

REVIEW | *Novel Mechanisms of Myocardial Ischemia, Ischemia-Reperfusion, and Protection by Myocardial Conditioning*

Inflammasome, pyroptosis, and cytokines in myocardial ischemia-reperfusion injury

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Toldo S, Mauro AG, Cutter Z, Abbate A. Inflammasome, pyroptosis, and cytokines in myocardial ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 315: H1553–H1568, 2018. First published August 31, 2018; doi:10.1152/ajpheart.00158.2018.—Myocardial ischemia-reperfusion injury induces a sterile inflammatory response, leading to further injury that contributes to the final infarct size. Locally released danger-associated molecular patterns lead to priming and triggering of the NOD-like receptor protein 3 inflammasome and amplification of the inflammatory response and cell death by activation of caspase-1. We review strategies inhibiting priming, triggering, or caspase-1 activity or blockade of the inflammasome-related cytokines interleukin-1 β and interleukin-18, focusing on the beneficial effects in experimental models of acute myocardial infarction in animals and the initial results of clinical translational research trials.

inflammasome; interleukin-1 β ; interleukin-18; ischemia-reperfusion injury; nucleotide-binding oligomerization domain-like receptor protein 3

THE INFLAMMASOME

The term inflammasome refers to a class of macromolecular structures that are formed from the activation of intracellular receptors for danger- or pathogen-associated molecular patterns (DAMPs or PAMPs, respectively) (15, 130), part of nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), retinoic acid-inducible gene I-like receptors (130), and absent in melanoma 2 (AIM2)-like receptor (123, 130). The evolutionary role of inflammasomes is to link detection of a “danger signal,” in the form of a plethora of molecular structures (microbial, cellular, and organic or inorganic simple molecules), to a specific cellular response that implies activation of the proinflammatory caspase-1 and release of the proinflammatory cytokines IL-1 β and IL-18 (Fig. 1) (123, 130). The various inflammasomes have different ligand specificity and, together, can recognize a wide range of DAMPs and PAMPs (106). As such, inflammasomes are central to innate immunity, a nonspecific first line of defense promptly activated during the course of infections, in immune and nonimmune cells, based on the chemical properties of the antigen. Innate immunity is different from adaptive immunity, which requires activity of specialized cells and produces an antigen-specific response. DAMPs are a class of endogenous (self) molecules, including nucleic acids, nucleotides, proteins,

fatty acids, and inorganic molecules, that, when in the extracellular space, in endocytotic vesicles, or free in the cytoplasm, trigger activation of the innate immune response independently from the presence of the infectious pathogen (112). The response to DAMPs in the absence of a microbial agent is, therefore, defined as “sterile immunity” (112). Congruent activation of the innate immune response in the presence or absence of a pathogen is due to the virtually indistinguishable chemical nature of DAMPs from PAMPs. The most studied inflammasome receptor is NACHT, leucine-rich repeat, and pyrin domain (PYD)-containing protein 3 [NLR protein 3 (NLRP3)], a pattern recognition receptor (PRR) that recognizes bacterial and viral PAMPs as well as numerous DAMPs involved in tissue injury (29, 93, 130). Several intracellular and extracellular stimuli that originate from disruption of cell homeostasis trigger NLRP3 activation, making this protein central in control of the inflammatory response to tissue injury (130). The focus of this review is to describe the activation of the NLRP3 inflammasome, its associated cytokines, and their consequences in response to myocardial ischemia-reperfusion (I/R) injury.

INJURY AND INFLAMMATION IN ACUTE MYOCARDIAL INFARCTION

Acute myocardial infarction (AMI) is one of the most common causes of morbidity, hospitalization, and mortality on a global scale (9). Sudden occlusion of a coronary artery due to rupture of the fibrous cap that overlays a lipid plaque and subsequent

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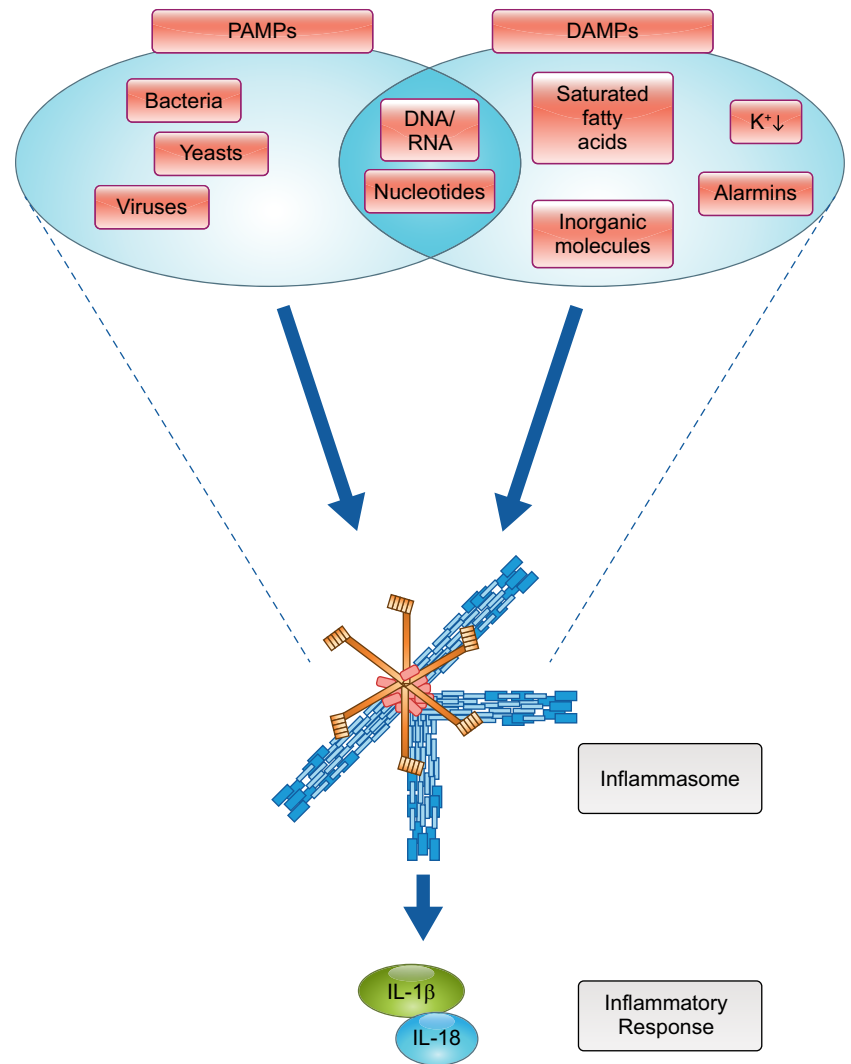


Fig. 1. Inflammasomes react to the presence of danger-associated molecule and activate a specific cytokine pathway. A plethora of molecular structures, stemming from pathogen invasion or host tissue/cell damage, result in a streamlined initiation and activation of the innate immune response. During pathogen invasion, bacterial and viral products, such as lipopolysaccharide, DNA, or RNA, serve as pathogen-associated molecular patterns (PAMPs) to the inflammasome. During tissue damage, molecules of intracellular origin serve as danger-associated molecular patterns (DAMPs) to also activate the subsequent immune response. Activation of the inflammasome induces production and release of active IL-1 β and/or IL-18.

thrombus formation results in immediate downstream reduction of oxygen delivery to the myocardium and intracellular energy that culminates in cardiomyocyte necrosis (26, 50). Restoration of blood flow to the ischemic myocardium, although a standard of care in AMI, can paradoxically compound injury to the myocardium through multiple mechanisms, deemed reperfusion injury (153). AMI represents a prototypical example of sterile injury, in which the NLRP3 inflammasome coordinates an inflammatory response in the absence of pathogens (130). While this response is essential to guarantee healing of the wounded tissue, uncontrolled or exuberant inflammation becomes a mechanism of disease (49). Prompted by local and systemic signals associated with cell damage and necrosis, leukocytes are recruited first to the region of ischemia and infarction (117). After AMI, there is no restitutio ad integrum. In fact, the resultant clearance of cellular debris, together with immune cell proliferation and fibrotic scar formation, leads to an incomplete functional recovery of the affected tissue, compounded by negligible cell cycle entry and regeneration of adult mammalian cardiomyocytes (48, 70, 117). Although the inflammatory response is needed to coordinate these healing phases, a chronic and unresolved inflammatory response leads to continual loss of functional myocytes,

deleterious geometrical abnormalities, and aneurysm formation, worsening the consequences of AMI (23, 52, 117). The activity of the NLRP3 inflammasome is a decisive component of the innate immune response to ischemia, repair, and perpetuation of injury (125, 130).

Danger-Associated Molecular Patterns

Prolonged ischemia induces a loss of membrane integrity, leading to necrosis, and is associated with the release of intracellular contents not found in the normal interstitium (21, 40). Several intracellular proteins with different biological functions are collectively named “alarmins” for their ability to become alarm or danger signals for nearby cells. Identified alarmins include IL-1 α (see below), high-mobility group box 1, S100 family proteins, some heat shock proteins (HSPs), and glucose-regulated proteins (GRPs) (10, 14, 77, 152). A common function of these alarmins is to activate NF- κ B (14). As a protein regulating transcription of pro- and anti-inflammatory genes, NF- κ B is a central orchestrator and key regulator of the inflammatory response and inflammasome pathway after AMI (137). One of the best-characterized alarmins is high-mobility group box 1. This nuclear protein is passively released during

necrosis, leading to paracrine diffusion and binding to Toll-like receptor (TLR)-4, a PRR of the TLR family, or the receptor for advanced glycation end products, increasing NF- κ B activation and perpetuating reperfusion injury (14, 42, 78). The S100 family of proteins also diffuse after necrosis, binding to the receptor for advanced glycation end products and TLR-4 and eliciting NF- κ B activation (41). A similar function has been observed for GRPs (e.g., GRP94/gp96 and GRP170) and HSPs (e.g., hsp70 and hsp90) (12, 83). Among other known DAMPs, extracellular ATP serves as a trigger for aggregation of the components of the NLRP3 inflammasome, leading to the active macromolecular structure (15, 21, 40, 130).

NLRP3: a Sensor That Links Injury to Inflammation

NLRP3 is a member of a family of 14 NLRPs that includes PRRs and proteins not involved in the inflammatory response. NLRP3 is structurally characterized by a domain of leucine-rich repeats assembled at the COOH terminus, a central NATCH domain (also known as NOD), and an NH₂-terminal effector PYD (130). The function of NLRP3 is mostly associated with the inflammasome; however, there have been reports of NLRP3 activity independent of the inflammasome pathway (146, 149). Upon ligand sensing, NLRP3 oligomerizes and,

through its NH₂-terminal PYD, interacts with the PYD of adaptor protein apoptosis-associated speck-like protein containing a COOH-terminus caspase activation and recruitment domain (ASC) (Fig. 2) (112, 130). This PYD-PYD interaction initiates polymerization of ASC into filamentous, insoluble structures that macroscopically distinguish the presence of the inflammasome as a large perinuclear speck (17). ASC is a scaffold protein that is necessary for recruitment of the effector enzyme pro-caspase-1 into the NLRP3 inflammasome (123, 130).

Initially named IL-1 β -converting enzyme for its recognized role in IL-1 β activation, caspase-1 is a cysteine proteinase synthesized as a zymogen (45). Although similar to other caspases, caspase-1 is not part of the apoptotic pathway, inasmuch as caspase-1-deficient mice remain sensitive to apoptotic stimuli and progress through normal development (72). Predominantly associated with the inflammasome and functioning to process cytokines, caspase-1 cleaves the inactive cytosolic pro-IL-1 β and pro-IL-18 into their respective active forms (45). Caspase-1 is also associated with a regulated form of cell death, termed pyroptosis (13). Morphologically and functionally distinct from apoptosis, which cleanly packages intracellular contents for immune cell phagocytosis, pyroptosis

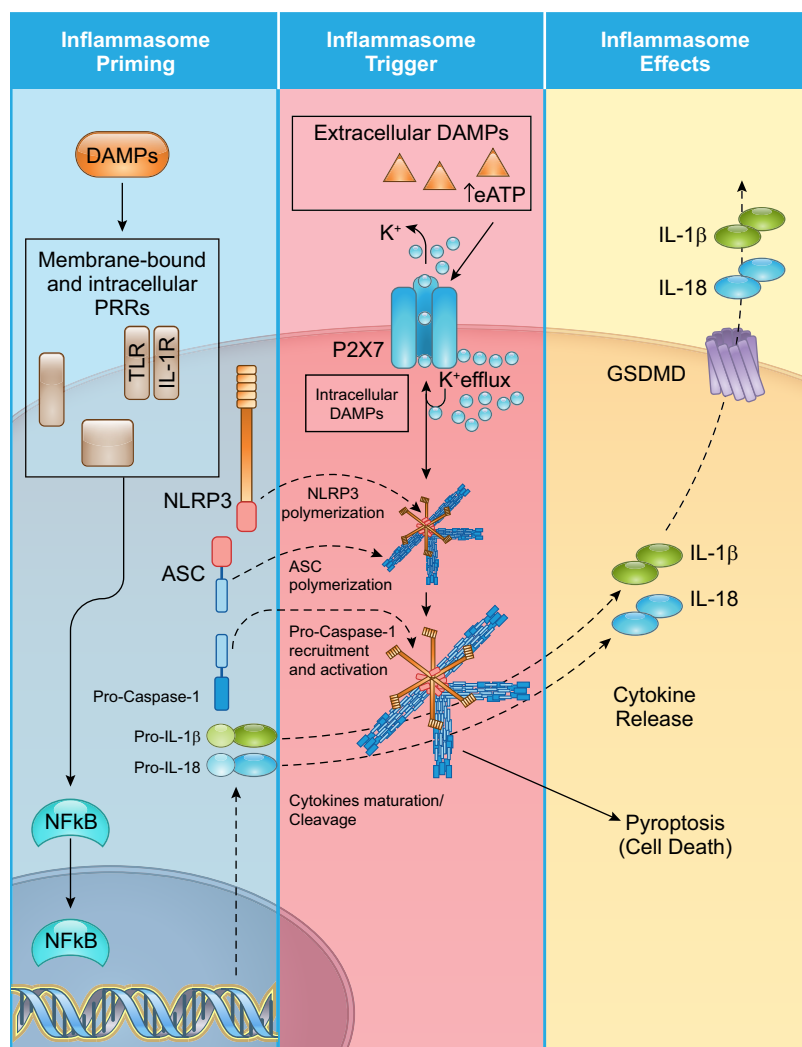


Fig. 2. Stages of NOD-like receptor protein 3 (NLRP3) inflammasome activation: priming, triggering, and downstream effects. NLRP3 inflammasome activation requires two independent steps. During tissue damage, the presence of damage-associated molecular patterns (DAMPs) stimulates pattern recognition receptors (PRRs) like Toll-like receptors (TLRs) or the IL-1 receptor, leading to translocation of NF- κ B into the nucleus. This event induces transcription of hundreds of pro-inflammatory genes, notably all components of the inflammasome (inflammasome priming). Sufficient translation of the inflammasome components NLRP3, apoptosis-associated speck-like protein containing a COOH-terminal caspase activation and recruitment domain (ASC), and pro-caspase-1 is a necessary step toward inflammasome formation but does not directly result in its activation. Extracellular DAMPs, like extracellular ATP (eATP), or intracellular DAMPs can induce NLRP3 activation (inflammasome trigger) through different mechanisms that involve K⁺ efflux. For example, eATP activates the purinergic P2X₇ receptor, resulting in K⁺ efflux and activation of NLRP3. Once active, NLRP3 oligomerizes into a platform for pyrin domain (PYD) interactions between itself and ASC. Subsequent polymerization of ASC into filamentous insoluble structures serves as a platform for pro-caspase-1 recruitment. Formation of the macromolecular structure is followed by autocatalytic activation of pro-caspase-1 into caspase-1 and cleavage of the pro-IL-1 β and pro-IL-18 into their active forms. Caspase-1-mediated cleavage of gasdermin-D (GSDMD), culminating in oligomerization of the NH₂-terminal fragment of GSDMD into a plasma membrane pore, mediates release of IL-1 β and IL-18 into the extracellular space for further autocrine, paracrine, and endocrine immune responses. Caspase-1 activity also mediates a form of regulated cell death termed pyroptosis.

features plasma membrane rupture and release of intracellular and proinflammatory contents (13). This inflammation-linked cell death is a driving factor of reperfusion injury (Figs. 2 and 3). NLRP3 can also serve as a platform for noncanonical activation on two other caspases, caspase-8 and caspase-11. However, it is not known whether these two caspases have a role in the response to tissue injury (57, 69).

NLRP3 INFLAMMASOME ACTIVATION CONTRIBUTES TO INFARCT SIZE

In the first report of inflammasome activation during AMI, Kawaguchi et al. (68) noted visible ASC aggregates in the human heart at autopsies. They subsequently showed smaller infarcts, reduced IL-1 β synthesis, and preservation of cardiac function in mice lacking ASC or caspase-1 (68). This is consistent with prior reports of the cardioprotective effect of caspase-1 deletion, preceding the notion of the inflammasome (53). Targeted deletion of caspase-1 reduces early mortality and left ventricular (LV) dilation after myocardial infarction (MI) (53, 105). Further evidence in support of a role of the NLRP3 inflammasome in AMI derived from gene silencing of NLRP3 in a different in vivo model of MI in the mouse by Mezzaroma et al. (94). These reports were followed by the work of Sandanger et al. (114), who found reduced infarct size in Nlrp3^{-/-} mice in an ex vivo Langendorff model of I/R.

Priming of the NLRP3 Inflammasome

Activation of the NLRP3 inflammasome involves two distinct and independent phases: inflammasome priming and triggering of NLRP3 (Fig. 2) (130, 131). NF- κ B drives transcription of hundreds of proinflammatory genes, including all the components of the NLRP3 inflammasome in leukocytes and cardiac resident cells, such as cardiomyocytes, fibroblasts, and endothelial cells (78, 123, 130). This step mediated by NF- κ B, termed priming, is necessary to produce a critical mass of

inflammasome components to suffice formation of the macromolecular complex (130, 131). The TLR signaling induced by DAMPs, including alarmins, and the activation of other cytokine receptors are the main factors that mediate the priming step (50, 130, 131).

Triggering of the NLRP3 Inflammasome

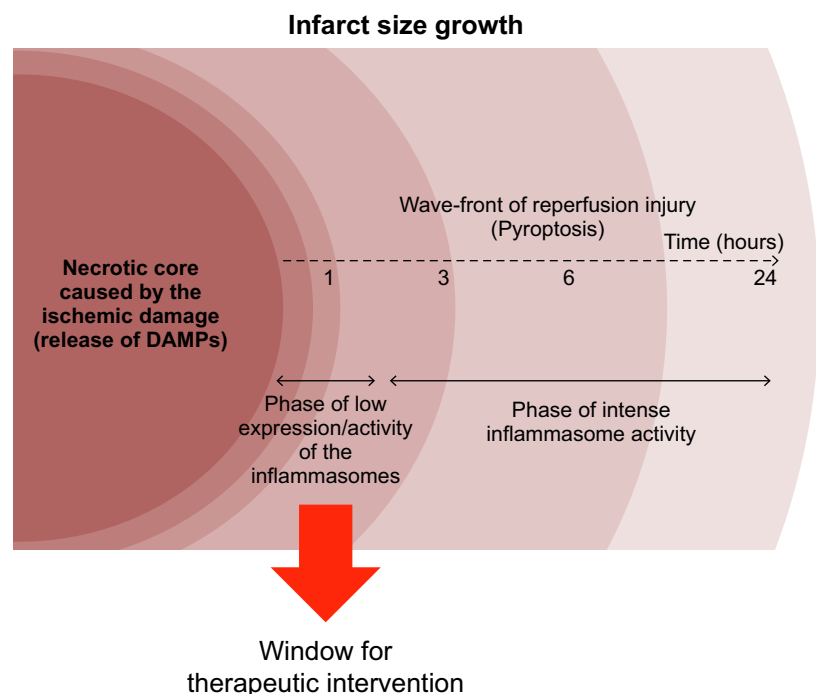
The presence of extracellular or intracellular triggers then leads to activation of NLRP3 (Fig. 2) (123). Many of these signals (see below) are associated with K⁺ efflux. Common trigger signals include extracellular ATP, reactive oxygen species (ROS), and impairment of the autophagy process, which has been shown to exacerbate the NLRP3 inflammasome-mediated response (150). When present in high extracellular concentration, ATP binds to the purinergic P2X₇ receptor, inducing K⁺ efflux and triggering the NLRP3 activation cascade (Fig. 2) (130).

In some instances, as in monocytes, the priming signal induced by lipopolysaccharide (LPS) is sufficient to induce release of IL-1 β (101). However, in the heart, the combination of the priming and triggering signals is necessary to elicit NLRP3 inflammasome activation (130, 131). The requirement for two signals broadens the therapeutic possibilities aimed at blocking inflammasome activation. The complexity of NLRP3 signaling and converging cellular processes continues to grow. Some of the mechanisms relevant to the sterile inflammatory response are described below.

ROLE OF THE INFLAMMASOME AND PYROPTOTIC CELL DEATH IN THE WAVEFRONT OF REPERFUSION INJURY

In the absence of priming, the concentration of inflammasome components in the heart is insufficient to respond to a trigger signal (131). This has been demonstrated using the mouse model of constitutively active mutant NLRP3, which

Fig. 3. Wavefront of reperfusion injury. Prolonged ischemia inevitably leads to death of cardiomyocytes mainly through necrosis. This initial area of necrotic cells represents only a part of the mature infarct measured hours after reperfusion. In the initial phases (<3 h) of reperfusion, activity of the inflammasome is negligible, but damage-associated molecular patterns (DAMPs) released by necrotic cells contribute to increase expression of inflammasome components. When levels of inflammasome proteins reach a threshold for activation, the high intensity of inflammasome activity becomes associated with a rapid growth of the infarct through pyroptosis, the inflammatory cell death mediated by the inflammasome. The first hours before the increase in this inflammasome activity represent a time window for successful therapeutic intervention.



fails to activate caspase-1 in the heart in the absence of priming (131).

I/R injury offers a combination of priming and triggering that activates the inflammasome pathway in the heart. We and others have shown that, after reperfusion, the size of the infarct increases in a time-dependent fashion (99, 127). Even if reperfusion reduces the total amount of myocardial necrosis, reperfusion fails to salvage all the myocardium that could be salvaged. Indeed, during the early phases of reperfusion, most of the infarct is represented by the necrotic core that is generated during ischemia plus a bordering portion that is part of the area at risk that is rescued from the hypoxic necrosis but has been exposed to sublethal injury and proinflammatory stimuli by DAMPs generated by the neighboring necrotic cells. Similar to the wavefront of ischemic injury during prolonged ischemia, this phenomenon represents a “wavefront of reperfusion injury,” whereby the size of the infarct continues to expand over the 3–6 h after reperfusion (Fig. 3) (109). The wavefront of reperfusion injury may, at least in part, be due to a propagation of cell damage, of an inflammatory nature, to cells that were previously harmed but not killed. Expression of NLRP3 and the inflammasome activation indeed increase over time after reperfusion, resulting in cell death (82, 127). In accordance with these dynamics, we have shown, in the mouse, a therapeutic intervention window of a few hours after reperfusion, characterized by a reduction in infarct size with inhibition of the NLRP3 inflammasome, that is lost if treatment is given 3 h after reperfusion (127). These results indicate that, in the mouse heart, a sustained NLRP3 inflammasome activation occurs between 1 and 3 h after reperfusion, and a therapeutic intervention before 3 h may improve the reperfusion outcome. As explained above, the mechanism by which the NLRP3 inflammasome induces cardiomyocyte death and infarct expansion is likely through pyroptosis and not through production of IL-1 β (94). Deletion or inhibition of the inflammasome components (NLRP3, ASC, or caspase-1) reduces infarct size, whereas IL-1 β blockade (see below) at the time of reperfusion fails to reduce infarct size (68, 84, 86, 113, 114, 127, 138). However, the mouse lacking IL-1 receptor type I (IL-1RI) or the mouse pretreated with an IL-1 blocker is protected, and this may be related to a further downregulation of the sensors of the inflammasome in the heart (133).

MECHANISMS OF REGULATION OF THE NLRP3 INFLAMMASOME

As described above, NLRP3 responds to a variety of different stimuli. The extensive research of the past few years has pointed out some key pathways and events that lead to the activation of NLRP3.

ROS and Mitochondrial Dysfunction

ROS, among the most common cellular byproducts produced during I/R injury, lead to oxidative stress and activation of the inflammasome cascade via multiple mechanisms (52, 108). Oxidative stress leads to an unfolded protein response that can cause detachment of thioredoxin from thioredoxin-interacting protein, which interacts with NLRP3 to promote activation of the inflammasome (82). siRNA against thioredoxin-interacting protein was shown to reduce inflammasome formation in the heart and reduce infarct size after I/R injury

(82). Mitochondria are responsible for cellular energy production and are actively involved in the induction of necrotic, apoptotic, and pyroptotic cell death (50). These organelles constitute one of the major sources of ROS after reperfusion, due to damage to the segment between complex III and cytochrome oxidase of the mitochondrial respiratory chain (22). ROS represent a potent trigger of NLRP3 and induce NLRP3-dependent lysosomal damage and inflammasome activation (62, 130). Another activator of NLRP3 of mitochondrial origin is cardiolipin, a phospholipid that is located in the inner membrane of the mitochondria and is responsible for several bioenergetic processes (65). In ROS-mediated mitochondrial dysfunction, exposed cardiolipin binds to and activates NLRP3 (65). Released cardiolipin, in combination with an ineffective autophagic clearance of damaged mitochondria, leads to NLRP3-mediated activation of caspase-1 and subsequent production of IL-1 β . Additionally, impaired mitochondrial fission can contribute to activation of NLRP3. This has been shown by deletion of dynamin-related protein 1, which leads to an increase in NLRP3-dependent caspase-1 activation and IL-1 β secretion (103).

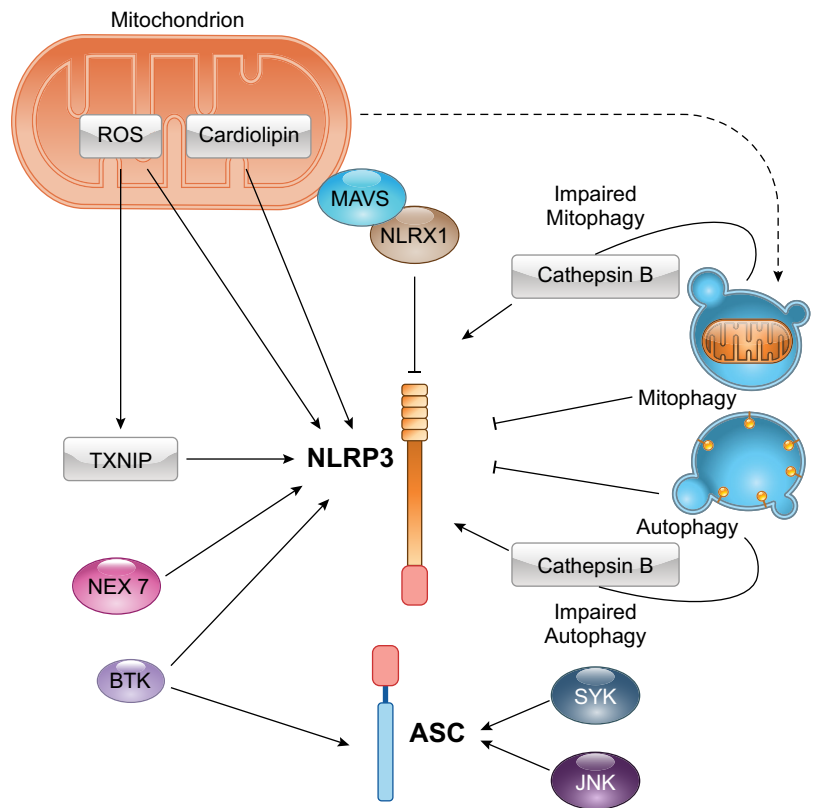
Autophagy

Autophagy is an additional important pathway that is linked to myocardial ischemia and inflammasome activation (122, 150). The primary function of autophagy is removal and recycling of misfolded or damaged proteins and organelles (97). Autophagy is, therefore, essential to maintain tissue homeostasis and becomes fundamental in limiting cellular damage (43, 97). Recent evidence has shown that autophagy is necessary to reduce myocardial damage after AMI (150). The autophagic process can limit activation of the NLRP3 inflammasome by removing damaged mitochondria (122). It has also been shown that physiological autophagy limits secretion of mature IL-1 β in macrophages in a mouse model of acute liver toxicity; however, this has not been shown in the heart (38, 124). The overall effect exerted on the inflammasome pathway by functional, physiological autophagy would be to minimize activation of the inflammasome and reduce secretion of cytokines. However, impairment of the autophagic pathways, as a consequence of extensive cell damage, would lead to ineffective or incomplete autophagy (and mitophagy), inducing activation of NLRP3 and exacerbation of myocardial damage.

Posttranslational Regulation of NLRP3 Inflammasome Activity

Activation of the inflammasome is also regulated by posttranslational modifications of its components (Fig. 4). In a model of stroke, Bruton's tyrosine kinase has been shown to interact with both NLRP3 and ASC, suggesting a potential role of Bruton's tyrosine kinase in the phosphorylation of ASC (64). Two other important kinases, spleen tyrosine kinase and c-Jun NH₂-terminal kinase, are involved in the response to oxidative stress and enhance ASC oligomerization through posttranslational modification of ASC (151). An additional kinase, NEK7, a member of the never in mitosis gene A-related kinase family, acts downstream of the P2X₇ receptor and binds to NLRP3, regulating its activation and oligomerization (61). NEK7 is highly expressed in heart muscle and might be a target for intervention in the heart (61).

Fig. 4. Molecular signaling of regulation of the NOD-like receptor protein 3 (NLRP3) inflammasome during tissue injury. Several stimuli regulate activation of NLRP3 [and apoptosis-associated speck-like protein containing a COOH-terminal caspase activation and recruitment domain (ASC)], controlling formation of the inflammasome. Mitochondria can stimulate NLRP3 activation through generation of reactive oxygen species (ROS). Although there are alternative sources of ROS, the mitochondrial origin of ROS is predominant during the reperfusion phase of injury. ROS also cause dissociation of thioredoxin-interacting protein (TXNIP) from thioredoxin. Free TXNIP mediates activation of NLRP3. Damaged mitochondria also release cardiolipin, a phospholipid present only in the inner mitochondrial membrane, but can be released in the cytoplasm and activate NLRP3 during damage. The mitochondrial antiviral signaling protein (MAVS) is also an important regulator of NLRP3 during viral infection and tissue damage. Interaction of MAVS with the nucleotide-binding oligomerization domain, leucine-rich repeat containing X1 (NLRX1) protein prevents NLRP3 activation. Damaged mitochondria enter autophagic vesicles for effective removal of the potentially dangerous organelle. Effective autophagy and mitophagy, leading to complete removal of defective proteins and intracellular organelles, prevent activity of the inflammasome. On the other hand, defective autophagy and mitophagy lead to the leak of the proteolytic enzyme cathepsin B inside the cytoplasm, leading to NLRP3 activation. NEK7, a member of the never in mitosis gene A (NIMA)-related kinases family, promotes NLRP3 activation after K^+ efflux. Bruton's tyrosine kinase (BTK) also activates the inflammasome by interacting with NLRP3 and ASC. Two other kinases, spleen tyrosine kinase (SYK) and c-jun NH₂-terminal kinase (JNK), enhance oligomerization of ASC.



Recently, a member of the NLR family has been found to act as a negative regulator of the inflammatory response during AMI. In an *in vitro* model of hypoxia-induced myocardial injury, NLRX1 has been found to inhibit NLRP3 activation mediated by mitochondrial antiviral signaling protein (121). Mitochondrial antiviral signaling protein is a mitochondrion-associated adaptor protein that is essential for recruitment of NLRP3 to the mitochondrial outer membrane (Fig. 4) (80, 121).

INFLAMMASOME-ASSOCIATED CYTOKINES

Inhibition of the NLRP3 inflammasome limits the inflammatory injury after myocardial I/R in the mouse. As described above, caspase-1 has a central role in the function of the inflammasome. This enzyme is fundamental for induction of pyroptotic cell death and is also necessary for processing and release of the inflammasome-associated cytokines IL-1 β and IL-18 (Fig. 5) (45, 46). The mechanism by which caspase-1 accomplishes these two functions is through cleavage of the protein gasdermin D (GSDMD) followed by oligomerization of the NH₂-terminal domain of GSDMD, to form a pore in the plasma membrane (36, 38, 61, 101). This pore disrupts the ionic gradients, causing Na⁺ and water influx, osmotic swelling, and membrane rupture (46, 119). Recent data hypothesized an intermediate phase between inflammasome activation and pyroptotic cell rupture, deemed "hyperactivated," where GSDMD pores permit IL-1 β (and IL-18) release in macrophages with activated inflammasomes and intact lipid bilayers (44, 74).

Interleukin-1 β

IL-1 β , the original member of the IL-1 family of cytokines, has been extensively studied because of its integral role in initiating and perpetuating physiological and pathological inflammation (31–33). IL-1 β is normally transcribed and translated in the cytosol at low basal levels in its inactive pro form; however, upon stimulation of TLRs or other cytokine receptors, transcription and translation are dramatically increased within 15 min (33). As previously reported, IL-1 β secretion in its active form requires the active form of caspase-1 (33). Therefore, the rate-limiting step in IL-1 β processing and secretion is inflammasome activation. Lacking a signal peptide for the classic endoplasmic reticulum-Golgi apparatus protein secretion pathway, IL-1 β secretion requires formation of a GSDMD pore (13, 44). Once released in the extracellular space, IL-1 β acts in an autocrine, paracrine, and endocrine fashion to initiate and sustain proinflammatory activity (33, 44).

The heterodimerization of two plasma membrane receptors, IL-1RI and IL-1 receptor accessory protein (IL-1RAcP), mediates signal transduction of the two isoforms of IL-1 (IL-1 β and IL-1 α) (16). This signaling involves interaction of the Toll-IL-1 receptor (TIR) domain of IL-1RI with the TIR domain of the myeloid differentiation factor 88 that activates a kinase-dependent signaling cascade (35). A second type II receptor, IL-1RII, is membrane bound but lacks the intracellular TIR domain and acts as a scavenger receptor (30, 35). The intracellular cascades [reviewed in detail elsewhere (123)] result in transcription of a multitude of chemokines, cytokines, and adhesion molecules that drive immune cell recruitment,

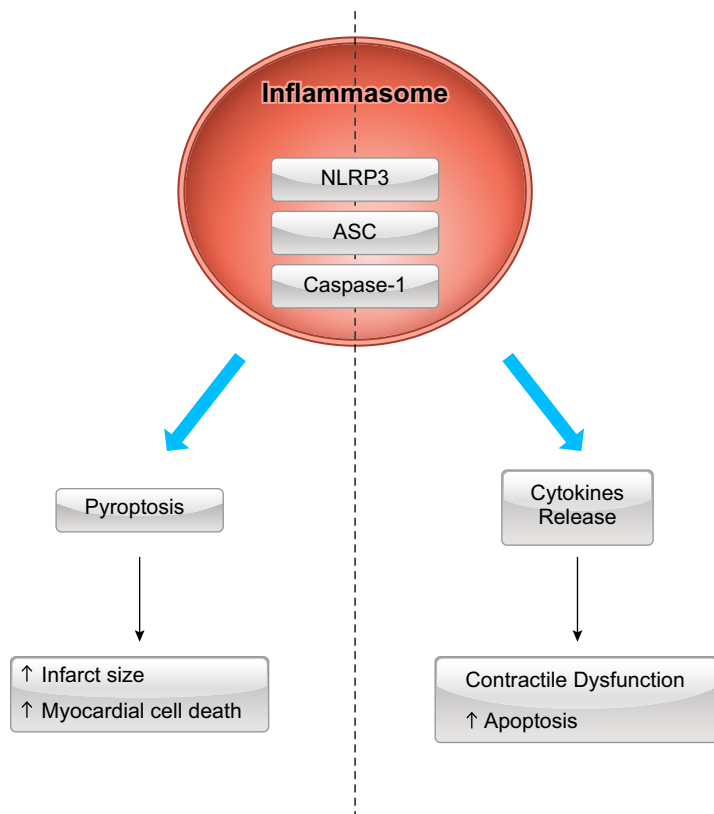


Fig. 5. Dual function of caspase-1 activation during myocardial infarction. Activation of the inflammasome leads to caspase-1 activation, which has two associated, but different, effects: 1) inflammatory cell death (pyroptosis), which fosters increased infarct size and myocardial cell death, and 2) release of cytokines IL-1 β and IL-18, which spur contractile dysfunction and regulated cell death through apoptosis. NLRP3, NOD-like receptor protein 3; ASC, apoptosis-associated speck-like protein containing a COOH-terminal caspase activation and recruitment domain.

activation, and differentiation (94). IL-1 signaling also alters intracellular protein expression, changing cellular function and metabolism (31). Sharing ~26% amino acid homology with IL-1 β is IL-1 receptor antagonist (IL-1Ra), an endogenous protein that tightly binds to and occupies IL-1RI, inhibits the binding of IL-1RAcP and subsequent receptor heterodimerization, and prevents the intracellular transduction of signals (130). Production of this high-affinity competitor is induced by several proinflammatory stimuli, including LPS, a complex molecule common on the wall of gram-negative bacteria that activates TLR4, and IL-1 β , presumably to balance a perpetual cycle of proinflammatory signaling (120, 126). Intracellular functions, specifically antiapoptotic, of IL-1Ra have also been noted in cardiomyocytes (144).

Interleukin-18

IL-18, also a member of the IL-1 family of cytokines, requires caspase-1-mediated cleavage to the active form (45). IL-18 is produced and stored in the cytoplasm, also in the absence of TLR or other proinflammatory stimulation, and is readily available for cleavage and secretion in case of inflammasome activation or by extracellular proteases in case of passive diffusion due to cell necrosis (102, 130). Receptor binding and signaling of IL-18 resemble receptor binding and signaling of IL-1, whereby a heterodimeric complex of IL-18R α and IL-18R β combine to evoke intracellular cascades, culminating in proinflammatory gene transcription (67, 102). A second IL-18 receptor, Na⁺-Cl⁻ cotransporter (NCC) was identified in 2015. NCC is a solute carrier symporter that is mainly present in the distal tubule of the glomeruli in the kidney (89). NCC becomes expressed in the atherosclerotic

plaques of apolipoprotein E-deficient mice and mediates IL-18 signaling in cytokine-primed macrophages (147). An endogenous circulating binding protein of IL-18 (IL-18BP) in healthy humans is normally present at levels 25–50 times higher than IL-18, with significantly higher affinity for membrane-bound IL-18R α (37).

Differences and Similarities Between IL-1 β and IL-18

The production and signaling of IL-1 β and IL-18 are similar, because both cytokines require caspase-1 and GSDMD for their secretion, bind a heterodimeric receptor of a similar structure, and share some intracellular downstream cascades involving myeloid differentiation factor 88 activation and convergence on NF- κ B (18, 45, 102). The specificity of the signal depends on the receptor and cell type; therefore, depending on the target, these two cytokines promote different biological effects (79). As previously mentioned, basal gene expression for IL-1 β in several cell types (e.g., blood mononuclear cells, hematopoietic cells, and endothelial cells) is extremely low (107). In contrast, the IL-18 precursor is present in blood monocytes from healthy subjects, unstimulated macrophages, and epithelial and endothelial cells (37). In addition, IL-1 induces fever and IL-18 does not. [IL-18 does not affect cyclooxygenase-2 or PGE₂ production (37, 79).] IL-1 (α or β) is active at concentrations severalfold lower than IL-18 (37). In addition, in some diseases, the activity of IL-1 and IL-18 is different. IL-1 is more involved in rheumatoid arthritis, whereas IL-18 is more involved in inflammatory bowel disease (34, 66). IL-18 is also involved in homeostasis. When fed a standard diet, mice lacking IL-18 develop obesity and hyperglycemia, a phenotype exacerbated by a high-fat diet (19, 100).

Finally, in the same cells, IL-1 and IL-18 can activate different responses: IL-1 β strongly induces NF- κ B, whereas IL-18 may promote a p38 MAPK-specific response (79).

IL-1 α , a Cytokine and an Alarmin

IL-1 α is also produced and stored in the cell (39). Different from IL-1 β and IL-18, IL-1 α translocates to the nucleus to influence transcription and is functionally active in its precursor form (14, 32). Therefore, pro-IL-1 α is capable of acting as an alarmin, initiating proinflammatory paracrine responses immediately after release from the cytosol after cellular necrosis (14, 36, 63), before IL-1 β becomes active and exerts its function (14, 36). IL-1 α is not recognized by caspase-1 and thus is not activated through the inflammasome (36), although it has been reported that active release of IL-1 α also necessitates inflammasome activation, likely through GSDM pore formation (14). The initial inflammatory signal that follows necrosis and recruitment of neutrophils has been shown to be due to the precursor of IL-1 α , whereas recruitment of macrophages to potentiate and maintain the inflammatory process was characteristic of IL-1 β (110).

CELL-SPECIFIC EFFECTS OF THE INFLAMMASOME AND ITS CYTOKINES ON AMI

Cell-Specific Effects of the Inflammasome

After AMI, ASC specks can be detected in most of the resident cells (endothelial cells, cardiomyocytes, and fibroblasts) and in cells infiltrating the infarct. In the subacute phase, the majority of the specks are observed in cells of the granulation tissue. However, during healing and maturation of the scar, as the infiltrate resolves, ASC positivity is detected mostly in isolated cardiomyocytes or fibroblasts (94, 130). The effects of NLRP3 inflammasome formation in the different cardiac resident cells are cell specific (125). IL-1 β production has been documented in cardiac fibroblasts and endothelial cells but not in cardiomyocytes, despite an increase in NLRP3 and caspase-1 activity in the latter (68, 82, 94, 114). Inflammasome and caspase-1 activation in cardiomyocytes, instead, induces loss of cell membrane integrity and death (94, 125).

Cell-Specific Effects of IL-1 β

After AMI, locally produced active IL-1 β also has regulatory and detrimental effects on cardiac resident and infiltrating cells. The initial release of active IL-1 β in the area of infarction creates homing signals for leukocytes and creates a "sticky" endothelium (48, 56). Abrupt chemokine release after ischemia creates a gradient that attracts leukocytes to the region (11, 51). Proinflammatory cytokines, including IL-1 α and IL-1 β , increase endothelial cell surface expression of adhesion molecules that bind to the recruited leukocytes and begin the process of leukocyte extravasation from the vessel into the injured area (52). IL-1 β is also capable of increasing chemokines, such as monocyte chemoattractant protein-1, responsible for mobilizing proinflammatory monocytes to the area (104). Resident cardiac fibroblasts are also important players in the post-AMI inflammatory response. In the initial phase after ischemic injury, IL-1 β has been shown to increase fibroblast expression of collagenases and inhibit transforming growth factor- β signaling, possibly to ensure that the injured matrix

area has been effectively broken down and cleared of additional damage signals before tissue reparative engines are fully engaged and fibroblast populations are converted to myofibroblasts (48, 115). Cardiomyocytes are also sensitive to IL-1 β . In fact, IL-1 β depresses contractility, in vitro and in vivo, via uncoupling of the L-type Ca²⁺ channel from the β -adrenergic receptor (81, 116), decreasing expression of phospholamban and the sarco(endo)plasmic reticulum Ca²⁺-ATPase and increasing local nitric oxide, which affects ATP production within the mitochondria (25). Moreover, IL-1 β induces apoptotic cell death of cardiomyocytes in the subacute and chronic phases after AMI (5, 132).

Cell-Specific Effects of IL-18

The cell-specific effects of IL-18 on myocardial cells post-AMI have been investigated to a lesser extent. IL-18 appears to induce angiogenesis but, at the same time, inhibits angiogenesis after ischemic damage (148). In vitro experiments using recombinant IL-18 proved that it induces apoptosis of cardiac endothelial cells in vitro (20). Cardiac fibroblasts exposed to IL-18 proliferate, produce collagen, and remodel the extracellular matrix (47). IL-18 depresses the contractility of cardiomyocytes in vitro and in vivo, and in an ex vivo model of hypoxia, IL-18 impairs the contractility of human cardiac strips (105).

PHARMACOLOGICAL INHIBITION OF THE NLRP3 INFLAMMASOME

Several NLRP3 inflammasome inhibitors have been identified; they include molecules already known and synthesized and newly designed compounds derived in attempts to increase specificity and successful clinical application (Tables 1 and 2).

Colchicine

Colchicine is one of the oldest anti-inflammatory drugs and, recently (2009), was found to inhibit NLRP3 activity by preventing its assembly and activation (87). Although historically used for treatment of Mediterranean fever, colchicine has been recently approved by the United States Food and Drug Administration for treatment of gout (27). Besides its effect on microtubule polymerization, colchicine inhibits NLRP3 activation, preventing the pore formation induced by the P2X₇ receptor and, therefore, limiting K⁺ efflux (87). Because of the integral role of microtubules in providing a platform for mediating intracellular trafficking and the known translocation and aggregation of NLRP3, ASC, and caspase-1, colchicine may inhibit the inflammasome by decreasing aggregation of its components. Colchicine suppressed the colocalization of ASC to NLRP3 and decreased amounts of mature IL-1 β in the peritoneal cavity of mice during acute gout (95). The novel understanding of the mechanism of action has opened the way to the investigation in the field of cardiology (27, 91). In a nonreperfused AMI mouse model, a high dose (0.1 mg·kg⁻¹·day⁻¹) of colchicine administered for 7 days significantly inhibited the increase in NLRP3 mRNA and caspase-1 activity at 24 h post-MI, inhibited expansion of LV scar size at 1 wk, attenuated ventricular remodeling at 7 days and 4 wk after MI, and improved 7-day survival (54). These findings show that colchicine inhibits the inflammasome pathway in the in vivo AMI model. Similarly, in a model of reperfused AMI

Table 1. *NLRP3 inflammasome inhibitors in experimental AMI*

Name(s)	Method(s)	Finding(s)	Reference(s)
Colchicine	0.1 mg/kg ip daily for 7 days to the mouse starting before ischemia in the nonreperfused AMI model	Smaller scar, reduced adverse remodeling, and heart failure and improved survival	8, 54
<i>N</i> -[(7 <i>S</i>)-1,2,3,10-tetramethoxy-9-oxo-6,7-dihydro-5 <i>H</i> -benzo[<i>a</i>]heptalen-7yl] acetamide Glyburide derivative; 16673-34-0	0.4 mg/kg ip 25 min before reperfusion in a model of transient I/R 100 mg/kg ip before surgery in a reperfused AMI model 100 mg/kg ip after ischemia in a model of nonreperfused AMI	Reduced infarct size and fibrosis in the chronic setting Reduced infarct size and improved LV function Improved cardiac remodeling and LV dysfunction	84, 86, 127
5-chloro-2-methoxy- <i>N</i> -[2-(4-sulfamoyl-phenyl) ethyl] benzamide MCC950	100 mg/kg ip up to 60 min after reperfusion 3–6 mg/kg iv 15 min before reperfusion and again after 24 h and following 5 days in the pig balloon angioplasty inflation ischemia model	Reduced infarct size at 24 h Reduced infarct size; improved cardiac remodeling and prevention of LV dysfunction	138
<i>N</i> -[(1,2,3,5,6,7-hexahydro- <i>s</i> -indacen-4-yl)carbamoyl]-4-(2-hydroxypropan-2-yl)furan-2-sulfonamide; <i>N</i> -[[1,2,3,5,6,7-hexahydro- <i>S</i> -indacen-4-yl]amino] carbonyl]-4-(1-hydroxy-1-methylethyl)-2-furansulfonamide BAY 11-7082	Treatment (dose not specified) before ischemia or before reperfusion in I/R models in mouse and rat	Reduced infarct size; preserved cardiac function	71
3-[(4-methyl-phenyl)-sulfonyl]-(2 <i>E</i>)-propenenitrile INF4E	50 μ M in an ex vivo myocardial ischemia model 20 min before ischemia 6–600 mg/kg ip before, or up to 60 min after, reperfusion in the mouse surgical coronary artery ligation model	Reduced infarct size at 60 min Reduced infarct size	88 128

AMI, acute myocardial infarction; I/R, ischemia-reperfusion; LV, left ventricular; NLRP3, NOD-like receptor protein 3.

induced by 45 min of transient ischemia, a single dose (0.4 mg/kg ip) of colchicine administered 25 min before reperfusion significantly reduced infarct size, troponin T levels, and inflammatory markers 24 h post-MI and significantly reduced fibrosis 10 wk after AMI (8). Recently, in a clinical trial, patients presenting with ST-segment elevation MI (STEMI) undergoing primary percutaneous coronary intervention were treated with colchicine (initial dose of 1.5 mg followed 1 h later by 0.5 mg and 0.5 mg twice daily for 5 days). The clinical trial results revealed a reduction in infarct size with colchicine, measured as area under the curve for creatine kinase myocardial band (28).

Glyburide Derivative

Glyburide, a sulfonyleurea used to increase pancreatic β -cell insulin secretion to treat type 2 diabetes mellitus, was shown to inhibit IL-1 β release in vitro by acting upstream of NLRP3 and downstream of the P2X₇ receptor (76). The use of glyburide at

a dose proven to inhibit the NLRP3 inflammasome would, however, cause lethal hypoglycemia in the mouse (84). A derivative of glyburide, 4-[2-(5-chloro-2-methoxybenzamido)ethyl]benzenesulfamide, which lacks the moiety responsible for inducing insulin secretion, was developed to maintain the inflammasome inhibitory effect and also to abolish the hypoglycemic effect of glyburide (84). This compound, known also as 16673-34-0, has been studied in a murine model of acute transient (30 or 75 min) coronary ligation and permanent ligation as well as in a model of cardiotoxicity induced by a single dose (10 mg/kg) of doxorubicin (86). Single-dose (100 mg/kg) glyburide analog treatment at reperfusion significantly decreased macromolecular aggregation of ASC, caspase-1 activity, plasma levels of cardiac troponin I, and infarct size, without effects on glycemia (84). Furthermore, when administered within 1 h of reperfusion injury, 16673-34-0 is as effective as when it is given at the time of reperfusion (127). This finding is particularly important, because it confirms the

Table 2. *Clinical trials of colchicine and IL-1 blockers in AMI and heart failure*

Disease	Clinical Trial Name	Study Design and Drug Regimen	Main Finding(s)	Reference(s)
ST-segment elevation AMI (<i>n</i> = 151)	Anti-Inflammatory Treatment With Colchicine in AMI	Randomization 1:1 Colchicine or placebo Loading dose of 2 mg (1.5 mg initially followed by 0.5 mg 1 h later) and continuing with 0.5 mg twice daily, or placebo, for 5 days	Reduced infarct size (cardiac magnetic resonance and cardiac biomarkers); reduced peak CRP	28
ST-segment elevation AMI (<i>n</i> = 10) (<i>n</i> = 30)	VCU-ART VCU-ART2	Randomization 1:1 Anakinra or placebo Anakinra 100 mg once daily for 14 days	Reduced peak CRP; trend toward reduced incidence of heart failure at 3 mo and at long-term followup with anakinra	2, 3, 6
Non-ST-segment elevation AMI (<i>n</i> = 182)	MRC-ILA Heart Study	Randomization 1:1 Anakinra or placebo Anakinra 100 mg once daily for 14 days	Reduced peak CRP; no differences in major adverse cardiac events at 30 days and 3 mo, but more events after 6 mo in the anakinra-treated group	98

AMI, acute myocardial infarction; CRP, C-reactive protein.

timing of activation of NLRP3 after I/R in AMI. Drugs that necessitate prompt administration after reperfusion are at higher risk of failure during testing in the clinical setting (136). Therefore, identification of a therapeutic target that can be blocked during an extended time window of intervention may lead to increased success during the clinical translation phase. After permanent ligation in the mouse, daily treatment with 16673-34-0 (100 mg/kg) for 7 days significantly preserved LV fractional shortening and LV end-diastolic and end-systolic diameters without affecting infarct size, indicating that some of the benefits for cardiac function are independent of infarct reduction, possibly due to reduced IL-1 β and IL-18 cytokine secretion (see below) (86). In the cardiotoxicity model, 16673-34-0 reduced myocardial fibrosis and preserved LV fractional shortening (84). The mechanism of action seems directed toward NLRP3 oligomerization downstream of activation, since 16673-34-0 inhibits macrophages with constitutively active mutant NLRP3 (86).

Recently, a compound derived from 16673-34-0, termed JC124, was tested in a setting of longer-term (75 min) ischemia; it was given after reperfusion and significantly reduced infarct size, indicating benefits also after more dramatic ischemic damage (55).

MCC950

A potent inhibitor of NLRP3, MCC950, is part of a class of drugs previously shown to inhibit NLRP3-induced ASC oligomerization, preventing formation of the specks (24, 75). MCC950 reduced IL-1 β production in vivo when given 1 h before LPS treatment and blocked ASC oligomerization in vitro without inhibiting NLRP3 ATPase activity (24). MCC950 has been shown to be effective also in a mouse model of experimental autoimmune encephalomyelitis in which NLRP3 is required for development of the disease (58). Administration of MCC950 suppressed T cell responses and attenuated the severity of the disease (58). Additionally, MCC950 has been effective in treating mice displaying Muckle-Wells syndrome, a cryopyrin-associated periodic syndrome. These mice, carrying a gain-of-function mutation of *Nlrp3*, are contradistinguished by neonatal mortality due to high IL-1 β and IL-18 plasma levels. Treatment for 28 days with MCC950 rescued these mice with constitutively active mutant NLRP3 (24).

In a porcine model of 75-min transluminal coronary artery balloon occlusion, MCC950 treatment (6 or 3 mg/kg) 15 min before ischemia and once daily after ischemia decreased infarct size, preserved LV ejection fraction, decreased myocardial IL-1 β levels, and attenuated myocardial neutrophil influx, validating the therapeutic target in the large-animal model (138).

BAY 11-7082

BAY 11-7082, previously known to inhibit NF- κ B, has been shown to directly inhibit NLRP3 ATPase activity in response to NLRP3-specific stimuli in vitro (71). In a rat model of I/R, pretreatment with BAY 11-7082 (130 μ g/kg) 30 min before reperfusion significantly reduced infarct size and cardiac fibrosis and improved LV fractional shortening (71).

INF4E

Acrylamide derivatives were developed by an Italian cohort as covalent inhibitors of NLRP3 and its ATPase activity required for inflammasome activation (88). An ex vivo model of I/R including pretreatment with the NLRP3 inhibitor ethyl-2-[[[(2-chlorophenyl)hydroxy]methyl]acrylate (INF4E) showed a significant reduction of infarct size and an improvement in LV developed pressure 60 min after ischemia (88).

Dapansutrile/OLT1177

Perhaps the NLRP3 inhibitor most advanced into the clinical testing realm is dapansutrile (or OLT1177). Preclinical models of 30 min of transient coronary ligation followed by OLT1177 administration (60 mg/kg) within 60 min at reperfusion in the mouse have shown significant reduction of infarct size, reduction of plasma cardiac troponin I, and preservation of LV fractional shortening (128). OLT1177 was tested as an orally active β -sulfonyl nitrile molecule in humans, and its safety has been shown at oral doses up to 1,000 mg/day for 8 days, with no clinical, hematological, or organ toxicities (85). Marchetti and colleagues (85) described an effect of OLT1177 on NLRP3 ATPase activity, leading to decreased NLRP-ASC oligomerization, but no effect on NLRC4 or AIM2 inflammasomes, K⁺ efflux, gene expression, or synthesis of IL-1 β precursor or on other kinases on a broad-range screen. The potential for OLT1177 as a treatment for acute gouty arthritis and osteoarthritis is under investigation in a phase II clinical trial (136a, 136b).

BLOCKADE OF IL-1 α AND IL-1 β IN AMI

The benefit of an anti-inflammatory strategy aimed at the inflammasome appears to extend beyond inhibition of inflammasome formation and to be, at least in part, related to reduced synthesis and secretion of proinflammatory cytokines.

Genetic deletion or pharmacological inhibition of IL-1 activity, via direct antibody neutralization/modulation of IL-1 β or IL-1 α , IL-1 receptor antagonism, or IL-1 β /IL-1 α sequestration via a chimeric protein made by the ectodomains of IL-1RI and IL-1RAcP (IL-1Trap), has been shown to lead to significantly reduced cardiomyocyte apoptosis and prevent LV dilation and systolic dysfunction (142, 143). One of the first reports of the use of anakinra, a recombinant IL-1Ra, given at different doses in an experimental AMI in mice and in rats, reduced the adverse remodeling process and myocardial apoptotic rate and preserved LV function (5). These data were supported by another study in which IL-1R1^{-/-} mice exhibited, after I/R injury, an attenuated proinflammatory and profibrotic response followed by a reduction of infarcted area and an attenuation of adverse remodeling of the LV (4). As shown in an elegant study from Sager et al. (113) using parabiotic bone marrow transplantation, release of IL-1 β after AMI fosters mobilization of inflammatory cells from the hematopoietic system, leading to granulation tissue and scar formation in the heart after AMI. Moreover, neutralization of the systemically released IL-1 β with an anti-IL-1 β treatment lowered monocyte and neutrophil infiltration and ameliorated adverse ventricular remodeling (113). As previously described, IL-1Ra prevents binding of both IL-1 α and IL-1 β to IL-1RI, making it impossible to distinguish the role of the two isoforms (32).

Anakinra is approved by the United States Food and Drug Administration for the treatment of rheumatoid arthritis and cryopyrin-associated periodic syndromes, diseases driven by enhanced IL-1 activity (73). Pilot clinical trials completed for the use of anakinra (100 mg sc daily) in patients with reperfusion STEMI and primary percutaneous coronary intervention ($n = 40$) noted that IL-1 blockade was safe, significantly limited the C-reactive protein (CRP) increase at 72 h, and lowered the rate of new onset heart failure after STEMI (Table 2) (2, 3, 6). The VCU-ART3 trial evaluating a higher dose of anakinra (100 mg twice daily) versus the standard dose (100 mg daily) versus placebo is ongoing (136c).

In the phase II clinical trial MRC-ILA heart study involving 182 patients with non-STEMI who were treated with anakinra (100 mg daily for 14 days), a similar reduction in the acute inflammatory response was observed (98). At 30 days and 3 mo, there was no change in clinical events, but by 12 mo, ischemic events were significantly increased in the anakinra-compared with placebo-treated patients.

Phase II clinical trials in patients with heart failure also support a beneficial role of IL-1 blockade with anakinra (Table 2) (139–141).

In additional studies conducted in mice after AMI, selective blockade of IL-1 β was explored using a monoclonal IgG2a antibody directed against IL-1 β , a mouse equivalent of canakinumab (7, 129). The antibody, given at reperfusion and repeated 1 wk later improved long-term survival after AMI; it inhibited myocardial apoptosis, limiting ventricular enlargement (7), independent of infarct size reduction (113, 132). In a recent study conducted on mice, administration of a polyclonal antibody against IL-1 α after I/R injury, on the other hand, limited inflammasome activation, decreased infarct size, and preserved LV function (90). This points to a different role and timing of action of the two isoforms of IL-1, IL-1 α and IL-1 β . In a recent preclinical study in diabetic rats, administration of gevokizumab, a monoclonal antibody against IL-1 β , limited the progression of HF after MI and reduced oxidative stress, ventricular remodeling, scar size, and coronary endothelium-dependent relaxation, independent of infarct size (60, 134).

There have been no clinical trials of selective IL-1 β or IL-1 α blockade in AMI. In a randomized, double-blind study involving 10,061 patients who had experienced AMI >30 days before enrollment and had serum CRP ≥ 2 mg/l, canakinumab, a human monoclonal antibody that inhibits IL-1 β , given subcutaneously once every 3 mo, decreased the incidence of nonfatal MI, nonfatal stroke, or cardiovascular death at a median followup of 3.7 yr. Administration of 150 mg of canakinumab every 3 mo significantly reduced the incidence of the primary end point (cardiac death, nonfatal MI, and nonfatal stroke) with a hazard ratio of 0.85. This outcome was largely due to a 24% reduction in MI. Interestingly, there was also a 36% reduction in urgent revascularization and a 37% reduction in cardiac arrest (1). In the patients who had a high-sensitivity CRP of <2.0 mg/l within 3 mo after the first dose of canakinumab, incidence of the primary end point was significantly decreased over time, whereas patients who had a high-sensitivity CRP of >2.0 mg/l did not benefit from the treatment. This signifies the importance of identifying specific responders to the anti-inflammatory intervention within the heterogeneous group of patients. Additionally, the decreased incidence of cardiovascular events was independent of lipid levels (111).

The effects on AMI-specific outcomes or HF have not been reported. A small single-center substudy of the Canakinumab Anti-Inflammatory Thrombosis Outcome Study in patients with systolic heart failure showed an improvement in peak aerobic exercise capacity in patients treated with canakinumab (135).

BLOCKADE OF IL-18 IN AMI

The role and blockade of IL-18 in myocardial ischemia and MI are less established. IL-18 expression and plasma levels increase after reperfusion in animal models, with myocardial levels peaking at 3 h after reperfusion and serum levels peaking at 6 h (145). Treatment with an IL-18-neutralizing antibody 1 h before I/R significantly decreased infarct size (145). The role of IL-18 has also been investigated in I/R injury in an experimental heart transplantation model. Using syngeneic heterotopic heart transplantation after myocardial ischemia, Gu et al. (59) explored the cardioprotective effects of IL-18BP that prevented IL-18 from binding its receptor. Pretreatment with IL-18BP before ischemia increased graft survival, reduced myocardial damage and leukocyte infiltration, and lowered expression of proinflammatory cytokines (59).

Clinical trials have been completed or are underway to prove the safety of intravenous infusion of GSK1070806, a humanized monoclonal antibody to IL-18, in patients with diabetes mellitus or inflammatory bowel disease (92, 96). A separate recombinant human IL-18BP (tadekinig alfa) phase III trial is underway in patients with NLRP4 macrophage activation syndrome (NLRP4-MAS) mutation or X-linked inhibitor of apoptosis deficiency (136d).

CONCLUSIONS

Myocardial I/R induces a sterile injury and inflammatory response, characterized by the priming and triggering of the NLRP3 inflammasome by locally released DAMPs. Strategies inhibiting the priming, triggering, or activity of the inflammasome or blockade of the inflammasome-related cytokines IL-1 β and IL-18 have shown beneficial effects in experimental models of AMI in animals (118, 134). None of the inflammasome inhibitors are approved for clinical use in myocardial I/R syndromes. IL-1 blockers have shown promising results in small studies of patients with STEMI and heart failure (anakinra) and in a large secondary prevention study in patients with prior AMI (canakinumab). NLRP3 inflammasome inhibitors are under development in phase I–II clinical programs (136a, 136b).

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AUTHOR CONTRIBUTIONS

S.T., A.G.M., Z.S.C., and A.A. drafted manuscript; S.T., A.G.M., Z.S.C., and A.A. edited and revised manuscript; S.T., A.G.M., Z.S.C., and A.A. approved final version of manuscript; A.G.M. and Z.S.C. prepared figures.

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