enalapril to mice exposed to MI reduced the splenic release and myocardial recruitment of monocytes, translating into a reduced inflammation and improved left ventricular function three weeks after MI (33).

The role of chemokines in post-MI inflammation has been extensively investigated in the past few years (see Table 1). They exist as 4 different subfamilies (CXC, CC, CX3C and XC chemokines) promoting the migration, growth and activation of leukocytes, with additional roles in the processes of angiogenesis and fibrosis (3). CXC chemokines bearing the ELR motif are PMNs attractants with pro-angiogenic properties, which comprise primarily IL-8 (CXCL8) and its murine counterparts MIP-2, KC and LIX (CXCL2, CXCL3, CXCL5) (3). ELR-negative CXC chemokines have divergent roles and include IP-10 (CXCL10) and Stromal cell-derived factor-1 (SDF-1, CXCL12). IP-10 is a potent angiostatic and antifibrotic factor transiently released in the first 24 hours after MI, which acts as an essential inhibitory signal regulating the cellular composition of the healing infarct and promoting wound contraction, thereby attenuating adverse remodeling (34). SDF-1 attracts CD34+ progenitor cells and has been associated with improved cardiomyocyte viability and angiogenesis after MI (3, 35). With specific respect to SDF-1, a recent experimental study reported that the administration of a protease-resistant form of SDF-1 after MI resulted in significant improvement in angiogenesis and ventricular function, suggesting that it might represent a useful strategy to prevent post-MI heart failure (36). Since SDF-1 binds to the receptor CXCR4, experiments have been conducted using mice with a conditional deletion of CXCR4 in cardiac myocytes. Interestingly, mice bearing such deletion displayed similar post-MI remodeling than WT animals, implying that the beneficial effects of SDF-1 do not depend on interactions with cardiomyocytes, but with other CXCR4-expressing cells, such as endothelial cells (37).

CC chemokines attract mononuclear cells, and have further actions in angiogenesis and in the modulation of fibroblast activity (29). The major member of this subfamily is MCP-1, a monocyte chemoattractant which also enhances the expression of cytokines and growth factors, and promotes the accumulation of myofibroblasts in the infarcted myocardium. Mice lacking MCP-1 or its receptor, CCR2, have reduced inflammation and adverse remodeling after MI (38, 39). In humans, MCP-1 appears to have a dichotomous role after MI, by helping early infarct healing but potentiating later remodeling (40). Finally, two recent experimental works have highlighted the key role of another member of the CC chemokine family, namely CCL5 or RANTES, in the development of post-MI heart failure (41). In a first study, mice with genetic deletion of CCR1, the CCL5 receptor, disclosed reduced neutrophil infiltration, decreased apoptosis and increased cell proliferation with earlier myofibroblast population in the infarcted tissue, together with an attenuated functional impairment of the left ventricle (42). In the second study, treatment of mice with a neutralizing anti-CCL5 monoclonal antibody during the early phases of myocardial ischemia reduced infarct size, decreased neutrophil and macrophage recruitment in the infarcted myocardium and promoted an increase in cardiomyocyte size at three weeks after MI, that translated into an improved left ventricle ejection fraction and survival rate (41).

4. RESOLUTION OF INFLAMMATION AND INDUCTION OF REPAIR PROCESSES

The ultimate goal of the early inflammatory response described above is to promote wound healing of the acutely injured myocardium. This process aims initially at the degradation of extracellular matrix (ECM) and the removal of dead cells and ECM debris. It is followed by a phase of inflammation resolution and tissue repair, characterized by increased synthesis of ECM and fibroblast proliferation leading to the formation of a scar (5). Mononuclear phagocytes play key roles in these processes, first to clear debris and secondly to promote angiogenesis and the accumulation of myofibroblasts within the injured tissue. A key mechanism initiating the switch between inflammation and repair is phagocytosis of apoptotic PMNs and clearing of the early granulation tissue, which convert cardiac macrophages from an inflammatory to a reparative state, characterized by the production of the potent anti-inflammatory cytokines IL-10 and TGF beta (3). Interestingly, the anti-inflammatory effects of apoptotic cells can be mimicked by pharmacological interventions using phosphatidylserine-presenting liposomes. Indeed, the uptake of such liposomes by cardiac macrophages triggers the secretion of high levels of TGF beta and IL-10, associated with a concomitant downregulation of proinflammatory mediator. In a rat model of acute MI, it has been thus recently reported that the intravenous injection of phosphatidylserine-presenting liposomes accelerated myocardial repair by the induction of angiogenesis, thereby preventing ventricular dilatation and adverse remodeling (43).

IL-10 represses the expression of inflammatory cytokines, and triggers the expression of the tissue inhibitor of metalloproteinases-1 (TIMP-1), which promotes stabilization of the ECM (3). TGF beta, produced as a latent complex cleaved by ECM proteases such as MMPs or the matrix protein thrombospondin-1, triggers intracellular signaling dependent on the Smad proteins (most notably Smad3) to suppress cytokine and chemokine expression, reduce leukocyte-endothelial interactions, promote context-dependent angiostatic or angiogenic effects, and favor the development of a fibrous scar, as extensively reviewed in (44). Mice with genetic deletion of Smad3 indeed display reduced interstitial fibrosis in the non-infarcted myocardium and attenuated cardiac remodeling after myocardial ischemia and reperfusion (see Table 1) (45).In fibroblasts, TGF beta reduces the expression of MMPs and enhances that of TIMP-1, thereby suppressing ECM degradation. Furthermore, TGF beta promotes the differentiation of fibroblasts into myofibroblasts, which are transiently present within the post-MI granulation tissue (see below) (44).

Another key aspect of myocardial repair is the induction of angiogenesis. An important network of immature capillaries develops early in the granulation tissue, stimulated by angiogenic factors such as VEGF (Vascular Endothelial Growth Factor) and angiopoietin, as well as TGF beta and chemokines, followed by the proliferation of mature neovessels with a muscular coat (3).

These sequential processes need to be particularly well controlled, both spatially and temporally, to avoid maladaptive responses with deleterious consequences on the heart. Sustained expression of inflammatory mediators, dysregulated angiogenesis and fibrosis, may promote inflammatory and fibrotic changes in the remote, non-infarcted regions, initiating progressive myocardial hypertrophy and chamber dilatation ultimately leading to heart failure (4).

5. INNATE IMMUNE MECHANISMS IN POST-ISCHEMIC MOYCARDIAL INFLAMMATION

The innate immune system functions as a sensor of danger signals from exogenous (pathogens) or endogenous sources, triggering immediate, non specific, inflammatory responses and the initiation of tissue repair processes, as summarized in Figure 1. Innate immunity involves a complex set-up of specialized cellular receptors (termed pattern-recognition receptors, or PRRs) able to recognize "danger signals", coupled to cell signaling pathways whose activation promotes non-specific inflammation. PRRs are broadly separated into membrane-associated receptors, primarily the Toll-like receptor family (TLRs), and intracellular receptors, primarily the nuclear oligomerization domain (NOD)-like receptors (NLRs), which induce the formation of multiprotein complexes termed inflammasomes, and the production of mature IL-1 beta and IL-18, as exposed below (46, 47).

5.1. Mechanisms of signal transduction in innate immunity

5.1.1. The toll-like receptor-nuclear factor kappa B signal transduction system

TLRs are type I transmembrane proteins composed of an extracytoplasmic, leucine-rich repeats (LRR) domain for ligand recognition, a short transmembrane domain, and a cytoplasmic Toll/interleukin-1 receptor (TIR) homology domain for signal transduction. So far, 10 and 12 functional TLRs have been identified in humans and mice, respectively (46). TLRs recognize both exogenous danger signals from pathogens (pathogen-associated molecular pattern, PAMPs), and endogenous danger signals emanating from host molecules (danger associated molecular patterns, DAMPs) (48). Prototypical PAMPs include bacterial endotoxin (the ligand of TLR4), peptidoglycan (the ligand of TLR2) or flagellin (which binds to TLR5). Endogenous ligands of TLRs include either normal cell constituents released by dying cells or components of the ECM released by the action of proteases at the site of tissue damage (see below) (49). Upon ligand binding, TLRs elicit complex intracellular signaling initiated by the recruitment of adaptor proteins, most notably the TIR domain–containing adaptor molecule MyD88 (myeloid differentiation primary response gene 88), which associates with proteins belonging to the IL-1 receptor-associated kinase (IRAK) family. As shown in Figure 1, Phosphorylation of IRAK proteins promotes downstream activation of further intermediates, leading to the activation of nuclear factor kappa B (NF-kappaB), mitogen-activated protein kinases (MAPKs: ERK, JNK, and p38) and the transcription factor activator-protein-1 (AP-1), which regulate a large number of genes involved in inflammation and immunity (46). TLR3 and TLR4 also trigger a MyD-88-independent pathway, which depends on the activation of the TIR domain-containing adaptor inducing interferon-beta (TRIF) and downstream signaling to the transcription factor interferon regulatory factor (IRF)3, which induces genes such as that encoding interferon (IFN)-beta. TRIF is used either directly in the case of TLR3, or indirectly in the case of TLR4, which uses TRIF-related adapter molecule (TRAM) protein to recruit TRIF (50).

NF-kappaB is a family of dimeric proteins that belong to the Rel family, including NF-kappaB1 (p50/p105), NF-kappaB2 (p52/p100), p65, RelB and c-Rel (51). The commonest dimer is formed from a p50 and a p65 subunit. NF-kappaB is normally retained in the cytoplasm, bound to an inhibitory protein, I-kappaB, the most common being I-kappaB alpha (52). NF-kappaB activation relies in its dissociation from I-kappaB, due to stimulus-induced I-kappaB serine phosphorylation, followed by its polyubiquitination and proteasomal degradation. In turn, NF-kappaB translocates into the nucleus to activate the transcription of multiple pro-inflammatory (as well as several anti-apoptotic) genes (53). The process of I-kappaB phosphorylation is governed by a protein kinase complex, I-kappaB kinase (IKK), composed of two catalytic subunits, IKKalpha and IKKbeta, and a regulatory subunit, IKKgamma (52). A considerable variety of stimuli activate IKK and downstream NF-kappaB signaling, comprising inflammatory cytokines such as TNF alpha and IL-1 beta, the TLR-MyD88 system, as well as genotoxic, physical or chemical stress factors, including oxidative stress, as exposed later in this review (54).

5.1.2. Signal transduction by nuclear oligomerization domain (NOD)-like receptors and inflammasomes

NLRs (at least 22 different human genes) represent a family of intracellular PRRs sensing both PAMPs and DAMPs. They share a common structure characterized by a C-terminal leucin-rich repeat domain (for ligand sensing), a central nucleotide-binding and oligomerization (NACHT) domain (for activation), and an N-terminal caspase-recruitment (CARD) or pyrin (PYD) domain (for downstream signaling) (55). NLRs are subdivided into 3 subfamilies, NODs, NLRPs, and IPAF. NODs activate NF-kappaB in response to intracellular bacterial breakdown products. NLRPs and IPAF members sense multiple microbial or endogenous signals, to trigger the assembly of multiprotein complexes termed "inflammasomes", which activate pro-inflammatory caspases, especially caspase-1, and promote the processing of pro-IL1 beta (and pro-IL-18) into mature IL-1 beta and IL-18 (47). The best characterized inflammasome is the NLRP3 (NALP3) inflammasome, a molecular platform comprising oligomerized NLRP3 assembled with the PYD and CARD-containing adaptor ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain), and pro-caspase-1. Subsequent autocleavage of pro-caspase-1 forms active caspase-1 (56). The NLRP3 inflammasome is activated in response to multiple pathogens (bacteria, viruses and yeasts), many endogenous

danger signals including extracellular ATP, high glucose, and hyaluronan, by exogenous irritants (eg. asbestos, silica), and endogenous crystal structures (e.g. monosodium urate and cholesteryl esters). Although debated, it is assumed that NLRP3 activation might be related to the generation of reactive oxygen species as a common mechanism (see below) (47, 57).

5.2. Major processes modulating innate immune responses in the infarcted myocardium

5.2.1. Activation of nuclear factor kappa B

Substantial evidence has accumulated that NF-kappaB activation is instrumental in post-MI inflammation, as extensively reviewed in (58). Morishita and coworkers were the first to identify a detrimental role of NF-kappaB in the infarcted heart. These authors transfected rats exposed to myocardial ischemia with synthetic double stranded DNA with high affinity for NF-kappaB (NF-kappaB cis element "decoys") and found a significant reduction of infarct size (59). Using the same approach, Sawa *et al.* demonstrated a reduced myocardial production of IL-8 and neutrophil trafficking, leading to an improved left ventricular function after coronary artery ligation in the rat (60). Comparable findings were then obtained by Moss *et al.*, who found that blockade of NF-kappa B signaling by an inhibitor of IKK activity reduced infarct size, improved cardiac function and decreased serum levels of TNF alpha and IL-6 in mice (61). In murine models of post-MI heart failure, the genetic deletion of the p50 subunit of NF-kappaB (see Table 1) protected from cardiac remodeling and left ventricular dysfunction up to 8 weeks after MI (62, 63). In line with these findings, transgenic mice with a myocyte-restricted overexpression of a phosphorylation-resistant IkappaB alpha displayed persistent myocyte NF-kappaB activation after MI and acceleration of adverse cardiac remodeling via pro-inflammatory, pro-fibrotic, and pro-apoptotic effects (64).

The detrimental role of NF-kappaB signaling exposed above has been recently challenged by a series of studies implicating NF-kappa B more as a protective than a cytotoxic signaling pathway in the injured myocardium. NF-kappaB not only activates genes involved in inflammation and hypertrophy, but is also an essential transcriptional activator of many anti-apoptotic genes, including cellular inhibitors of apoptosis (c-IAPs), and Bcl2 (65), while being a suppressor of pro-apoptotic genes such as BNIP3 (66). *In vitro*, NF-kappaB is essential to prevent apoptosis of hypoxic cardiomyocytes (66, 67). *In vivo*, transgenic mice with defective NF-kappaB activation produced by cardiac restricted overexpression of a mutated I kappaB alpha, exhibited larger infarct size due to massive cardiomyocyte apoptosis (68). Furthermore, targeted deletion of NF-kappaB p50 in mice resulted in enhanced matrix remodeling and myocardial inflammation with functional deterioration following MI, (69), in striking contrast with the previously discussed studies using the same mouse model (62, 63). Although such contradictory and discrepant roles of NF-kappaB appear difficult to reconcile, a plausible explanation, relying on distinct time-courses of NF-kappaB activation within the infarcted myocardium was recently proposed by Gordon *et al* (58). The early, acute NF-kappaB activation, might be essential to prevent apoptosis and to preserve myocyte number, whereas its persistence would contribute to the perpetuation of the inflammatory process and the promotion of maladaptive ventricular remodeling (58).

5.2.2. Activation of toll-like receptor signaling

The heart expresses several TLRs, including TLR 2, 3, 4, 5, 7 and 9, and pathogen-derived ligands of TLR 2, 4, 5 and 9 can activate NF-kappaB-dependent inflammatory signaling in cardiomyocytes and promote cardiac dysfunction *in vivo*, indicating that the heart possesses an intact TLR-dependent signaling machinery (6, 70-73). Cardiac TLRs are also increasingly contemplated as central actors modulating NF-kappaB -dependent immune responses in the heart, independently from any infectious process, most especially following MI (see (48, 74) for extensive recent reviews).

A seminal work by Frantz *et al.* indicated that cardiac TLR4 expression is upregulated after MI in mice (75), particularly in remodeling myocardium remote from sites of ischemic injury. In the same study, the authors also provided evidence of intense TLR4 expression in focal areas of cardiac tissue obtained from patients with dilated cardiomyopathy (75). In another human study, TLR4 expression, as well as expression of TNF alpha, IL-1 beta and IL-6, were significantly greater in the myocardium of rapidly deteriorating patients with heart failure in comparison to more stable patients (76). In a recent experimental work in a pig model of MI, TLR4 expression and TLR4-dependent signaling were upregulated both in the ischemic and non ischemic myocardium up to 6 days after MI (77).

A direct participation of TLR4 to the elaboration of post-ischemic inflammation has been proven by the use of various strategies blocking TLR4 signaling (Table 1). Mice expressing a non functional TLR4 (C3H/HeJ mice) (78, 79), like mice treated with the TLR4 antagonist eritoran (80), showed reduced myocardial NF-kappaB activation and cytokine production after MI. Two studies in TLR4 KO mice further demonstrated reduced cardiac remodeling with an improved cardiac function and increased survival after MI (81, 82). In a model of cardiac hypertrophy induced by aortic banding, it is also noticeable that TLR4 deficient mice disclosed much less NF-kappaB-dependent cardiac hypertrophy than TLR4 competent animals (83).

Besides TLR4, TLR2 has also been implicated in the process of myocardial damage and adverse remodeling after MI (Table 1). TLR2 KO mice displayed reduced myocardial injury, decreased production of reactive oxygen species and leukocyte infiltration, and were protected against postischemic coronary endothelial dysfunction (84), myocardial inflammatory changes and left ventricular remodeling after MI (85, 86). These beneficial consequences appeared essentially related to the suppression of TLR2 signaling in circulating, but not parenchymal (i.e. cardiac/endothelial) cells, as demonstrated by the use of chimeric mice (87).

As stated above, TLR signaling depends on the recruitment of downstream adapters, most especially MyD88, and, to a lesser extent, TRIF (in the case of TLR3 and TLR4). With this respect, experimental evidence exists implicating MyD88 in the pathogenesis of MI (88), whereas the responsibility of Trif has not been established so far (89). Adenoviral transfer of dominant negative MyD88 to rats significantly reduced infarct size, markedly reduced NFkappaB activity and increased the anti-apoptotic protein BCL-2, resulting in a 60% reduction of cardiac myocyte apoptosis (90). Suppression of MyD88 by pharmacological inhibitors, or by MyD88-targeted siRNA, also protected against left ventricular dilatation and hypertrophy in mice following MI (91). In fact, signaling through MyD88 appears critical for the early inflammatory changes after MI, as indicated by the marked reduction of neutrophil recruitment in MyD88 KO mice subjected to myocardial ischemia-reperfusion (92). Importantly, reduced infarct size and neutrophil infiltration could be reproduced in chimeric mice lacking MyD88 in bone marrow-derived circulating cells but with normal MyD88 signaling in the heart (89). By contrast, these chimeric mice had similar increases in the expression of various chemokines in the myocardium when compared to wild-type animals. Therefore, MyD88 signaling in circulating cells appears essential to the development of myocardial I/R injury by maintaining normal neutrophil migratory function, whereas chemokine production in the heart appears mainly determined by myocardial MyD88 expression (89).

5.2.3. Role of endogenous danger signals (danger associated molecular patterns, DAMPs)

As mentioned previously, the passive release of components (termed Danger Associated Molecular Patterns, or DAMPs) from necrotic cells or from the ECM might serve as a diffusible signal to activate TLR-dependent signaling in the infarcted myocardium (93) (see figure 1). The availability of DAMPs for immune activation may result from three basic mechanisms, including cell necrosis and passive DAMP release, regulated cell secretion of DAMPs, and liberation of ECM components by enzymes (49). Recent evidence has indicated that the protein high mobility group box-1 (HMGB1) is an essential danger signal molecule regulating innate immune responses after MI (94). HMGB1 is a nuclear protein implicated in the binding of transcription factors to their cognate DNA sequences. HMGB1 also acts as a cytokine secreted by monocytes, and is passively released from necrotic cells to promote NF-kappaB-dependent inflammatory responses in neighbor cells (95). Such responses are related to HMGB1 binding to several classes of cell receptors, including TLR2 and TLR4, as well as RAGE (receptor for advanced glycation end-products) (96).

In a murine model of MI, Andrassy *et al.* showed that HMGB1 levels in the myocardium were already elevated 30 minutes after the induction of ischemia. Treatment of mice with a functional antagonist of HMGB1 reduced, whereas the systemic administration of HMGB1 enhanced, myocardial inflammation, cardiac dysfunction and adverse remodeling (94). These effects were not observed in RAGE KO mice, substantiating the essential role of this receptor in conveying the pro-inflammatory actions of HMGB1 (Table 1) (94). A further role of HMGB1 during MI has been recently proposed by Xu *et al.*, who found that HMGB1 served the purpose to amplify apoptotic cell death in cardiomyocytes, by a mechanism of facilitation of TNF alpha-dependent activation of the pro-apoptotic JNK signaling pathway (97). The latter was dependent on HMGB1 interacting with TLR4, as identified in experiments using TLR4-deficient myocytes (97). It is also worth to mention that plasma HMGB1 levels rise after MI in humans, and that the highest levels appear correlated with the worse prognosis in this setting (98).

In contrast to the above observations, Kitahara *et al.* reported that transgenic mice with cardiac overexpression of HMGB1 exhibited smaller infarcts, improved cardiac function and survival rates after MI (99), whereas Kohno *et al.* showed that a neutralizing HMGB1 antibody given to rats after MI aggravated left venricular remodeling (98). Several plausible mechanisms may explain these results in favor of a beneficial, instead of detrimental role of HMGB1 after MI. First, HMGB1 represents an important stimulus for angiogenesis and for the proliferation and differentiation of cardiac stem cells, by promoting the synthesis of various cytokines and growth factors by cardiac fibroblasts (100, 101) (see below). Secondly, HMGB1 can reduce cardiac fibrosis and cardiomyocyte hypertrophy after MI by attenuating the recruitment of dendritic cells in the peri-infarct area (102). The reasons for the contrasted roles of HMGB1 remain so far incompletely understood, although the different experimental models used (ischemia-reperfusion or permanent ischemia) may be partly responsible. It is also highly likely that the time window of HMGB1 inhibition may critically influence the outcome. On the one hand, the early “burst” of HMGB1 appears mainly associated with detrimental pro-inflammatory actions that would be favorably influenced by anti-HMGB1 strategies (94). On the other hand, at later time-points, HMGB1 might principally promote “reparative” actions, by favoring cardiac regeneration (100, 101)and should therefore not be suppressed by therapeutic interventions. Future studies will be critical to address these issues and provide answers regarding the potential therapeutic application of pro- or anti-HMGB1 therapy after MI.

Besides HMGB1, many additional molecules may act as DAMPs activating TLR (or RAGE)-dependent innate immune response after MI. The calcium regulating protein S100B is upregulated in cardiac myocytes of the peri-infarct area in close relationship with RAGE, its cellular receptor, and is passively released in the extracellular milieu. In such conditions, S100B has been shown to promote myocyte apoptosis in an ERK- and p53-dependent manner (103). Several heat shock proteins (HSPs), which naturally function as molecular chaperones, are also released by damaged cells and may act as danger signals. The ischemic myocardium notably releases large amounts of HSP 60 and HSP 70, which have been identified as endogenous TLR ligands. Indeed, recent

studies have identified HSP 60 as a potent mediator of TLR4-dependent apoptosis and inflammation in cardiac myocytes under ischemic conditions (104, 105) and HSP 70 has been recently shown to elicit myocardial inflammatory responses and cardiac dysfunction after global ischemia-reperfusion in the mouse heart, in a TLR4-dependent manner (106). The myofibrillar protein myosin is a further intracellular component able to promote innate immune responses in cardiac myocytes, via mechanisms dependent on TLR2 and TLR8 signaling (107). Various ECM components, including fibronectin EDA, fibrinogen, hyaluronic acid and biglycan may also contribute to activate innate immune mechanisms in the infarcted heart following their binding to TLR2 and/or TLR4 (108, 109). For instance, recent studies have indicated that fibronectin EDA, a particular form of the extracellular matrix protein fibronectin, was markedly overexpressed in the heart in murine models of MI, and that EDA KO mice exhibited reduced inflammation, attenuated MMP 2 and 9 activity, decreased fibrosis and less contractile dysfunction than WT animals, after MI (108). Finally, self nucleic acids and ribonucleoprotein complexes, including mitochondrial–derived DNA such as unmethylated cytosine−phosphate−guanine (CpG) dinucleotides, which can bind TLR3 or TLR9, should also be considered as possible activators of innate immune mechanisms in the ischemic myocardium (48, 72, 110).

5.2.4. Activation of the inflammasome

The NLRP3 inflammasome can be activated by necrotic cells to promote a localized inflammation (111), and it may facilitate the release of HMGB1 by necrotic cells (112), which suggests that it might greatly contribute to the sterile inflammation initiated by tissue injury such as MI. It is also noteworthy that the NLRP3 inflammasome is activated in the wall of blood vessels by cholesterol crystals, promoting inflammation and atherogenesis (113), substantiating a major role of NLRP3 in pathophysiological processes in the cardiovascular system. Experimental studies investigating the role of inflammasome activation in ischemic cardiac diseases are scarce. Mice deficient in caspase-1, a fundamental component of the inflammasome, disclosed decreased rate of left ventricle dilatation after MI (114) and blocking IL-1 beta signaling (the final product of inflammasome activation) with recombinant IL-1 receptor antagonist (anakinra) or by IL-1 receptor deletion, reduced myocardial injury, inflammation and remodeling after MI in animals (15). Treatment with anakinra has been also reported to favorably affect left ventricular remodeling after MI in a small pilot clinical study (20), which hopefully will be confirmed in large scale randomized trials. The most convincing report supporting the involvement of the inflammasome has been recently published by Kawakuchi and co-workers (115). Using *in vivo* and *in vitro* approaches, these investigators found that inflammasome activation was restricted to cardiac fibroblasts, but did not affect cardiomyocytes, and was mediated through reactive oxygen species production and potassium efflux. In mice deficient for apoptosis-associated speck-like adaptor protein and caspase-1, two key components of the inflammasome, myocardial inflammation, fibrosis, and cardiac dysfunction were all markedly diminished (115). Therefore, these observations indicate that cardiac fibroblasts may mediate inflammation after MI, and support a crucial role of the inflammasome in this setting. Additional studies, using NLRP3 deficient cells and animals, will help to gain further insights into such roles of the inflammasome and to determine whether it may become a suitable therapeutic target in ischemic cardiac diseases.

5.2.5. Role of oxidative stress

Free radicals have an unpaired valence shell electron and are thus highly reactive, by substracting electrons from other molecules to stabilize their conformation (116). Two main families of radicals exist, oxygen-centered (reactive oxygen species, ROS) or nitrogen-centered (reactive nitrogen species, RNS) (117). During MI, especially following reperfusion, a burst of superoxide radical ($O2^{-}$) arises from mitochondria, NADPH oxidases, cyclooxygenase, activated neutrophils, and xanthine oxidase (118). RNS are also formed in excess, due to an enhanced generation of nitric oxide ($NO^{\cdot}$) by distinct NO synthases (NOS), including endothelial (eNOS), and inducible NOS (iNOS) (119). During ischemia and early reperfusion, $NO^{\cdot}$ mainly derives from stimulated eNOS activity as a result of acute increases in intracellular calcium, whereas it is mainly formed from iNOS in the delayed phase of injury, consecutively to NF-kappaB-dependent iNOS induction by infiltrating leukocytes and other cell types including cardiac myocytes (120, 121). The simultaneous generation of $NO^{\cdot}$ and $O2^{-\cdot}$ triggers the formation of a more cytotoxic oxidant, peroxynitrite, which promotes myocardial necrosis by activating poly(ADP-ribose) polymerase (PARP) (118), and apoptosis via activation of caspase-3 (122). Peroxynitrite (and other oxidants as well) also activates MMPs, promoting detrimental ECM remodeling (123), and can precipitate cardiac dysfunction by nitrating tyrosine residues within metabolic enzymes, ion channels and structural protein in cardiomyocytes (118, 123). In addition to these direct damaging potential, ROS and RNS can also promote a pro-inflammatory state. Although the mechanisms linking oxidative stress to inflammation remain only partly understood, three main principles can be postulated and will be briefly exposed below, namely (a) the direct activation of pro-inflammatory signal transduction cascade, especially NF-kappaB and the MAP kinases, (b) the promotion of cellular release of DAMPs, (c) the activation of the NLRP3 inflammasome.

Reactive oxidants act as potent second messengers in signal transduction, a concept termed “redox signaling” (124). NF-kappaB is generally viewed as such a redox-sensitive signaling pathway (125). During MI, anti-oxidants reduce NF-kappaB activation and decrease myocardial inflammation (126), supporting a link between ROS/RNS and NF-kappaB in this setting. It is worth to mention however that the redox regulation of NF-kappaB may be more complex than initially anticipated, as under certain conditions, oxidants inhibit, instead of activate, NF-kappaB (124). Using cardiac and endothelial cell lines, we thus showed that oxidative stress prevented NF-kappaB activation in response to an immune stimulus by inactivating IKK, the upstream kinase in the NF-kappaB

activation cascade (see above) (127). More recently, we demonstrated that the redox regulation of NF-kappaB is, in fact, totally context-dependent. In unstimulated cells, oxidants promote IKK inactivation, resulting in NF-kappaB inhibition, whereas in cells primed by an immune stimulus, oxidants amplify and prolong NF-kappaB activation via a mechanism involving the inactivation of the phosphatase PP2A. Therefore, oxidants may either inhibit or amplify NF-kappaB activation, depending on its initial state of activation (117).Whether a similar context-dependent regulation of NF-kappaB by oxidants exists during MI *in vivo*, remains to be investigated.

Oxidants may also indirectly trigger innate immune responses by promoting the release of DAMPs by stressed cells. We recently showed that peroxynitrite elicited significant cardiomyocyte necrosis *in vitro*, which was associated with the loss of intracellular HMGB1 and its passive release into the medium (96). In an *in vivo* model of myocardial ischemia-reperfusion in rats, we then found that peroxynitrite, generated in the reperfused myocardium, was responsible for a marked overexpression of HMGB1. Indeed, suppression of peroxynitrite accumulation with various pharmacological scavengers completely prevented HMGB1 upregulation (96). In an unrelated study performed in hypoxic hepatocytes, Tsung *et al.* also reported that the generation of ROS in response to hypoxia triggered the mobilization and release of HMGB1, which appeared consecutive to ROS-dependent activation of calcium/calmodulin-dependent protein kinases (CaMKs) (128). Interestingly, it has been proposed that TLR activation might amplify ROS generation through direct interactions between TLRs and the superoxide producing enzyme NADPH oxidase (128). The produced ROS would then favor the release of additional HMGB1, leading to further TLR activation, thereby forming a paracrine loop of progressive amplification of inflammation (49).

Finally, oxidants may also be linked to inflammation through their role in the process of NLRP3 inflammasome activation. Indeed, all NLRP3 activators promote the generation of ROS in target cells, through mechanisms involving potassium efflux, NADPH oxidase activation, and the release of cathepsin B by damaged lysosomes. In addition, it has been established that NLRP3 inflammasome assembly can be abrogated by antioxidant strategies (57, 129). The mechanisms underlying NLRP3 inflammasome activation by ROS may include signaling through the phosphatidyl inositol-3 kinase (PI3K) pathway, as well as the association of NLRP3 with thioredoxin-interacting protein, following its dissociation from its inhibitor thioredoxin upon an increase in cellular ROS concentration (57, 129). Although not yet investigated, it is a likely hypothesis that such interactions between oxidants and NLRP3 inflammasome may represent an important mechanism linking the generation of ROS/RNS with innate immune response in the infarcted myocardium.

6. ROLE OF CARDIAC FIBROBLASTS IN POST-ISCHEMIC MOYCARDIAL INFLAMMATION

The adult heart consists of ~ 30% cardiomyocytes and 70% non myocytes (fibroblasts, endothelial and vascular smooth muscle cells). Cardiac fibroblasts (CFs), account for ~ 60% of all cardiac cells. CFs arise during embryonic development from epicardial-derived mesenchymal cells and from hematopoietic stem cells (130). In the adult heart, CFs are replaced by endogenous CFs, but may also arise from epithelial cells through mesenchymal transformation, from bone-marrow progenitors termed fibrocytes and from pericytes surrounding endothelial cells (130). The primary function of CFs is to maintain the integrity of the ECM, a three dimensional network containing collagen, proteoglycans, glycoproteins, and proteases, which surrounds and connects the cellular components, and which maintains the structural, electrical and mechanical stability of the heart (131). Furthermore, CFs are an essential sources of multiple cytokines and growth factors which are critical in the process of ECM turnover (131). ECM homeostasis is ensured by a coordinated balance between proteolytic MMPs and their natural inhibitors, the TIMPs, both expressed by the CFs. CFs interact with the ECM through integrins and the cell surface receptor DDR2 (discoidin domain receptor 2), and they establish intercellular communications with other CFs, as well as with cardiomyocytes, through connexins and cadherins (130). It has been proposed that these cell-cell interactions especially occur within a fundamental functional unit, termed the “cardiovascular unit”, which includes cardiomyocytes, CFs and endothelial cells in a proportion of 2/1/1. In such unit, the CF can dynamically respond to many chemical, mechanical and electrical signals to maintain the proper form and function of the heart (132).

The inflammatory environment which builds up after MI promotes the proliferation and activation of CFs, which undergo transformation into myofibroblasts. Myofibroblasts exhibit phenotypic characteristics of smooth muscle cells, notably expressing α-smooth muscle actin (3). Key mechanisms leading to myofibroblasts differentiation include the secretion of TGF beta (see above), mechanical stress and the return to normoxic conditions after the initial ischemic hypoxia (3, 133). Myofibroblasts become highly proliferative and invasive, secrete multiple cytokines, growth factors and actively remodel the ECM by increasing secretion of MMPs and collagen, as extensively reviewed in (133). Myofibroblasts are abundant within the early granulation tissue, but they undergo apoptosis during maturation of the scar, through yet poorly defined mechanisms (130, 131). In the early phase of healing, CFs have reduced collagen synthesis with increased expression of MMPs, whereas in the later phase of healing, proliferation of fibroblasts occur together with increased TIMP expression, allowing scar stabilization. At the end of the healing process, fibroblasts are cleared and leave a collagen-based scar (133). These processes may become maladaptive in conditions of persistent or uncontrolled inflammation, overcoming the regulatory mechanisms of healing. In turn, persistent activation of CFs and myofibroblasts leads to adverse remodeling and an extension of the fibrotic changes in areas remote from the initial site of injury (130, 131).

The involvement of activated CFs and myofibroblasts in the elaboration of the sterile inflammation following MI has only recently began to attract attention. These cells can secrete large amounts of cytokines, such as TNF alpha, IL-1 beta, IL-6 and TGF beta, as well as growth factors and vasoactive peptides including angiotensin II, endothelin-1, natriuretic peptides and VEGF in response to cytokines, hypoxia, and mechanical stress (130, 131). Furthermore, CFs appear particularly sensitive to the pro-inflammatory signals triggered by HMGB1, as shown by their stimulated production of a large amount of cytokines and growth factors in response to HMGB1 *in vitro* (101), suggesting that they might represent key components of the response to danger molecules released by necrotic cardiomyocytes during MI. In addition, recent evidence indicates that inflammasome activation in cardiac fibroblasts is a critical pro-inflammatory mechanism after myocardial ischemia and reperfusion (see above) (115). Altogether, these results clearly indicate that CFs play a much more active role in the development of myocardial inflammation after MI than previously anticipated. Novel studies are under way to precisely delineate this emerging role of CFs, and therapeutic strategies targeting specifically this cell population might be developed in the future to modulate post-ischemic immune response in the heart.

7. INFLAMMATION AND CARDIAC REGENERATIVE PROCESSES AFTER MYOCARDIAL INFARCTION

The regenerative capacities of the heart, once believed a terminally differentiated organ, have been unraveled in the past decade (134). The heart is indeed in continuous turnover, and cycling myocyte precursors undergoing mitosis and cytokinesis can be detected both under normal and pathologic conditions (135, 136). The mechanisms involved in the formation of new myocytes are not yet understood but re-entry of the terminally differentiated myocytes into the cell cycle, as well as differentiation of stem cells into cardiomyocytes, might both contribute to this process (137-139). Although a detailed presentation of the mechanisms involved in cardiac regeneration is beyond the scope of this review, some key issues regarding the interrelations between inflammation and regenerative processes following MI will be briefly developed in the next paragraphs.

Stem cells may originate from the heart itself or from sources outside from the heart. Endogenous cardiac progenitor cells are located in cardiac niches and/or in the epicardium, and they express some “hematopoietic stem cell” markers, such as c-kit (the receptor of the so-called stem cell factor-SCF), and the stem cell-antigen-1 (Sca-1). In addition, cells expressing the transcription factor *islet1*, may represent an additional population of cardiac progenitor cells in the adult heart (140, 141). There is only little information regarding the mechanisms implicated in the proliferation and differentiation of endogenous cardiac stem cells after MI, but it is likely that multiple mediators produced during the post-MI inflammation modulate such responses. Such is the case for HMGB1, which has been shown to act as a potent inducer of resident cardiac c-kit+ cell proliferation (100), as well as for the fibroblast growth factor FGF-2, whose expression is upregulated in the injured myocardium. The latter might indeed play an essential role in the process of differentiation of endogenous stem cells, as suggested by the inability of cardiac progenitors isolated from FGF-2 deficient mice to differentiate into cardiomyocytes *in vitro* (142).

Exogenous cardiac stem cells originate essentially from the bone marrow and belong to the hematopoietic and/or mesenchymal stem cells (143). The chemokines SDF-1 and MCP-1, both markedly upregulated after MI, are essential for the mobilization of exogenous cardiac stem cells and their homing to the damaged heart, by interacting with their receptors, respectively CXCR4 and CCR2 (144-147). Homing of exogenous stem cells to the heart is further promoted by the action of inflammatory cytokines such as TNF alpha, which fosters the expression of functionally active CXCR4 receptors on the surface of mesenchymal stem cells (146, 147). After homing, the exogenous precursors migrate from the blood across endothelial cells into the sites of injury, through mechanisms comprising rolling, adhesion and transmigration, which are governed by the upregulated expression of integrins, selectins and chemokine receptors expressed at the surface of mesenchymal stem cells (148). The adhesion molecule VCAM-1 expressed both on endothelial and mesenchymal stem cells is a further key mediator of these processes, the expression of which is stimulated by the inflammatory cytokines TNF alpha and IL-1 beta (149). Following transmigration within the infarct and peri-infarct myocardium, precursor cells differentiate into mature cardiomyocytes and/or vascular cells through mechanism remaining not elucidated, but seemingly dependent on the expression of c-kit (150, 151). Besides their differentiation capacities, mesenchymal stem cells exert paracrine effects by secreting large amounts of growth factors and cytokines which favor endogenous regeneration by activating resident cardiac stem cells (152, 153). They also promote potent anti-inflammatory and anti-apoptotic effects, notably by secreting IL-10 and prostaglandin E2 which inhibit T cell proliferation and which are essential for the survival of the engrafted cells (154, 155).

The therapeutic use of stem cells is increasingly considered as a potential alternative to drug therapy for ischemic heart diseases. However, isolation and re-injection of some of these precursors in human patients led to disappointing results due to their very low survival and differentiation rates (156). Many problems therefore remain to be solved before such cell-based therapy becomes a clinical standard to reduce the adverse consequences of MI. What is the most suitable cell population, which should be the administration dose, which would be the best timing for cell-based therapy, are some of the key issues that need now to be addressed in preclinical and clinical investigations (156). Future developments will also require a much better understanding of the molecular mechanisms governing the fate of stem/progenitor cells, in order to devise novel strategies able to induce their proliferation,

migration and differentiation within the injured myocardium.

8. SUMMARY AND PERSPECTIVES

The central role of inflammation in the process of myocardial damage, repair and remodeling has been highlighted in the past 15 years. It is now established that such inflammation is orchestrated by the activation of innate immune defenses in response to the severe tissue injury initiated by myocardial ischemia and reperfusion. Still, much remains to be discovered regarding the mechanisms underlying these processes, in order to envision potential innovative therapies for the future. Especially, the sources and nature of the danger molecules triggering innate immune defenses, the cellular receptors and signal transduction pathways targeted by these danger molecules, and the potential role of cardiac fibroblasts in the orchestration of this inflammatory response are major issues requiring investigation. Furthermore, it will be critical to gain further insights into the ramifications between the inflammatory network building up in the injured myocardium and the modulation of cardiac and extra-cardiac stem cells, in order to propose innovative strategies for cardiac regeneration. These tasks must be undertaken now, in view of the worldwide rising epidemics of ischemic heart diseases and chronic heart failure, and they will require, more than ever, the close collaboration between molecular biologists, physiologists, clinical cardiologists and epidemiologists, so that basic discoveries be rapidly translated into therapeutic innovations.

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Abbreviations: CCL: chemokine (C-C motif) ligand; CCR: chemokine (C-C motif) receptor; CXCR: chemokine (C-X-C motif) receptor; DAMP: danger-associated molecular pattern; ECM: extra-cellular matrix; EDA: extra-domain A; ERK: extracellular signal-regulated protein kinase; FGF: fibroblast growth factor; HMGB1: high-mobility group box 1 protein; ICAM-1: inter-cellular adhesion moelcule-1; IkappaB: inhibitor of KappaB; IKK: inhibitor of kappaB kinase; IL: interleukin; IL-1 Ra: interleukin-1 receptor antagonist; IP-10: Interferon-inducing protein 10; JAK-STAT : Janus-activated kinase-signal transducer and activator of transcription; JNK: c-Jun NH2-terminal kinase; MAP kinases: mitogen-activated protein kinases; MCP-1: monocyte chemoattractant protein-1; MMP: matrix metalloproteinase; MyD88: myeloid differentiation primary response gene 88; NF-kappaB: nuclear factor kappaB; NLR: NOD-like receptor; PAMP: pathogen-associated molecular pattern; PMN: ploymorphonuclear cell; PRR: pattern recognition receptor; RAGE: receptor for advanced glycation end-products; RNS: reactive nitrogen species; ROS: reactive oxygen species; SCF: stem cell factor; SDF-1: stromal cell-derived factor-1; TGF beta: transforming growth factor beta; TIMP: tissue inhibitor of metalloproteases; TLR: toll-like receptor; TNF alpha: tumor necrosis factor alpha; VCAM-1: Vascular cell adhesion molecule-1; VEGF : vascular endothelial growth factor

Key Words: Myocardial infarction, Heart, Inflammation, Innate immunity, Toll-like receptor, NOD-like receptor, Inflammasome, Nuclear factor kappaB, Oxidative stress, Remodeling, Cytokine, Chemokine, High-mobility group box-1, Review

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